March 2013 Epidemics and AIDS Update

1. HIV and Gay Media: The Vanishing Virus
2. Portable Device Detects HIV with Push of a Button
3. New York City Mayor Bloomberg Pledges $100M To Global Polio Eradication Initiative
4. Haiti Launches $2.2B, 10-Year Plan To Eradicate Cholera
5. Poor Sanitation In Africa Causing Hundreds Of Thousands Of Deaths, WaterAid Report Says
7. MIMR researchers find a protein link to STI susceptibility
8. Wolf in sheep's clothing: Uncovering how deadly bacteria trick the immune system
9. Strains of antibiotic-resistant 'Staph' bacteria show seasonal preference; children at higher risk in summer
10. Mayo Clinic finds steroids may shorten hospital stay for pneumonia patients
11. Mutation altering stability of surface molecule in acid enables H5N1 infection of mammals
12. 'Defective' virus surprisingly plays major role in spread of disease, UCLA life scientists report
13. Viruses Can Have Immune Systems: A Pirate Phage Commandeers the Immune System of Bacteria
14. Important Control Mechanism Behind Autoimmune Diseases Discovered
15. Three Overstretched DNA Structures Confirmed
16. New Method for Researching Understudied Malaria-Spreading Mosquitoes
17. Case report of a 'functional' HIV cure in a child *****
18. Dangerous TB Patient Detained on U.S. Border
19. Strategy To Prevent HIV In Newborns Sparks Enthusiasm And Skepticism
20. Macrophages allow entry of HIV in the urethra
21. Is the HIV 'functional cure' the breakthrough it seems?
22. Reports of 'HIV cure' are premature
23. HIV Trial Yields Disappointing Results
24. UB Invention Leads to Discovery of Novel Pathway for TB Vaccine
25. Women's Low Adherence To Daily-Dose Products In HIV Prevention Trial Suggest Different Approach Needed, Researchers Say
26. Media Examine Reaction To, Potential Implications Of 'Functional Cure' Of Infant Born With HIV
27. Prevention Remains Best Method To Stop Mother-To-Child HIV Transmission
28. Green tea extract interferes with the formation of amyloid plaques in Alzheimer's disease
29. Very early antiretroviral treatment limits the size of HIV reservoir
30. Monthly injectable drug offers 100% protection against HIV in monkeys – could be dosed every three months
31. Consistent condom use in anal sex stops 70% of HIV infections, study finds, but intermittent use has no effect
33. High Humidity Leads to Loss of Infectious Influenza Virus from Simulated Coughs
34. Using Routine Surveillance Data to Estimate the Epidemic Potential of Emerging Zoonoses: Application to the Emergence of US Swine Origin Influenza A H3N2v Virus
35. Macrophage-expressed IFN-β Contributes to Apoptotic Alveolar Epithelial Cell Injury in Severe Influenza Virus Pneumonia
36. Infections With 'Nightmare Bacteria' Are On The Rise In U.S. Hospitals
37. Human Y Chromosome Much Older Than Previously Thought
38. AIDS Journal Publishes Findings of Two Important Studies in March 2013 Issue
39. How Do Bacteria Clog Medical Devices? Very Quickly
40. 'Defective' Virus Surprisingly Plays Major Role in Spread of Disease
41. Order in the Chaos of a Cell Membrane
42. Deadly Bacteria That Resist Strongest Drugs Are Spreading
43. Patients Face More Lethal Infections from CRE
44. Dosing of key TB drug rifampicin could go higher
45. New pro-drug tenofovir alafenamide appears equally effective but better tolerated
46. Can people with resistant HIV omit NRTIs when switching from a failing regimen?
47. Long-term efavirenz linked to worse neurocognitive function in US CHARTER group
48. HIV exploits a human cyt Circuitry of Cells Involved in Immunity, Autoimmune Diseases Exposed: Connections Point to Interplay Between Salt and Genetic Factors
49. okine in semen to promote its own transmission
50. Transmission of Resistant HIV Steady
51. HIV 'Cure' in Toddler Offers 'Global Hope'
52. HIV Linked to Higher Chance of Heart Attack
53. A new hypothesis has been formulated on why bacteria are becoming increasingly more resistant to antibiotics
54. Behind the miracle child a broken system lurks NIH study sheds light on role of climate in influenza transmission
56. Michigan's HIV Testing Scandal
57. Putting the Nail in the Coffin of Condom-Only HIV Prevention
58. **Link between Violence and HIV Must Be Made Explicit, Say African Ministers**
59. **More MSM with HIV Aware of their Infection**
60. **Dually Active Antiretroviral Therapy Protects Against Hepatitis B Infection**
61. **U.N. Warns Of Little Progress Curbing Child Marriage Rates; Human Rights Watch Report Shows Practice Widespread In South Sudan**
62. **WHO Announces 8th Death From Novel Coronavirus**
63. **A*STAR Scientists Discover "Switch" Critical to Wound Healing**
64. New Study Validates Longevity Pathway: Findings Identify Universal Mechanism for Activating Anti-Aging Pathway
65. **Bee Venom Destroys HIV and Spares Surrounding Cells**
66. **Drug To Treat Leishmaniasis Fails In 20% Of Patients, Study Shows**
67. **Listen To Women's Needs When Designing HIV Prevention Strategies**
68. **Evolution in the antibody factory**
69. **Study: Antibiotics are unique assassins**
70. **No increase in risk of death for patients with well-controlled HIV, reports AIDS journal**
71. **Fatty Acids Could Lead to Flu Drug**
72. **Virus and Genes Involved in Causation of Schizophrenia**
73. **High Viral Load, HBeAg Positivity Increased Risk for Mother-to-Infant HBV Transmission**
75. **Haiti Fighting Cholera By Recycling Human Waste Into Fertilizer**
76. **Preventing HIV infection with anti-HIV drugs in people at risk is cost-effective**
77. **Some bacteria may protect against disease caused by stomach infection**
78. **Discovery May Explain How Prion Diseases Spread Between Different Types of Animals**
79. **Happy Birthday, Dr Snow**
80. **MRSA in the Groin of HIV Patients Ups Infection Risk**
81. **Rapid hearing loss may be a symptom of rare Creutzfeldt-Jakob Disease**
82. **Immune finding aids quest for vaccines to beat tropical infections**
83. **Current HIV Screening Guidelines Are Too Conservative**
84. **New Sensor Developed for Methylated DNA**
85. **Post-Treatment HIV-1 Controllers with a Long-Term Virological Remission after the Interruption of Early Initiated Antiretroviral Therapy ANRS VISCONTI Study**
86. Are we underestimating the proportion of virally-suppressed patients in the US?
87. **Plan to Fight Deadly TB Strain Advances in India**
88. **Bill Gates Says Capitalism Funnels More Resources Into Minor Ailments Than Infectious Diseases**
89. **Nigerian Polio Vaccinators Face Challenges But Aim For Eradication By 2018**
90. **More parents say they won't vaccinate daughters against HPV, researchers find**
91. **Probiotics Reduce Stress-Induced Intestinal Flare-Ups, Study Finds**
92. **Swarm Intelligence: New Collective Properties of Swarm Dynamics Uncovered**
93. **Synthetic Peptide Fools Immune System**
94. **Circular RNA Surprise**
95. **Natural STD Protection for Women?**
96. **Prion-like Proteins Cause Disease**
97. **Bedeviled by Dengue**
98. **Going viral**
99. **Immune to Failure**
100. **Salt at Fault?**
101. **Novel Virus Entry Portal Found**
102. **High Maternal Viral Load Key to Vertical Transmission of Hepatitis B**
103. **Mozambique Completes First HIV Vaccine Clinical Trial**
104. **HIV Treatment Should Be Started Earlier For Most, But Challenges Remain**
105. **Philippines Government Must Support Reproductive Health Law**
106. **Sex between monogamous heterosexuals rarely source of hepatitis C infection**
107. **We Are Still at Risk of the Plague, New Study Says; Historical Review Provides Lessons for the Control of the 'Black Death'
February 27th, 2013

**HIV and Gay Media: The Vanishing Virus**

The turning point could be traced to August of 1998. It was the month that, for the first time in well over a decade, the Bay Area Reporter did not have a single AIDS obituary submitted for publication. The promise of protease inhibitor medications had been realized, and it felt for many that our long community nightmare was coming to a close.

The milestone in the life of San Francisco’s LGBT newspaper was celebrated around the country and became a media story unto itself. “AIDS Deaths Take Holiday,” trumpeted the Pittsburgh Post-Gazette. “For Once, No AIDS,” said the Wilmington Morning Star. The headline in the Spokesman Review assured us that “No News is Good News.” The Bay Area Reporter’s own front page carried two words in enormous type: “No Obits.”

That could be seen as the moment in which coverage of HIV in gay media began to fade.

Today, the LGBT community is celebrating other milestones with joyful regularity. The right to serve openly in the military. Marriage. Growing acceptance and political muscle.

HIV/AIDS has largely moved off the front page and out of public consciousness. Despite newsworthy data such as increased HIV transmission among gay men and the ongoing slaughter of gay black men in particular, those stories feel stale. It has all been said so many times before. Even new storylines, such as Pre- and Post-Exposure Prophylaxis, cure research advocacy, and tools on the horizon such as rectal microbicides, it’s become harder to capture the imagination or interest of the gay community. When new data was reported recently showing that half of the 20-year-old gay men today will have HIV by the time they’re 50 (and if they’re black, that figure rises to a whopping 70 percent), the news barely rated a tweet or newspaper item.

What, then, is the responsibility of LGBT media in this climate of rising infection rates and a bored readership? Are they simply reflecting the community’s waning interest, or do they have a responsibility to keep HIV in the headlines, to serve as advocates for better public awareness?

I was just in the perfect place to ask these questions: The 2013 LGBT Media Journalists Convening, held in Philadelphia and sponsored by the National Lesbian and Gay Journalists Association. About 100
media professionals, including a healthy dose of bloggers like myself, attended the event, which educates LGBT journalists on various issues so they might report on them with more authority. Those issues this year were transgenders, immigration, aging, labor, and international rights.

The absence of HIV/AIDS wasn’t lost on me, I assure you (AIDS activists called them out about this in real time in the event’s Twitter feed at #LGBTmedia13) and it became the topic of my interviews with various people in attendance. Their very personal answers – and undeniable passion for the cause of HIV in many cases – sure made it a little easier to understand the tough choices they are making every day. I will be very interested in your reaction.

Aside from my griping over HIV coverage, it really was terrific to be in the company of a lot of dedicated journalists, and I appreciate very much the work done to mount the event, including the contributions of Bil Browning of The Bilerico Project (pictured with me above, at right).

Is sparse HIV coverage just a sign of the times? Is it progress? And what can we do to increase visibility again?

The journalists in my video provide some answers, but I especially liked the observation by gay political activist David Mixner, who reminded me that coming out, whether as gay men or as someone living with HIV, is the greatest tool in fighting stigma and helping people see the importance of the issue. I’m glad I have some company in the poz blogosphere, but we can always use more voices. Anyone who has the ability to share their story, online or across the dinner table, can make an awesome contribution.

Meanwhile, I’m going to keep nudging my LGBT media colleagues, and I encourage you to do the same.

Thanks for watching, and please be well.

**Portable Device Detects HIV with Push of a Button**

*Futurity.org* (02.27.2013)  Caltech

After approximately 10 years of work, researchers at the California Institute of Technology (Caltech) have built an inexpensive, portable, easy-to-use device to quickly diagnose HIV/AIDS and other diseases. Axel Scherer and George Maltezos began investigating how to manipulate biological fluids on a chip in 2004. Maltezos then started working on applying these techniques to real-world problems. They applied the technology to diagnosing H5N1 with satisfactory results. Maltezos built a prototype of a less expensive polymerase chain reaction (PCR) machine that performed well in H5N1 diagnostic field tests in Thailand. However, it did not give results quickly enough to make it a commercial success.

Maltezos and Scherer teamed up with David Baltimore, professor of biology, to work on an improved device that would detect other viruses or diseases. By the end of 2006, a newer version of the device could evaluate a sample in 94 seconds, compared to 45 minutes with regular PCR machines. A company, Helixis, was formed to manufacture and sell the device. The first Helixis product was a pathogen-detection PCR instrument called the Eco, which cost $13,000. It was fast and relatively cheap, but its size—about that of a microwave—made it too bulky to be easily carried to rural areas of developing countries.

Maltezos teamed up with Baltimore’s and Scherer’s labs to build a new-generation PCR machine specifically meant for use in remote areas of the developing world. The newest prototype is a push-button model that uses a rechargeable battery. It consists of a chip that can analyze a blood sample to detect different pathogens, including TB, HIV, acute lower-respiratory diseases, diarrheal diseases, malaria, and other conditions. The latest goal is to bring the machine’s cost below $1,000 and the cost of each test below $5. According to Maltezos, preliminary results of clinical tests show the device is working well. The next step is to move beyond laboratory testing and into real-world use for those who need it.

**New York City Mayor Bloomberg Pledges $100M To Global Polio Eradication Initiative**

"New York [City] Mayor Michael Bloomberg has pledged $100 million to help the Bill & Melinda Gates Foundation and others to fight polio around the world," the *Associated Press* reports (2/28). Bloomberg "plans to announce Thursday that he is giving $100 million through the philanthropic arm of his foundation to the Global Polio Eradication Initiative," the *Wall Street Journal* writes, noting, "The donation from Bloomberg Philanthropies, to be made over six years, gives a big boost to an effort by fellow billionaire philanthropist [Bill] Gates"—co-chair of the Gates Foundation—"to raise $5.5 billion for a new GPEI ‘polio eradication and endgame strategic plan’ to wipe out the virus by 2018" (McKay, 2/28).

"GPEI’s Polio Eradication and Endgame Strategic Plan 2013-2018 is spearheaded by WHO, UNICEF,
Rotary International and the U.S. Centers for Disease Control," and it "addresses all aspects of ending polio, including stopping transmission, strengthening routine immunization, addressing challenges such as insecurity and access, and preparing the polio infrastructure to reach children with other health services," a Bloomberg Philanthropies press release states (2/28).

**Haiti Launches $2.2B, 10-Year Plan To Eradicate Cholera**

"The Haitian government's $2.2 billion, 10-year plan to eradicate cholera was launched on Wednesday against the backdrop of the U.N.'s rejection of a legal claim from more than 5,000 victims" that sought compensation for the cholera epidemic, which "has killed more than 8,000 people and infected nearly 648,000" and is thought to have been started by a U.N. peacekeeping mission, the Guardian reports. "The first two years of the plan call for an investment of almost $500 million," the newspaper notes, adding, "Over 10 years, the aim is to increase access to potable water from 69 percent of the population to 85 percent; to toilets and latrines from 27 percent to 90 percent, and to health care from 54 percent to 80 percent—while strengthening education, infrastructure and government capacity."

"However, the plan is being launched amid waning commitments from international donors, in a country with fragile government capacity, and in the midst of a crisis," the Guardian continues and includes comments from "Daniele Lantagne, a U.S. cholera expert specializing in emergency water and sanitation interventions in developing countries"; Nigel Fisher, head of the U.N. mission in Haiti; and Ralph Ternier, director of community care and support at Partners in Health (Doucet, 2/28).

**Poor Sanitation In Africa Causing Hundreds Of Thousands Of Deaths, WaterAid Report Says**

"Poor sanitation is causing hundreds of thousands of deaths a year in Africa, where 600 million people—about 70 percent of the population—do not have a safe toilet," and that number is up from 210 million in 1990 and continues to increase as the population grows and people move into poor urban areas, according to a report (.pdf) from WaterAid, titled "Keeping promises: why African leaders need now to deliver on their past water and sanitation commitments," the Guardian reports.

"John Garrett, senior policy analyst at WaterAid, said one of the problems was that governments were prioritizing other areas of need, such as health and education," the newspaper writes, adding, "However, when people do not have access to adequate sanitation and clean water, money spent on health and education is often wasted because people fall ill from preventable diseases such as diarrhea." The newspaper continues, "Donor funding for sanitation amounts to about $9 billion annually, but WaterAid is urging donor countries to double those sums" in order to "help African countries' economies to progress, and save money in health and education, the [non-governmental organization (NGO)] argues" (Harvey, 2/27).


"GlaxoSmithKline’s HIV/AIDS drugs business is to share intellectual property rights on children’s medicine in a patent pool designed to make treatments more widely available in poor countries," Reuters reports. "ViiV Healthcare, majority-owned by GSK, is the second research-based pharmaceutical business to sign up to the new Medicines Patent Pool, following a lead set in 2011 by Gilead Sciences," the news service adds. "The Medicines Patent Pool (MPP), launched in 2010 by the UNITAID health financing system that is funded by a levy on airline tickets, aims to address the remaining gap [in treatment coverage] by getting patent holders to share know-how with makers of cheap generic drugs," the news service notes.

"In the case of ViiV, a key pediatric medicine known as abacavir will be made available to generic manufacturers which will be able to take a license to make and sell it in 118 poor countries, the patent pool said on Wednesday," according to Reuters. "ViiV and the patent pool have also agreed to negotiate further licenses that will allow generics firms to manufacture low-cost versions of an experimental drug, dolutegravir, that is currently awaiting regulatory approval in Western markets," the news service writes, adding, "ViiV—which is owned 76.5 percent by GSK, 13.5 percent by Pfizer and 10 percent by Shionogi—only signed up to the patent pool after lengthy negotiations" (Hirschler, 2/27).
**MIMR researchers find a protein link to STI susceptibility**

Melbourne, AUSTRALIA—Monash Institute of Medical Research scientists have found a protein in the female reproductive tract that protects against sexually transmitted diseases (STIs) such as chlamydia and herpes simplex virus (HSV).

It is estimated that 450 million people worldwide are newly infected with STIs each year. Chlamydia has the highest infection rate of all the STIs reported in Australia.

The research, published today in the prestigious journal, *Science*, was led by Prof Paul Hertzog, Director of MIMR's Centre for Innate Immunity and Infectious Diseases, and his team including, Ka Yee Fung and Niamh Mangan.

The team discovered a protein, which they called Interferon epsilon (IFNe), and showed it plays an important role in protecting females against infections. It could have clinical potential to determine which women may be more or less susceptible to disease such as STIs or to boost protective immunity.

IFNe could also be used potentially to treat STIs or other inflammatory diseases.

"One way this protein is unusual is because of the way it's produced," Prof Hertzog said. "Most proteins protecting us against infection are produced only after we’re exposed to a virus or bacteria.

"But this protein is produced normally and is instead regulated by hormones so its levels change during the oestrous cycle (an animal's menstrual cycle) and is switched off at implantation in pregnancy and at other times like menopause," Prof Hertzog said.

"Some of these times when normal IFNe is lowest, correlate with when women are most susceptible to STIs so this might be an important link to new therapeutic opportunities – IFNe follows different rules to normal immuno-modulatory proteins, and therefore this might also be important to vaccines and the way they're formulated to boost our protective immunity.

"Since this protein boosts female reproductive tract immune responses, it's likely, although we haven't addressed it directly, that this finding will be important for other infectious diseases like HIV and HPV and other diseases."

Prof Hertzog said STIs are a critical global health and socioeconomic problem.

According to the 2011 Australian Bureau of Statistics, chlamydia has the highest infection rates of the notifiable STIs, and infection rates have more than tripled over the past decade. Men and women in the 15-19-year age group saw the largest increase in infection rates. According to these statistics, chlamydia affects more women than men, with 46,636 women aged over 15 diagnosed compared with 33,197 men aged 15 and over.

Prof Hertzog said the next step for this research would be to work towards clinical studies within the next five years. He is also keen to see whether this work can be applied across other diseases including cancer, female reproductive tract related disorders including endometriosis and pelvic inflammatory disease, as well as other non reproductive tract diseases.

**Wolf in sheep's clothing: Uncovering how deadly bacteria trick the immune system**

**UCLA study could provide insight into recent TB outbreak in L.A.'s skid row**

An outbreak of tuberculosis in the skid row area of downtown Los Angeles may have exposed up to 4,500 individuals to the bacterium that causes the deadly disease and has left federal officials scrambling to intervene.

The outbreak is occurring during winter, when homeless individuals are driven to crowded shelters, when influenza is peaking and when people's vitamin D levels, typically boosted by sunlight exposure, are low. A new UCLA study offers critical insight into how various bacteria may manipulate such factors to their advantage.

In a study published online Feb. 28 in the journal *Science*, UCLA researchers demonstrate that certain cunning bacteria — including the type that causes tuberculosis — can pretend to be viruses when infecting humans, allowing them to hijack the body's immune response so that they can hide out, unhindered, inside our
cells. The findings may also help explain how viral infections like the flu make us more susceptible to subsequent bacterial infections such as pneumonia.

The study is particularly relevant to tuberculosis, which kills 1.4 million people worldwide each year. In the case of the recent Los Angeles outbreak, the findings could provide clues as to how the flu and a lack of vitamin D may have given the tuberculosis bacterium an edge.

"With 8.7 million in the world falling ill with tuberculosis each year, a better understanding of how these bacteria avoid our immune system could lead to new ways to fight them and to better, more targeted treatments," said senior author Dr. Robert L. Modlin, chief of dermatology at the David Geffen School of Medicine at UCLA and a professor of microbiology, immunology and molecular genetics in the UCLA Division of Life Sciences.

The protection our immune system provides against bacteria-based diseases and infections depends on the critical response of T cells — white blood cells that play a central role in fighting infections — and in particular on the release of a protein called interferon-gamma. Interferon-gamma utilizes the vitamin D hormone to alert and activate cells to destroy invading bacteria.

The research team found that bacteria can pretend to be viruses, triggering the immune system to launch an attack with a different protein, called interferon-beta, which is designed to fight viruses, not bacteria. Not only is interferon-beta ineffective against bacteria, but it can also block the action of interferon-gamma, to the advantage of bacteria. Further, if a real virus were to infect the body, triggering interferon-beta, it would divert the attention of the immune response, preventing an attack on the bacterial invader. The researchers say this may explain why the flu can lead to a more serious bacterial-based infection like pneumonia.

"Like a wolf in sheep's clothing, the bacteria can fool the immune system into launching an attack against the wrong type of infection, thus weakening the response against the bacteria," said first author Rosane M. B. Teles, a researcher in the dermatology division at the Geffen School of Medicine.

For the study, the team examined the mechanisms by which the virus-fighting interferon-beta protein suppresses the interferon-gamma defense response to bacterial infections, tricking the immune system into making the wrong defense choices.

The researchers studied leprosy as a model and then applied what they learned to understand tuberculosis, given that leprosy and tuberculosis are caused by related bacteria. Modlin noted that leprosy is an outstanding model for studying immune mechanisms in host defense since it presents as a clinical spectrum that correlates with the level and type of immune response of the pathogen.

The scientists first compared the genetic expression of the virus-fighting interferon-beta protein and the bacteria-fighting interferon-gamma protein in skin lesions from leprosy patients. They found that interferon-gamma was expressed in patients with the milder form of the disease and that interferon-beta was significantly increased in those with the more serious, progressive form of leprosy.

The researchers then compared the genes triggered by interferon-beta in these leprosy skin lesions with those found by two other groups of investigators in the blood of tuberculosis patients. Remarkably, there was a significant overlap. The interferon-beta genes were more frequent in both the skin lesions of leprosy patients with extensive disease and the blood of tuberculosis patients with more severe disease.

"We found this common interferon-beta gene pattern correlated with the greater extent of disease in both leprosy and tuberculosis, which are two very distinct diseases," Teles said.

Previous work by the UCLA team demonstrated that the interferon-gamma defense pathway relies on a specific mechanism involving vitamin D, a natural hormone that plays an essential role in the body's
fight against infections. The current study found that interferon-beta suppressed elements involved in the interferon-gamma–triggered vitamin D pathway, preventing the immune system from killing the bacteria.

"The study raises the possibility that a decrease or increase of one of these two interferon proteins could shift the balance from mild to more serious disease," Modlin said. "We may find that therapeutic interventions to block or enhance specific interferon responses may be an effective strategy to alter the balance in favor of protection against bacterial diseases."

The new findings may indicate why, in winter, Los Angeles skid row residents are at an added disadvantage in dealing with tuberculosis — for at least three reasons. First, because of colder weather at night, indigent homeless people tend to stay in shelters, where they live in close proximity with others, facilitating the spread of the infection. Second, due to the seasonal rise in influenza, the body's immune system could be diverted by the flu virus to produce interferon-beta, blocking an effective immune response to the tuberculosis bacteria. And finally, the drop in vitamin D levels associated with a decrease in exposure to sunlight during the winter months could diminish the ability of individuals' immune systems to kill the tuberculosis bacteria.

"With TB on the rise, this scenario could play out not only in cities in the United States but all over the world," Modlin said. "We hope that our findings may provide insight into harnessing new methods to combat TB and other bacterial infections as well."

Modlin noted that 8.7 million become ill with tuberculosis each year, and 1.4 million die from the disease. He added that an increase or decrease in one of the two interferon proteins could help explain why some people may be more resilient against or susceptible to the infection or have a more serious course of the disease.

The next step, according to Teles, is to further understand the mechanisms that bacterial pathogens use to activate interferon-beta and how bacteria can manipulate the immune system to block the potent interferon-gamma host antimicrobial responses in human infections.

**Strains of antibiotic-resistant 'Staph' bacteria show seasonal preference; children at higher risk in summer**

Strains of potentially deadly, antibiotic-resistant *Staphylococcus aureus* bacteria show seasonal infection preferences, putting children at greater risk in summer and seniors at greater risk in winter, according to results of a new nationwide study led by a Johns Hopkins researcher.

It's unclear why these seasonal and age preferences for infection with methicillin-resistant *Staph aureus* (MRSA) occur, says Eili Klein, Ph.D., lead author on the study and a researcher at the Johns Hopkins Center for Advanced Modeling in the Social, Behavioral and Health Sciences.

But he says that increased use of antibiotics in the winter may be one of the reasons. The winter strain that infects seniors at a greater rate is generally acquired in the hospital and resistant to more antibiotics. On the other hand, the summer strain of MRSA, which is seen with growing frequency in children, is largely a community-transmitted strain that is resistant to fewer antibiotics.

"Overprescribing antibiotics is not harmless," Klein notes. "Inappropriate use of these drugs to treat influenza and other respiratory infections is driving resistance throughout the community, increasing the probability that children will contract untreatable infections."

In fact, the study found that while MRSA strains exhibit a seasonal pattern, overall MRSA infections have not decreased over the last five years, despite efforts to control their spread.

A report on the study, which used sophisticated statistical models to analyze national data for 2005-2009, appears today in the online issue of the *American Journal of Epidemiology*.

As the researchers report, hospitalizations from infections tied to MRSA doubled in the United States between 1999 and 2005. The ballooning infection numbers were propelled by MRSA acquired in community settings, not hospital or other health care settings, as had been the case prior to 1999.

Specifically, the study found that a strain of MRSA typically seen in community settings is more likely to cause infection during the summer months, peaking around July/August. The authors' data analysis showed children were most at risk of becoming infected with this strain, typically from a skin or soft tissue wound or ailment.

In fact, in examining data for one year — 2008 — the research team found that 74 percent of those under the age of 20 who developed an infection with MRSA had a community-associated MRSA infection.

Meanwhile, the health care-associated MRSA strain, which is typically seen in hospitals, nursing homes and other health care settings, was found to be most prevalent in the winter months, peaking in February/March. Patients aged 65 or older are more likely to acquire a MRSA infection from this strain.
"Our analysis ... shows significant seasonality of MRSA infections and the rate at which they affect different age groups," write the authors of the report titled "The changing epidemiology of methicillin-resistant Staphylococcus aureus in the United States: A national observational study."

Klein said additional research on seasonal patterns of MRSA infections and drug resistance may help with developing new treatment guidelines, prescription practices and infection control programs.

Mayo Clinic finds steroids may shorten hospital stay for pneumonia patients

ROCHESTER, Minn. — Patients with pneumonia may spend fewer days in the hospital if they are given steroids along with antibiotics and supportive care. That's the finding of a Mayo Clinic analysis of eight randomized-controlled clinical trials involving more than 1,100 patients. The results appear in the March issue of the Journal of Hospital Medicine.

"Given that the average hospital stay for community-acquired pneumonia can range from nine to 23 days, the prospect of speeding recovery, even by a day or two, is helpful," says co-author M. Rizwan Sohail, M.D., a Mayo infectious disease specialist.

Pneumonia is a major health risk, especially in the elderly, the very young and those with chronic lung diseases. Five percent to 15 percent of pneumonia patients die from it, depending on its severity and the treatment administered, recent studies report.

Mayo researchers reviewed eight clinical trials conducted from 2000 to 2011. Most of the research studied patients between 60 and 80. While steroid use didn't prevent deaths, for those who survived the pneumonia, it reduced their hospital stays an average of 1.21 days.

The researchers say that while the findings are significant, the data were not strong enough for them to recommend routine use of steroids for pneumonia patients; more study is needed. They add that continuing any steroids patients may already be taking is reasonable.

The study was led by Majid Shafiq, M.D., a former Mayo Clinic researcher currently at Johns Hopkins University.

A similar, but unrelated study by A.R. Khan, M.D., involving researchers at Mayo Clinic and other institutions, found that use of certain lipid-lowering medicines, commonly referred to as statins, may have a role in reducing the risk of developing community-acquired pneumonia and associated mortality. That study, published last month in PLOS ONE, reviewed 18 clinical trials. The evidence was considered less reliable due to design issues, bias and other factors in the original studies.

Vaccination remains the most powerful tool in infection prevention, researchers say. The Centers for Disease Control and Prevention recommends that all adults 65 or older should receive the pneumonia vaccine and that anyone 19 or older with risk factors such as immune deficiency or chronic lung disease also should get it.

Mutation altering stability of surface molecule in acid enables H5N1 infection of mammals

A single mutation in the H5N1 avian influenza virus that affects the pH at which the hemagglutinin surface protein is activated simultaneously reduces its capacity to infect ducks and enhances its capacity to grow in mice according to research published ahead of print today in the Journal of Virology.

"Knowing the factors and markers that govern the efficient growth of a virus in one host species, tissue, or cell culture versus another is of fundamental importance in viral infectious disease," says Charles J. Russell of St. Jude Children's Research Hospital, Memphis, TN, an author on the study. "It is essential for us to identify influenza viruses that have increased potential to jump species, to help us make decisions to cull animals, or quarantine humans." The same knowledge "will help us identify targets to make new drugs that stop the virus... [and] engineer vaccines."

Various influenza viruses are spreading around the globe among wild birds, but fortunately, few gain the ability to jump to humans. However, those that do, and are able to then spread efficiently from person to person, cause global epidemics, such as the infamous pandemic of 1918, which infected one fifth and killed an estimated 2.7 percent of the world’s population. Occasionally, one of these viruses is exceptionally lethal. For example, H5N1 has killed more than half of the humans it has infected. The specter of such a virus becoming easily transmissible among humans truly frightens public health officials. But understanding the mechanisms of transmission could help microbiologists find ways to mitigate major epidemics.

When influenza viruses infect birds, the hemagglutinin surface protein of the virus is activated by acid in the entry pathway inside the host cell, enabling it to invade that cell. In earlier work, Russell and
collaborators showed that a mutant version of the influenza H5N1 virus called K58I that resists acid activation, loses its capacity to infect ducks. Noting that the upper airways of mammals are more acidic than infected tissues of birds, they hypothesized, correctly, that a mutation rendering the hemagglutinin protein resistant to acid might render the virus more infective in mammals.

In this study the investigators found that K58I grows 100-fold better than the wild-type in the nasal cavities of mice, and is 50 percent more lethal. Conversely, the mutant K58I virus failed completely to kill ducks the investigators infected, while the wild-type killed 66 percent of ducks, says Russell. "A single mutation that eliminates H5N1 growth in ducks simultaneously enhances the capacity of H5N1 to grow in mice. We conclude that enhanced resistance to acid inactivation helps adapt H5N1 influenza virus from an avian to a mammalian host."

"These data contribute new information about viral determinants of influenza virus virulence and provide additional evidence to support the idea that H5N1 influenza virus pathogenesis in birds and mammals is linked to the pH of [hemagglutinin] activation in an opposing fashion," Terence S. Dermody of Vanderbilt University et al. write in an editorial in the journal accompanying the paper. "A higher pH optimum of [hemagglutinin] activation favors virulence in birds, whereas a lower pH optimum... favors virulence in mammals."

Based on this and another study, "...surveillance should include phenotypic assessment of the [hemagglutinin] activation pH in addition to sequence analysis," Dermody writes. The journal carefully considered whether to publish the paper, because it raised issues of "dual use research of concern" (DURC), writes Dermody. DURC is defined as "Life sciences research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products, or technologies that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security," according to a US government policy document. However, both the National Institute of Allergy and Infectious Diseases and the St. Jude Institutional Biosafety Committee concluded that the study failed to meet the definition of DURC. Clinching the case, "the addition of the key mutation in the Russell paper to other previously reported mutations would not result in an even more virulent H5N1 influenza virus," says Dermody.

A copy of the research manuscript can be found online at http://bit.ly/asmpr0213b. The manuscript of the accompanying editorial can be accessed at http://bit.ly/asmpr0213c. Both are scheduled to be formally published in the May 2013 issue of the Journal of Virology.


'Defective' virus surprisingly plays major role in spread of disease, UCLA life scientists report

Defective viruses, thought for decades to be essentially garbage unrelated to the transmission of normal viruses, now appear able to play an important role in the spread of disease, new research by UCLA life scientists indicates.

Defective viruses have genetic mutations or deletions that eliminate their essential viral functions. They have been observed for many human pathogens and are generated frequently for viruses that have high mutation rates. However, for some 40 years, it was believed that they were unimportant in natural settings.

In findings published Feb. 28 in the journal PLoS Pathogens, UCLA scientists and their colleagues report for the first time a significant link between a defective virus and an increased rate of transmission of a major disease.

"The idea has always been that defective viruses are either meaningless or detrimental," said James O. Lloyd-Smith, a UCLA assistant professor of ecology and evolutionary biology and the senior author of the research. "We have found the opposite of that — that the defective virus is actually helping the normal, functional virus. This finding is bizarre and hard to believe, but the data are the data."

"We have shown that the defective virus not only transmits with the virus but increases the transmission of the functional virus," said Ruian Ke, a UCLA postdoctoral scholar in the department of ecology and evolutionary biology and the lead author of the study.

Defective viruses cannot complete their life cycle on their own, but if they're able to get into the same cell with a non-defective virus, they can "hitchhike" with the normal virus and propagate, Lloyd-Smith said. Biologists had thought that defective viruses interfered with normal versions of the virus, "clogging up the gears of viral replication," he said.
The life scientists studied DENV-1, one of four known types of the dengue virus that infect humans. Dengue viruses are transmitted by several species of mosquitoes and cause dengue fever, which is characterized by fever, joint pain and a skin rash similar to measles. Dengue hemorrhagic fever, a more severe form of dengue infection, can cause death. The dengue virus infects between 50 million and 100 million people each year in Southeast Asia, South America, parts of the United States and elsewhere.

The life sciences team — which also included John Aaskov, a virologist and professor of health at Australia’s Queensland University of Technology in Brisbane, and Edward Holmes, a professor of biological sciences at Australia’s University of Sydney — found that the presence of a defective DENV-1 virus may have led to large increases in dengue fever cases in Myanmar in 2001 and 2002, when that country experienced its most severe dengue epidemics on record.

The scientists describe when and how the defective "lineage," or series of very closely related defective DENV-1 viruses, emerged and was transmitted between humans and mosquitoes in Myanmar, as well as what the public health implications are.

For the study, Ke designed a mathematical model to analyze the data to learn how the defective DENV-1 virus interacted with the normal virus. Aaskov and Holmes collected genetic sequences from 15 people in Myanmar sampled over an 18-month period, all of whom were infected with the DENV-1 virus and nine of whom were also infected with the defective version.

Ke discovered that the lineage of defective viruses emerged between June 1998 and February 2001 and that it was spreading in the population until at least 2002. (The following year, the lineage appeared on the South Pacific island of New Caledonia, carried there by either a mosquito or a person.) The scientists analyzed the genetic sequences of both the defective and normal dengue viruses to estimate how long the defective virus had been transmitting in the human population.

"We can see from the gene sequence of the defective version that it is the same lineage and is a continued propagation of the virus," said Lloyd-Smith, who holds UCLA’s De Logi Chair in Biological Sciences. "From 2001 to 2002, it went from being quite rare to being in all nine people we sampled that year; everybody sampled who was getting dengue fever was getting the defective version along with the functional virus. It rose from being rare to being very common in just one year."

Most surprisingly, Lloyd-Smith said, the combination of the defective virus with the normal virus was "more fit" than the normal dengue virus alone.

"What we have shown is that this defective virus, which everyone had thought was useless or even detrimental to the fitness of the functional virus, actually appears to have made it better able to spread," he said. "Ruian [Ke] calculated that the defective virus makes it at least 10 percent more transmissible, which is a lot. It was spreading better with its weird, defective cousin tagging along than on its own.

"This study has shown that the functional virus and defective virus travel in unison. The two transmit together in an unbroken chain, and that's not just a matter of getting into the same human or the same mosquito — they need to get into the same cell inside that human or mosquito in order to share their genes and for the defective version to continue 'hitchhiking.' We are gaining insights into the cellular-level biology of how dengue is infecting hosts. It must be the case that frequently there are multiple infections of single cells.

"Ruian showed the defective virus appeared one to three years before these major epidemics," Lloyd-Smith added. "One could imagine that if you build an understanding of this mechanism, you could measure it, see it coming and potentially get ahead of it."

Might defective viruses play a role in the transmission of influenza, measles and other diseases?

"There are a few signs that this phenomenon may be happening for other viruses," Lloyd-Smith said. "We may be cracking open the book on the possible interactions between the normal, functional viruses and the defective ones that people thought were just dead-ends. These supposedly meaningless viruses may be having a positive impact — positive for the virus, not for us. There is great variation, year to year, in how large dengue epidemics are in various locations, and we don’t understand why. This is a possible mechanism for why there are large epidemics in some years in some places. We need to keep studying this question."

The research points to implications for how mutations might allow a new non-human virus to become a human virus.

"Different strains of a virus with different genetic properties may be interacting more frequently than we thought," said Lloyd-Smith, who studies how ecology, evolution and epidemiology merge to drive the emergence of new pathogens, including new strains with important properties like drug resistance.

Why would a defective virus increase transmission of a disease?
Lloyd-Smith offers two hypotheses. One is that the presence of the defective virus with the functional virus in the same cell makes the functional virus replicate better within the cell by some unknown mechanism. "It might give the virus a bit of flexibility in how it expresses its genes and may make it a bit more fit, a bit better to reproduce under some circumstances," he said.

A second idea is that the defective virus may be interfering with the disease-causing virus, making the disease less intense; people then have a milder infection, and because they don't feel as sick, they are more likely to go out and spread the disease.

"Normally, biologists test for how well a virus can replicate in a cell, but what we have shown here is even a genotype that cannot replicate in a cell can have an impact on transmission," Ke said.

In conducting the research, Lloyd-Smith and Ke combined genetic sequence analysis with sophisticated mathematical models and bioinformatics.

Genetic sequencing technology has "exploded," Lloyd-Smith said, providing a wealth of data on genetic sequences of pathogens and the evolution of viruses, leading to major new insights into the transmission of viruses.

"We were able to show that this defective virus transmitted in an unbroken chain across this population for a year-and-a-half," Lloyd-Smith said. "Without gene sequencing, we would not have been able to establish that."

**Viruses Can Have Immune Systems: A Pirate Phage Commandeers the Immune System of Bacteria**

Feb. 27, 2013 — A study published today in the journal *Nature* reports that a viral predator of the cholera bacteria has stolen the functional immune system of bacteria and is using it against its bacterial host. The study provides the first evidence that this type of virus, the bacteriophage ("phage" for short), can acquire a wholly functional and adaptive immune system.

The phage used the stolen immune system to disable—and thus overcome—the cholera bacteria's defense system against phages. Therefore, the phage can kill the cholera bacteria and multiply to produce more phage offspring, which can then kill more cholera bacteria. The study has dramatic implications for phage therapy, which is the use of phages to treat bacterial diseases. Developing phage therapy is particularly important because some bacteri, called superbugs, are resistant to most or all current antibiotics.

Until now, scientists thought phages existed only as primitive particles of DNA or RNA and therefore lacked the sophistication of an adaptive immune system, which is a system that can respond rapidly to a nearly infinite variety of new challenges. Phages are viruses that prey exclusively on bacteria and each phage is parasitically mated to a specific type of bacteria. This study focused on a phage that attacks *Vibrio cholerae*, the bacterium responsible for cholera epidemics in humans.

Howard Hughes Medical Institute investigator Andrew Camilli, Ph.D., of Tufts University School of Medicine led the research team responsible for the surprising discovery.

First author Kimberley D. Seed, Ph.D., a postdoctoral fellow in Camilli's lab, was analyzing DNA sequences of phages taken from stool samples from patients with cholera in Bangladesh when she identified genes for a functional immune system previously found only in some bacteria (and most Archaea, a separate domain of single-celled microorganisms).

To verify the findings, the researchers used phage lacking the adaptive immune system to infect a new strain of cholera bacteria that is naturally resistant to the phage. The phage were unable to adapt to and kill the cholera strain. They next infected the same strain of cholera bacteria with phage harboring the immune system, and observed that the phage rapidly adapted and thus gained the ability to kill the cholera bacteria. This work demonstrates that the immune system harbored by the phage is fully functional and adaptive.

"Virtually all bacteria can be infected by phages. About half of the world's known bacteria have this adaptive immune system, called CRISPR/Cas, which is used primarily to provide immunity against phages. Although this immune system was commandeered by the phage, its origin remains unknown because the cholera bacterium itself currently lacks this system. What is really remarkable is that the immune system is being used by the phage to adapt to and overcome the defense systems of the cholera bacteria. Finding a CRISPR/Cas system in a phage shows that there is gene flow between the phage and bacteria even for something as large and complex as the genes for an adaptive immune system," said Seed.

"The study lends credence to the controversial idea that viruses are living creatures, and bolsters the possibility of using phage therapy to treat bacterial infections, especially those that are resistant to
antibiotic treatment," said Camilli, professor of Molecular Biology & Microbiology at Tufts University School of Medicine and member of the Molecular Microbiology program faculty at the Sackler School of Graduate Biomedical Sciences at Tufts University.

Camilli's previous research established that phages are highly prevalent in stool samples from patients with cholera, implying that phage therapy is happening naturally and could be made more effective. In addition, a study published by Camilli in 2008 determined that phage therapy works in a mouse model of cholera intestinal infection.

The team is currently working on a study to understand precisely how the phage immune system disables the defense systems of the cholera bacteria. This new knowledge will be important for understanding whether the phage's immune system could overcome newly acquired or evolved phage defense systems of the cholera bacteria, and thus has implications for designing an effective and stable phage therapy to combat cholera.

Journal Reference:

*Important Control Mechanism Behind Autoimmune Diseases Discovered*

May 4, 2010 — Researchers at the Swedish medical university Karolinska Institutet have discovered a new control mechanism in our immune system. The discovery is of potential significance to the treatment of serious diseases such as MS (multiple sclerosis), rheumatoid arthritis, and SLE (Systemic lupus erythematosus).

"Now that we've started to understand the regulatory mechanisms involved in these autoimmune diseases, we are hopeful that new treatments can be found," says Mikael Karlsson, associate professor at the Department of Medicine at Karolinska Institutet in Solna, and one of the team behind the study now published in the highly reputed periodical, The *Journal of Experimental Medicine*.

An important component of our immune defence is a type of cell called a B cell. Normally, the job of these cells is to produce antibodies, which in turn bind to and neutralise invasive microorganisms, such as bacteria and viruses. In people with an autoimmune disease, explains Dr Karlsson, these B cells actually have an injurious effect and instead of serving the body, are activated against its own tissues, which they start to break down.

Patients with SLE and other autoimmune diseases have lower levels of so-called NKT cells. Previously, it was not known what part these cells play in the origin and development of the disease; now, however, the research group at KI has shown that this deficiency is a contributory pathogenic factor.

"We've demonstrated that NKT cells can regulate how B cells become activated against healthy tissue, and that a lack of NKT cells results in greater misguided B cell activation," says Dr Karlsson. "So now we can mechanically link the NKT cell defect in patients to the disease."

The study also shows that the NKT cells directly impede faulty B cell activation, and that they do so early in the misdirected process. The team managed to inhibit the activity of pathogenic B cells by adding NKT cells—a result that may one day lead to new types of treatment.

"This means that new treatments specifically targeting the protective NKT cells can help this patient group," concludes Dr Karlsson.

Journal Reference:

*Three Overstretched DNA Structures Confirmed*

-An illustration of three distinct elongated DNA structures produced by mechanical stretching. (Credit: NUS)
Feb. 28, 2013 — A novel discovery brings a close to a 17-year-old scientific debate about the impact of mechanical stretching on the structure of DNA.

A team of researchers led by Associate Professor Yan Jie from the Department of Physics at the National University of Singapore (NUS) Faculty of Science has identified three new distinct overstretched deoxyribonucleic acid (DNA) structures caused by mechanical stretching. This discovery provides a clear answer to a long-running debate among scientists over the nature of DNA overstretching.

**Debate on Possible DNA Structural Transitions**

Recent single-molecule studies revealed that mechanical stretching could induce transitions to elongated DNA structures. Three possible elongated DNA structures have been proposed, namely: a single-stranded DNA under tension, DNA bubbles consisting two parallel, separated single-stranded DNA under tension, and a new form of base-paired double-stranded DNA. The existence of the three transitions has been heavily discussed among scientists for some 17 years.

To fully understand the nature of DNA overstretching, the team led by Assoc Prof Yan, which comprises members from NUS, the University of Minnesota and the Massachusetts Institute of Technology, explored the possible structural transitions.

**Three Distinct Transitions Revealed**

In their recent study, the researchers systematically investigated the three possible transitions induced by mechanical stretching, with methods to control DNA construct, temperature, force and salt concentration. Their data successfully identified all the three proposed structures and fully characterised their respective thermo-mechanical properties. These findings were first published on the online version of the Proceedings of the National Academy of Sciences on 19 February 2013. These findings complete the picture about the structures of DNA under tension, providing a conclusion to the 17-year-old debate.

**Biological Implications and Potential Applications**

As forces over a wide range are present in the DNA of cells, the researchers' findings provide new perspectives of possible force-dependent regulations of critical biological processes, such as DNA damage repair and gene transcriptions.

In addition, as many recently developed DNA devices are based on thermo-mechanical properties of various DNA structural motifs, these findings may also have potential applications in designing new DNA devices for the future.

**The Next Step**

To further their research, Assoc Prof Yan and his team will study the physiological functions of the three overstretched DNA structures, and investigate the presence of any new DNA structures under other mechanical constraints.

**New Method for Researching Understudied Malaria-Spreading Mosquitoes**

Feb. 28, 2013 — Researchers at the Johns Hopkins Malaria Research Institute have developed a new method for studying the complex molecular workings of Anopheles albimanus, an important but less studied spreader of human malaria. An. albimanus carries Plasmodium vivax, the primary cause of malaria in humans in South America and regions outside of Africa. Unlike Anopheles gambiae, the genome of the An. albimanus mosquito has not been sequenced and since these two species are evolutionarily divergent, the genome sequence of An. gambiae cannot serve as an appropriate reference.

The researchers' findings were published online in the journal Molecular & Cellular Proteomics.

"Technologies and platforms are needed to bridge the scientific gaps that could eventually spur the development of novel interventions to combat all human malaria," said study author, Rhoel Dinglasan, PhD, MPH, an assistant professor with the W. Harry Feinstone Department of Molecular Microbiology and Immunology at the Johns Hopkins Bloomberg School of Public Health. "To our knowledge, no approaches have been published that address this issue."

For the study, Dinglasan and his colleagues developed a method to compare proteins of the midgut of An. albimanus and An. gambiae. The mosquito midgut is a critical stage in the lifecycle of the malaria parasite and in the transmission of malaria to people. For An. albimanus, the researchers developed a seamless, integrated transcriptomic-to-proteomic approach involving assembly of the An. albimanus midgut transcriptome followed by acquisition of the luminal midgut microvilli proteome.

Dinglasan added, "This comparative proteomic analysis of the midgut brush borders of two important malaria vectors, An. gambiae and An. albimanus, which we envision will be one of many comparative studies, will help researchers develop new mosquito-based targets for drugs, vaccines or other interventions that would theoretically work in blocking both P. falciparum and P. vivax."
Malaria sickens more than 250 million people worldwide resulting in over 800,000 deaths, mostly African children.

Case report of a 'functional' HIV cure in a child ******

Researchers in the United States today said that they have identified a case of a 'functional' HIV cure in a child infected with HIV who began antiretroviral treatment within days of birth. The child has now been off treatment for over a year, and although HIV DNA has been detected at very low levels in the child’s cells, the virus is not reproducing.

Further follow-up will be required to determine whether this state persists, or whether viral replication resumes, but researchers involved in the case are optimistic that what they have found represents a functional cure – a state in which HIV remains in the body, but no longer replicates. One functional cure in an adult has been reported previously – the so-called 'Berlin patient' (see below and related news story).

The findings were presented at a press conference on the opening day of the 20th Conference on Retroviruses and Opportunistic Infections (CROI 2013) in Atlanta.

At the press conference, Deborah Persaud of Johns Hopkins University School of Medicine, Baltimore, reported on the case of a child treated with antiretroviral therapy from 30 hours after birth, almost immediately after testing for HIV DNA and RNA on the second day of life. The child was born to a mother with detectable viral load at the time of delivery who had not been in care prior to the time she presented at the hospital, in labour.

The child was tested for HIV very quickly after a premature delivery in hospital, and tests revealed detectable HIV DNA and RNA. RNA tests continued to be positive up until 20 days of life, indicating that this was not an isolated false positive result and that viral replication was taking place.

Viral load tests were carried out at 7, 12 and 20 days of age that showed detectable virus after the initiation of treatment, before the virus became undetectable on a test with a lower limit of detection of 20 copies/ml at day 29. Subsequent testing until month 26 showed that viral load remained persistently undetectable after this point, despite the fact that treatment was stopped after 18 months.

In line with recommended practice in cases where mothers have detectable viral load at the time of delivery, the infant was initiated on a regimen of AZT/3TC and nevirapine, as prophylaxis against infection. In cases of prophylactic treatment to prevent infection in HIV-exposed infants, this regimen would be given for 4 weeks. In this case, because virus was detected and the infant had confirmed HIV infection, treatment continued until the age of 18 months, at which point the child’s caregiver withdrew the child from treatment and the child was lost to follow-up for nearly six months.

When the child returned to care at 23 months of age and it became apparent that viral load was not detectable in the absence of treatment, clinicians at the University of Mississippi Medical Center sought advice from research groups outside the state, including Dr Katherine Luzuriaga at the University of Massachusetts.

Collaborating laboratories at the National Institutes of Allergy and Infectious Diseases (NIAID) and the University of California San Diego carried out ultrasensitive tests to determine whether HIV had been eliminated, or whether any traces of the virus persisted in any cell types. They found that a single copy of viral RNA (indicating viral replication) could be detected in tests carried out when the child was two years of age. Co-culture of 22 million resting CD4 cells failed to identify any replication-competent HIV.

However, testing at 24 and 26 months of age found a reservoir of presumably latently infected cells: HIV DNA was detected in peripheral blood mononuclear cells at a frequency of 37 and 4 copies per million cells. The investigators also looked for 2-LTR circles, fragments of unintegrated HIV DNA that might have the potential either to influence the way in which the infected cell evades immune surveillance, or to establish a prolonged state of latency. They found no 2-LTR circles within the cells containing HIV DNA, suggesting that HIV is completely quiescent, and not replicating – a functional cure of HIV infection.
Speaking at the press conference, Deborah Persaud rejected suggestions that the case might represent an episode of successful post-exposure prophylaxis, noting that several blood samples taken during the first week of life had tested positive for viral RNA, indicating that infection had already become established. However, further testing of these samples is not possible because the samples had not been stored, their future significance not being appreciated at the time the child was diagnosed.

Dr Persaud said that the findings represented a "proof of concept". Referring to the case of a Berlin man who was declared cured of HIV infection following a bone marrow transplant from a donor with genetically conferred resistance to HIV infection, she said: "We believe this is our Timothy Brown moment."

She said that trials to investigate whether a functional cure is possible for larger numbers of infants are now in design, and that if successful, the challenge will be to replicate the results through the existing platform of services for prevention of mother to child transmission.

**Note:** the full presentation of these findings will take place on Monday 4th March and this report will be updated to include further details after the presentation. This report is based on an abstract of the presentation and on details released at a press conference on the opening day of the conference. It is published in advance of the full presentation due to the high level of interest in this case.

**Reference**

---

**Dangerous TB Patient Detained on U.S. Border**

By **Betsy Mckay**

In medical isolation in South Texas, 100 miles or so from Mexico’s border, is a man who embodies one of U.S. health officials’ greatest worries: He is the first person to cross and be held in detention while infected with one of the most severe types of drug-resistant tuberculosis known today.

Multi-drug-resistant tuberculosis has been a growing problem in India for years. Now an even more extreme strain of the deadly disease—resistant to all of the drugs normally used to treat it—is causing concern. WSJ's Natacha Butler reports from Mumbai.

His three-month odyssey through 13 countries—from his homeland of Nepal through South Asia, Brazil, Mexico, and finally into Texas—shows the way in which dangerous new strains of the disease can migrate across the world unchecked.

Tuberculosis, an ancient, fatal airborne disease, has been treatable for decades with a cocktail of drugs. However, shoddy medical practices world-wide have enabled the bacteria to mutate and, in some cases, become all but untreatable. In recent months The Wall Street Journal has exposed widening TB drug resistance in hot spots like India, and shown that the U.S. is surprisingly unprepared for the growing global problem. Most U.S. cases of drug-resistant TB occur in people who were born abroad, according to the Centers for Disease Control and Prevention.
The Nepalese man detained at the U.S. border carries a particularly deadly strain—XDR, "extensively drug-resistant" TB. His TB is resistant to at least eight of the 15 or so standard drugs, according to a U.S. government description of the case reviewed by the Journal. His XDR strain has been seen only once before in the U.S., in another patient of Nepalese origin, according to the government description.

The Nepalese patient was taken into custody by the U.S. Border Patrol in late November as he tried to cross the border illegally near McAllen, Texas, according to Department of Homeland Security officials. The government declined to name him.

He was transferred five days later to an Immigration and Customs Enforcement detention facility in Los Fresnos, Texas, and put into "medical isolation" with suspected tuberculosis, according to ICE. He has since been moved to another ICE detention facility, in Pearsall, Texas, with more medical staff, ICE said. He is the first XDR-case in ICE custody.

**In-Depth: A Killer Quietly Gains Strength**
The Wall Street Journal is chronicling the world's imperfect response to the rise of drug-resistant tuberculosis, an ancient disease that modern medicine, until recently, could defeat.

A selection of reports:
- Deadly unintended consequences: The global TB-fighting strategy helped allow the spread of new, all-but-untreatable strains. (11/23/12)
- Exclusive numbers suggest more than 25% of patients at one Indian TB clinic don't respond to the primary treatment. (11/23/12)
- One woman’s case of nearly incurable tuberculosis echoes around the world. (9/8/12)
India’s slow reaction appears to be nurturing an all-but-untreatable strain of TB, raising the prospect of a global health hazard. (6/20/12)

A top doctor in Mumbai reports finding 12 cases of tuberculosis that are all but untreatable by current methods. (1/19/12)

Twelve Border Patrol agents were tested for the disease, but none contracted it from the patient, a Customs and Border Protection official said. Casual contact doesn’t necessarily lead to infection, though it depends in part on how much time is spent in tight quarters with a patient, and how much the patient coughs, spreading bacteria into the air.

It remains unclear whether other people in custody with the Nepalese detainee might have been infected. By the time the Border Patrol learned of his infection, other people detained with him would have been transferred elsewhere, the CBP official said. Detainees who are suspected of being ill are placed in cells by themselves.

Given how far and wide the patient ventured—he took a flight of more than eight hours to Brazil, and also traveled by car, boat and on foot—his case was reported to the World Health Organization as having potentially widespread public-health impact. Now, officials in the 13 countries the man visited along the way must try to track down thousands of people he likely came into contact with, to see if any were infected.

That will be a challenge. "We will try to investigate where he was," said Martin Castellanos, director of Mexico’s national TB program. But reconstructing his precise route through Mexico, or any country, will be difficult and perhaps impossible, he said.

Dr. Castellanos says he was told the man spent time in a migrant community in Reynosa, across the border from Texas. But migrants typically linger there only "for a week, two weeks," he said, before moving on. "For sure, no one who was there in November is there now," he said.

The WHO's Stop TB Department said it is working with the CDC to inform affected countries about people who may have been exposed to the man. It is also trying to get more details on potentially infected people in those countries who have been reached by local authorities.

DHS and the CDC declined to discuss details of the man’s case, citing patient privacy. The man declined an interview request from the Journal made through ICE. He also declined to sign a privacy waiver allowing officials to release details of his treatment and his immigration case, ICE said.

XDR-TB is a particularly dangerous form of the disease that is resistant not only to the two most potent TB drugs, but also a handful of second-line drugs. It is rare in the U.S.: Only six cases were reported in 2011, according to the CDC.

But it is a growing threat in countries including India and South Africa, where it has been found all over the country. The risk to the world is that the disease will migrate outward from these hot spots. Treatment options for XDR-TB are limited and can themselves be toxic.

ICE officials screen patients for TB—both regular and multidrug-resistant varieties—when they arrive at a detention facility. "We prepare for it and look for it,” an ICE medical official said. They find one or two cases of multidrug-resistant TB a year, the official said.

How long the man will remain in care in the U.S. is unclear. Treatment can last for years, but TB patients aren’t infectious for the entire course of treatment.

Detainee patients aren’t normally kept until they are completely cured. However, infectious patients aren’t deported on commercial flights or by any other means that “could be a danger to anyone,” the official said.

One risk, of course, is that a patient won’t have enough drugs or medical expertise to complete the treatment he or she needs once deported to another country. TB strains can become increasingly drug-resistant if a patient’s treatment regimen is interrupted, even briefly. This is one way that drug-resistant TB has emerged over time.

The Migrant Clinicians Network, an Austin, Texas, nongovernmental organization, helps arrange for deported patients to continue their treatment in their home countries. U.S. officials also often send patients home with a supply of the TB drugs they need, particularly to countries where supplies are uneven.

Arranging care for drug-resistant patients is complicated, said Ed Zuroweste, the Migrant Clinicians Network’s chief medical officer. "XDR is hugely difficult," he said. "You really have to have experts to treat someone like that."
Nepal is known for innovative health programs, including some to fight TB. But like many countries, it has struggled with drug-resistant forms. Nepal reported more than 35,000 TB cases in 2011, and 2.9% of new and 12% of previously-treated TB cases are multidrug-resistant, according to WHO data.

Strategy To Prevent HIV In Newborns Sparks Enthusiasm And Skepticism
by Richard Knox
February 28, 2013 5:05 PM
There's great enthusiasm among some global health leaders about a bold – some say radical – strategy to prevent pregnant women from transmitting HIV to their newborns.

But skeptics worry that the approach, dubbed Option B+, will pit pregnant women with HIV against others infected with the virus, diverting resources from the broader struggle against the pandemic.

The goal of Option B+ is to make serious inroads in reducing a stubborn and heart-breaking problem. Every year about 300,000 babies in sub-Saharan Africa are born with HIV.

The new strategy aims to put every pregnant woman with HIV on triple-drug treatment and keep her on it for the rest of her life — even if the virus has not yet damaged her immune system to the point where she needs medications to preserve her own health.

Universal treatment of HIV-positive pregnant women avoids the need to do expensive and often hard-to-access testing of a woman's level of CD4 immune cells to determine if she's sick enough to need long-term antiretroviral drugs.

Where is Option B+? Several countries in sub-Saharan Africa, like Zambia, Kenya and Tanzania, are already planning to implement the program. The U.S. government funds Option B+ through the President’s Emergency Plan for AIDS Relief.

Centers for Disease Control and Prevention.

The name Option B+ distinguishes the strategy from two earlier approaches, called A and B, which have failed so far to eliminate mother-to-child transmission of HIV in most-affected countries. Those other options either reserve triple-drug treatment for pregnant women with severely compromised immune systems or provide it only temporarily around the time of childbirth to those whose immunity is still relatively robust.

A report, published Thursday by scientists at the Centers for Disease Control and Prevention, finds that when Malawi, a small country in southeast Africa, made a big push to implement Option B+, the payoff was impressive: a sevenfold increase in the number of pregnant and breast-feeding women starting anti-HIV treatment in only a year.

"I think this is actually a big deal," CDC director Dr. Thomas Frieden tells Shots. He says the results not only demonstrate that it's possible to implement Option B+ across an entire country, "but that it makes a huge difference. The data are really remarkable."

In the space of about a year, Malawi trained 5,000 health care workers to give Option B+. It doubled clinics offering triple-drug treatment and went from having less than 1,300 pregnant women on HIV treatment to nearly 11,000.

Frieden estimates Option B+ prevented 7,000 infants from getting HIV from their mothers in its first year of operation.
"I think Option B+ is absolutely crucial," Frieden says, "because it gets to people who account for 60 percent of new HIV infections – women of childbearing age."

He predicts Option B+ will have ripple effects that benefit families and communities by sharply reducing transmission of HIV from women to their uninfected partners.

But not everyone is convinced. Among them is Dr. Hoosen Coovadia, a leading South African HIV specialist, who chaired the International AIDS Conference in 2000, which helped focus the world’s attention on the pandemic’s staggering toll in Africa.

Coovadia tells Shots that he had "a gut reaction" against Option B+ last July "because of the way it was presented" at the International AIDS Conference in Washington, D.C. "It looked like everyone had been tutored to say this was a great thing," he says. "Hillary Clinton mentioned it in her speech. Almost everyone spoke about B+ as though it was going to change the direction of the AIDS epidemic. But it didn't look to me that there had been adequate consultation. This was a pet program of the U.S. government."

After the Washington conference, Coovadia says he and his colleagues debated the strategy back in South Africa. Recently he coauthored a sharply worded commentary in The Lancet calling Option B+ "extreme.

"The strong push for countries to switch to B+ is premature," they write. "A switch now would be dangerous, ignoring severe ethical, safety, feasibility and economic concerns."

The CDC's report on Option B+ in Malawi this week "is not a convincing argument that B+ is worth all the potential problems," Coovadia says.

South Africa, he says, has been able to lower the rate of mother-to-child HIV transmission from 33 percent to less than 3 percent by treating pregnant women with simpler, cheaper regimens and reserving triple-drug treatment for those who need it.

One big concern is that Option B+ will create tensions in households and communities when it becomes known that pregnant women with HIV are preferentially getting triple-drug therapy whatever their immune status, while other infected people with deteriorating health may not.

"South Africa is already a fractious community," Coovadia says. "If it got out that of two women with the same CD4 count, one was treated and the other was not, the scope for tensions would be great."

Frieden's response to that: "Why should being pregnant get you to the front of the line? The answer to that, I think, is first, that it's more likely to get the baby protected ... It also saves the lives of the mother, the child, the family and it stops the spread of HIV."

And the critics? "Frankly, I think they're mistaken," Frieden says.

Coovadia calls for more study of Option B+ before implementing it aggressively. More data will be forthcoming. Rwanda and Haiti have already adopted the strategy, and seven other sub-Saharan countries are actively implementing it or preparing to.

**Macrophages allow entry of HIV in the urethra**

Published on March 1, 2013 at 7:21 AM

Having suggested in 2011 that the urethra is a novel entry site for HIV, a team from the Institut Cochin (CNRS/Inserm/Université Paris Descartes, with the support of Anrs), has now confirmed this hypothesis and identified the cells and mechanisms brought into play: the immune system cells macrophages, present in the epithelium of the urethra, allow the entry of HIV. This work, published online on the website of the journal Mucosal Immunology, could make it possible to test novel HIV/AIDS prevention strategies.

While the mechanisms of rectal or vaginal infection in women are quite well described, penile infection in men remains poorly understood. Clinical studies conducted in the 2000s showed that circumcision could reduce the risk of infection in men by 60% during sexual intercourse. Following this work, the Institut Cochin team demonstrated that the mucous membrane on the inner layer of the foreskin was one of the main entry sites for HIV. However, since circumcision does not provide complete protection, it remained to be determined what other mucous sites in the penis could facilitate HIV infection.

To localize these entry sites, the researchers used penis tissue taken from healthy adult males during transgender surgery. HIV can, a priori, penetrate via three areas of the penis: the glans, the end of the urethra known as the fossa navicularis and the part of the urethra located inside the penis. By placing the mucous membranes covering these three areas in contact with the HIV virus, the researchers observed that the glans and the fossa navicularis resist infection. On the other hand, the virus efficiently penetrates
the penis through the urethra, which is also an entry site for many other sexually transmitted pathogens, such as gonococci or chlamydia (these results were presented in part at the international Conference on Retroviruses and Opportunistic Infections (CROI) in 2011).

The researchers are now focusing on molecular and cellular infection mechanisms. They have demonstrated that, in the urethra, the immune system cells responsible for the phagocytosis of pathogenic agents, known as macrophages, are the first to be invaded by HIV. This had never been observed in this type of mucous membrane. At the same time, the cells of the epithelium stop secreting the signals retaining the macrophages. Consequently, the infected macrophages leave the epithelium, allowing HIV to propagate. The researchers thus observed that, in the mucous membrane of the urethra, the TCD4+ lymphocytes—the main target of the virus—are not infected because they are immature. They could be infected later, after migration of HIV to the ganglia. The researchers now seek to determine whether the macrophages of the urethra constitute reservoirs preventing the virus from being completely eliminated by tritherapy treatment. This work is important from a fundamental viewpoint and it makes it possible to shed light on how the urethra can be an entry site for HIV in men, whether they are circumcised or not. It could also lead to the development of new prevention strategies.


Is the HIV 'functional cure' the breakthrough it seems?

Mississippi baby now effectively free of the virus will cause excitement, but is likely to mean little for those already infected

Scientists on a quest to cure HIV will be enormously encouraged, as well as intrigued, by the reports from Mississippi in the US of a two-year-old child who had the virus at birth but who is now apparently free of it.

It sounds like one of those serendipitous breakthroughs that have characterised the fight against HIV and Aids, such as the discovery that some African sex workers are resistant to the virus and the realisation that people taking antiretroviral drugs, which suppress the levels of HIV in the body, are unlikely to infect their partners.

But is this the big one? Have doctors stumbled across the cure for HIV? Unfortunately not. This is progress and will open up new avenues for scientists to explore, but the implications for those already infected or even the still significant numbers of babies born with the virus in the developing world are sadly probably slight.

The Mississippi baby became infected because the mother had not been tested in early pregnancy. If she had, the woman would have been put on antiretroviral drugs, the baby would have been delivered by caesarean section and then given a short course of drugs — all of which would almost certainly have prevented transmission of HIV from mother to child.

When doctors realised the mother had HIV, it was too late for the standard prevention package, so they implemented plan B, which was to put the baby on the full three-drug cocktail straight away. It is already known that the sooner after infection an adult goes on the drugs, the better the outcome. But here, it seems, the drugs hit the virus so hard and so early that it all but disappeared.

This is what scientists call a "functional cure". Traces of the virus remain, but they are inactive even though the mother disappeared from follow-up and the baby was off drugs for five months. This was the serendipitous event — other babies will have been treated the same way but remain on the drugs, so it is impossible to know whether they are HIV-free or their HIV is just drug-suppressed. And the scientists are anxious they should not stop the drugs now as a result of this case. One HIV-free baby may be exceptional. There could be some reason, as yet unknown, why this baby is different from others.

Hopefully, scientists will establish that any newborn baby can be functionally cured in this way. But they do not expect the same to be true of children whose HIV infection is discovered later — let alone adults. They think this has to do with hitting the virus at the earliest possible moment after birth, before it has reached the CD4 cells in the immune system which harbour a reservoir of HIV in adults that the drugs are never quite able to wipe out.

The Mississippi baby was unusual, because the vast majority of pregnant women in wealthy countries are tested for HIV and most infections in babies are prevented — in the UK as many as 98%.

That is not so in poorer countries. In the developing world, there is still a big and tragic problem. In 2008, the latest year for which there are figures, 430,000 babies were infected at birth. That is a drop in the numbers, but far too high and desperately sad for parents and child.
It might seem as though the Mississippi baby breakthrough will, therefore, save thousands of lives in the 25 countries in sub-Saharan Africa, where most of these infections are taking place. But there is already a way of preventing these infections using drugs – which is far better than a functional cure using similar drugs. The problem is not how to do it – it is to ensure the drugs and the medical staff are in the right place at the right time to treat mother or baby or both. There are plenty of pregnant women in Africa known to have HIV who cannot get the treatment they want and need to protect their child. It is not very likely that the clinics they attend will instead have the three-drug combinations that the Mississippi baby received from skilled nursing staff within hours of birth.

Real excitement is justified by the Mississippi discovery – but it is what it tells scientists still trying to figure out how to defeat HIV that matters. Any practical applications are a long way further down the line.

**Reports of 'HIV cure' are premature**

Monday March 4 2013

Global news coverage has been dominated by the potentially groundbreaking news that a child born with HIV appears to have been ‘cured’ of the infection.

The Guardian reports that US doctors have made medical history with a ‘first functional cure’ of an unnamed two-year-old girl born infected with HIV and ‘who now needs no medication’. BBC News quotes researcher Dr Deborah Persaud, who presented the news to a medical conference, as saying, “This is a proof of concept that HIV can be potentially curable in infants”.

The researchers report that the baby was started on antiretroviral (anti-HIV) treatment at two days of age and continued on this to 18 months. By one month old, HIV could no longer be detected in the baby’s blood using standard laboratory tests, and the virus continued to be undetectable up to 26 months of age. However, highly sensitive laboratory tests could still detect the presence of HIV at very low levels.

This means that scientists have not found a complete cure for HIV. However, as The Guardian clarifies, they have found a ‘functional cure’, in which the girl is still infected, but currently requires no treatment. This means the disease is less likely to progress in the girl, potentially giving her a good life expectancy.

It is not yet possible to say whether this child’s viral levels will remain low, or whether she will need further antiretroviral therapy.

These findings therefore do not mean that a complete cure for HIV has been discovered.

**What have the scientists discovered about HIV treatment?**

It is now unusual for babies to be born with HIV in developed countries due to advances in treatment and care. These advances mean it is usually possible to prevent an HIV-infected mother from passing the infection on to her baby. However, infant HIV remains a significant problem in many developing countries.

Researchers from several US medical institutions have presented the findings from a case of a 26-month-old child who was born with HIV and had anti-HIV treatment started when she was just 30 hours old. The findings were announced at the Conference on Retroviruses and Opportunistic Infections in Atlanta, US, on March 4 2013.

Dr Deborah Persaud and colleagues say the baby girl was born to a mother confirmed (apparently at a late stage) to be HIV positive. Two separate blood samples were taken from the newborn when she was two days old, confirming that she was also infected.

The baby was started on antiretroviral treatment (ART), and further blood samples were taken to test for the HIV virus when she was seven, 12 and 20 days old. These blood samples were all positive for HIV, but a further sample taken at 29 days did not detect levels of the virus. ART was continued until 18 months of age.

Standard laboratory tests could then not detect any levels of the virus in 16 further blood samples taken between one and 26 months of age. Highly sensitive laboratory tests for HIV were also performed at 24 and 26 months of age. At 24 months, these sensitive tests identified a single copy of HIV RNA in the blood, and 37 copies of HIV DNA per million of a particular type of white blood cell. However, the virus did not appear to be able to replicate itself. By 26 months, highly sensitive tests revealed only four copies of HIV DNA per million of the white blood cell.

Therefore, though the virus was still detectable with highly sensitive blood tests, the virus was undetectable with standard clinical tests, which the researchers say ‘confirms a state of functional HIV cure’. They conclude that ‘this is the first well-documented case of functional cure in an HIV positive child.
and suggests that very early ART may prevent establishment of a latent reservoir and achieve a cure in children’.

**What is antiretroviral therapy?**

HIV is treated with a combination of antiretroviral (anti-HIV) drugs, known as ‘ART’. These drugs are not a ‘cure’ for HIV, doctors give them to patients with HIV to try and stop the virus replicating and to reduce the levels of virus. Reducing the amount of viruses in a person’s body can help limit the harm done to the body’s immune system by HIV.

Doctors measure the success of ART treatment by how much it reduces the viral load (the number of particles of HIV present in a volume of blood) to levels that can no longer be detected by standard blood tests (‘undetectable levels’). Doctors hope that by using ART treatment, they can prolong life and reduce the risk of disease progression and associated complications. A person with HIV normally has to continue on ART for the rest of their life to prevent viral levels from increasing again.

However, as was demonstrated in the case of this young child, even if the HIV is at undetectable levels, it doesn’t mean the virus has completely gone. It can still be detected on highly sensitive tests. For this reason, the researchers in the current study were careful to call this a ‘functional cure’ because the virus was undetectable on standard tests but had not gone completely.

**How is HIV passed from a mother to her baby?**

HIV is a bloodborne virus and can be passed on through blood and other bodily fluids. If an HIV positive woman is pregnant there is a small risk of the virus being passed to the baby during pregnancy, during birth, or through breastfeeding. Doctors will make every effort to prevent HIV being transmitted from mother to baby. This is usually attempted by:

- giving the mother ART during her pregnancy
- taking special care around the time of delivery
- using formula rather than breast milk

However, if the baby is infected and starts treatment early, and treatment is taken when needed, then the outlook for the child is good.

**Conclusion**

We are still a long way short of a ‘cure’ for HIV.

The potential outcome of treatment for the baby girl in the current US case is unclear. She is likely to need further blood tests as she grows up, to keep a check on the levels of HIV in her blood. Hopefully, she will continue to grow healthily into adulthood with the virus at undetectable levels. However, it is possible that she may need further ART if her viral levels begin to rise again.

It is impossible to say how or why this particular child has achieved a ‘functional cure’. It could be the fact that she had very early treatment with ART, or it could be due to the biology of this individual child.

The next step for researchers is to see whether the ART regime used for this child causes a similar outcome for other high-risk newborns.

It is currently uncertain whether the information contained in this case report will lead to any advances in the treatment of older children or adults with HIV. ART is prescribed on an individual basis according to clinical tests, response and adverse effects. Anyone taking ART should continue to take the treatment as prescribed by their specialist.

The findings do not mean that a new complete cure for HIV has been found.

However, if the results can be replicated in other newborns, it may offer the hope of reducing the number of cases of infant HIV in the developing world.

**HIV Trial Yields Disappointing Results**

*Voice of America News*, (03.04.2013) Joe DeCapua

A large-scale HIV prevention trial, called Vaginal and Oral Interventions to Control the Epidemic (VOICE), comprised of African women has produced disappointing results; however, the results may be based more on the behavior rather than the prevention methods utilized in the study. VOICE trial results were announced at the 20th Conference on Retroviruses and Opportunistic Infections in Atlanta.

Executive Director Mitchell Warren of AVAC, a nonprofit HIV/AIDS advocacy group, stated that the study centered on PrEP—preexposure prophylaxis—and examined three different options to help African women prevent HIV infection. VOICE was funded by the US National Institutes of Health and was conducted with more than 5,000 women in Zimbabwe, South Africa, and Uganda. The women used the following prevention methods: a daily oral dose of tenofovir, a daily oral dose of a combination pill known as Truvada, and a daily 1-percent vaginal tenofovir gel. These methods previously had been shown to
provide protection, but in the VOICE trial, the results were disappointing. Warren declared that “They were disappointing, and pivotal studies are not just ones that tell us the answers we want. A pivotal study is one to help give us answers to the questions we have. And this study showed that none of the three study products provided additional protection. They were safe, but not effective.” He said that the products will only succeed if they are used as prescribed.

Warren noted that even though the women were dedicated to the trial and returned to the clinic every month, they did not actually use the products, further saying that behavior is more important than biomedicine. Even though the daily pill and daily vaginal gel were convenient, the study participants did not respond. Warren declared one of the ideas the trial provided was that “while women may be at risk of HIV, they may not perceive themselves on a daily basis to be at risk.” Warren said that the African women in the study were concerned about contraception, so future research could involve using birth control pills with an antiretroviral drug. Warren stated that the VOICE study conveys to researchers the importance of listening to what women say and giving them something that they will use.

**UB Invention Leads to Discovery of Novel Pathway for TB Vaccine**
*University at Buffalo*, (03.01.2013)
Researchers have figured out how to use a University of Buffalo (UB)-patented mucosal adjuvant, LT-IIb, to dramatically strengthen TB vaccines delivered through the mucous membranes. The discovery, which clarified that the IL-17 pathway is the best route for TB vaccines, was part of a study focused on “bacterial proteins in the type II family of bacterial heat-labile enterotoxins” (HLT), according to Terry D. Connell, PhD, UB professor of microbiology and immunology.

Connell explained that LT-IIb and similar type II adjuvants can boost the body’s ability either to make antibodies or to strengthen a cellular response, depending on which type II adjuvant is selected. The research team’s next step is to understand how LT-IIb works to create IL-17 immune response. The team can then “engineer” HLT to maximize the adjuvants’ capacity to stimulate immune response.

Mucosal adjuvant-based TB vaccines can be dried in powdered form and stored without refrigeration until needed. The vaccine powder then can be sterilized with boiling water, and used as a nasal spray. In contrast, most injectable vaccines must be refrigerated constantly to remain effective and safe, which is not always possible in developing countries.

As drug-resistant strains of Mycobacterium tuberculosis appear, researchers are eager to find better ways to immunize people from TB, which kills more than 1.7 million people each year. Earlier TB vaccines targeted the IFN-y and T helper 1 pathways, which are still essential pathways for curing TB infections. The full report, “Interleukin-17-dependent CXCL13 Mediates Mucosal Vaccine-induced Immunity Against Tuberculosis,” was published online in the journal *Mucosal Immunology* (2013; doi: 10.1038/mi.2012.135).

**Women’s Low Adherence To Daily-Dose Products In HIV Prevention Trial Suggest Different Approach Needed, Researchers Say**
"Results of a major HIV prevention trial suggest that daily use of a product—whether a vaginal gel or an oral tablet—does not appear to be the right approach for preventing HIV in young, unmarried African women,” a press release from the Microbicide Trials Network reports (3/4). "Adherence among the women in the study was ‘very low,’ a researcher from the University of Washington said at the Conference on Retroviruses and Opportunistic Infections in Atlanta, where the results were presented,” the *New York Times* writes. "The study, known as VOICE, for Vaginal and Oral Interventions to Control the Epidemic, followed more than 5,000 women in South Africa, Uganda and Zimbabwe" who were given tenofovir gel, oral tenofovir or oral Truvada, and found that, "[a]lthough 95 percent of the women in the study made their monthly clinic visits, and 70 percent said they were using the pills or gel, blood tests suggested that only 25 percent actually were," according to the newspaper (McNeil, 3/4).

"HIV/AIDS experts said the results showed how important a factor human behavior is when devising ways to prevent HIV,” *Reuters* reports. "Clinicians and public health professionals will have to further assess and better understand how to promote and support the high levels of adherence necessary," Jonathan Mermin, an HIV/AIDS prevention expert at the CDC, said, the news agency notes (Herskovitz/Kelland, 3/4). "The VOICE study also found that single women under 25 were the least likely to use the product and the most likely to acquire HIV," a CONRAD press release states (3/4). Mitchell Warren, executive director of AVAC, "said one of the things African women are very concerned about is contraception," VOA News writes, adding, "Future research, therefore, could involve combining birth control pills with an antiretroviral drug" (DeCapua, 3/4). Writing in the Bill & Melinda Gates...
Foundation's "Impatient Optimists" blog, Stephen Becker, deputy director of the HIV program at the foundation, says the trial results "provide an opportunity to address fundamental factors that influence prevention product preferences and behaviors. To turn the tide we must stay committed to discovering and developing HIV prevention and treatment tools that those at risk will want and use" (3/4).

**Media Examine Reaction To, Potential Implications Of 'Functional Cure' Of Infant Born With HIV**

"The remarkable case of a baby being cured of HIV infection in the United States using readily available drugs has raised new hope for eradicating the infection in infants worldwide, but scientists say it will take a lot more research and much more sensitive diagnostics before this hope becomes a reality," Reuters reports (Steenhuysen, 3/4). "The toddler's case, if confirmed in further research, could have important implications for treatment of more than 300,000 babies born with the virus each year—mostly in the developing world," the Wall Street Journal writes, noting, "The baby is the second person ever documented to be cured of the virus during the 32-year global AIDS epidemic" after "a man named Timothy Brown and known as the Berlin patient, was cured as an adult as a result of a bone-marrow transplant he received to treat his leukemia" (Winslow, 3/4).

Pediatrician Deborah Persaud of Johns Hopkins Bloomberg School of Public Health "acknowledges that, like Brown, this is an n=1 finding, and says the child may still harbor an infection, which is why they're referring to the case as a 'functional cure' rather than the complete eradication of HIV, called a 'sterilizing' cure," Wired notes, adding, "This is one case and we definitely need to have more, and hopefully we can have more,' she said" (Cohen, 3/4). "If, on the other hand, what happened to the baby ... proves to be replicable, the implications for treatment of children born with the virus are vast," the Center for Global Health Policy's "Science Speaks" blog writes (Barton, 3/5). The Wall Street Journal in a separate video report examines some of the potential implications of the case on broader treatments (3/4). In an audio report from PRI's "The World," anchor Marco Werman "gets reaction from Dr. Donald Thea, Dr. Julie Herlihy, and Leoda Hamomba, of Boston University's 'Preventing Mother to Child Transmission Project' in Zambia" (Crossan, 3/4).

**Prevention Remains Best Method To Stop Mother-To-Child HIV Transmission**

"If confirmed by further analysis," the case of a Mississippi infant being cured of HIV "would be the first time a person has been cured with simple drug treatments, making a lifetime of antiviral therapy unnecessary," a New York Times editorial states. Born to an HIV-positive mother who did not receive HIV treatment during her pregnancy, the newborn was aggressively treated for HIV infection beginning 30 hours after birth, but the mother stopped treatment after 18 months, the editorial notes, adding, "The baby, now two and a half years old, has been free of the active virus ever since." The editorial adds, "Although very sophisticated tests can find traces of the virus, it is not able to replicate and spread. This is described as a 'functional cure.'"

"Researchers must still demonstrate conclusively that the baby had truly been infected and was not simply prevented from absorbing its mother's infection—a process achieved routinely in many babies," the New York Times writes, adding, "They must also show that this is not an exceptional, nonreplicable case with an atypical baby, but that the same treatment would work in other newborns." The editorial concludes, "If the stronger early treatment is confirmed to work, it could become the standard of care around the world. ... [But e]ven if the treatment works, the best defense against mother-to-child transmission is still prevention, which will always be preferable to treatment after an infection has set in" (3/4).

**Green tea extract interferes with the formation of amyloid plaques in Alzheimer's disease**

ANN ARBOR—Researchers at the University of Michigan have found a new potential benefit of a molecule in green tea: preventing the misfolding of specific proteins in the brain. The aggregation of these proteins, called metal-associated amyloids, is associated with Alzheimer's disease and other neurodegenerative conditions.

A paper published recently in the Proceedings of the National Academy of Sciences explained how U-M Life Sciences Institute faculty member Mi Hee Lim and an interdisciplinary team of researchers used green tea extract to control the generation of metal-associated amyloid-β aggregates associated with Alzheimer's disease in the lab.
The specific molecule in green tea, (−)-epigallocatechin-3-gallate, also known as EGCG, prevented aggregate formation and broke down existing aggregate structures in the proteins that contained metals—specifically copper, iron and zinc.

"A lot of people are very excited about this molecule," said Lim, noting that the EGCG and other flavonoids in natural products have long been established as powerful antioxidants. "We used a multidisciplinary approach. This is the first example of structure-centric, multidisciplinary investigations by three principal investigators with three different areas of expertise."

The research team included chemists, biochemists and biophysicists.

While many researchers are investigating small molecules and metal-associated amyloids, most are looking from a limited perspective, said Lim, assistant professor of chemistry and research assistant professor at the Life Sciences Institute, where her lab is located and her research is conducted.

"But we believe you have to have a lot of approaches working together, because the brain is very complex," she said.

The PNAS paper was a starting point, Lim said, and her team's next step is to "tweak" the molecule and then test its ability to interfere with plaque formation in fruit flies.

"We want to modify them for the brain, specifically to interfere with the plaques associated with Alzheimer's," she said.

Lim plans to collaborate with Bing Ye, a neurobiologist in the LSI. Together, the researchers will test the new molecule's power to inhibit potential toxicity of aggregates containing proteins and metals in fruit flies.

Very early antiretroviral treatment limits the size of HIV reservoir
People treated very early may be "prime candidates" for HIV cure studies
Keith Alcorn
Published: 05 March 2013
Very early antiretroviral treatment may limit the size of the HIV reservoir in adults and children, according to studies presented on 4 March at the 20th Conference on Retroviruses and Opportunistic Infections (CROI) in Atlanta.

Other research presented at the meeting suggests that too little is known about either the size or the cell types that constitute the HIV reservoir to be confident that early assessments will be a reliable guide to the potential for viral eradication.

Background: the problem of HIV persistence in the human body
The primary obstacle to the elimination of HIV from the human body is the ability of the virus to persist in a latent form within CD4 cells that are in a resting state. These are cells which become infected with HIV and where the virus is incorporated into the cell’s genetic material (genome), after which the virus remains in a latent state. A variety of triggers can cause the cell to become activated and to begin producing virus, but the virus may remain integrated within the cell, undetected by the immune system, for many years. It is only when the virus begins to reproduce that the cell's surface will display proteins that cause the immune system to target that cell for destruction.

Latently infected cells form a 'reservoir' of cells that are constantly being activated to produce HIV, which then goes on to establish latent infections in other cells. In people taking antiretroviral therapy, that process of activation causes a very low level of viral replication, below the limits of detection of all but the most sensitive experimental tests, because antiretroviral therapy shuts down this re-seeding process. Yet, only a very small number of latently infected cells are needed in order to establish a detectable level of virus production within days of halting antiretroviral therapy. For this reason, studies of treatment interruption have consistently shown that viral load rebounds to a level corresponding to the pre-treatment peak within a few weeks of stopping treatment.

It has been proposed previously that very early antiretroviral treatment in infants might offer the best prospect of HIV elimination using antiretroviral therapy alone. This is because antiretroviral therapy started early enough, in an infant infected with HIV during delivery, might have the potential to restrict the establishment of long-lasting reservoirs of latently infected cells. One case report also presented at this conference, of an infant with confirmed infection treated from 31 hours after delivery, and now off treatment for one year with no signs of viral replication, offers proof of concept. Further studies are planned to test whether this 'functional cure' can be replicated in other infants.
Limiting the size of the reservoir by early treatment

Another question for future HIV eradication studies will be: does a smaller reservoir of latently infected cells afford a better prospect of curing HIV infection, and if so, is it possible to limit the size of the reservoir by treating a person very soon after HIV infection?

Researchers in Thailand have been attempting to do this, by offering a process of very fast diagnosis and treatment initiation, in a setting with a high incidence of HIV infection. People who presented to the Thai Red Cross HIV testing centre in Bangkok were tested for HIV RNA, p24 antigen and HIV antibodies, using a third-generation enzyme-linked immunosorbent assay (EIA).

Diagnostics were completed within a median of three days, after which people diagnosed with HIV were asked if they would be willing to undergo leukopheresis for CD4 cell counting and enumeration of total and integrated HIV DNA in peripheral blood mononuclear cells (PBMCs). (Integrated HIV DNA indicates that, in theory, a cell would be capable of producing replication-competent virus in the future.) Participants were also asked if they would be willing to undergo sigmoid colon sampling in order to obtain samples from any potential HIV reservoirs in the gut. A median of two days after diagnosis, subjects started antiretroviral therapy with a regimen that included raltegravir in order to achieve very rapid viral load reduction.

The study recruited 75 participants, 91% of them men who have sex with men. Data were presented on 68 subjects for whom quantification of HIV DNA had taken place. These included 21 subjects in whom sigmoid biopsies had also been carried out. Just over one-third (37%) were in Feibig 1 of acute HIV infection, that is to say, they were HIV RNA positive but negative for p24 antigen and negative for HIV antibody by third-generation ELISA test. The median time between enrolment and exposure was 15 days in this sub-set of patients, said Dr Jintanat Ananworanich.

Patients in this sub-set had the lowest HIV DNA levels prior to treatment; 92% had undetectable integrated HIV DNA in PBMCs and 88% had undetectable integrated HIV DNA in the sigmoid colon, indicating that a reservoir of detectable infected cells had not yet been established (although it should be noted that this study sampled a limited number of cell types).

Patients in Feibig 2 (HIV RNA+, p24+) and Feibig 3 (ELISA+, western blot negative) stages of acute infection showed substantial reductions in HIV DNA within 12 weeks of starting antiretroviral treatment, and reached undetectable levels of integrated HIV DNA in PBMCs by week 24. Seven out of ten patients (3 Feibig 1 and 4 Feibig 2/3 patients) who underwent sigmoid biopsy, and who had detectable integrated HIV DNA at baseline, had undetectable HIV DNA by week 24 of treatment.

Analysis of CD4 central memory cells (the key reservoir for infection) showed very limited infection compared to transitional and effector memory CD4 cells at baseline, and this trend persisted after 24 weeks of treatment.

Patients treated early in acute infection, whether in stages Feibig 1 or 3, showed similar characteristics to ‘elite’ HIV controllers – a small or undetectable reservoir of HIV DNA, and a bias towards infection of transitional and effector cells rather than central memory cells, concluded Dr Ananworanich. These patients may be ideal candidates for future cure studies which look at the use of therapeutic vaccines in combination with agents that can deplete the HIV reservoir. In due course, treatment interruptions might also be attempted in order to determine whether any of these patients is functionally cured, and if so, what might be the immunologic correlates of a functional cure.

Dr Katherine Luzuriaga also presented data on the characteristics of the HIV reservoir after early treatment, this time in five adolescents with a median age of 16 who had received antiretroviral treatment since soon after birth (median 2 months of age). It was impossible to isolate replication-competent HIV DNA from any of these patients, although proviral DNA was detectable at a low level, and they had no HIV-specific antibody or CD8+ T-cell responses. In comparison, four age-matched young people who had begun HIV treatment in later childhood, and who had sustained undetectable viral load ever since, had detectable HIV RNA (8 copies/ml) by ultrasensitive assay and HIV antibody and CD8+ T-cell responses to a broad range of HIV genes, indicating ongoing replication.

Dr Luzuriaga’s group suggested that these young people, like the acutely infected Thai patients described by Dr Ananworanich, could be “prime candidates for interventions to achieve functional cure or eradication.”

In contrast, data presented by collaborators from the University of Pittsburgh and Harvard University, show that in adults treated with fully suppressive antiretroviral therapy for at least ten years, but commenced in advanced HIV disease (median CD4 cell count 193 cells/mm³), HIV DNA declines during treatment, but remains detectable after ten years, with higher levels correlated with older age and
higher baseline viral load. These findings suggest a much more well-established reservoir of HIV infection in chronically infected adults.

**Where is the reservoir, and what's in it?**

Further grounds for caution regarding the stubborn persistence of the HIV reservoir came in a series of presentations from research groups that had evaluated a variety of cell types, and also questioned the assays used to assess reservoir size, and come up with some dispiriting assessments of the complexity and size of the reservoir.

Maria Buzon and colleagues at the Ragon Institute, Harvard University, identified a pool of long-lasting cells, memory T-cell stem cells, which harbour high levels of HIV DNA despite long-term antiretroviral treatment, and which become more important as a proportion of all infected cells as time goes on. Natalia Soriano-Sorabia of University of North Carolina, Chapel Hill, identified gamma-delta T-cells as an important and hitherto unmeasured reservoir.

Another problematic issue for HIV eradication is to determine what to measure. Assays which seek to measure replication-competent HIV in the laboratory from cells harvested from patients — viral outgrowth — may under-count cells containing HIV DNA that might only be induced to replicate in certain restricted circumstances.

Ya-Chi Ho of Johns Hopkins University, Baltimore, described an analysis of the gene sequences of HIV proviruses that could not be induced to replicate after one round of activation in a viral outgrowth assay. 88% were defective, but 12% had an intact viral genome, suggesting that they might have the potential to infect other cells if they could be triggered to replicate. This finding led Ho and colleagues to estimate that the average latent reservoir could be 48 times larger than currently estimated.

**References**

1. Ananworanich J et al. Early ART intervention restricts the seeding of the HIV reservoir in long-lived central memory CD4 T cells. 20th Conference on Retroviruses and Opportunistic Infections, Atlanta, abstract 47, 2013. [View abstract 47 on the conference website](#).


**Monthly injectable drug offers 100% protection against HIV in monkeys — could be dosed every three months**

Gus Cairns

Published: 04 March 2013

**Injectable integrase inhibitor protects monkeys against rectal exposure**

An injectable, long-lasting integrase inhibitor drug, when given to rhesus macaques exposed to a monkey-adapted version of HIV, completely protected them against the virus. This drug, an injectable version of GSK1265744 (GSK744), has already been given as a single dose to HIV-negative human volunteers, and has a half-life of 21 to 50 days. Levels stayed above the IC<sub>90</sub> (the level necessary to suppress 90% of HIV replication) for six months, and above four times this level for four months.

This means that if it proves safe and effective in humans, it could be given as an injection as little as four times a year, though individual variations seen in this study mean that monthly dosing may be safer.

GSK744 is similar to dolutegravir, which is already nearing approval as an anti-HIV drug in Europe and the US. It is effective against HIV, though, at lower concentrations in the body, which means manufacturers GlaxoSmithKline have been able to formulate it as a nanoparticle suspension — tiny 'packets' of the drug floating in fluid, which provide a long-lasting supply of the drug when injected.

Scientists at the Aaron Diamond AIDS Research Institute injected GSK744 into eight male macaques and then a week later started 'challenging' them by introducing SHIV, a monkey adapted version of HIV as weekly doses (eight in total) in the rectum, to simulate anal sex. At the same time they challenged eight control monkeys without giving them GSK744 first. The monkeys on GSK744 were given a second dose four weeks after the first.

All the monkeys not given GSK744 became infected, on average after two challenges, though one took seven challenges. In contrast, none of the monkeys given GSK744 became infected or have shown any sign of virus in their blood up to three weeks after the last challenge.

Levels of GSK744 seen in these monkeys' rectal tissues were equivalent to a level that would be expected to be protective in humans, and stayed above the four-times-IC<sub>90</sub> level for the full eight weeks of viral challenge in six monkeys. In the other two levels, it fell below the four-times IC<sub>90</sub> level at week seven, in other words, just before the last viral challenge.
Given that adherence is turning out to be a major barrier to the effectiveness of pre-exposure prophylaxis – see this report and this one on the VOICE trial – HIV drugs that can be given by injection at quarterly sexual health check-ups could, in the long term, be a more feasible way of offering biomedical protection against HIV. The researchers are studying the protected monkeys to see if there is any sign of virus in their systems and to determine the minimum effective dose.

**Tenofovir vaginal ring protects monkeys against vaginal exposure**

In a separate monkey study, researchers from the US Centers for Disease Control protected female monkeys using another long-lasting PrEP method – a ring impregnated with tenofovir that could be inserted into the vagina. In this case, they used a polyurethane ring that was replaced every four weeks over a 16-week period.

Six monkeys exposed to SHIV were protected against infection, whereas eleven out of twelve not given the ring became infected. Unlike the injectable formulation, human research into vaginal rings is already well advanced, with the International Partnerships for Microbicides’ RING and ASPIRE phase III studies of an intravaginal ring impregnated with the NNRTI drug dapivirine well underway and rings containing the CCR5 inhibitor maraviroc in phase I trials too.

In this case the researchers, for the first time, used the tenofovir prodrug that is actually used in the oral tenofovir pill – tenofovir disoproxil fumarate or TDF. In previous microbicides, they have used the biologically active tenofovir molecule, but this is not as well-absorbed and does not produce such high concentrations in tissues as TDF. However TDF is not as stable, and the team had to develop a new polyurethane ring rather than the silicone ring used in other vaginal-ring studies.

This is the first time it has been shown that a ring can deliver enough of the widely used NRTI drug tenofovir to completely protect monkeys, and is the first time a ring has been demonstrated to protect monkeys who are repeatedly vaginally challenged with virus. It will help to advance development of this option in humans.

**References**

3. A webcast of the session in which this research was presented, [HIV prevention: ARV, counseling, contraception, and condoms](https://vimeo.com/51391954), is available on the conference website.

**Consistent condom use in anal sex stops 70% of HIV infections, study finds, but intermittent use has no effect**

*Only one-in-six men reported 100% condom use during three to four years of follow-up*

Gus Cairns
Published: 04 March 2013

An analysis by Dawn Smith of the US Centers for Disease Control (CDC) reported at the 20th Conference on Retroviruses and Opportunistic Infections (CROI 2013) on 4 March has provided the first estimate of the efficacy of condoms in preventing HIV transmission during anal sex since 1989. It found condoms stop seven out of ten anal transmissions – the same efficacy found by the 1989 study.

However, it also found that sometimes using condoms is not effective at preventing HIV infection, and that long-term 100% condom use is a minority behaviour: only one-in-six gay men actually managed to maintain it over the three- to four-year time frame of the analysis.

One ongoing problem in assessing the effectiveness of different HIV prevention methods is that anal sex is under-studied. We do not have enough data on rectal viral loads and their effect on transmission, or on whether HIV treatment reduces transmission via anal sex as well as it does for vaginal sex.

We are also unclear about to what extent condoms actually prevent HIV transmission in anal sex. This last fact may seem surprising, given that condoms have been recommended since the mid-1980s as the only effective HIV prevention method for gay men who have anal sex.

In fact, there is only one large study in gay men, dating from 1989. In this study of 2914 gay men, HIV incidence among those who said they used condoms 100% of the time was 70% lower than in men who did not use them at all. There has been one small study in the era of antiretroviral treatment (ART), which found an efficacy of approximately 75%.
These are somewhat lower than efficacies computed for vaginal sex, which is in the order of 80 to 85%, and may reflect both that HIV is at least ten times more easily transmitted via anal than vaginal sex, and also that condoms may be more likely to fail during anal sex.

So a new study estimating condom efficacy in anal sex is very overdue and, given the need to compare condom efficacy against newer, biomedical prevention methods, very useful.

**The studies analysed**

Researchers from the US Centers for Disease Control retrospectively analysed condom use and HIV infection data from two different studies of US HIV-negative gay men: the **VAX004 study, the first efficacy trial of an HIV vaccine**, conducted between 1998 and 1999, and **EXPLORE, one of the largest studies ever conducted of a behavioural intervention in HIV**, conducted between 1999 and 2001.

There were some differences between the trial populations. Men in EXPLORE were twice as likely to be black and 50% more likely to have a college or university degree, and were younger, with fewer than half of the men in VAX004 aged 35 or under but nearly two-thirds in EXPLORE.

There were 1323 out of 3102 men in EXPLORE who said that they had had at least one episode of unprotected sex with an HIV-positive partner (43% of the men in the trial) and 2167 out of 4264 in VAX004, or 51% of all men in that trial. Altogether then, the CDC studied 3490 men who had had serodiscordant unprotected anal sex out of a total of 7366. There were 154 HIV infections in men in VAX004 and 71 in EXPLORE.

The total follow-up period in VAX004 was three years, and four years in EXPLORE. Participants were tested for HIV every six months and asked whether they had always used a condom for anal sex in the previous six months, sometimes used one, or never used one.

**Condom efficacy with 100% use**

Amongst all men having anal sex, men who said they used condoms 100% of the time were 67% less likely to acquire HIV than men who never used condoms, and 64% less likely than men who said they sometimes used them.

Condom efficacy was consistently higher in EXPLORE: it was 86% for all anal sex, 87% for receptive anal sex and 76% for insertive anal sex compared with 59, 63 and 55% in VAX004.

Why the big difference? One possibility is that because EXPLORE was a behavioural intervention, it may have helped participants use condoms better and have fewer ‘accidents’ than in VAX004, which monitored condom use but did not intervene.

These figures are derived by comparing HIV incidence in men who said they *always* used condoms with men who *never* used them. What about the men who sometimes used them?

**Condom efficacy with intermittent use**

This analysis also shows that sometimes using condoms is no better than not using them at all. Overall, men who said they sometimes used condoms were only 4.4% less likely to acquire HIV than men who never used them. This difference was statistically insignificant; the margin of uncertainty means that, statistically, the ‘true’ efficacy of ‘sometimes’ versus ‘never’ using condoms could be anything between 29% fewer infections to 29% more infections, which is as good as saying that intermittent condom use essentially has zero efficacy.

There were 26% fewer infections in EXPLORE in men who used condoms intermittently than in men who never used them, but this was not statistically significant.

In VAX004 ‘sometimes’ using condoms was, if anything slightly less effective than never using them, with nearly 10% more infections in ‘sometimes’ versus ‘never’ users. How could this be? In the 1989 study, gay men who said they ‘sometimes’ used condoms were no less than 70% more likely to acquire HIV than men who said they never used them. The researchers at the time hypothesised that this because men who never used condoms might be more likely to be in monogamous relationships.

The researchers are now going to do further research to split ‘sometimes’ into different frequencies of use to find out below which level condom use ceases to be protective.

**Consistency of condom use**

How consistent was consistent condom use? Over the whole length of the studies, not very. Two-thirds of men reported using condoms 100% of the time for at least one six-month slot during the two trials: but only 16.4% reported using them in every single six-month slot, i.e. truly consistently. Conversely, while only 5% of men reported never using condoms for the whole length of the studies, 40% reported never using them for at least one six-month period.

In the same session, Bob Grant, lead investigator of the **iPrEx PrEP trial**, showed an interesting slide showing selected ‘condom careers’ in individual trial participants and showed a whole variety of different use patterns over time, with the only consistent factor being no consistency.
There are a couple of important considerations to apply the CDC study's conclusions. One is that social desirability bias almost certainly means that men's reported use of condoms was higher than it really was. This would mean the figures would tend to underestimate condom efficacy in the men who really did use condoms 100% of the time.

On the other hand, since only a minority of men in the studies did use condoms 100% of the time, these computed efficacy figures for condoms have to be divided by the fraction who actually did use them, resulting in lower effectiveness than this in actually preventing HIV on a population level.

Reference
2. A webcast of the session in which this research was presented, HIV prevention: ARV, counseling, contraception, and condoms, is available on the conference website.

U.S. "Stalling" Could Force Acceptance of Onerous TPP
WASHINGTON, Mar 5 2013 (IPS)—Civil society opposition here has strengthened against a U.S.-proposed free trade zone that would include some dozen countries around the Pacific Rim.

As negotiators head into a 16th round of talks this week in Singapore, around 400 organisations are urging the U.S. Congress to demand greater transparency in the proceedings.

On Monday, the first day of the negotiations, Medecins Sans Frontieres (MSF), a humanitarian group, called on President Barack Obama's administration to “end its stall tactics and revise its proposals for what otherwise promises to be the most harmful trade deal ever for access to medicines in developing countries.”

Look at who has a seat at the table, with the public shut out and more than 600 corporate lobbyists...

The Singapore talks will extend through Mar. 13. Critics say civil society and other critical stakeholders have been systematically shut out of the negotiations, supplanted by corporate interests.

The proposal, known as the Trans-Pacific Partnership (TPP), currently comprises 11 countries (a 12th, Japan, is also contemplating joining). But the Obama administration has been clear that if passed, the zone would be open-ended in terms of future expansion.

That broad geographical sweep, together with the simultaneous negotiation of a lengthy but highly secretive list of contentious issues not necessarily related to trade, is leading critics to warn that the scope of any pending agreement could negatively impact on nearly half the globe.

And with the Obama administration now saying it wants to wrap up the negotiations by October, some TPP negotiators are reportedly worried that some of the most controversial issues up for discussion are being pushed to the very end in an attempt to “run out the clock”.

According to a new brief released by MSF, U.S. TPP negotiators are pushing for rules that would “enhance patent and data protections for pharmaceutical companies, dismantle public health safeguards enshrined in international law and obstruct price-lowering generic competition for medicines”.

The result could be restrictions on access to affordable generic medicines for “millions” of people.

Judit Rius Sanjuan, U.S. manager for MSF’s Access Campaign, says her office heard that the last time the TPP negotiations included substantive talks on access to medicines was a year ago. At that time, nearly all negotiating partners reportedly rejected a draft chapter on intellectual property rights, which includes the patent provisions.

And while the White House has stated that it would be resubmitting a revised chapter on this issue, Sanjuan says it appears that access to medicines is once again not on the agenda this week in Singapore. “We are hearing from other negotiating teams that the pressure to finalise this agreement by October is rising, and they fear that if there is not more time for substantive discussion, this chapter could stand,” she told IPS.

“We share the concern that this delay in presenting an alternative text is a U.S. strategy to focus instead on the less controversial chapters and leave behind debate over access to medicines. But doing so would have huge consequences for developing countries.”

In fact, imposing these types of new restrictions would run counter to previous international agreements and national legislation under which Washington has pledged to expand access to generic medicines.

Any restriction in access to such medicines would also affect the United States’ own global health goals. According to Sanjuan, generics make up some 98 percent of the medicines used by PEPFAR, the United States’ flagship anti-HIV/AIDS programme and the world’s largest.

Half the world
Global health wouldn’t be the only sector impacted by the TPP’s passage. Also on Monday, coinciding with the first day of negotiations in Singapore, around 400 groups from a broad range of backgrounds sent an open letter to the U.S. Congress opposing the abnormally secretive way in which negotiations for the trade area have been run.

“This agreement will impact on how trade and investment are conducted in the Pacific Rim for decades, yet the ramifications aren’t fully understood even by people who know about the TPP,” Arthur Stamoulis, executive director of the Washington-based Citizens Trade Campaign, an advocacy group, and an organiser of the letter, told IPS.

“This is an agreement that wouldn’t just affect the economy and sustainability in these 11 countries, but has the potential to impact the economy and environment for literally half the world.”

In lieu of official consultation, the groups are offering recommendations for draft language on issues from environmental standards and human and labour rights to financial regulation and national sovereignty. Yet the central complaint has to do with lack of oversight and transparency.

“We find it troubling that … U.S. negotiators still refuse to inform the American public what they have been proposing,” the letter states. “Shielding not only proposals but agreed-upon texts from public view until after negotiations have concluded and the pact is finalized is not consistent with democratic principles.”

The groups are calling for an opening-up of the talks to both the U.S. Congress and the public at large. They’re also urging lawmakers not to authorise new “fast track” powers that would allow the president to send Congress trade pacts for straight votes without the possibility of amendments.

Free trade advocates tend to suggest that such powers are necessary to get other countries to agree to large-scale trade agreements in the first place, but President Obama had allowed the “fast track” legislation to lapse. On Friday, however, the administration’s new trade policy agenda noted that the president would work with Congress to re-authorise that authority.

The administration has used similar concerns to rationalise the high level of secrecy surrounding the negotiations, saying that greater transparency would upset delicate discussions.

Yet critics point out that draft trade texts at this point in negotiations are often made public, including by the World Trade Organisation. Similar precedent exists from the Free Trade Area of the Americas, the trade zone agreed to in 2001 covering 34 countries, including the United States.

“There’s a real reason why the draft has been kept secret from the U.S. public – Americans wouldn’t support a huge amount of the agenda that the [Obama administration] has been pushing,” Citizens Trade’s Stamoulis says.

“If they were to negotiate an agreement that put human rights ahead of corporate profit, creating more just and sustainable social policy, the TPP could be a tool for incredible good. But if you look at who has a seat at the table, with the public shut out and more than 600 corporate lobbyists included, there is nothing to indicate that’s the deal we’re going to get.”

High Humidity Leads to Loss of Infectious Influenza Virus from Simulated Coughs
John D. Noti

Background
The role of relative humidity in the aerosol transmission of influenza was examined in a simulated examination room containing coughing and breathing manikins.

Methods
Nebulized influenza was coughed into the examination room and Bioaerosol samplers collected size-fractionated aerosols (<1 µM, 1–4 µM, and >4 µM aerodynamic diameters) adjacent to the breathing manikin’s mouth and also at other locations within the room. At constant temperature, the RH was varied from 7–73% and infectivity was assessed by the viral plaque assay.

Results
Total virus collected for 60 minutes retained 70.6–77.3% infectivity at relative humidity ≤23% but only 14.6–22.2% at relative humidity ≥43%. Analysis of the individual aerosol fractions showed a similar loss in infectivity among the fractions. Time interval analysis showed that most of the loss in infectivity within each aerosol fraction occurred 0–15 minutes after coughing. Thereafter, losses in infectivity continued up to 5 hours after coughing, however, the rate of decline at 45% relative humidity was not statistically different than that at 20% regardless of the aerosol fraction analyzed.

Conclusion
At low relative humidity, influenza retains maximal infectivity and inactivation of the virus at higher relative humidity occurs rapidly after coughing. Although virus carried on aerosol particles <4 µM have the potential for remaining suspended in air currents longer and traveling further distances than those on larger particles, their rapid inactivation at high humidity tempers this concern. Maintaining indoor relative humidity >40% will significantly reduce the infectivity of aerosolized virus.

**Using Routine Surveillance Data to Estimate the Epidemic Potential of Emerging Zoonoses: Application to the Emergence of US Swine Origin Influenza A H3N2v Virus**
Simon Cauchemez

**Background**
Prior to emergence in human populations, zoonoses such as SARS cause occasional infections in human populations exposed to reservoir species. The risk of widespread epidemics in humans can be assessed by monitoring the reproduction number \( R \) (average number of persons infected by a human case). However, until now, estimating \( R \) required detailed outbreak investigations of human clusters, for which resources and expertise are not always available. Additionally, existing methods do not correct for important selection and under-ascertainment biases. Here, we present simple estimation methods that overcome many of these limitations.

**Methods and Findings**
Our approach is based on a parsimonious mathematical model of disease transmission and only requires data collected through routine surveillance and standard case investigations. We apply it to assess the transmissibility of swine-origin influenza A H3N2v-M virus in the US, Nipah virus in Malaysia and Bangladesh, and also present a non-zoonotic example (cholera in the Dominican Republic). Estimation is based on two simple summary statistics, the proportion infected by the natural reservoir among detected cases \( G \) and among the subset of the first detected cases in each cluster \( F \). If detection of a case does not affect detection of other cases from the same cluster, we find that \( R \) can be estimated by \( 1-G \); otherwise \( R \) can be estimated by \( 1-F \) when the case detection rate is low. In more general cases, bounds on \( R \) can still be derived.

**Conclusions**
We have developed a simple approach with limited data requirements that enables robust assessment of the risks posed by emerging zoonoses. We illustrate this by deriving transmissibility estimates for the H3N2v-M virus, an important step in evaluating the possible pandemic threat posed by this virus.

Please see later in the article for the Editors' Summary

**Editors' Summary**

**Background**
When a virus emerges in the human population, such viruses can cause global epidemics potentially harming large numbers of people. Zoonotic viruses are viruses that are transmissible from animals to humans; the global health threat of zoonotic viruses was recently demonstrated by the 2009 H1N1 influenza pandemic and the SARS epidemic in 2003. Many zoonotic viruses are transmitted by means of an infected vector, while others can be transmitted by inhalation, contact with infected excretions, or by direct contact with an infected animal. Zoonotic viruses primarily cause occasional infections in human populations exposed to reservoir species (the animal species harboring the virus) because the pathogens are usually poorly adapted for sustained human-to-human transmission. However, zoonotic viruses are under strong selective pressure to acquire the ability for human-to-human transmission.

**Why Was This Study Done?**
The highly pathogenic H5N1 avian influenza epidemic was alarming to many because of the high mortality rate in humans and its rapid spread in avian populations. Public health response to outbreaks such as those of H5N1 avian influenza and SARS required reliable estimates of transmissibility (how easily it spreads between people) and severity (the proportion of infected people who needed hospital treatment). For efficient prevention and control of the emerging epidemic, quantitative and rigorous assessment of the associated risks is needed. Specifically, health officials and researchers need fast, reliable methods for estimating the extent to which a virus has acquired the ability to transmit from person to person. In this study, the authors developed a novel method to estimate a standard measure of transmissibility, the human-to-human reproduction number \( R \) (average number of persons infected by a human case) of a zoonotic virus, which overcomes many of the limitations of existing methods.
What Did the Researchers Do and Find?
The authors developed a simple method to estimate the reproduction number of emerging zoonoses from routine surveillance data. By using two simple summary statistics, the proportion infected by the natural reservoir among detected cases (G) and among the subset of the first detected cases in each cluster (F), the authors estimated R, the reproduction number of zoonoses in humans. The authors then applied their new approach to assess the human-to-human transmissibility of swine-origin influenza A variant (H1N1v, H1N2v, and H3N2v) virus, in particular that of the H3N2v-M virus, from US surveillance data for the period December 2005–December 2011, Nipah virus in Malaysia and Bangladesh, as well as to a non-zoonotic pathogen Vibrio Cholerae in the Dominican Republic. This study demonstrates the applicability of this novel approach to estimating R during zoonotic and certain non-zoonotic outbreaks.

What Do These Findings Mean?
Cauchemez and colleagues show that their new approach will be useful in assessing human-to-human transmissions during zoonotic outbreaks. The authors show that their new method does not require as much of an investigation effort as existing methods, the statistical treatment of the data is extremely simple, and the robustness of the method is demonstrated even if larger clusters are more likely to be detected and if the ability to detect all cases in a cluster once a cluster is identified is low. This method of estimating R is designed for the context of subcritical outbreaks, i.e., $R<1$. However if $R\geq1$, other estimation methods will be needed.

Macrophage-expressed IFN-β Contributes to Apoptotic Alveolar Epithelial Cell Injury in Severe Influenza Virus Pneumonia
Katrin Högner

Abstract
Influenza viruses (IV) cause pneumonia in humans with progression to lung failure and fatal outcome. Dysregulated release of cytokines including type I interferons (IFNs) has been attributed a crucial role in immune-mediated pulmonary injury during severe IV infection. Using ex vivo and in vivo IV infection models, we demonstrate that alveolar macrophage (AM)-expressed IFN-β significantly contributes to IV-induced alveolar epithelial cell (AEC) injury by autocrine induction of the pro-apoptotic factor TNF-related apoptosis-inducing ligand (TRAIL). Of note, TRAIL was highly upregulated in and released from AM of patients with pandemic H1N1 IV-induced acute lung injury. Elucidating the cell-specific underlying signalling pathways revealed that IV infection induced IFN-β release in AM in a protein kinase R- (PKR-) and NF-kB-dependent way. Bone marrow chimeric mice lacking these signalling mediators in resident and lung-recruited AM and mice subjected to alveolar neutralization of IFN-β and TRAIL displayed reduced alveolar epithelial cell apoptosis and attenuated lung injury during severe IV pneumonia. Together, we demonstrate that macrophage-released type I IFNs, apart from their well-known anti-viral properties, contribute to IV-induced AEC damage and lung injury by autocrine induction of the pro-apoptotic factor TRAIL. Our data suggest that therapeutic targeting of the macrophage IFN-β-TRAIL axis might represent a promising strategy to attenuate IV-induced acute lung injury.

Author Summary
Acute lung injury induced by influenza virus (IV) infection has been linked to an unbalanced release of pro-inflammatory cytokines including type I interferons (IFN) causing immune-mediated organ damage. Using ex vivo and in vivo IV infection models, we demonstrate that alveolar macrophage-expressed IFN-β induces alveolar epithelial cell injury by autocrine induction of the pro-apoptotic TNF-related apoptosis-inducing ligand (TRAIL). Elucidating the cell-specific underlying signalling pathways revealed that IV-induced IFN-β release from alveolar macrophages (AM) strictly depended on protein kinase R- (PKR-) and NF-kB-signalling. Autocrine activation via the macrophage type I IFN receptor (IFNAR) resulted in increased expression and release of TRAIL which caused apoptosis of IV-infected and non-infected alveolar epithelial cells and promoted alveolar barrier dysfunction as demonstrated in ex vivo co-cultures and in bone marrow chimeric mouse models in vivo. Importantly, we found TRAIL highly upregulated in and released from AM of hospitalized patients with pandemic H1N1-induced lung failure. Therapeutic targeting of the macrophage IFN-β-TRAIL axis might therefore represent a promising strategy to attenuate IV-induced acute lung injury.
Infections With 'Nightmare Bacteria' Are On The Rise In U.S. Hospitals

by Rob Stein
March 05, 2013 2:56 PM

Federal officials warned Tuesday that an especially dangerous group of superbugs has become a significant health problem in hospitals throughout the United States.

These germs, known as carbapenem-resistant Enterobacteriaceae, or CRE, have become much more common in the last decade, according to the Centers for Disease Control and Prevention. And the risk they pose to health is becoming evident.

"What's called CRE are nightmare bacteria," Dr. Thomas Frieden, director of the CDC, tells Shots. "They're basically a triple threat."

First of all, they are resistant to virtually all antibiotics, including the ones doctors use as a last-ditch option.

They can transfer the invincibility to other bacteria. "The mechanism of resistance to antibiotics not only works for one bacteria, but can be spread to others," Frieden says.

Third, the bacteria can be deadly. Infection with the bacteria "have a fatality rate as high as 50 percent," Frieden says.

Although the resistant bacteria potentially pose a risk to anyone, people whose immune systems are weaker, such as elderly people, children and people who have other health problems, tend to be most susceptible to infection.

If the drug-resistance starts to spread from bacteria that are usually a problem in hospitals to much more widespread causes of infections, the risk could rise even more. "If it spread to things like E. coli, which is a common urinary tract infection, it would be a very serious problem," Frieden says.

The CDC sounded the alarm because of data that show the proportion of bacteria that have this resistance to many drugs has quadrupled in the last decade or so.

CRE cases were reported by 4 percent of hospitals in 2012, up from about 1 percent from about a decade earlier, according to the report. In long-term care hospitals the situation is even worse — about 18 percent have reported cases, the CDC says.

In addition, the proportion of Enterobacteriaceae bacteria that were resistant increased from 1.2 percent in 2001 to 4.2 percent in 2011, the CDC reported.

"And that's for the whole family," says Dr. Arjun Srinivasan, the CDC's associate director for health care-associated infection-prevention programs. "When we look at one member of this family, a bacteria called Klebsiella, which is the most common type of CRE that we see in the United States, resistance there has gone from about 2 percent to over 10 percent. So a dramatic increase in the frequency with which these organisms are being seen in our hospitals in the United States."

Infectious disease specialist Dr. Brad Spellberg, of the Los Angeles Biomedical Research Institute at the Harbor-UCLA Medical Center, likens the situation to the Titanic's ill-fated voyage. "We're not talking about an iceberg that's down the line," he says. "The ship has hit the iceberg. We're taking on water. We already have people dying. Not only of CRE, but of untreatable CRE."

So far, these infections are still relatively rare. And they have been seen only in hospitals.

The big fear is that they'll start to move out of hospitals and into the communities around them. "If CRE spreads out of hospitals and into communities, that's when the ship is totally underwater and we all drown," Spellberg says.

To prevent that from happening, the CDC and others are calling on hospitals to contain CRE. "We can nip this in the bud. But it's going to take a lot of effort on the part of hospitals," Frieden says.

The first thing hospitals need to do is test patients to see if they have these bugs. "The basic steps are finding patients with CRE and making sure they are isolated so that they don't spread it to others," Frieden says.
That includes common-sense things like keeping them away from other patients and sterilizing everything they come into contact with.

"We know that this can stop outbreaks. It has helped in Florida. It's helped in other countries," Frieden says. "And the good news about this is that it still hasn't spread so widely that we can't stop it."

And doctors have to use antibiotics more carefully to prevent more germs from developing into dangerous superbugs.

**Human Y Chromosome Much Older Than Previously Thought**

Mar. 4, 2013 — The discovery and analysis of an extremely rare African American Y chromosome pushes back the time of the most recent common ancestor for the Y chromosome lineage tree to 338,000 years ago. This time predates the age of the oldest known anatomically modern human fossils.

University of Arizona geneticists have discovered the oldest known genetic branch of the human Y chromosome—the hereditary factor determining male sex.

The new divergent lineage, which was found in an individual who submitted his DNA to Family Tree DNA, a company specializing in DNA analysis to trace family roots, branched from the Y chromosome tree before the first appearance of anatomically modern humans in the fossil record.

The results are published in the *American Journal of Human Genetics*.

"Our analysis indicates this lineage diverged from previously known Y chromosomes about 338,000 ago, a time when anatomically modern humans had not yet evolved," said Michael Hammer, an associate professor in the University of Arizona's department of ecology and evolutionary biology and a research scientist at the UA's Arizona Research Labs. "This pushes back the time the last common Y chromosome ancestor lived by almost 70 percent."

Unlike the other human chromosomes, the majority of the Y chromosome does not exchange genetic material with other chromosomes, which makes it simpler to trace ancestral relationships among contemporary lineages. If two Y chromosomes carry the same mutation, it is because they share a common paternal ancestor at some point in the past. The more mutations that differ between two Y chromosomes the farther back in time the common ancestor lived.

Originally, a DNA sample obtained from an African American living in South Carolina was submitted to the National Geographic Genographic Project. When none of the genetic markers used to assign lineages to known Y chromosome groupings were found, the DNA sample was sent to Family Tree DNA for sequencing. Fernando Mendez, a postdoctoral researcher in Hammer's lab, led the effort to analyze the DNA sequence, which included more than 240,000 base pairs of the Y chromosome.
Hammer said "the most striking feature of this research is that a consumer genetic testing company identified a lineage that didn't fit anywhere on the existing Y chromosome tree, even though the tree had been constructed based on perhaps a half-million individuals or more. Nobody expected to find anything like this."

About 300,000 years ago, the time the Neanderthals are believed to have split from the ancestral human lineage. It was not until more than 100,000 years later that anatomically modern humans appear in the fossil record. They differ from the more archaic forms by a more lightly built skeleton, a smaller face tucked under a high forehead, the absence of a cranial ridge and smaller chins.

Hammer said the newly discovered Y chromosome variation is extremely rare. Through large database searches, his team eventually was able to find a similar chromosome in the Mbo, a population living in a tiny area of western Cameroon in sub-Saharan Africa.

"This was surprising because previously the most diverged branches of the Y chromosome were found in traditional hunter-gatherer populations such as Pygmies and the click-speaking KhoeSan, who are considered to be the most diverged human populations living today."

"Instead, the sample matched the Y chromosome DNA of 11 men, who all came from a very small region of western Cameroon," Hammer said. "And the sequences of those individuals are variable, so it's not like they all descended from the same grandfather."

Hammer cautions against popular concepts of "mitochondrial Eve" or "Y chromosome Adam" that suggest all of humankind descended from exactly one pair of humans that lived at a certain point in human evolution.

"There has been too much emphasis on this in the past," he said. "It is a misconception that the genealogy of a single genetic region reflects population divergence. Instead, our results suggest that there are pockets of genetically isolated communities that together preserve a great deal of human diversity."

Still, Hammer said, "It is likely that other divergent lineages will be found, whether in Africa or among African-Americans in the U.S. and that some of these may further increase the age of the Y chromosome tree."

He added: "There has been a lot of hype with people trying to trace their Y chromosome to different tribes, but this individual from South Carolina can say he did it."

The study came about by combined efforts of a private business, Family Tree DNA, the efforts of a citizen scientist, Bonnie Schrack, and the research capabilities at the UA.

**Journal Reference:**

---

**AIDS Journal Publishes Findings of Two Important Studies in March 2013 Issue**

Mar. 4, 2013 — The results of two important studies have been published in the March issue of AIDS, the official journal of the International AIDS Society. One study notes that screening for HIV should be performed more frequently — up to every three months for the highest-risk patients, while low-risk groups to be tested every three years. A second study demonstrates a link between heavy drinking and risky behaviors for men who have sex with men (MSM). AIDS is published by Lippincott Williams & Wilkins, a part of Wolters Kluwer Health.

**Northwestern University Study Researches the Most Cost-Effective Approach to HIV Screening**

The mathematical modeling study was performed to assess "optimal testing frequencies" for HIV screening in different risk groups. Current Centers for Disease Control and Prevention (CDC) guidelines recommend annual testing for high-risk groups, such as people with HIV-positive partners, people with multiple partners, injection drug users, and sex workers and once-in-a-lifetime testing for low-risk groups (whose annual risk of acquiring HIV is only one-hundredth of one percent).

The researchers modeled various scenarios in an attempt to "optimize the tradeoff" between the societal costs of testing versus the benefits of earlier HIV diagnosis over a patient's lifetime. Frequent testing is shown to be an effective method for identifying new HIV infections. In the past, people with new HIV infections weren't treated until they had significant declines in immune functioning, as measured by the CD4 cell count. But there's a growing consensus that antiretroviral treatment is beneficial for all HIV-infected patients, regardless of CD4 count. Starting treatment immediately after diagnosis also reduces the risk of transmitting HIV.
Within its limitations, the study suggests that current recommendations for HIV testing are “too conservative, especially for low risk groups who would benefit from more frequent testing,” according to Lucas and Armbruster. They conclude, “These results should encourage policymakers and medical professionals to reconsider how often adolescents and adults should be tested for HIV.”

The full article is available on the AIDS journal homepage (http://www.aidsonline.com/) and in the March 13 print and online edition.

**University of North Carolina Study Reveals Heavy Drinking Affects HIV Risk for MSM**

Researchers analyzed long-term follow-up data on MSM from a large study of HIV risk factors. All men were HIV-negative at the beginning of the study. Using special statistical weighting techniques, Dr Cole and colleagues analyzed the joint effects of alcohol consumption and sexual behavior on the risk of acquiring HIV (seroconversion) during follow-up. Findings show that for men who have sex with men (MSM), heavy drinking may lead to an increased risk of acquiring HIV.

Overall, 529 cases of HIV seroconversion were identified during follow-up. Thirty percent of the men reported having unprotected sex with multiple partners in the two years before the study. Risk is especially high for men who are heavy drinkers and have multiple partners, suggests the study by Stephen R. Cole, PhD, of University of North Carolina, Chapel Hill, and colleagues. They write, “These findings suggest that alcohol interventions to reduce heavy drinking among MSM should be integrated into existing HIV prevention activities.”

The full article can be read by visiting the AIDS journal homepage (http://www.aidsonline.com/) and can also be found in the March 13 print edition.

**Journal References:**


**How Do Bacteria Clog Medical Devices? Very Quickly**

Mar. 1, 2013 — A new study has examined how bacteria clog medical devices, and the result isn’t pretty. The microbes join to create slimy ribbons that tangle and trap other passing bacteria, creating a full blockage in a startlingly short period of time.

The finding could help shape strategies for preventing clogging of devices such as stents—which are implanted in the body to keep open blood vessels and passages—as well as water filters and other items that are susceptible to contamination. The research was published in *Proceedings of the National Academy of Sciences.*

Click on the image to view movie. Over a period of about 40 hours, bacterial cells (green) flowed through a channel, forming a green biofilm on the walls. Over the next ten hours, researchers sent red bacterial cells through the channel. The red cells became stuck in the sticky biofilm and began to form thin red streamers. Once stuck, these streamers in turn trapped additional cells, leading to rapid clogging. (Credit: Knut Drescher)

Using time-lapse imaging, researchers at Princeton University monitored fluid flow in narrow tubes or pores similar to those used in water filters and medical devices. Unlike previous studies, the Princeton experiment more closely mimicked the natural features of the devices, using rough rather than smooth surfaces and pressure-driven fluid instead of non-moving fluid.

The team of biologists and engineers introduced a small number of bacteria known to be common contaminants of medical devices. Over a period of about 40 hours, the researchers observed that some of the microbes—dyed green for visibility—attached to the inner wall of the tube and began to multiply,
eventually forming a slimy coating called a biofilm. These films consist of thousands of individual cells held together by a sort of biological glue.

Over the next several hours, the researchers sent additional microbes, dyed red, into the tube. These red cells became stuck to the biofilm-coated walls, where the force of the flowing liquid shaped the trapped cells into streamers that rippled in the liquid like flags rippling in a breeze. During this time, the fluid flow slowed only slightly.

At about 55 hours into the experiment, the biofilm streamers tangled with each other, forming a net-like barrier that trapped additional bacterial cells, creating a larger barrier which in turn ensnared more cells. Within an hour, the entire tube became blocked and the fluid flow stopped.

The study was conducted by lead author Knut Drescher with assistance from technician Yi Shen. Drescher is a postdoctoral research associate working with Bonnie Bassler, Princeton's Squibb Professor in Molecular Biology and a Howard Hughes Medical Institute Investigator, and Howard Stone, Princeton's Donald R. Dixon '69 and Elizabeth W. Dixon Professor of Mechanical and Aerospace Engineering.

"For me the surprise was how quickly the biofilm streamers caused complete clogging," said Stone. "There was no warning that something bad was about to happen."

By constructing their own controlled environment, the researchers demonstrated that rough surfaces and pressure driven flow are characteristics of nature and need to be taken into account experimentally. The researchers used stents, soil-based filters and water filters to prove that the biofilm streams indeed form in real scenarios and likely explain why devices fail.

The work also allowed the researchers to explore which bacterial genes contribute to biofilm streamer formation. Previous studies, conducted under non-realistic conditions, identified several genes involved in formation of the biofilm streamers. The Princeton researchers found that some of those previously identified genes were not needed for biofilm streamer formation in the more realistic habitat.

**Journal Reference:**

---

'Defective' Virus Surprisingly Plays Major Role in Spread of Disease

Feb. 28, 2013 — Defective viruses, thought for decades to be essentially garbage unrelated to the transmission of normal viruses, now appear able to play an important role in the spread of disease, new research by UCLA life scientists indicates.

Defective viruses have genetic mutations or deletions that eliminate their essential viral functions. They have been observed for many human pathogens and are generated frequently for viruses that have high mutation rates. However, for some 40 years, it was believed that they were unimportant in natural settings.

In findings published Feb. 28 in the journal *PLoS Pathogens*, UCLA scientists and their colleagues report for the first time a significant link between a defective virus and an increased rate of transmission of a major disease.

"The idea has always been that defective viruses are either meaningless or detrimental," said James O. Lloyd-Smith, a UCLA assistant professor of ecology and evolutionary biology and the senior author of the research. "We have found the opposite of that—that the defective virus is actually helping the normal, functional virus. This finding is bizarre and hard to believe, but the data are the data."

"We have shown that the defective virus not only transmits with the virus but increases the transmission of the functional virus," said Ruian Ke, a UCLA postdoctoral scholar in the department of ecology and evolutionary biology and the lead author of the study.

Defective viruses cannot complete their life cycle on their own, but if they’re able to get into the same cell with a non-defective virus, they can "hitchhike" with the normal virus and propagate, Lloyd-Smith said. Biologists had thought that defective viruses interfered with normal versions of the virus, "clogging up the gears of viral replication," he said.

The life scientists studied DENV-1, one of four known types of the dengue virus that infect humans. Dengue viruses are transmitted by several species of mosquitoes and cause dengue fever, which is characterized by fever, joint pain and a skin rash similar to measles. Dengue hemorrhagic fever, a more severe form of dengue infection, can cause death. The dengue virus infects between 50 million and 100 million people each year in Southeast Asia, South America, parts of the United States and elsewhere.
The life sciences team—which also included John Aaskov, a virologist and professor of health at Australia’s Queensland University of Technology in Brisbane, and Edward Holmes, a professor of biological sciences at Australia’s University of Sydney—found that the presence of a defective DENV-1 virus may have led to large increases in dengue fever cases in Myanmar in 2001 and 2002, when that country experienced its most severe dengue epidemics on record.

The scientists describe when and how the defective "lineage," or series of very closely related defective DENV-1 viruses, emerged and was transmitted between humans and mosquitoes in Myanmar, as well as what the public health implications are.

For the study, Ke designed a mathematical model to analyze the data to learn how the defective DENV-1 virus interacted with the normal virus. Aaskov and Holmes collected genetic sequences from 15 people in Myanmar sampled over an 18-month period, all of whom were infected with the DENV-1 virus and nine of whom were also infected with the defective version.

Ke discovered that the lineage of defective viruses emerged between June 1998 and February 2001 and that it was spreading in the population until at least 2002. (The following year, the lineage appeared on the South Pacific island of New Caledonia, carried there by either a mosquito or a person.) The scientists analyzed the genetic sequences of both the defective and normal dengue viruses to estimate how long the defective virus had been transmitting in the human population.

"We can see from the gene sequence of the defective version that it is the same lineage and is a continued propagation of the virus," said Lloyd-Smith, who holds UCLA’s De Logi Chair in Biological Sciences. "From 2001 to 2002, it went from being quite rare to being in all nine people we sampled that year; everybody sampled who was getting dengue fever was getting the defective version along with the functional virus. It rose from being rare to being very common in just one year."

Most surprisingly, Lloyd-Smith said, the combination of the defective virus with the normal virus was "more fit" than the normal dengue virus alone.

"What we have shown is that this defective virus, which everyone had thought was useless or even detrimental to the fitness of the functional virus, actually appears to have made it better able to spread," he said. "Ruian Ke calculated that the defective virus makes it at least 10 percent more transmissible, which is a lot. It was spreading better with its weird, defective cousin tagging along than on its own.

"This study has shown that the functional virus and defective virus travel in unison. The two transmit together in an unbroken chain, and that’s not just a matter of getting into the same human or the same mosquito—they need to get into the same cell inside that human or mosquito in order to share their genes and for the defective version to continue ‘hitchhiking.’ We are gaining insights into the cellular-level biology of how dengue is infecting hosts. It must be the case that frequently there are multiple infections of single cells.

"Ruian showed the defective virus appeared one to three years before these major epidemics," Lloyd-Smith added. "One could imagine that if you build an understanding of this mechanism, you could measure it, see it coming and potentially get ahead of it."

Might defective viruses play a role in the transmission of influenza, measles and other diseases?

"There are a few signs that this phenomenon may be happening for other viruses," Lloyd-Smith said. "We may be cracking open the book on the possible interactions between the normal, functional viruses and the defective ones that people thought were just dead-ends. These supposedly meaningless viruses may be having a positive impact—positive for the virus, not for us. There is great variation, year to year, in how large dengue epidemics are in various locations, and we don't understand why. This is a possible mechanism for why there are large epidemics in some years in some places. We need to keep studying this question."

The research points to implications for how mutations might allow a new non-human virus to become a human virus.

"Different strains of a virus with different genetic properties may be interacting more frequently than we thought," said Lloyd-Smith, who studies how ecology, evolution and epidemiology merge to drive the emergence of new pathogens, including new strains with important properties like drug resistance.

Why would a defective virus increase transmission of a disease?

Lloyd-Smith offers two hypotheses. One is that the presence of the defective virus with the functional virus in the same cell makes the functional virus replicate better within the cell by some unknown mechanism. "It might give the virus a bit of flexibility in how it expresses its genes and may make it a bit more fit, a bit better able to reproduce under some circumstances," he said.
A second idea is that the defective virus may be interfering with the disease-causing virus, making the disease less intense; people then have a milder infection, and because they don't feel as sick, they are more likely to go out and spread the disease.

"Normally, biologists test for how well a virus can replicate in a cell, but what we have shown here is even a genotype that cannot replicate in a cell can have an impact on transmission," Ke said.

In conducting the research, Lloyd-Smith and Ke combined genetic sequence analysis with sophisticated mathematical models and bioinformatics.

Genetic sequencing technology has "exploded," Lloyd-Smith said, providing a wealth of data on genetic sequences of pathogens and the evolution of viruses, leading to major new insights into the transmission of viruses.

"We were able to show that this defective virus transmitted in an unbroken chain across this population for a year-and-a-half," Lloyd-Smith said. "Without gene sequencing, we would not have been able to establish that."

---

**Order in the Chaos of a Cell Membrane**

Mar. 1, 2013 — An explanation has been proposed for the way in which ordered structures arise in cell membranes. Scientists from the Max Planck Institute of Colloids and Interfaces in Potsdam have discovered how complex compounds of sugar and lipids—known as glycolipids—order themselves in cell membranes into rafts, namely small, highly organised domains. The arrangement of glycolipids on the surface of plant and animal cell membranes regulates numerous cellular processes. If errors occur in this process, diseases like PNH and BSE can arise.

Lipids, i.e. fats and fat-like substances, arise all over the human body. They are the body's most important energy storage system and are crucial structural components of cell membranes. Compounds formed from complex sugar components and fats are known as glycolipids. Those are vital communicators found in the membranes of every human cell, and constantly exchange information about the type and state of the cell. Numerous metabolic processes depend on glycolipids and their recognition. Even the immune system identifies and combats many pathogens using certain sugar structures located on the surface of the pathogen cells.

**Glycosylphosphatidylinositols (GPIs)** belong to the group of natural glycolipids. They are found on the surface of plant and animal cell membranes, where they appear either as free molecules or as membrane anchors for various proteins. The arrangement in clusters and their preference for denser and, in part, highly-organised micro-domains in the membrane are seen as essential for the effective functioning of a cell. These minuscule clusters are extremely important for the regulation of many cellular processes, and their malfunction can have very serious consequences. For example, it has been proven that the accumulation, missing or alteration of GPI-anchored molecules can trigger the development of serious diseases like BSE and paroxysmal nocturnal hemoglobinuria (PNH). Scientists at the Max Planck Institute of Colloids and Interfaces in Golm near Potsdam have gained new insight into how GPIs structure themselves in membranes.

**Crystalline lipid areas never previously observed in membranes**

It was assumed up to now that the arrangement of the GPIs in clusters and rafts was determined by the water-repelling section of the glycolipids embedded in the cell membrane. The chemical structure of the hydrophobic ends is actually responsible for strong interactions with similarly rigid neighbouring molecules. If the number of the molecules that interact with each other is big enough, rigid and partly organised domains may arise like icebergs on the surface of the ocean.

Cristina Stefaniu and her colleagues have now discovered that, in addition to the hydrophobic ends, the large GPI head groups, which contain sugar, mainly contribute to the formation of the rafts. This means that the hydrophylc part of the molecule is able to build strong interactions with the neighbouring GPI molecules. This part of the molecule is located precisely on the boundary between the membrane surface and the liquid medium. "The interactions between neighbouring GPI molecules result in the formation of crystalline orders that have not previously been observed for other membrane lipids," says Cristina Stefaniu.
Hydrogen bonds connect the hydrophylic head groups
The scientists reached this new conclusion about the order in membranes by studying a model molecule. This is a GPI fragment that was synthesised by the groups headed by Peter Seeberger and Daniel Varón Silva and that imitates the behaviour of entire GPIs. It forms a very thin film, just one molecule thick, on the surface of the water. This so-called monolayer is the simplified model of a half cell membrane which the researchers analysed using synchrotron x-ray scattering. "Surprisingly, the highly ordered structure in the GPI monolayer is predominantly determined by the bulky hydrophilic head groups that connect through hydrogen bonds," says Stefaniu. A hydrogen bond is a relatively weak chemical bond and usually links two molecules through the bonding of a hydrogen atom from one molecule with an oxygen or nitrogen atom from the other molecule. Thus the monolayers of the GPI fragment are characterised by both the order of the hydrophilic lipid chains and the crystalline arrangement of the GPI head groups. "The molecular lattices observed here have not yet been described for lipid monolayers," says Cristina Stefaniu. "A similar order forms in lipid bilayers if they are stored at temperatures close to zero degrees Celsius." The strong interactions between the head groups can only be disrupted and the molecular lattice destroyed through the addition of a highly concentrated urea solution, which breaks the hydrogen bonds, eliminates the strong interactions of the head groups and destroys the molecular lattice. In addition, the scientists proved that ordered clusters can arise in mixtures of the GPI fragment with typical membrane lipids, which only form unordered films. Thus, the GPls are able to generate order in the chaos of a membrane. This special skill could be very important for the GPI interactions in real cell membranes.

Journal Reference:

NYTimes, March 5, 2013
Deadly Bacteria That Resist Strongest Drugs Are Spreading
By Denise Grady
Deadly infections with bacteria that resist even the strongest antibiotics are on the rise in hospitals in the United States, and there is only a “limited window of opportunity” to halt their spread, health officials warned Tuesday.

The bacteria, normally found in the gut, have acquired a lethal trait: they are unscathed by antibiotics, including carbapenems, a group of drugs that are generally considered a last resort. When these resistant germs invade parts of the body where they do not belong, like the bloodstream, lungs or urinary tract, the illness may be untreatable. The death rate from bloodstream infections can reach 50 percent.

Dr. Thomas R. Frieden, director of the Centers for Disease Control and Prevention, called the organisms “nightmare bacteria” during a telephone news conference, and noted that they could pass their trait for drug resistance — encoded in a scrap of genetic material called a plasmid — along to other bacteria.

Most people who contract these infections already have other serious illnesses that require complicated treatment and lengthy stays in hospitals, nursing homes or long-term care facilities. One bit of good news, Dr. Frieden said, is that the infections do not seem to have spread beyond hospitals into the community at large. But that could easily happen, he warned.

According to a new report by the disease centers, among all infections with gut bacteria, the proportion caused by carbapenem-resistant types rose to 4 percent in 2012, from 1 percent in 2001; among infections caused by one type of bacteria, Klebsiella, 10 percent have become resistant, compared with 2 percent a decade ago.

Drug-resistant Klebsiella, traced to one patient, caused a notorious outbreak in 2011 at a hospital at the National Institutes of Health. Seventeen other people were infected, and six of them died.

Forty-two states have had cases of carbapenem-resistant infection. The problem is most common in the Northeast, particularly in hospitals in New York City, officials said. Nationwide, about 4 percent of short-stay hospitals reported such infections in the first half of 2012, but the rate was much higher — 18 percent — among long-term acute-care hospitals, which treat people who need ventilators for a long time or who have other chronic problems.

The disease centers recommended a variety of ways to try to stop the infections from spreading. The advice includes the usual call for ruthless scrubbing of all surfaces and relentless handwashing.
But hospitals are also urged to find out whether patients are infected, isolate those who are, and assign dedicated-care teams and equipment to infected people only, to avoid spreading the bacteria to others. Catheters and intravenous lines should be removed as quickly as possible, because they can be avenues of infection, and doctors should prescribe antibiotics only when they are truly needed. Health officials also urge patients and their loved ones to insist that medical personnel wash their hands before touching a patient.

**Patients Face More Lethal Infections from CRE**

A new Vital Signs report shows that antibiotics are being overpowered by lethal germs called carbapenem-resistant Enterobacteriaceae (CRE). These germs cause lethal infections in patients receiving inpatient medical care, such as in hospitals, long-term acute care facilities, and nursing homes.

In their usual forms, germs from the Enterobacteriaceae family (e.g. *E. coli*) are a normal part of the human digestive system. However, some of these germs have developed defenses to fight off all or almost all antibiotics we have today. When these germs get into the blood, bladder or other areas where germs don’t belong, patients suffer from infections that are difficult, and sometimes impossible, to treat.

While CDC has warned about CRE for more than a decade, new information shows that these germs are now becoming more common. One type of CRE has been detected in medical facilities in 42 states. Even more concerning, this report documents a seven-fold increase in the spread of the most common type of CRE during the past 10 years.

**Why are CRE so alarming?**

Even though these infections are not common, their rise is alarming because they kill up to half of people who get severe infections from them. In addition to causing lethal infections among patients, CRE are especially good at giving their antibiotic-fighting abilities to other kinds of germs. This means that in the near future, more bacteria will become immune to treatment, and more patients’ lives could be at risk from routine bladder or wound infections. Without serious efforts to stop CRE in medical facilities, and without rapid improvement in the way doctors everywhere prescribe antibiotics, CRE will likely become a problem in the community, among otherwise healthy people not receiving medical care.

**How can CRE be stopped?**

There have been major successes in stopping CRE in medical facilities in the United States, and nationally in other countries. Stopping CRE will take a rapid, coordinated, and aggressive "Detect and Protect" action that includes intense infection prevention work and antibiotic prescribing changes. CDC released a CRE prevention toolkit in 2012 reiterating practical CRE prevention and control steps. Leadership and medical staff in hospitals, long-term acute care hospitals, nursing homes, health departments, and even outpatient practices must work together to implement these recommendations to protect patients from CRE.

**Dosing of key TB drug rifampicin could go higher**

Keith Alcorn

Published: 07 March 2013

Rifampicin, a key drug in tuberculosis (TB) treatment, can be tolerated at much higher doses than used in current clinical practice – suggesting that much higher drug levels may lead to a more rapid treatment response, allowing the treatment course to be shortened without the need for new drugs in first-line treatment, according to findings presented at the 20th Conference on Retroviruses and Opportunistic Infections (CROI 2013) in Atlanta this week.

Rifampicin is used at a dose of 600mg per day throughout the six-month TB treatment course. Yet the maximum-tolerated dose of rifampicin has never been defined. When the drug was being developed in the 1960s, it was extremely expensive to synthesise, and on the basis of anecdotal reports it was also assumed at the time that the toxicities of the drug were likely to be dose-related. This led investigators to settle on a dose of 600mg as adequate to achieve minimum inhibitory concentrations for 24 hours. Indeed, early studies of short-course treatment either limited the dose and duration of rifampicin treatment, or avoided use of the drug altogether, in order to test regimens that would be affordable in low-resource settings.

A literature review by Martin Boeree and colleagues at the St Radboud University, Nijmegen, subsequently found that there was little evidence to support assumptions about dose-related toxicity. Furthermore, they argued that previous investigators had made the wrong assumption about the most relevant pharmacokinetic parameter for assessment in studies of rifampicin as a TB treatment. Rather
than looking at the ratio of the trough level ($C_{\text{min}}$) to the minimum inhibitory concentration, which shows whether adequate blood levels are maintained throughout a 24-hour period, Boeree and colleagues argued that what mattered was the total drug exposure achieved during a 24-hour period. Higher total drug exposure would be more likely to reduce bacterial load, and higher peak concentrations would be needed to inhibit bacterial replication in epithelial fluids within the lungs.

The study, conducted by Boeree and colleagues in the PanACEA consortium in South Africa, recruited people with smear-positive TB in five consecutive groups, each of which received a higher dose of rifampicin for the first seven days of TB treatment, after which isoniazid, pyrazinamide and ethambutol were added for a further seven days. The first group received a dose of 10mg/kg, and subsequent groups received 20, 25, 30 or 35 mg/kg doses. Dose increments were approved after reviewing data on adverse events in each cohort of patients. All participants received rifampicin in combination with standard doses of isoniazid, ethambutol and pyrazinamide.

The study measured rifampicin levels at days 7 and 14 of treatment. *Mycobacterium tuberculosis* colony-forming units were measured by culture on solid media at baseline and days 1 to 7, and days 12 and 14. Time to culture positivity was measured in liquid media at the same time intervals. Rifampicin was well-tolerated at higher doses, with no grade 4 adverse events and only four grade 3 adverse events reported, with no association between increasing dose and incidence of grade 3 events. On the basis of these results, the researchers concluded that they had failed to identify a maximum tolerated dose, and research will now go forward to test a 35mg dose for safety and efficacy in a phase II study, and to test doses of 40 and 45mg for safety.

There was also a significant trend towards a reduced number of colony-forming units of m.TB at higher doses, and a sevenfold increase in total rifampicin exposure at the highest dose when compared to the standard dose. This increase in exposure was disproportionate to the increase in dose. However, very substantial variations in exposure were seen between individuals at each dose, indicating that pharmacogenomic factors such as genetic markers for rapid clearance of rifampicin will require attention in future research into the optimisation of TB regimens.

Another factor that will require attention is the interaction between rifampicin and the anti-HIV drug efavirenz (*Sustiva*, Stocrin, also in the combination drug *Atripla*). At a dose of 600mg, the effect of rifampicin on efavirenz levels is enough to persuade US physicians that the efavirenz dose should be increased to 800mg. What is unclear at present is whether the increase in rifampicin exposure is accompanied by a corresponding increase in the induction of efavirenz metabolism, or whether this effect hits a plateau at some point, beyond which the efavirenz dose would not need to be increased any further.

**Reference**


---

**New pro-drug tenofovir alafenamide appears equally effective but better tolerated**

Liz Highleyman
Published: 07 March 2013

Tenofovir alafenamide fumarate or TAF (formerly GS-7340), a new pro-drug of the widely used NRTI **tenofovir**, reaches cells harbouring HIV more easily than the older tenofovir disoproxil fumarate (TDF), allowing for similar antiviral efficacy with smaller doses and with less adverse effect on kidneys and bones, researchers reported at the **20th Conference on Retroviruses and Opportunistic Infections (CROI 2013)** this week in Atlanta.

Gilead Sciences’ tenofovir is highly effective and a recommended component of first-line HIV treatment, but it can cause impaired kidney function and bone loss in some people. Moreover, it is not very bioavailable. TDF (*Viread*, also in the **Truvada**, *Atripla*, *Emtriva*/Complera and *Stribild* co-formulations) is a pro-drug that delivers tenofovir diphosphate, the active form, efficiently to blood plasma.

TAF has a different structure to TDF and is metabolised by a protein known as cadapsin A, reaching higher concentrations in lymphoid cells such as CD4 cells. With the new formulation, adequate tenofovir concentrations in cells can be achieved using a much lower dose, which has less potential to harm kidney and bone tissue.

Andrew Zolopa from Stanford University and colleagues conducted a phase II study to compare the safety and efficacy of TAF vs TDF in 170 previously untreated people with HIV. At study entry they had normal kidney function and no known resistance to tenofovir or **emtricitabine** (*FTC*, *Emtriva*).
Almost all participants in Study 102 were men, two-thirds were white, most of the rest were black, and about 20% were of Hispanic/Latino ethnicity. The mean age was approximately 35 years. The median CD4 cell count was just under 400 cells/mm³ and about 20% had high baseline viral load (HIV RNA >100,000 copies/ml). None had hepatitis B or C co-infection.

The 58 participants randomly assigned to the TDF arm used the *Stribild* single-tablet regimen, which also contains the integrase inhibitor elvitegravir, the boosting agent cobicistat and emtricitabine. The 112 people in other arm used a similar once-daily ‘Quad’ coformulation with TAF replacing TDF. *Stribild* contains 300mg TDF whilst the comparator formulation contains just 10mg TAF; doses of the three components are the same.

Zolopa reported primary endpoint data on the proportions of participants with undetectable HIV viral load (<50 copies/ml) at week 24 of treatment. Follow-up will continue through to week 48.

Results showed that people using the TAF coformulation had about five-fold higher concentrations of tenofovir diphosphate in peripheral blood mononuclear cells (PBMCs) but about 90% lower plasma levels compared with those taking TDF.

In an intent-to-treat analysis at 24 weeks, 87% of participants taking the TAF coformulation pill achieved HIV RNA <50 copies/ml compared with 90% of those taking *Stribild*, not a statistically significant difference.

Virological failure at week 24 was observed in 13% of TAF recipients and 10% of TDF recipients, again not a significant difference. Among three participants eligible for resistance testing, one was found to have NRTI resistance mutation.

Mean CD4 cell count increase were also similar in both treatment arms, 163 and 177 cells/mm³, respectively.

Treatment was generally safe and well tolerated with similar overall drug safety profiles. Most side-effects were mild-to-moderate. No treatment-related serious adverse events were reported in either treatment arm. Although four TAF recipients discontinued treatment prematurely due to adverse events compared with none in the TDF arm, only one such event (photosensitivity) was considered related to study drugs.

The most common adverse events reported by at least 10% of participants included nausea (18% with the TAF coformulation vs 12% with *Stribild*), diarrhoea (12% in both arms), fatigue (12 vs 9%), headache (10% in both arms) and upper respiratory tract infections (7 vs 12%).

There were no significant differences in the frequency or type of grade 3 and 4 laboratory abnormalities. Levels of total, LDL (‘bad’) and HDL (‘good’) cholesterol rose across the board, but significantly more so in the TAF arm. Levels of triglycerides and glucose were about the same in both arms.

Turning to kidney-related side-effects, participants in both the TAF and TDF arms saw increases in serum creatinine (0.07 vs 0.12 mg/dl, respectively) and declines in estimated glomerular filtration rate (eGFR) (-4.9 vs -11.8 ml/min, respectively), but both were significantly smaller with TAF. These changes were mostly evident by week 2 but stabilised by week 24. Although creatinine changes can be markers of impaired kidney function, Zolopa described them as a known effects of cobicistat blocking tubular secretion of creatinine. Plasma albumin and retina binding protein levels were both lower in the TAF arm. TAF recipients were about two-thirds as likely to have protein in their urine (14 vs 21%, respectively). There were no cases of proximal renal tubulopathy and no discontinuations due to kidney problems in either treatment arm.

DEXA measurements performed at baseline and week 24 showed significantly smaller decreases in bone mineral density in the TAF arm compared with the TDF arm at both the spine (mean -0.8 vs -2.5%) and the hip (mean -0.3 vs -2.0%). Furthermore, considerably more people taking TAF showed no decrease in bone density at the spine (8 vs 12%) or hip (41 vs 23%).

In this first phase II study comparing two tenofovir pro-drugs, the TAF-containing coformulation "demonstrated comparable efficacy and a statistically significant improvement in the renal and bone safety profile at 24 weeks” relative to the TDF-containing regimen, the researchers concluded.

These results support further evaluation of the TAF coformulation in phase III clinical trials, they added. Gilead announced in January that one such trial (Study 104) is now underway and another (Study 111) is expected to start soon.

"I'm not so worried about virus [developing resistance], we have to worry more about long-term consequences to our patients” of remaining on treatment, Zolopa emphasized at a CROI press conference discussing new antiretroviral agents and approaches to therapy.
Can people with resistant HIV omit NRTIs when switching from a failing regimen?
Liz Highleyman
Published: 07 March 2013

Omitting nucleoside reverse transcriptase inhibitors (NRTIs) when switching from a non-suppressive regimen to a new combination with at least two active agents can reduce pill burden and side-effects without compromising effectiveness, researchers reported yesterday at the 20th Conference on Retroviruses and Opportunistic Infections (CROI 2013) in Atlanta.

Individuals with hard-to-treat HIV – for example, those who received suboptimal therapy during the pre-HAART era and have extensive drug resistance – often include multiple drugs in their antiretroviral regimen in the hope of maximizing potency.

In the early years of combination therapy, many experts thought that even partially active NRTIs could make an important contribution to total antiviral efficacy of a marginal regimen. Furthermore, research showed that HIV can become less ‘fit’ and lose the ability to replicate efficiently as it accumulates resistance mutations for certain NRTIs such as lamivudine (3TC, Epivir).

As treatment has improved, however, a growing proportion of people with HIV can now construct fully suppressive regimens using new types of agents. In addition, the long-term toxicities of some NRTIs are better recognised, giving further impetus to avoid these drugs.

If someone plans to switch from a failing regimen to a new combination that includes two or more active agents from other classes, is there any need to retain potentially ineffective NRTIs?

Findings from the ACTG OPTIONS trial suggest the answer is no.

Karen Tashima from Brown University and fellow investigators enrolled 360 study participants between February 2008 and May 2011. To be eligible they had to be on a failing protease inhibitor regimen with viral load of at least 1000 copies/ml and have experience using or evidence of resistance to NRTIs and non-nucleoside reverse transcriptase inhibitors (NNRTIs).

Three-quarters of participants were men, about 40% were black and one-quarter were Hispanic. The median age was 46 years and they had been on antiretroviral therapy for 12 years on average. At study entry they had a low median CD4 cell count of approximately 200 cells/mm³ and viral load of 4.2 log₁₀.

Half had exclusively CCR5-tropic virus, making then eligible to use maraviroc (Selzentry or Celsentri).

Investigators helped participants put together new regimens guided by resistance and tropism testing. They could choose among 20 potential three- or four-drug combinations including ritonavir–boosted darunavir (Prezista), enfuvirtide (T-20, Fuzeon), etravirine (Intelenz), maraviroc, raltegravir (Isentress) or boosted tipranavir (Aptivus).

The most common regimen – used by 56% – consisted of raltegravir, boosted darunavir and etravirine. That is, these participants opted to combine an integrase inhibitor, a modern protease inhibitor and a second-generation NNRTI.

Patients and their clinicians then selected which NRTIs they would like to use – with tenofovir plus emtricitabine (the drugs in Truvada) or lamivudine being most common by far at 82% – but an open-label randomisation was done to determine whether they would actually start or omit these NRTIs.

Results after one year showed that omitting NRTIs was not inferior to adding NRTIs to an otherwise optimised regimen. Overall regimen failure occurred in 30% of NRTI-omitters and 26% of NRTI-adders, not a statistically significant difference. Rates of virological failure were 25% in both groups.

Similar proportions of NRTI-omitters and NRTI-adders achieved undetectable plasma viral load (<50 copies/mL) and CD4 cell gains were also statistically similar.

Looking at safety and tolerability, the time to first sign or symptom of side-effects was the same regardless of NRTI use or non-use. Eleven people who omitted NRTIs withdrew from the study prematurely, as did five who added NRTIs. According to the researchers, there was “no statistically significant difference in primary safety when considering both symptoms and labs”.

Interestingly, six people who used NRTIs died during follow-up, compared with none in the NRTI-free group – a significant difference. Causes of death included heart, kidney and liver failure, bacterial meningitis, sepsis, and progressive multifocal leukoencephalopathy (an opportunistic infection of the...
brain). In a couple of cases, investigators could not rule out that study drugs may have been a contributing factor.

"In this population, NRTIs can be safely omitted without compromising regimen failure," the researchers concluded. "Potential benefits of omitting NRTIs include reduced pill burden and cost."

This study is a "game-changer", Andrew Zolopa from Stanford University opined during the discussion after the presentation. "Many of us are recycling nukes, but it looks quite convincing that we don’t have to do this."

"You don’t need to include NRTIs when new active agents are onboard," Tashima concurred at an accompanying press conference. "We don’t need to hang on to this old class. We’ve become quite comfortable with them, but they add to pill burden and toxicity."

CROI vice-chair Scott Hammer from Columbia University noted that the OPTIONS study illustrated the evolution of HIV treatment: "We’re making a judgment that toxicity and pill burden are more of a risk than a benefit in this situation because now we can suppress almost everyone."

Reference
Tashima K et al. Omitting NRTI from ARV regimens is not inferior to adding NRTI in treatment-experienced HIV+ subjects failing a protease inhibitor regimen: The ACTG OPTIONS Study. 20th Conference on Retroviruses and Opportunistic Infections, Atlanta, abstract 153LB, 2013.

Long-term efavirenz linked to worse neurocognitive function in US CHARTER group

Long-term treatment with an efavirenz-based regimen correlated with worse neurocognitive function than did treatment with lopinavir/ritonavir, according to results of a retrospective case-control comparison within the US CHARTER cohort [1]. Worse performance with efavirenz held true in subgroups without hepatitis C virus (HCV) coinfection or with a plasma viral load below 50 copies. In contrast, HCV-positive cohort members had worse neurocognitive performance if taking lopinavir/ritonavir.

Scott Letendre (University of California, San Diego) and CHARTER colleagues cautioned that "the complexity of these data is substantial and differences would best be evaluated in a randomized clinical trial."

HIV-associated neurocognitive disorders (HAND) remain prevalent in people responding well to current antiretroviral regimens. Antiretroviral neurotoxicity may be one factor accounting for persistence of these disorders. Because efavirenz has well-documented neurologic side effects, the CHARTER team planned this retrospective case-control analysis of people currently taking efavirenz or lopinavir/ritonavir with at least two other antiretrovirals for at least 12 weeks.

No study participants had severe neuropsychiatric morbidities, and all underwent comprehensive assessment of seven neurocognitive domains. Average age was similar in the 272 people taking efavirenz (43.9) and the 173 taking lopinavir (45.1). The efavirenz and lopinavir groups did not differ much in years of education (12.8 and 12.6), gender (81% and 79% men), or ethnicity (40% and 36% white). Duration of treatment was similar in the efavirenz and lopinavir groups (27.6 and 25.1 months).

The efavirenz cohort had a lower proportion of people coinfected with HCV (23% versus 32%, \( P = 0.046 \)), a lower proportion with AIDS (64% versus 82%, \( P < 0.001 \)), a shorter duration of HIV infection (109.8 versus 144 months, \( P < 0.001 \)), a higher current CD4 count (472 versus 381, \( P = 0.01 \)), a higher nadir CD4 count (166 versus 88, \( P < 0.001 \)), a lower current plasma viral load (1.7 versus 1.8 log, \( P < 0.001 \)), and a higher proportion with a cerebrospinal fluid load at or below 1.7 log (92% versus 74%, \( P < 0.001 \)). The central nervous system penetration effectiveness score was significantly lower (worse) with efavirenz and lopinavir/ritonavir with at least two other antiretrovirals for at least 12 weeks.

To compare neurocognitive test results, the CHARTER team divided study participants into those with and without HCV. In 328 people without HCV infection, a higher proportion of efavirenz takers had impairment in every domain assessed, and the difference was significant for executive functioning (\( P = 0.05 \)), speed of information processing (\( P = 0.04 \)), and a combined global functioning score (about 50% versus 40% impaired, \( P = 0.02 \)). Verbal functioning was marginally worse in the efavirenz group (\( P = 0.08 \)).

Among the 117 HCV-positive cohort members, the proportion with neurocognitive impairment was consistently higher with lopinavir/ritonavir and significantly higher for learning (\( P = 0.04 \)), memory (\( P = 0.01 \)), and the combined global functioning score (about 80% versus 40% impaired, \( P = 0.04 \)). Motor functioning was marginally worse with lopinavir than efavirenz (\( P = 0.06 \)).

In 269 people with a plasma viral load below 50 copies, efavirenz users had worse executive functioning (\( P = 0.03 \)) and worse speed of information processing (\( P = 0.02 \)) than lopinavir users.
The researchers noted that factors possibly affecting susceptibility to HIV-associated neurocognitive disorders differed substantially between the efavirenz and lopinavir groups. Some of those differences, they speculated, could reflect clinicians' tendency to prescribe lopinavir/ritonavir for people with more advanced HIV infection or as second-line therapy.

"Despite these differences," the CHARTER team concluded, "efavirenz users had worse neurocognitive functioning."

By Mark Mascolini

Reference


HIV exploits a human cytokine in semen to promote its own transmission


A new report suggests that the concentration of one human cytokine, interleukin 7 (IL-7), in the semen of HIV-1-infected men may be a key determinant of the efficiency of HIV-1 transmission to an uninfected female partner. In their study published February 7 in the Open Access journal PLOS Pathogens, a research group from the Eunice Kennedy-Shriver National Institute of Child Health and Human Development (NICHD) led by Leonid Margolis report that the increased IL-7 concentration in semen facilitates HIV transmission to cervical tissue ex vivo.

Semen is a complex biological fluid containing not only spermatozoa but also cytokines, a group of extracellular proteins that modulate immune responses. As a result of HIV infection, the concentrations of various cytokines in semen is profoundly modified, in particular the concentration of interleukin 7 (IL-7) is greatly increased. Despite this evidence of strikingly elevated IL-7 levels in seminal plasma, there was limited knowledge about any effects this cytokine might have on HIV-1 sexual transmission.

To investigate the question about the effects of this increased IL-17 on HIV-1 sexual transmission, Andrea Introini and colleagues from the Margolis lab developed a system of explants of cervico-vaginal tissue that can be maintained outside of the body in culture for up to two weeks while preserving the cytoarchitecture of the tissue. In this system, HIV transmission can be simulated and studied under controlled laboratory conditions. When researchers added IL-7 in concentrations comparable to that found in the semen of HIV-1 infected men, HIV was transmitted more efficiently and replicated to a higher level than without IL-7. Normally, HIV-1-infected cells quickly die as the result of apoptosis, a programmed death triggered by HIV infection. IL-7 inhibits apoptosis of infected cells, allowing them to produce more virus and thus increasing the chances of the incoming virus to disseminate through the tissue. Also, IL-7 stimulates T cell proliferation, thereby also providing to HIV even more potential targets to infect.

The authors speculate that IL-7, together with other cytokines, may determine sexual transmission rates of HIV-1 and that changes in the seminal cytokine load may explain differences in HIV transmission from different individuals. However, whether the effect of IL-7 that has been demonstrated ex vivo occurs also for sexual partners in vivo, is a subject for future research. If this increase does occur in vivo, then it should be investigated whether HIV-1 infected individuals that have been treated systemically with IL-7 in order to increase their T cell counts may have also resulted in the unintended increase of their seminal IL-7 levels. Finally, this study suggests that seminal cytokines may become new targets for HIV-preventive strategies.


Transmission of Resistant HIV Steady

By Michael Smith, North American Correspondent, MedPage Today

Published: March 06, 2013

Reviewed by Robert Jasmer, MD: Associate Clinical Professor of Medicine, University of California, San Francisco

Action Points

- Note that this study was published as an abstract and presented at a conference. These data and conclusions should be considered to be preliminary until published in a peer-reviewed journal.
- The rate of drug-resistant HIV transmission in the U.S. appears to be holding steady.
- Point out that about 16% of the HIV genetic sequences analyzed from newly diagnosed HIV-1 infected patients from 2007 through 2010 showed transmitted drug resistance mutations.
ATLANTA – The rate of drug-resistant HIV transmission in the U.S. appears to be holding steady, according to the most recent CDC figures.

About 16% of the HIV genetic sequences analyzed from newly diagnosed patients from 2007 through 2010 showed transmitted drug resistance mutations, according to David Kim, MD, of the CDC.

But the rates have not increased significantly from 2007—when the CDC found a rate of 15%—to 2010, when the rate reached 16.7%, Kim reported here at the Conference on Retroviruses and Opportunistic Infections.

On the other hand, he told reporters after his oral presentation, transmitted resistance to one class of drugs – the non-nucleoside reverse transcriptase inhibitors (NNRTIs) – is rising, with an estimated annual percentage change of 5.2%, which was significant at $P=0.03$.

The reasons for that need more study, he said, but he told MedPage Today the two facts together don't necessarily imply that transmitted resistance to other classes of drugs is on the wane.

Indeed, he reported, the estimated annual percentage changes for transmitted resistance to nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs) were not significant over the 4 years.

Measuring transmitted drug resistance is a complicated issue, although the most recent figures are reassurance that rates are not skyrocketing, commented Scott Hammer, MD, of Columbia University in New York City, who was not involved in the study but who moderated a press conference at which it was presented.

Hammer noted that some resistance mutations can be transmitted, but then are outcompeted by other variants as HIV diversifies in its new host. Those mutations would be “archived” — still present but not detectable — and so would not be picked up by surveillance.

Equally, he noted, transmission of resistance would be low if a large proportion of the HIV-positive population had never been treated. Much of the transmission then would be "wild-type" HIV, as is seen in some European countries, he told MedPage Today.

Finally, he said, the effect of modern highly effective treatments, combined with better testing and linkage to care, would be to reduce both the rise of resistance in the first place and overall transmission in the second, he said.

But Hammer said in his own practice, he assumes that about one in 10 newly diagnosed patients will already have resistance to at least one drug, so the CDC estimate is not out of line.

The findings come from a convenience sample of 18,144 HIV sequences collected from 77,887 new diagnosed HIV patients at 10 U.S. surveillance sites over the 4-year study period, Kim reported.

Of those sequences, 2,932—or 16.2%—had one or more resistance mutations. Since the patients were newly diagnosed and had never had antiretroviral therapy, the resistance could not have arisen from their own exposure to medication and must have been transmitted.

Of the resistance sequences, Kim reported, 2,461 conveyed resistance to a single class of drugs, 386 were resistant to two classes, and 85 to three.

There were also 1,464 mutations that delivered resistance to NNRTIs, 1,206 to NRTIs, and 818 to PIs, he said.

For healthcare providers, the findings are a reminder to continue genetic testing of newly diagnosed patients in order to make sure resistance doesn’t compromise the effectiveness of their antiretroviral regimen, Kim said.

HIV ‘Cure’ in Toddler Offers ‘Global Hope’

CNN.com, (03.05.2013) Jen Christiansen

Johns Hopkins Children’s Center pediatrician Dr. Deborah Persaud reported at the 2013 Conference on Retroviruses and Opportunistic Infections that a Mississippi toddler is “functionally cured” of HIV.

Duplicating the functional cure with other HIV-infected infants would offer hope to other children whose mothers transmitted the virus during pregnancy or while giving birth or breast-feeding. A “functional cure” means that, although testing reveals HIV traces in the toddler, highly sensitive tests have been unable to detect fragments of HIV virus that can replicate.

In the Mississippi case, the HIV-infected mother received no prenatal care and was diagnosed during labor. Doctors typically give an HIV-infected newborn a prophylactic combination of two drugs. In this case, Dr. Hannah Gay gave the Mississippi newborn a three-drug mixture within 30 hours of birth, without waiting for HIV test results. Gay suspects the timing of treatment was the curative factor. She

CDC reports that the number of HIV-infected infants born in the United States dropped by 90 percent since the 1990s, when HIV testing became a routine part of prenatal care. Doctors are able to suppress the virus enough in mothers to prevent them from transmitting it to their babies. UNAIDS Global Report estimates that 1,000 HIV-infected infants are born each day; there are 330,000 HIV-infected children, living mostly in developing countries where expectant mothers are less likely to be tested and treated for HIV. For example, only three percent of HIV-infected pregnant women in North Africa and the Middle East and 23 percent of women in West and Central Africa received antiretroviral therapy.

Current World Health Organization (WHO) pediatric treatment guidance will remain in place pending results of future studies and clinical trials, according to Dr. Meg Doherty, coordinator of treatment and care for WHO’s Department of HIV/AIDS.

**HIV Linked to Higher Chance of Heart Attack**

*Reuters* (03.05.2013) Genevra Pittman

The risk of heart attack goes up almost 50 percent for HIV-infected people, even when other risk factors are considered, according to University of Pittsburgh School of Medicine researcher Dr. Matthew Freiberg. The increased risk probably results from the combination of the HIV virus’s effects and antiretroviral therapy (ART).

ART has extended life expectancy for HIV-infected people, so researchers are now exploring health threats facing HIV-infected people as they age. The current study analyzed six years of data from more than 82,000 US veterans, a third of whom were HIV-infected. Of the 871 study participants who had a heart attack, HIV-infected participants were 48 percent more likely to have a heart attack than vets ages 40 to 69 who did not have HIV. The study controlled for other factors—high blood pressure, diabetes, and drug and alcohol use—also associated with higher heart attack risk.

Since past studies show that HIV increases the risk of heart disease, Freiberg theorized that the presence of the virus stimulates an “inflammatory response” that increases heart attack risk. Hepatitis C and kidney disease also are associated with increased risk of heart attack. University College of Dublin School of Medical Science’s Dr. Patrick Mallon stated that the veterans’ study clears up questions about whether HIV and HIV medications increase heart attack risk or other factors like smoking and high cholesterol are responsible for higher risk.

Mallon and Freiberg recommended that HIV-infected people make lifestyle changes such as quitting smoking and take preventive measures, including regular blood pressure and cholesterol checks, to prevent heart attack.


**A new hypothesis has been formulated on why bacteria are becoming increasingly more resistant to antibiotics**

A University of Granada researcher has formulated a new hypothesis concerning an enigma that the scientific community has still not been able to solve and which could revolutionise the pharmaceutical industry: Why are bacteria becoming increasingly more resistant to antibiotics? His work has revealed that the use of antibiotics can even cause non-resistant bacteria to become resistant because they take up the DNA of others that are already resistant.

Mohammed Bakkali, a scientist in the Genetics Department at the Faculty of Science of the UGR, maintains that our abuse of antibiotics "forces" the bacteria to take up the DNA of other bacteria that are resistant tosaid antibiotics, since the presence of antibiotics exposes them to great stress. According to the researcher, “In this way, thenon-resistant bacteria become resistant completely by accident oningesting this DNA and can even become much more virulent, partly dueto the stress we subject them to when we make an abusive use ofantibiotics".

For decades, scientists from all over the world have been researching into when, how and why bacteria take up DNA from other antibiotic-resistant bacteria, thus becoming also resistant. The answers as to when there is DNA uptake (in unfavourable or stressful circumstances) and as to how the bacteria take it up are clear, but, up until now, "nobody has pinpointed the reason why bacteria ingest this genetic material", as Bakkali points out in an article published in the latest edition of the journal "Archives of Microbiology".
Under normal conditions, a bacterium could have a lot to lose if it 'decides' to take up DNA, since it does not have a 'DNA reader' enabling it to take up only those molecules that are of use to it and the most likely is that this DNA will be dangerous, or even lethal.

**They do not want that DNA, because they break it up**

In his article, Mohammed Bakkali argues that, in reality, bacteria do not look for DNA to takeup (they appear not to 'want' this DNA, since they are constantly degrading it; in other words, breaking it up) and that this uptake is a chance event and the sub-product of a type of bacterial motility that is part of its response to the stress that the bacteria may be subjected to.

Therefore, our current indiscriminate use of antibiotics "not only selects theresistant bacteria, but also means that the bacteria take up more DNA, due to their increased motility in response to the stress that the antibiotic subjects them to". The result is that the stress caused by the antibiotic itself induces the uptake of genetic material that can bring about resistance to the antibiotic bybacteria that, otherwise, would not have taken up that DNA nor become resistant to the antibiotic. Furthermore, this effect is strengthened by its lack of specificity, since it occurs both in the target pathogen and in other bacteria.

The UGR researcher states that, when a bacterium takes up DNA from another antibiotic-resistant one (and which could have died due to another environmental factor), the bacterium that takes it up becomes resistant to that antibiotic."Thus, the bacteria can go on adding to their arsenal of resistance to antibiotics and end up being resistant to a wide range of them, such as is the case of the multi-resistant strain of *staphylococcus*, called *Staphylococcus aureus*, which creates havoc in many operating theatres.

**Reference:**
Could DNA Uptake Be a Side Effect of Bacterial Adhesion and Twitching Motility?
The article is available online via the following link: [http://link.springer.com/article/10.1007/s00203-013-0870-1](http://link.springer.com/article/10.1007/s00203-013-0870-1)

**Circuitry of Cells Involved in Immunity, Autoimmune Diseases Exposed: Connections Point to Interplay Between Salt and Genetic Factors**

Mar. 6, 2013 — New work from the Broad Institute's Klarman Cell Observatory, Brigham and Women's Hospital, Harvard University, MIT, and Yale University expands the understanding of how one type of immune cell—known as a T helper 17 or Th17 cell—develops, and how its growth influences the development of immune responses. By figuring out how these cells are "wired," the researchers make a surprising connection between autoimmunity and salt consumption, highlighting the interplay of genetics and environmental factors in disease susceptibility.

The results of their work appear in three companion papers in *Nature* this week.

The researchers concentrated on T cells because of their important roles in clearing foreign pathogens and in various autoimmune diseases. "The question we wanted to pursue was: how does the highly pathogenic, pro-inflammatory T cell develop?" said Vijay Kuchroo, co-director of the Center for Infection and Immunity at Brigham and Women's Hospital's Biomedical Research Institute and a Broad associate member. Kuchroo is also a professor of neurology at Harvard Medical School. "Once we have a more nuanced understanding of the development of the pathogenic Th17 cells, we may be able to pursue ways to regulate them or their function."

The human immune system is in a state of delicate balance: too little activity leaves a person vulnerable to foreign invaders, but too much activity threatens to harm the body it ought to protect. When this delicate balance is broken, it can lead to autoimmune diseases. But little is known about the molecular circuitry that maintains—or upsets—such a fine equilibrium.

"We wanted to understand how the body gets the right kinds of immune cells in the right amount, and how it keeps those cells at the right activity level so that they are not too active but also not underactive," said Avi Regev, a Broad Institute core member and an associate professor of biology at MIT. Regev is also an Early Career Scientist at Howard Hughes Medical Institute and the director of the Klarman Cell Observatory at the Broad. "The value in doing an unbiased analysis is that we're able to understand a lot more about the molecular biology at play and identify novel players in this process."

Th17 cells can promote inflammation that is important for protection against pathogens, but they have also been implicated in diseases like multiple sclerosis, psoriasis, rheumatoid arthritis, and ankylosing spondylitis. Treatment options for some of these diseases, such as psoriasis, include manipulating T cell function.

David Hafler's group at Yale University studies human autoimmune diseases in general and the role of Th17 cells in particular, and has collaborated with Kuchroo's group for many years. "These are not diseases of bad genes alone or diseases caused by the environment, but diseases of a bad interaction..."
between genes and the environment," said Hafler, Gilbert H. Glaser Professor of Neurology, professor of immunobiology, chair of Department of Neurology, and senior author of one of this week's *Nature* papers.

Some genes have been previously tied to Th17 development, but the research team wanted a more comprehensive view. One of the challenges of studying cell development, however, is that cells, particularly immune cells, change and evolve over time. The researchers chose to take frequent snapshots—18 over the course of three days—to see what was happening within the T cells as they grew from naïve cells into more specialized Th17 cells. From these snapshots, they used computational algorithms to begin to stitch together a network of molecular changes happening as the cells matured.

With this initial information in hand, the researchers systematically tested their model by silencing genes one-by-one, which could help reveal the most important points in the network and untangle their biological meaning.

To do so, they needed a technology that would allow them to silence genes without perturbing the cells in the process. Although RNA interference (RNAi) is a powerful way to turn off individual genes, most RNAi techniques rely on viruses as delivery vehicles. When scientists tried to perturb the T cells using these traditional techniques, cells either changed or died, limiting the effectiveness of these strategies.

"This was a real challenge," said Kuchroo. "Every time we tried to downregulate a gene with existing technologies, the cell would change. We didn't know if we were looking at the right thing. We needed a new technology—something that could have a dramatic but precise effect."

A solution came from an unlikely source. Harvard professor and Broad associate member Hongkun Park and his lab in the departments of chemistry and chemical biology and of physics had been working on a computer-chip-like structure to interact with brain cells. Co-first authors Alex Shalek and Jellert Gaublomme along with other lab members had developed a bed of silicon nanowires—miniscule needles designed to pierce cells.

"We learned that we could use these needles to deliver molecules into cells in a minimally invasive fashion," said Park. "And as Vijay and Aviv taught me, there are lots of things that this allows you to do that you could not do before. It's been an eye-opening experience."

Just as the thin needle of a syringe can be inserted into the skin and cause no more than a small pinching sensation, nanowires can be inserted into cells, causing minimal disruption. Using this new technology, the team teased apart the network, piece by piece, by deleting each of the key genes required in the development of Th17 cells.

With the help of co-first author Nir Yosef, a postdoc at the Broad and Brigham and Women's Hospital, the team found that Th17 cells are governed by two networks, seemingly at odds with each other: one network positively regulates the cells, coaxing them to increase in number while suppressing the development of other cells. The other negatively regulates them, having the opposite effect.

"It's a system in perfect tension," said Regev. "It both suppresses and promotes Th17 cell creation, keeping the cells at equilibrium."

Through this analysis, one particular gene stood out to the researchers: SGK1. The gene plays an important role in the cells' development, and when turned off in mice, Th17 cells are not produced. SGK1 had not been described in T cells before, but it has been found in cells in the gut and in kidneys, where it plays a role in absorbing salt.

Based on this, two teams of researchers set out to test the connection between salt and autoimmunity—Kuchroo, Regev, and their colleagues working with mouse cells and mouse models, and Hafler's team working with human cells.

Through efforts led by co-first author and Brigham and Women's Hospital postdoc Chuan Wu, the team found that they could induce more severe forms of autoimmune diseases, and at higher rates, in mice fed a high-salt diet than in those that were fed a normal mouse diet. Kuchroo notes though that the high-salt diet alone did not cause autoimmune diseases—the researchers had to induce disease, in this case by injecting a self-antigen to prompt the mouse immune system to respond.

"It's not just salt, of course," Kuchroo said. "We have this genetic architecture—genes that have been linked to various forms of autoimmune diseases, and predispose a person to developing autoimmune diseases. But we also suspect that environmental factors—infestation, smoking, and lack of sunlight and Vitamin D—may play a role. Salt could be one more thing on the list of predisposing environmental factors that may promote the development of autoimmunity."

"One important question is: how can one think of these results in the context of human health?" said Regev. "It's premature to say, 'You shouldn't eat salt because you'll get an autoimmune disease.' We're
putting forth an interesting hypothesis—a connection between salt and autoimmunity—that now must be tested through careful epidemiological studies in humans."

The researchers plan to harness the cell circuitry data to identify and follow up on potential drug targets. Kuchroo notes that the published work and future studies are only possible because of the interdisciplinary team brought together by shared questions about cell circuitry.

"We often work in isolation in our areas of expertise, but this is the kind of work I could not have done in my own lab, and that Hongkun and Aviv could not have done in their respective labs," said Kuchroo.

"We needed this unique combination of tools and technologies to come together around this problem. Looking forward, we'll need the tools and intellect of different disciplines in order to solve big problems in biology and medicine."

**Journal References:**


**Behind the miracle child a broken system lurks**

By Jim Merrell

ATLANTA, GA—The stories we tell ourselves about the world we live in matter. While we celebrate this week’s story of an HIV-miracle cure, let’s not forget the story of injustice that made it possible.

There have been a lot of stories about this week’s “Mississippi Miracle”—the apparent functional cure of a two-year-old girl who was born with HIV.

But I’d like to tell you another story: one that’s based on the few details that have emerged about the Mississippi case and the hard truths we know about the challenges facing many people living with HIV in the U.S.

It’s a story about our health care system:

A young mother in Mississippi goes into labor early. Her arrival at the hospital is the first time she has received medical care during her pregnancy.

She has no documented HIV status, prompting hospital staff to test her for the virus.

The test comes back positive. Her baby is born prematurely, also infected with HIV.

In a matter of hours, the life of the young mother has been turned upside down. She is now forced to confront the reality of living a lifetime with an incurable disease, one that could have been prevented.

Whatever life circumstances prevented her from accessing pre-natal care are likely left unaddressed. And then, at 18 months after birth, mother and baby are disconnected from care for a period of six months.

Sadly, if the story ended here, it would be unremarkable. Despite over a decade of knowledge on preventing mother-to-child-transmission of HIV and the amazing success of public health programming, the CDC reports that about 300,000 children are still born with HIV each year globally and about 200 here in the U.S.

Of course, this particular story has quite a silver lining. Defying all previous scientific knowledge and after a six-month gap in care, the toddler appears to have achieved a “functional cure” of her HIV infection.

Media attention has understandably focused on this historic observation and its potential implications for research that seeks a cure for HIV and mother-to-child-transmission.

But lurking behind this feel-good narrative are several heart-wrenching questions:

- How is it possible for a pregnant woman in the world’s wealthiest country to not receive prenatal care?
- Why is it that our medical care system did not detect the HIV status of this young woman until she was about to delivery her baby?
- Why is it that even after the mother and child were diagnosed with HIV that they were “lost to care” for over 6 months?
- Why are we not asking these questions?
The fact of the matter is this “natural experiment” was brought about by a failure of our health care system to protect the most vulnerable (and often most invisible) members of our communities. It should never have happened.

Our excitement and thrill over the breakthrough made by this accident of injustice should be matched by an equal sense of shame that we continue to allow our fellow human beings to slip through the cracks of a broken health care system.

However, the little girl blessed with a miracle need not be left to the mercy of the same social safety net that clearly failed her mother. This is a story we can still change. I hope you will stand with HIV Prevention Justice Alliance as we speak truth to power, putting pen to paper and bringing our bodies to streets, to write the story of a more just future for all.

NIH study sheds light on role of climate in influenza transmission

Two types of environmental conditions—cold-dry and humid-rainy—are associated with seasonal influenza epidemics, according to an epidemiological study led by researchers at the National Institutes of Health’s Fogarty International Center. The paper, published in PLOS Pathogens, presents a simple climate-based model that maps influenza activity globally and accounts for the diverse range of seasonal patterns observed across temperate, subtropical and tropical regions.

The findings could be used to improve existing current influenza transmission models, and could help target surveillance efforts and optimize the timing of seasonal vaccine delivery, according to Fogarty researcher Cecile Viboud, Ph.D., who headed the study. "The model could have a broader application, encouraging researchers to analyze the association between climatic patterns and infectious disease across a wide range of diseases and latitudes,” said Viboud.

Human influenza infections exhibit a strong seasonal cycle in temperate regions, and laboratory experiments suggest that low specific humidity facilitates the airborne survival and transmission of the virus in temperate regions. Specific humidity is the ratio of water vapor to dry air in a particular body of air while relative humidity—commonly used in weather forecasts—is the amount of water vapor in the air relative to its capacity to hold water vapor, and is primarily a function of temperature.

Data from animal studies indicate low temperature and humidity increase the duration of the virus's reproduction and expulsion in infected organisms and virus stability in the environment, increasing the probability of transmission through coughing, sneezing or breathing. In contrast, high temperature seems to block airborne transmission.

According to James Tamerius, Ph.D., a geographer at Columbia University, New York City, and the first author of the study, the effect of low specific humidity on influenza could cause annual winter epidemics in temperate areas. "However, this relationship is unlikely to account for the epidemiology of influenza in tropical and subtropical regions where epidemics often occur during the rainy season or transmit year-round without a well-defined season,” he said.

After assessing the role of local climatic variables on virus seasonality in a global sample of study sites, Viboud and her colleagues found that temperature and specific humidity were the best individual predictors of the months of maximum influenza activity, known as influenza peaks. The team discovered that in temperate regions, influenza was more common one month after periods of minimum specific humidity. These periods happen to coincide with months of lowest temperature. In contrast, sites that maintained high levels of specific humidity and temperature were generally characterized by influenza epidemics during the most humid and rainy months of the year. ”The models we used predicted the timing of peak influenza activity with 75 to 87 percent accuracy,” said Viboud.

"Anecdotal evidence suggests that colder climates have winter flu while warmer climates that experience major fluctuations in precipitation have flu epidemics during the rainy season, and the current study fits that pattern,” said Viboud. "In contrast, the seasonality of influenza is less well-defined in locations with little variation in temperature and precipitation, and is a pattern that remains poorly understood. One hypothesis that is often used to explain tropical influenza activity is that people congregate indoors more frequently during the rainy season, increasing contact rates and disease transmission. There is little data to confirm this, however, and it’s an interesting area for future research.”

To reach these conclusions, the researchers used a recently developed global database that provides information on influenza peaks from 1975-2008 for 78 sites worldwide. The study spanned a range of latitude that was between 1 and 60 degrees, with 39 percent of the sites located in the tropics. Additionally, epidemiological data from nine countries participating in FluNet, the World Health Organization’s global influenza surveillance program, was used to ensure independent validation. The
nine countries—including Spain, Tunisia, Senegal, Philippines, Vietnam, Colombia, Paraguay, South
Africa and Argentina—were not represented in the original 78-location database and were chosen
because each country provided several years of data.

"We've shown the importance of thresholds in humidity and temperature which are predictive of
whether influenza activity occurs during winter months, the rainy season or throughout the year," said
Viboud. "The predictions of our climate-based models compared favorably to epidemiological information
collected independently of the dataset used for the model-building exercise."

Though the study offers researchers a new tool in the global effort to track the spread of influenza,
climate is only one of several potential drivers of influenza seasonality. "Further work should focus on
examining the role of population travel and other factors in influenza transmission," notes Mark Miller,
M.D., director of Fogarty's Division of International Epidemiology and Population Studies. "

More broadly, additional analysis of the link between climate and infectious diseases is needed—
particularly for respiratory and intestinal pathogens that display marked seasonality." The authors
conclude, "A better understanding of the environmental, demographic and social drivers of infectious
disease seasonality is crucial for improving transmission models and optimizing interventions."
The study was conducted in the context of the Multinational Influenza Seasonal Mortality Study, an ongoing international
collaborative effort led by Fogarty to better understand the epidemiological and evolutionary patterns of influenza. A link to the
paper can be found at http://dx.plos.org/10.1371/journal.ppat.1003194.

**Michigan's HIV Testing Scandal**

**Abstract**

In researching alleged 2011 Michigan Department of Community Health (MDCH) data breaches,
investigative reporter Todd Heywood discovered that MDCH "secretly collected" personal information—
names, dates of birth, risk categories, and other demographic data—on people (and their partners) who
got to government grant-funded organizations for confidential testing. Heywood reports that the state is
using the private information in civil and criminal cases against HIV-infected people who present a
"health threat." To learn about MDCH collection of confidential HIV testing information, Heywood
submitted multiple Freedom of Information Act (FOIA) requests, and he also asked to attend a March 6
mandatory meeting for all Michigan agencies that receive federal funding for HIV prevention and testing
through MDCH grants. Although meeting organizers initially denied Heywood's request because he is not
a grantee, he eventually received permission to attend. Heywood states his 300-hour investigation has
encompassed 60 e-mail interviews and phone calls, FOIA requests and appeals, and document review.

**Source**

http://detnews.com/

**Putting the Nail in the Coffin of Condom-Only HIV Prevention**

Part 16 in a Series on PrEP by PositiveFrontiers.com

Jake Sobo, 3/7/2013

Dearly beloved, we gather here to say our goodbyes. For over thirty years, condoms have been our only
lifesavers in the face of HIV. Gay men invented their promotion at a time when death was the only
seeming alternative. When treatment did not exist. When Kaposi's sarcoma was a visible reminder of the
epidemic. It was a different world. To quote Mr. Kushner, "You can never make that crossing that she
made, for such great voyages in this world do not any more exist."

Weep not, dear friends, for the passing of our friend. For a new era is dawning in HIV prevention.
If we needed any additional evidence of the need to turn the page, it came in the form of a seemingly banal
conference presentation this week in Atlanta at the 20th Conference on Retroviruses and Opportunistic
Infections. While CDC staff scientist’s Dawn Smith analysis of data from two previous HIV clinical trials
seems at first glance to be of little import, its findings scream a different truth. (You can watch her
presentation here – it’s the last paper in the session.)

The study’s aim may seem modest to many readers: to estimate how effective condoms are at
reducing the risk of HIV transmission among men who have sex with men. But you may be surprised to
learn that we have surprisingly little evidence to support the promotion of condoms to gay men for HIV
prevention.

Don’t get me wrong. I’m no denialist. We have good reason to believe they work – all the evidence
strongly suggests that they do. But how effective are they precisely? And in what context? Well, for many
years, your guess was as good as mine. The last time a major study attempted to tackle this question, the
year was 1989 — nearly a quarter-century ago. *When Ronald Reagan was President.*
But while the aim of the CDC’s study itself makes it important, it is what they found that should have every _condom-thumping_ prevention activist in the country questioning their strategies. While most condom studies just look at whether guys have used them in the past six months, this analysis based on two independent, large studies employs data collected over a three to four year period. And rather than just asking guys how often they used a rubber, researchers asked them every six months.

Since I began writing this column, I have said more than once that the condom use of most gay men I knew could only be said to be “inconsistent” – at best. But this study puts a more precise figure to my anecdotal evidence. Let’s start with the basic question: How many guys said they used a condom every time during every single six-month check-in? While over two-thirds of participants said they used a condom every time during at least one of the six month intervals, just 16.4% said they used a condom every time every single time they were interviewed. Not 50%. Not even 25%. 16.4%.

Now the more complicated question: By how much did reported use of a condom every time reduce the risk of HIV infection? This is sticky. As you know, guys lie left and right about how often they use condoms. Social scientists call this “social desirability bias.”

We tell docs what they wanna hear. So any estimate of condom efficacy based on self-report is bound to skew lower than what it probably is in real life. With that in mind, the CDC found that guys who reported using a condom every time were 70% less likely to contract HIV than guys who never used them, and 68% less likely than guys who said they sometimes used them.

Now here comes the rub: guys who reported using a condom “sometimes” were almost just as likely to contract HIV as guys who said they never used them. In other words, sometimes using condoms is no better than not using them at all.

So what does this study tell us? Over the long haul, the vast majority of gay men aren’t using condoms every time. And if you’re not using a condom every time, your risk of infection is similar to guys who fuck bare every single time.

Of course, these findings do not mean we should throw out condoms entirely from our toolbox. They are a part of the puzzle, and should continue to be. But we can no longer pretend that they are the only tool worthy of our time, energy, and money. These findings are clear evidence that we cannot rely on condoms alone to end the epidemic among gay men.

In the new era of HIV prevention, condoms will be one of many technologies in our arsenal. PrEP and treatment as prevention for HIV-positive people will be recognized as important tools as well, as will serosorting and other harm reduction strategies. These technologies will be understood not as implements that exist apart from sexual behavior, but as _integrated within the social landscape_ in which people navigate, understand, and practice HIV risk.

We have the tools to build a new framework for HIV prevention. What stands between us and a new era is not science, but politics. Sexual shame. HIV stigma. Misinformation. Let us tear down those walls that separate us from a new tomorrow.

**Link between Violence and HIV Must Be Made Explicit, Say African Ministers**

_The Guardian (London)_ (03.07.2013) Liz Ford

During a side event at the 57th UN Commission on the Status of Women (CSW) on March 4, ministers from Ghana, Liberia, and Zimbabwe elucidated upon the problem of violence and infection in their countries. They recommended to advocates that the UN’s CSW outcome document should emphasize the link between gender-based violence and HIV infections. The ministers urged that this document also should include information on governments providing effective measures to prevent violence against women as well as information on treating women who have been infected with HIV through sexual abuse. The inclusion of such passages in the final CSW document is still under discussion.

Julia Duncan-Cassell, Liberia’s gender minister, declared that the CSW afforded a chance to lobby for action. She stated, "Increasingly, violence is being recognized as a cause and consequence of HIV, and that's important, particularly for women and girls."

Nana Oye Lithur, Ghana’s gender minister, stated that, in spite of education, some Ghanaian women still find it challenging to negotiate contraception with their partners. Lithur said that there are still traditional practices in existence that compel women to marry a brother-in-law if her husband has died, as well as the belief that a woman needs to have sex with a stranger to expel the disease of a dead husband. Women are thus powerless and HIV is easily spread.

Zimbabwe Deputy Prime Minister Thokozani Khupe spoke of the importance of education in addressing violence against women, emphasizing the needs of women living with HIV. She noted that...
rural women are especially vulnerable and need better support. In Zimbabwe, women carry babies on their backs and farm the fields using primitive tools for most of the day. They need to be empowered. Khupe declared, "Once you empower [women], issues of HIV and gender-based violence will be a thing of the past."

Sheila Tlou, director of the UNAIDS regional support team for east and southern Africa, told event attendees that gender equality was still a faraway dream for many. She exclaimed, "We need to change attitudes, even among women themselves. Women need to know their human rights." She declared that, unless gender equality is achieved, the UNAIDS concept of zero new HIV infections, zero discrimination, and zero AIDS-related deaths will remain an unfulfilled ambition.

**More MSM with HIV Aware of their Infection**

*Healio*, (03.06.2013)
The number of US HIV-infected men who have sex with men (MSM) who are aware of their HIV infection increased from 2008 to 2011, while the proportion of US HIV-infected men remained approximately the same, according to data collected for CDC’s National HIV Behavioral Surveillance System (NHBS). CDC Division of HIV/AIDS Prevention’s Cyprian Wejnert, PhD, suggested the change in HIV awareness may be attributed to efforts to increase HIV testing and reduce HIV stigma.

To reach their conclusions, CDC epidemiologists reviewed NHBS data collected from 20 cities: 19 percent of 7,847 MSM tested in 2008 was HIV positive, and 18 percent of the 8,423 men tested in 2011 was HIV positive. In 2008, only 56 percent of those tested was aware they had HIV, compared to 66 percent of those tested in 2011. Awareness increased most among MSM under age 25 (49 percent in 2011 compared to 31 percent in 2008). MSMs ages 40 and older had the least change in awareness (69 percent in 2008 compared to 76 percent in 2011). HIV prevalence continued to be highest among US blacks, who also reported less awareness of their HIV infections.

Wejnert stated that people who are not aware they have HIV transmit more than half of new HIV infections.


**Dually Active Antiretroviral Therapy Protects Against Hepatitis B Infection**

*AIDSMAP*, (03.07.2013)  Liz Highleyman

Formulations of antiretroviral therapy (ART) that also are effective against hepatitis B protect HIV-infected patients from primary hepatitis B infections, according to a study led by Kees Brinkman at Amsterdam’s Onze Lieve Vrouwe Gasthuis. Brinkman’s team undertook the retrospective study because the rate of new hepatitis B infections in Amsterdam’s largest HIV clinic had dropped to “very low” levels; they suspected ART with “dual” activity against HIV and hepatitis B might have reduced new hepatitis B infections. ART combinations effective against both HIV and hepatitis B include tenofovir (Viread, also in Truvada, Atripla, Complera, and Stribild), 3TC (lamivudine, Epivir), and FTC (emtricitabine, Emtriva).

The study included nearly 3,000 clinic patients, 2,280 of whom were men who have sex with men. At baseline, 51 percent were HIV- and hepatitis B-coinfected, 13 percent had been vaccinated for hepatitis B, and 30 percent were still susceptible to hepatitis B. Second samples of the participants who were hepatitis B-susceptible indicated that 530 remained hepatitis B-susceptible, 171 had been vaccinated for hepatitis B, and 35 patients had new hepatitis B infections. Researchers zeroed in on 350 HIV-infected patients who were not yet infected with hepatitis B, and for whom subsequent samples were available, to determine whether their hepatitis B status had changed.

Brinkman reported that HIV-infected people who did not receive dually active ART became infected with hepatitis B much sooner than people taking ART that also acts against hepatitis B. ART containing tenofovir was the combination most effective in preventing hepatitis B. Brinkman presented the study results during the 20th Conference on Retroviruses and Opportunistic Infections.

A webcast of the presentation, “Protective Effect of Hepatitis B Virus-active cART Against Primary Hepatitis B Virus Infection,” was published online by the 20th Conference on Retroviruses and Opportunistic Infections at http://webcasts.retroconference.org/console/player/19410?mediaType=podiumVideo.
U.N. Warns Of Little Progress Curbing Child Marriage Rates; Human Rights Watch Report Shows Practice Widespread In South Sudan

"If current child marriage rates continue, more than 140 million girls will become child brides between 2011 and 2020, the United Nations said [Thursday], warning that little progress has been made towards ending this harmful practice," the U.N. News Centre reports. "Of these 140 million girls, 50 million will be under the age of 15, according to the U.N. Population Fund (UNFPA), which added that young girls who marry before the age of 18 have a greater risk of becoming victims of intimate partner violence than those who marry later," the news service writes, noting, "Child marriage is increasingly recognized as a violation of the rights of girls as it interferes with their education, blocks their opportunity to gain vocational and life skills, and increases their risk to sexual violence as well as their chances to contract HIV" (3/7).

In related news, "Human Rights Watch says widespread child marriage in South Sudan is violating girls' rights, limiting female education and contributing to soaring maternal mortality rates," VOA News reports. "David Mepham of Human Rights Watch said the child marriage report is based on more than 80 interviews with girls and women," according to the news service, which notes, "The Human Rights Watch report released Thursday says this has major implications for the education of girls and women, and for the overall well-being of the country. It says many girls are leaving school early, some as young as 11, to marry." The news service adds, "According to government statistics, just under half of girls aged between 15 and 19 in South Sudan are married. Some marry as early as 12" (Hennessy, 3/7).

WHO Announces 8th Death From Novel Coronavirus

"A 69-year-old man in Saudi Arabia died after being infected with a new SARS-related virus, becoming the 14th confirmed case and the eighth death, the World Health Organization said" on Wednesday, Bloomberg reports. "Preliminary investigations indicated the patient had no contact with previously reported cases of infection with the novel coronavirus," the news agency writes (Bennet, 3/7).

"The patient was a 69-year-old man who was hospitalized on February 10 and died on February 19," CIDRAP News notes, adding, "His illness raises the global number of infections from the new virus to 14, including eight deaths."

"The wide illness spectrum has left health officials wondering if current surveillance for NCoV is missing mild or asymptomatic infections and if new strategies are needed," the news service continues (Schnerring, 3/6). The WHO "has asked countries to be alert to unusual patterns of disease among patients who fall ill with respiratory infections, but has not recommended travel or trade restrictions on any of the countries involved," the Guardian reports (Sample, 3/7). The CDC "on Thursday warned state and local health officials about potential infections from" the coronavirus, Reuters/Chicago Tribune notes (3/7).

Friday, March 08, 2013

A*STAR Scientists Discover “Switch” Critical to Wound Healing

Patients with diseases such as diabetes suffer from painful wounds that take a long time to heal making them more susceptible to infections that could even lead to amputations. A*STAR’s discovery paves the way for therapeutics to improve healing of such chronic wounds, which are a significant burden to patients.

1. Scientists from A*STAR’s Institute of Medical Biology (IMB) have identified a molecular “switch” that controls the migration of skin cells necessary for wounds to close and heal. This is especially significant for diabetics and other patients who suffer from chronic wounds, wounds that do not heal or take years to do so, which are vulnerable to infections and could lead to amputations. This switch mechanism may hold the key to developing therapeutics that will reduce or prevent chronic wounds.

2. The scientists discovered that a tiny “micro-RNA” molecule, called miR-198, controls several different processes that help wound healing, by keeping them switched off in healthy skin. When skin is wounded, the manufacture of miR-198 quickly stops and the levels of miR-198 drop, switching on many wound healing processes.

3. In the non-healing wounds of diabetics, miR-198 does not disappear and wound healing remains blocked. This therefore identifies miR-198 as a potential diagnostic biomarker for non-healing wounds. These findings were recently published in the prestigious journal Nature [1].

4. The research leading to this discovery was carried out in collaboration with A*STAR’s Bioinformatics Institute (BII), National University Hospital (NUH), Singapore and Jnana Sanjeevini Diabetes Center, Bangalore, India.
Importance of this discovery
1. Chronic wounds in patients with diabetes are a major global health burden and the most common cause of lower extremity amputations. In Singapore, diabetes is the fifth most common medical condition diagnosed and one in nine people aged 18 to 69 has diabetes. Unfortunately, chronic wounds are currently poorly understood and insufficiently treated. Chronic wounds also tend to affect the elderly and disabled patients, especially those confined to a wheelchair or bed-bound.
2. Dr. Prabha Sampath Moving forward, we hope to translate this research into improved patient outcomes. We can now build on this research, to see how we can modulate the defective switch in chronic wounds by targeting miR-198 and its interacting molecules, to develop new strategies for treating chronic wounds.” , principal investigator at IMB and lead author of the paper, said, “Our research provides a comprehensive understanding of the mechanism of the wound healing process.
3. Professor Birgitte Lane, Executive Director of IMB, said, “This switch appears to be an entirely new regulatory component in wound healing, and probably a very important one. Poor wound healing is a major healthcare burden, and this discovery is particularly timely in the face of aging populations and the sharp global rise in diabetes. The finding gives us a platform from which to develop therapies that could significantly reduce chronic wounds and improve healthcare.”

An FSTL1-miR-198 molecular ‘see-saw’ switch
1. The information necessary to express microRNA-198 (miR-198) and follistatin-like 1 (FSTL1) protein are found in a single “message” produced by the cell. However, miR-198 and FSTL1 protein cannot be produced at the same time – it can only be one or the other. These two molecules also have opposite roles: miR-198 (found in unwounded skin) inhibits skin cell migration and wound healing, whereas FSTL1 protein (expressed after injury) promotes skin cell migration and wound healing. A regulatory switch dictates their expression, and hence controls the “see-saw” between inactive resting skin cells and the cell migration necessary for wound healing.
2. Dr. Sampath and her team showed that healthy unwounded skin contained high levels of miR-198 but no FSTL1 protein. They demonstrated that these high levels of miR-198 prevent skin cell migration by suppressing several genes, such as PLAU, LAMC2 and DIAPH1, which are needed for different aspects of the wound healing process. However upon injury, miR-198 is switched off in the wound by a signal from transforming growth factor β1 (TGF-β1). This allows FSTL1 to now be made instead, and the skin migration genes to be unblocked, promoting migration of skin cells into the wound area to drive skin wound healing.
3. The scientists further examined skin samples of chronic non-healing ulcer wounds from patients with diabetes mellitus. They observed that, unlike healthy skin that had been injured, there remained high levels of miR-198 (inhibiting skin cell migration and wound healing) and an absence of FSTL1 protein (promoting skin cell migration upon wounding), indicating that this “switch” is defective in chronic wounds. (Annex A)

Notes for editor:
The research findings described in this news release can be found in Nature under the title “‘See-saw’ expression of microRNA-198 and FSTL1 from a single transcript in wound healing” by Gopinath M. Sundaram1,*, John E. A. Common1,*, Felicia E. Gopal1, Satyanarayana Srikanta2, Krishnaswamy Lakshman2, Declan P. Lunny1, Thiam C. Lim2,4, Vivek Tanavde1,5, E. Birgitte Lane1,6,7 & Prabha Sampath1,7. Doi:10.1038/nature11890
New Study Validates Longevity Pathway: Findings Identify Universal Mechanism for Activating Anti-Aging Pathway

Mar. 7, 2013 — A new study demonstrates what researchers consider conclusive evidence that the red wine compound resveratrol directly activates a protein that promotes health and longevity in animal models. What’s more, the researchers have uncovered the molecular mechanism for this interaction, and show that a class of more potent drugs currently in clinical trials act in a similar fashion. Pharmaceutical compounds similar to resveratrol may potentially treat and prevent diseases related to aging in people, the authors contend.

These findings are published in the March 8 issue of Science.

For the last decade, the science of aging has increasingly focused on sirtuins, a group of genes that are believed to protect many organisms, including mammals, against diseases of aging. Mounting evidence has demonstrated that resveratrol, a compound found in the skin of grapes as well as in peanuts and berries, increases the activity of a specific sirtuin, SIRT1, that protects the body from diseases by revving up the mitochondria, a kind of cellular battery that slowly runs down as we age. By recharging the batteries, SIRT1 can have profound effects on health.

Mice on resveratrol have twice the endurance and are relatively immune from effects of obesity and aging. In experiments with yeast, nematodes, bees, flies and mice, lifespan has been extended.

"In the history of pharmaceuticals, there has never been a drug that binds to a protein to make it run faster in the way that resveratrol activates SIRT1," said David Sinclair, Harvard Medical School professor of genetics and senior author on the paper. "Almost all drugs either slow or block them."

In 2006, Sinclair’s group published a study showing that resveratrol could extend the lifespan of mice, and the company Sirtris Pharmaceuticals, which was started by HMS researchers, was founded to make drugs more potent than resveratrol. (Sinclair is a co-founder of Sirtris, a GlaxoSmithKline company, and remains a scientific advisor. Sirtris currently has a number of sirtuin-activating compounds in clinical trials.)

But while numerous studies, from Sinclair’s lab and elsewhere, underscored a direct causal link between resveratrol and SIRT1, some scientists claimed the studies were flawed.

The contention lay in the way SIRT1 was studied in vitro, using a specific chemical group attached to the targets of SIRT1 that fluoresces more brightly as SIRT1 activity increases. This chemical group, however, is synthetic and does not exist in cells or in nature, and without it the experiments did not work. As a response to this, a paper published in 2010 surmised that resveratrol’s activation of SIRT1 was an experimental artifact, one that existed in the lab, but not in an actual animal. SIRT1 activity in mice was, the paper claimed, at best an indirect result of resveratrol, and perhaps even a sheer coincidence.

As a result, a debate erupted over the particular pathway that resveratrol and similar compounds affected. Does resveratrol directly activate SIRT1 or is the effect indirect? "We had six years of work telling us that this was most definitely not an artifact," said Sinclair. "Still, we needed to figure out precisely how resveratrol works. The answer was extremely elegant."

Sinclair and Basil Hubbard, then a doctoral student in the lab, teamed up with a group of researchers from both the National Institutes of Health and Sirtris Pharmaceuticals to address this question.

First, the team addressed the problem of the fluorescent chemical group. Why was it required for resveratrol to rev up SIRT1 in the test tube? Instead of dismissing the result as an artifact, the researchers surmised that the chemical might be mimicking molecules found naturally in the cell. These turned out to be a specific class of amino acid, the building blocks of proteins. In nature, there are three amino acids that resemble the fluorescent chemical group, one of which is tryptophan, a molecule abundant in turkey and notable for inducing drowsiness. When researchers repeated the experiment, swapping the
fluorescing chemical group on the substrate with a tryptophan residue, resveratrol and similar molecules were once again able to activate SIRT1.

"We discovered a signature for activation that is in fact found in the cell and doesn't require these other synthetic groups," said Hubbard, first author of the study. "This was a critical result, which allowed us to bridge the gap between our biochemical and physiological findings.

"Next, we needed to identify precisely how resveratrol presses on SIRT1's accelerator," said Sinclair. The team tested approximately 2,000 mutants of the SIRT1 gene, eventually identifying one mutant that completely blocked resveratrol's effect. The particular mutation resulted in the substitution of a single amino acid residue, out of the 747 that make up SIRT1. The researchers also tested hundreds of other molecules from the Sirtris library, many of which are far more powerful than resveratrol, against this mutant SIRT1. All failed to activate it.

The authors propose a model for how resveratrol works: When the molecule binds, a hinge flips, and SIRT1 becomes hyperactive.

Although these experiments occurred in a test tube, once the researchers identified the precise location of the accelerator pedal on SIRT1—and how to break it—they could test their ideas in a cell. They replaced the normal SIRT1 gene in muscle and skin cells with the accelerator-dead mutant. Now they could test precisely whether resveratrol and the drugs in development work by tweaking SIRT1 (in which case they would not work) or one of the thousands of other proteins in a cell (in which they would work). While resveratrol and the drugs tested revved up mitochondria in normal cells (an effect caused activating by SIRT1), the mutant cells were completely immune.

"This was the killer experiment," said Sinclair. "There is no rational alternative explanation other than resveratrol directly activates SIRT1 in cells. Now that we know the exact location on SIRT1 where and how resveratrol works, we can engineer even better molecules that more precisely and effectively trigger the effects of resveratrol."

Journal Reference:

Bee Venom Destroys HIV and Spares Surrounding Cells
Medical News Today (03.10.2013) Christian Nordquist
Washington University School of Medicine researchers describe the bee venom toxin melittin as a promising HIV prevention and treatment measure, because melittin can disrupt the protective double-layered membrane surrounding the HIV virus. In laboratory studies, scientists loaded melittin onto nanoparticles and then added protective bumpers that cause the nanoparticles to bounce off normal cells, which tend to be much larger. The HIV virus, which is much smaller than nanoparticles, slips between the nanoparticle bumpers and comes into direct contact with melittin. The bee venom then fuses with the HIV viral envelope, ruptures it, and strips the vital structure from the virus. Thus, the melittin prevents HIV infection.

The research team is exploring two possible therapies based on the melittin-loaded nanoparticles: a vaginal gel to prevent HIV infection, and therapy for existing HIV infections. Research Instructor Joshua L. Hood, MD, PhD, said the vaginal gel could help couples in which one person is HIV-infected and the other is not, when the couple wants to have a baby, because the nanoparticles are safe for vaginal cells and sperm. Other HIV therapies aim to disrupt virus replication in HIV-infected people, but some HIV strains have been able to reproduce in spite of the anti-replication therapies.

Hood said the nanoparticles were originally developed as an “artificial blood product,” but nanoparticles were not effective in delivering oxygen. However, the nanoparticles circulate well and safely through the body and provide an adaptable “platform” for delivering therapies for HIV and other infections. Since melittin attacks double-layered membranes indiscriminately, it also could be useful in treating viruses like hepatitis B and C.

The full report, “Cytolytic Nanoparticles Attenuate HIV-1 Infectivity,” was published online in the journal Antiviral Therapy (2012; doi: 10.3851/IMP2346).

Drug To Treat Leishmaniasis Fails In 20% Of Patients, Study Shows
"One in five people treated for a serious form of leishmaniasis in Nepal relapse after a year," Nature reports, adding, "The finding, published in Clinical Infectious Diseases last month, is ‘an alarming signal’ for campaigns to eliminate the neglected disease, say researchers.” The news service notes, "The orally
administered drug miltefosine emerged as the treatment of choice a decade ago, taking over from injections of the highly toxic, antimony-based drug sodium stibogluconate, which had started showing failure rates of 65 percent in India's northern state of Bihar." However, "reports are now emerging of miltefosine failure, which scientists worry will narrow down the pipeline of drugs available for treatment," the news service adds.

"In the latest study, a team of researchers from Belgium, Nepal and the Netherlands identified 187 cases of visceral leishmaniasis in Nepal, of whom 120 were treated with miltefosine," Nature writes, noting, "Six months after treatment with miltefosine, 10 percent of cases had relapsed; after a year, that had doubled to 20 percent." The magazine continues, "The cause of the relapses is unknown: they were not attributable to fresh infections; to co-infection with HIV, which reduces the host's immunity; to low drug quality; to poor adherence to the treatment regime; or to drug resistance in the parasite." Nature adds, "Whatever the causes of the failure, 'these new results support the recommendation to discontinue miltefosine monotherapy,' [Manica Balasegaram of Médecins Sans Frontières] says" (3/8).

**Listen To Women's Needs When Designing HIV Prevention Strategies**

According to "results from a large HIV prevention trial among African women" known as VOICE and released last week, "none of the prevention methods tested in the study made a difference in HIV infection rates, because few women actually used them as directed," AVAC Executive Director Mitchell Warren writes in an opinion piece published in the Huffington Post's "Global Motherhood" blog. "The women in the trial are telling us something that is true for every group at risk for HIV: to help more people avoid infection, we need to offer prevention tools they will actually want, demand and use," he states. "After years of exciting news on the biomedical prevention front, the VOICE results underscore that it is time to get serious about the behavioral side of new HIV prevention options," Warren writes.

"First, it means figuring out how to identify those who are most likely to use and benefit from [pre-exposure prophylaxis (PrEP)] and other emerging options," he states, adding, "Second, we need to redouble research into additional options that women can control, want and use," such as vaginal rings or injections. "In addition, research and development resources are urgently needed for combined contraceptive and HIV prevention methods, which would address many women's needs more comprehensively," and "research to find HIV vaccines, which would overcome many of the issues around adherence, also needs an aggressive push," he continues. Warren adds, "We need to listen to the women of VOICE and other recent studies. That means designing prevention options based on a deeper understanding of women's reproductive and sexual health needs and desires, their perceptions of their personal risk for HIV infection, and their willingness and ability to use the products on offer" (3/8).

**Evolution in the antibody factory**

*How immune cells are able to advance their own evolution*

11.03.2013

Theory of colours for immunologists – sophisticatedly-designed microscopy technologies offer scientists insights into the evolution of antibody producers in the lymph node. Antibodies and immune cells are marked with different colours.

Immune system B cells play a crucial role in the defence of pathogens; when they detect such an intruder, they produce antibodies that help to combat the enemy. They concurrently and continuously improve these molecules to more precisely recognize the pathogens. A team of scientists with participation of the Helmholtz Centre for Infection Research (HZI) has discovered that during this process the cells are able to advance their own evolution themselves by increasing the selection pressure through previously-produced antibodies. The results are also significant for the development of new vaccination strategies.

The principle of evolution signifies the competition for limited resources and a reaction to changeable environmental conditions. This selection pressure is virtually produced by the B cells on their own; they subject themselves to an optimization cycle in the lymph node, a process which only a few of them survive,
i.e. particular cells that are able to produce “better” antibody molecules as compared to those that already exist within the body. The quality of these antibodies is tested in the lymph nodes, and only those cells that are able to prove themselves here receive signals from other immune cells that assure their survival.

Every B cell carries a specific defence molecule on its surface. It recognizes certain structures of pathogens – so-called antigens – similar to the way a key fits into one specific lock. This molecule is furthermore produced in a certain form that does not remain on the cell surface; rather, it travels with blood and lymph throughout the body. If the antibody encounters an antigen, it either binds it to neutralise it, or it sends out an alarm to other players within the immune system.

At the beginning of an infection there are, figuratively speaking, several keys that do not yet fit properly. This changes in the course of a process that immunologists refer to as “somatic hypermutation”; B cells mutate those gene segments that determine the design of both the surface molecule and the soluble variation – thus influencing how strongly the antibodies attach themselves to the pathogens. Those cells, in which the optimal fit of the key increases, survive and multiply. They then produce the desired molecule in large quantities and thus help us to get healthy again.

But how do the immune cells know that they are on the right way with this arbitrary mutation process, i.e. that the key will fit better later on? Scientists from England, Germany and Switzerland have now been able to answer this question jointly in a collaborative project between Dr. Kai-Michael Toellner, University of Birmingham, and Prof. Michael Meyer-Hermann, Head of the Department Systems Immunology. They published their findings in the renowned Journal of Experimental Medicine. Meyer-Hermann makes use of mathematical models to understand diseases more thoroughly and quicker. “Systems immunology enables us to simulate, in a short amount of time, numerous experimental conditions,” he describes his area of expertise. With the aid of such mathematical simulations followed by experimental examinations, the researchers discovered that the antibody producers advance their own evolution, which represents without a doubt an alignment with the enormous selection pressure that we are subject to due to a constant threat from pathogens.

The stage for this process is the so-called germinal centres within the lymph nodes. Here, the maturing B cells encounter the antigens. The researchers’ results suggest that completed antibodies from all germinal centres re-appear at the sites of antibody production and bind there to pathogen fragments as well. They represent competition thereby for those cells that are still in the process of refining the optimal fit of their surface molecules. Once the immune cells with their “surface-key” are able to bind to the “antigen locks” more readily than the finished antibodies, they receive survival signals and their key-form asserts itself.

“This is the ‘survival of the fittest’ as previously described by Charles Darwin on a molecular level,” compares Meyer-Hermann. Studies with mice were able to be confirmed in computer simulations only under the assumption that the B cells compete with their own products – namely the antibodies – for the right to bind to antigens.

This astounding mechanism could, in the future, improve conventional vaccination methods. “It is plausible that patients could be administered, in addition to a vaccine, sufficiently-strong-binding antibodies,” explains Meyer-Hermann. “Our models constructed in the computer suggest that this method accelerates the process of identifying optimal antibodies.” The scientists suspect that the addition of antibodies manipulates the reaction to vaccination, since the newly-generated antibodies are now in competition with the externally-introduced molecules. The conditions for selection are thus intensified and the B cells react by producing optimal antibodies earlier on. The result is that vaccinations could take effect quicker.

Original publication:
Germainal center B cells govern their own fate via antibody feedback, Journal of Experimental Medicine, 2013, doi: 10.1084/jem.20120150

Study: Antibiotics are unique assassins
March 9, 2013 by Angela Herring
In recent years, a body of publications in the microbiology field has challenged all previous knowledge of how antibiotics kill bacteria. “A slew of papers came out studying this phenomenon, suggesting that there is a general mechanism of killing by antibiotics,” said Kim Lewis, University Distinguished Professor in the Department of Biology and director of Northeastern’s Antimicrobial Discovery Center.
The standard thinking at the time was that the three main classes of bactericidal antibiotics each had a unique way of killing bacterial cells like specialized assassins each trained in a single type of weaponry. But this new research suggested that all antibiotics work the same way, by urging bacterial cells to make compounds called reactive oxygen species, or ROS, which bacteria are naturally susceptible to.

“If they were right it would have been an important finding that could have changed the way we treat patients,” said Iris Keren, a senior scientist in Lewis’ lab.

And that’s exactly how science usually works, said Lewis—through challenges to mainstream thinking. But recent results reported by Lewis, Keren, and their research partners in an article published Friday in the journal Science suggest that this alternative hypothesis doesn’t hold up. For example, even bacteria that are incapable of making ROS are still vulnerable to antibiotics. Further, some antibiotics can work their fatal magic in both aerobic and anaerobic conditions—but reactive oxygen species can only form when there’s oxygen to fuel them.

“We chose to do the simplest and most critical experiment aimed at falsifying this hypothesis,” said Lewis. “Killing by antibiotics is unrelated to ROS production,” the authors wrote. The findings were corroborated by University of Illinois researchers in another study released on Friday.

The team treated bacterial cultures with antibiotics in both the presence and absence of oxygen. Other than the gaseous environment, the two treatments were identical. There was no difference in cell death between the two populations.

Before performing these experiments, Lewis’ team first looked at signals of a fluorescent dye, which previous researchers had used as an indicator for ROS levels. The team treated bacterial cells with a variety of antibiotics and measured the strength of this signal. Since antibiotics were presumed to increase ROS levels, one would have expected increased concentrations of antibiotics to correlate with stronger signals. However, Lewis’ group saw no such correlation.

“But there’s a difference between correlation and direct observation,” Keren said. In order to support their observations with unequivocal data, the team members physically separated the cells that had stronger fluorescent signals from those with weak signals and treated them both with the same antibiotics. Both populations suffered equivalent cell death.

“The research from Dr. Lewis’ group demonstrates that, contrary to current dogma, antibiotics apparently do not kill bacteria through induction of reactive oxygen species,” said Steven Projan, vice president for research and development at iMed and head of Infectious Diseases and Vaccines at MedImmune, both subsidiaries of AstraZeneca. “The results shown are rather clear but still leave us with the mystery as to how antibacterial drugs help infected people clear bacterial infections. At this point, we should probably dispense with the ‘one size fits all’ approach to bacterial killing by antibiotics,” said Projan, who was not involved in the research.

With these results, Lewis and Keren hope the field will be able to focus its efforts on understanding the true mechanisms of how antibiotics wipe out bacteria in order to effectively address chronic bacterial infections, one of the most pressing issues facing public health today.

No increase in risk of death for patients with well-controlled HIV, reports AIDS journal

With undetectable virus and normal immune function, mortality risk no higher than in general population, reports AIDS journal

Philadelphia, Pa. (March 11, 2013) – For HIV-infected patients whose disease is well-controlled by modern treatment, the risk of death is not significantly higher than in the general population, according to a study published in AIDS, official journal of the International AIDS Society. AIDS is published by Lippincott Williams & Wilkins, a part of Wolters Kluwer Health.

The study suggests that patients with undetectable viral loads and near-normal levels of immune cells on state-of-the art antiretroviral therapy (ART) can expect to have about the same risk of death as people without HIV. The article is available on the AIDS journal homepage and in the March 13 print edition.

What’s the Risk of Death with Well-Controlled HIV?

Dr Alison Rodger of University College London and colleagues assessed mortality rates in a group of patients with “optimally treated” HIV, drawn from two major trials of treatment for HIV infection: the ESPRIT and SMART trials. The analysis included nearly 3,300 patients who were not injecting drug users and who received continuous ART. On treatment, all had achieved undetectable HIV levels and had relatively high levels of CD4+ cells, a key population of immune cells—at least 350 cells/mm3. (A CD4+ cell count of 500 to 1,000 cells/mm3 is considered normal.)
The patients' average age was 43 years; 80 percent were men. Rates and causes of death in these patients with well-controlled HIV were compared with those in the general population.

During a median follow-up of about three years, 62 of the patients died. The most common causes of death were cardiovascular disease or sudden death, responsible for 31 percent of deaths; and non-HIV-related cancers, 19 percent. Only two deaths (three percent) were considered AIDS-related.

Patients with below-normal CD4+ cell counts (350 to 499 cells/mm³) were at elevated risk of death. Based on the standardized mortality ratio, the risk of death in this group was 77 percent higher than in the general population.

**With Normal CD4+ Cell Counts, No Increase in Mortality**

However, in HIV-infected patients with a CD4+ cell count of 500 cells/mm³ or higher, the risk of death was not significantly higher than in the general population. For this group, the risk of death was essentially normal regardless of how low the CD4+ cell count dipped during treatment, as long as it returned to normal.

Over the years, effective ART regimens for HIV infection have become simpler, less toxic, and more effective. "Due to the success of ART, it is relevant to ask if death rates in optimally treated HIV are higher than the general population," the researchers write.

Previous studies have suggested that, with successful treatment, mortality risk approaches that of people without HIV. However, these studies have had important limitations, including a lack of complete information on patient outcomes. The use of comprehensive follow-up data from the ESPRIT and SMART trials overcomes this limitation.

The new study provides the best evidence yet that, with effective ART that achieving good disease control, the mortality rate for people with HIV is essentially the same as in the general population. Dr Rodger and colleagues conclude, "Our data support the importance of early diagnosis and treatment to improve clinical outcomes and it is likely that much of the excess mortality associated with HIV would be preventable with timely diagnosis of HIV and initiation of ART."

Further studies will be needed to clarify the implications for HIV treatment, including the best time to start ART based on CD4+ cell counts. The researchers also note that other causes of illness or death emerge as the current generation of treated HIV-infected people continues to age.

"Rodger and colleagues add to the considerable body of evidence on which early treatment initiation guidelines are based," commented Veronica Miller, PhD, Director of the Forum for Collaborative HIV Research. "Together with studies indicating equal benefit across risk groups, including injecting drug users, as long as individuals are maintained in care, this study further validates universal testing with immediate linkage and retention in care policies."

**Fatty Acids Could Lead to Flu Drug**

Mar. 7, 2013 — Flu viruses are a major cause of death and sickness around the world, and antiviral drugs currently do not protect the most seriously ill patients. A study published March 7th by Cell Press in the journal *Cell* reveals that a compound derived from fats found in fish oils prevents death in influenza-virus-infected mice, even at advanced stages of disease. The study offers a promising strategy for the treatment of patients with severe influenza virus infections.

"Given the potential for future lethal pandemics, effective drugs are needed for the treatment of severe influenza, such as that caused by H5N1 viruses," says senior study author Yumiko Imai of Akita University. "We have identified a novel therapeutic target for the treatment of severe influenza that is effective under conditions where known antiviral drugs fail to protect from death."

Currently available antiviral drugs inhibit influenza virus replication, but they are not typically effective when given to patients as little as 2 days after infection. In an attempt to discover more effective drug targets for influenza, scientists have recently identified several genes and molecules that are crucial for influenza virus replication. However, until now it was not known whether naturally occurring lipids, such as those derived from omega-3 polyunsaturated fatty acids (PUFAs) found in fish oils, might also be involved in influenza virus infections.

To answer this question, Imai and her team screened for PUFA-derived lipids in influenza-virus-infected human lung cells. When they treated infected cells with these lipids, they found that protectin D1 (PD1) was the most effective at inhibiting the replication of viruses, including H5N1.

In addition, low levels of PD1 in the lungs of influenza-virus-infected mice were associated with severe infection and highly pathogenic viruses, such as H5N1. Treatment with PD1 in combination with an approved antiviral drug improved the survival of influenza-virus-infected mice and prevented death, even
when given 2 days after infection. "Our findings suggest that PD1 could serve as a biomarker as well as a much needed antiviral drug for severe and lethal influenza virus infections," Imai says.

**Journal Reference:**

**Virus and Genes Involved in Causation of Schizophrenia**

Mar. 8, 2013 — Viruses and genes interact in a way that may increase the risk of developing schizophrenia significantly. This happens already in the developing fetus.

An international team of scientists led by Aarhus University, Denmark, has made this discovery. As the first in the world, they scanned the entire genome of hundreds of sick and healthy people to see if there is an interaction between genes and a very common virus—cytomegalovirus—and to see whether the interaction influences the risk of developing schizophrenia.

And it does.

Women that have been infected by the virus—and around 70 % has—will have a statistically significant increased risk of giving birth to a child who later develops schizophrenia if the child also has the aforementioned gene variant. This variant is found in 15 percent. The risk is five times higher than usual, the researchers report in *Molecular Psychiatry.*

**No cause for alarm**

People infected with cytomegalovirus most often do not know it, as the infection by the virus, which belongs to the herpes virus family, is usually very mild. But the researchers stress that there is no cause for alarm—even if both risk factors are present in mother and child, there may be a variety of other factors that prevents disease development in the child.

But as schizophrenia affects 1 per cent of the global population, this new knowledge is very important. "In the longer term, the development of an effective vaccine against cytomegalovirus may help to prevent many cases of schizophrenia," says Professor of Medical Genetics at Aarhus University, Anders Børglum. "And our discovery emphasizes that mental disorders such as schizophrenia may arise in the context of an interaction between genes and biological environmental factors very early in life."

**Journal Reference:**

**High Viral Load, HBeAg Positivity Increased Risk for Mother-to-Infant HBV Transmission**

*Healio* ©, (03.08.2013)

Babies born to women who have a high hepatitis B viral load—especially if the mothers also test positive for hepatitis B e antigen (HBeAg)—are more likely to contract hepatitis B, even when vaccinated against the disease.

The study focused on 303 hepatitis B-infected mothers and their babies’ risk of contracting the virus during the first three years of life. Researchers first established the maternal viral load and HBeAg status—81 women were HBeAg-positive—then gave initial and follow-up hepatitis B tests to all of the babies. All babies received complete doses of hepatitis B immunization, and the babies born to HBeAg-positive mothers also received hepatitis B immunoglobulin within the first 24 hours of life. The study results controlled for confounding factors, including age, birth type, gender, weight, gestational age, and feeding practices.

Ten children in the study, all of whom were born to HBeAg-positive mothers, developed chronic hepatitis B, in spite of prophylactic measures. To lower risk of hepatitis B infection, researchers recommended that future screening and treatment interventions incorporate the study results.

**Antibiotic Resistance Poses 'Catastrophic Threat' To Public Health, England’s Top Health Official Warns In Report**

In her first annual report, "England’s top health official said Monday that antibiotic resistance poses a 'catastrophic threat' to public health," GlobalPost reports (DeFraia, 3/11). "Sally Davies ... said global action is needed to fight antibiotic, or antimicrobial, resistance and fill a drug 'discovery void' by researching and developing new medicines to treat emerging, mutating infections," Reuters writes (Kelland, 3/10). "If tough measures are not taken to restrict the use of antibiotics and no new ones are discovered, [Davies said], 'we will find ourselves in a health system not dissimilar to the early 19th century at some point,'" the Guardian notes (Boseley, 3/10). Davies "said the problem is 'as important as climate change for the world' and urged the [British] government to raise the issue when meeting political leaders at the G8 summit in London in April," according to The Telegraph (3/10). A related post in the Guardian's "Data Blog" provides an infographic comparing antibiotic use globally (Rogers, 3/11).

**Haiti Fighting Cholera By Recycling Human Waste Into Fertilizer**

The Guardian describes a program being run by Soil (Sustainable Organic Integrated Livelihoods) in Haiti, in which human waste is being collected and recycled "into fertile soil, simultaneously helping to fight cholera and deforestation, and revive food production." The newspaper notes, "Haiti is trying to fight what has exploded into the worst cholera epidemic in modern history, with 57 percent of global cholera cases last year concentrated on this tiny half-island." In addition to Soil's program, "[t]he Haitian government recently built several sewage treatment plants that process traditional pit latrine waste in open-air stabilization ponds. [The government] and sewage treatment companies such as Jedco are experimenting with the alchemy of transforming a potentially deadly substance into a rich and much-needed fertilizer," the Guardian writes (Doucet, 3/10).

**Preventing HIV infection with anti-HIV drugs in people at risk is cost-effective**

**Press release from PLOS Medicine**

An HIV prevention strategy in which people at risk of becoming exposed to HIV take antiretroviral drugs to reduce their chance of becoming infected (often referred to as pre-exposure prophylaxis or PrEP), may be a cost-effective method of preventing HIV in some settings, according to a study by international researchers published in this week's PLOS Medicine.

In an analysis of 13 modelling studies led by Gabriela Gomez from the Department of Global Health, Academic Medical Centre, University of Amsterdam/AIGHD in The Netherlands, the authors evaluated the impact of pre-exposure prophylaxis in different populations (heterosexual couples, men who have sex with men, and people who inject drugs) in different regions and countries, such as southern Africa, Ukraine, the US, and Peru.

They found that in every setting, the cost of antiretroviral drugs was an important factor influencing the affordability of effective prevention programmes but delivery of pre-exposure prophylaxis to populations at higher risk of HIV exposure appeared to be the most cost-effective strategy. The authors also found that both behavioural changes and adherence to the pre-exposure prophylaxis drug regimens affected programme effectiveness.

The authors say: "Our findings show that pre-exposure prophylaxis has the potential to be a cost-effective addition to HIV prevention programmes in some settings."

They continue: "However, the cost-effectiveness of pre-exposure prophylaxis is likely to depend on considerations such as cost, the epidemic context, pre-exposure prophylaxis programme coverage and prioritisation strategies, as well as individual adherence levels and pre-exposure prophylaxis efficacy estimates."

The authors add: "Given that our review shows that both the setting and which population is prioritised for pre-exposure prophylaxis are critical drivers of cost-effectiveness, the next step is to conduct context-specific demonstration studies, including comprehensive cost analyses, of different prioritisation and adherence promotion strategies to ensure that the maximum benefit from the introduction of pre-exposure prophylaxis is realised within combination HIV prevention programmes."
Some bacteria may protect against disease caused by stomach infection
Other stomach microbes influence whether Helicobacter pylori infection causes inflammation leading to ulcers and cancer, study finds

Half of the world’s human population is infected with the stomach bacteria called Helicobacter pylori, yet it causes disease in only about 10 percent of those infected. Other bacteria living in the stomach may be a key factor in whether or not H. pylori causes disease, according to a new study led by scientists at the University of California, Santa Cruz.

"People tend to think of the stomach as a relatively sterile environment, but it’s actually populated with microbes," said Karen Ottemann, professor and chair of microbiology and environmental toxicology at UC Santa Cruz.

Researchers in Ottemann's lab were studying H. pylori infections in mice when they noticed that mice from two different suppliers had different responses to the infection, even though they were the same mouse strain and therefore genetically identical. Examining the bacteria in the stomachs of the mice (the stomach "microflora"), they found differences between the mice from different suppliers. They then used antibiotics to alter the stomach microflora in mice from a single supplier and again found changes in the response to H. pylori.

"We found that something about the preexisting microflora, before H. pylori comes into the mouse, changes the mouse's response to the infection," Ottemann said.

The findings, published in the journal Infection and Immunity, have potential implications for treating human infections. The bacteria in the stomachs of mice and humans are broadly the same—not necessarily at the species level, but the same types of bacteria are present in both, Ottemann said.

H. pylori infections can cause ulcers and stomach cancer, but most infected people don't develop any disease. Furthermore, there is evidence that H. pylori infection can protect against diseases such as esophageal cancer and asthma. For these reasons, people are only treated for the infection if they develop symptoms. With a better understanding of the effects of the stomach microflora, it might be possible to predict whether someone is likely to develop disease and should be treated for an H. pylori infection.

"It would be nice if we could predict who would get disease," Ottemann said. "The other possibility is that we might be able to identify some bacteria that could be used as a probiotic to dampen H. pylori disease."

At this point, it is not clear which bacteria are responsible for changing the response to H. pylori infection in mice. Focusing on mice from one supplier, Ottemann's team used genetic profiling techniques to identify more than 10,000 different types of bacteria present in mouse stomachs, of which about 2,000 were found in all the mice sampled.

The researchers treated some of the mice with antibiotics, which did not eliminate stomach bacteria but substantially changed the composition of the gut microflora. The altered microflora dampened the inflammatory response to H. pylori infection. When they looked for differences in the stomach microfloras of mice with and without inflammatory disease, the researchers found more than 4,000 differences—either species present in one group and not in the other, or differences in the abundances of certain species.

More work is needed to identify which differences in bacterial composition are responsible for the differences in response to H. pylori, Ottemann said. "The results do point to some potential candidates for a protective effect, such as Clostridium species, some of which are known to influence inflammation in the intestine," she said.

Discovery May Explain How Prion Diseases Spread Between Different Types of Animals

Mar. 11, 2013 — Medical researchers at the University of Alberta have made a discovery that may explain how prion diseases, like chronic wasting disease and mad cow disease, adapt in order to spread between various types of animals.

The research team, led by neurologist Valerie Sim, discovered that a miniscule change in the prions' makeup appears to give the disease the ability to adapt—to mimic and recreate new strains with which it comes into contact. The team has been studying this area for two years.

"Prion diseases don't always successfully go from one animal to another, but when they do, the process is called adaptation. And we want to figure out what triggers that process to happen, what changes happen within prions to allow the disease to spread," says Sim, a researcher with the Faculty of Medicine
& Dentistry, whose discovery was recently published in the peer-reviewed *Journal of Biological Chemistry*.

“One of the important things researchers in this field have realized is that if you pass certain strains of prion disease through a number of different hosts, the disease can adapt along the way and increase the number of susceptible hosts. That's the big concern right now.

"We want to determine why one prion disease might be able to spread from one type of animal to another and why another strain of the disease can’t."

For instance, if a deer with chronic wasting disease is scavenged by another animal, could the prion disease cross into that intermediate host, evolve and then infect animals or species typically not at direct risk for the disease.

"We hope to understand how these bigger issues develop," says Sim. "We need to pay attention to chronic wasting disease in particular because it has the ability to spread in a different way than mad cow disease. Chronic wasting disease prions can be deposited into the soil and stay there for years, and could be eaten by another animal. How does it evolve from there then?"

Sim and her team are continuing their research in this area and are seeing impressive results in the lab—reconfirming their findings through the testing of additional models.

**Journal Reference:**

---

**Happy Birthday, Dr Snow**
Howard Markel, MD, PhD

March 15, 2013, marks the 200th birthday of John Snow, the singular genius who created the modern science of epidemiology. Without fear of historical hyperbole, the occasion merits a global pause of reflection and honor.

Born in York, England, John Snow chose a life in medicine at a relatively young age. At 16, he began an apprenticeship under William Hardcastle, a surgeon who practiced in Newcastle-upon-Tyne. A few years later, in 1831, Snow first encountered cholera, which entered Newcastle via the seaport of Sunderland and decimated his town.

By 1836, Snow moved to London, where he furthered his medical studies at Westminster Hospital and earned his membership in the Royal College of Surgeons and as a licentiate of the Society of Apothecaries. In 1843, he took his bachelor’s in medicine and, the following year, received his doctorate in medicine, both at the University of London. He “hung his shingle” or, as the British like to say, “nailed up his colours” in Soho, a raucous neighborhood where he cared for working-class patients for the rest of his career.

After its demonstration at the Massachusetts General Hospital in 1846, ether anesthesia became the surgical rage on both sides of the Atlantic. Snow wrote a superb book on its use, *On Ether*, in 1847 before focusing his attention on another anesthetic called chloroform. Since at least the days of the Old Testament, medical doctrine assumed that childbirth was destined to be a painful event. Yet many physicians like Snow insisted this need not be the case. Snow’s opinion became common practice on April 7, 1853, when he was chosen to administer chloroform to Queen Victoria as she delivered Prince Leopold.

It is difficult, if not impossible, for most modern readers to fathom how badly London smelled in the mid 19th century. Every day was a constant negotiation against the odiferous waste left behind by more than 300 000 horses, hundreds of thousands of pigs, sheep, cows, and other livestock waiting to become somebody’s meal, and 2.4 million Londoners. With the paucity of modern sewage systems, water closets in wealthier homes expelling streams of stool and urine into the streets and the Thames River, and countless outhouses, privies, and cesspools, the city positively stunk.

When confronted with cholera epidemics in the years before medical scientists elucidated the role microbes play in infectious diseases, many of Britain’s finest medical minds took a page from Hippocrates and associated cholera with the foul-smelling gases produced by mounting piles of rotting garbage and raw sewage. Miasma (from the Greek, for *pollution*) was thought to contaminate the atmosphere. When inhaled, this noxious air upset the balance of an individual’s body humors and led to an abundance of *choler*, or yellow bile, which the body did its best to expel, even if it meant overwhelming dehydration and death.

Snow’s genius was his uncanny ability to connect the dots, so to speak, of disease causation. A keen observer of the diffusion of gases, gained from his work on ether and chloroform, Snow began to doubt
the miasma theory during the 1848 cholera epidemic that ruthlessly carted off thousands of Londoners to the graveyard. If foul-smelling gases caused cholera, he queried in his landmark 1849 book, On the Mode of Communication of Cholera, why were those closest to the emanations, such as garbage removal workers and night-soil men (those who emptied privies), not disproportionately affected? Conversely, given that the concentration of a gas tended to dissipate and decline as it traveled over a distance, how could one miasmic source infect people living far from it, let alone an entire city? The accepted dogma that cholera was inhaled through the air via the respiratory tract—even though the disease clearly struck the gastrointestinal tract with an ugly vengeance—simply made no sense to the inquiring Dr Snow.3

Although there were many miasmatists and anticontagionists who scoffed at Snow’s thesis, the good doctor seized his opportunity during London’s 1854 cholera epidemic. He began by meticulously surveying every case and their contacts, even to the point of verifying their water source by checking each home’s water bills. Snow discovered that Londoners who drew water from the Southwark and Vauxhall Water Company, which came from the fecal-contaminated Thames River, were infected nine times more than those living in areas supplied by the Lambeth Company, whose water originated from an upstream, and less contaminated, source.4

Snow’s greatest scientific moment, however, resulted from an even more detailed study of the cholera’s spread in Soho. After carefully charting some 500 cases in his district, Dr Snow noted that most of the cholera victims had been consuming water from a well located in Broad Street. Unfortunately for those frequenting the hand pump—operated well, it was contaminated with sewage from a nearby house where cholera had previously visited. Snow convinced the parish councilors to remove the well’s pump handle, thus making it inoperable. Soon after disabling the pump, the cholera rate plummeted, allowing Snow a well-deserved quod erat demonstrandum. The doctor concluded that a specific water-borne “poison” capable of self-reproduction was in the excreta of cholera patients who, in turn, tainted the water supply. His solution: careful washing of the hands, decontaminating soiled linens, and boiling all drinking water.5

John Snow died of a cerebral hemorrhage on June 16, 1858. He was 45. Twenty-five years later, in 1883, Robert Koch, who along with Louis Pasteur is credited with demonstrating the germ theory of disease, proved Snow correct. While battling a cholera epidemic in Egypt, Koch identified Vibrio cholerae, teeming in fecal-contaminated water supplies, as the microbial cause of cholera. Yet even before Koch’s great discovery, Snow was on an upward trajectory toward permanent, albeit posthumous, acclaim in the history books.6

Last summer, I had the pleasure of taking my 12-year-old daughter, Bess, on a trip to London. A few hours before a much-anticipated high tea at the Ritz Hotel, Bess asked, “Dad, if you could take me to the most important historical sight in London, where would we go?”

With a spring to my middle-aged step, I escorted her across Piccadilly, past the Royal Academy of Art, and through Soho to Broadwick Street, as Broad Street is presently known. In front of a facsimile of the Broad Street pump, I told Bess how Snow helped usher in the modern world by insisting that we clean up after our own excrement. Yet as great as that contribution was, I added, this basic health requirement has still not been met for, at least, 783 million people living in the developing nations of Africa, Asia, and South America who do not have daily access to clean drinking water; 2.6 billion people do not have adequate sanitation. Every year, more than 1.5 million people, mostly children younger than 5 years, die because of water-borne diarrhea, including cholera, which modern medicine has known how to prevent, or at least attenuate, since the mid 19th century.7

Bess’s eyes opened widely as she exclaimed an impressed “Wow!”

Wow, indeed.

Thank you, Dr Snow. Two centuries after your birth, you still have the power to change the world. If only everyone had the access and wherewithal to follow your life-saving prescriptions.

MRSA in the Groin of HIV Patients Ups Infection Risk
Steven Fox
Mar 13, 2013
Colonization of methicillin-resistant Staphylococcus aureus (MRSA) in the groin area of HIV-infected adults increases risk for subsequent clinical infection, according to results of a prospective study carried out by the Centers for Disease Control and Prevention.

Phillip J. Peters, MD, Medical Officer, Division of HIV/AIDS Prevention, Centers for Disease Control and Prevention, and colleagues published their results online March 13 and in the April issue of Emerging Infectious Diseases.

"Data on the interaction between [MRSA] colonization and clinical infection are limited," the authors write. They say that colonization with S aureus is a risk factor for subsequent clinical infection and that the site of colonization may also play a role in subsequent risk.

For example, they note that although the anterior nares is usually thought of as the primary reservoir of MRSA, some variants of the bacteria may more often colonize the buttocks, genitals, and perineum.

"Improving our understanding of the interaction between MRSA colonization and clinical infection among persons with HIV is necessary so that effective prevention strategies can be developed for this population," they write.

Therefore, the researchers conducted a prospective cohort study of 600 HIV-infected adults (98% men) recruited from the Veterans Affairs (VA) Medical Center in Atlanta, Georgia. All patients enrolled in the study received outpatient care at the center's HIV clinic from September 2007 through April 2008.

The researchers took swabs of the nares and groin and cultured them for S aureus. The cultures were performed at enrollment and again at 6 months and 12 months.

The cultures showed evidence of MRSA colonization in 13% to 15% of the participants at baseline, 6 months, and 12 months. Of those participants colonized with MRSA, 41% had colonization in the nares only, 21% had colonization in the groin only, and 38% exhibited colonization in both sites.

During a median follow-up of 2.1 years, 25 patients in the cohort developed 29 MRSA clinical infections.

In a multivariate analysis, the authors found that MRSA clinical infection was significantly associated with MRSA colonization of the groin (adjusted risk ratio, 4.8) and a history of MRSA infection (adjusted risk ratio, 3.1). The analysis adjusted for a variety of factors, including CD4 cell count, history of an abscess, renal insufficiency, a history of syphilis, use of certain antistaphylococcal drugs in the previous year, contact with a prison or jail, and certain hygienic factors.

"Given the frequency of MRSA colonization in the groin and its association with clinical infection, MRSA prevention strategies (both hygienic practices and decolonization treatments) with HIV-infected adults should be used to prevent or eliminate colonization at this anatomic site to reduce MRSA clinical infections in this population," they conclude.

Rapid hearing loss may be a symptom of rare Creutzfeldt-Jakob Disease
DETROIT – Rapid hearing loss in both ears may be a symptom of the rare but always-fatal Creutzfeldt-Jakob Disease and should be considered a reason for clinicians to test for the disorder.

That was the conclusion of Henry Ford Hospital researchers after encountering a 67-year-old patient who had been progressively losing hearing in both ears for two months and was eventually diagnosed with the disease.

Creutzfeldt-Jakob Disease, or CJD, is often confused with so-called "mad cow disease," and though they are in the same family of disorders, are not the same.

However, both are always fatal and share such symptoms as impaired thinking, jerky body movements, memory loss and dementia. Once infected with CJD, the brain develops holes, resulting in tissue which resembles a sponge.

The report will be presented March 19 during the annual scientific meeting of the American Academy of Neurology in San Diego.

According to Ahmad Riad Ramadan, M.D., a Henry Ford neurologist and lead author, when the patient sought treatment he had no significant medical history and was complaining only of a continuing, rapid loss of hearing in both ears, and tinnitus – a "ringing in the ears" – that is a common side effect of hearing loss.
"This was followed by the kind of cognitive decline that is typical of CJD," Ramadan said. "During the patient's hospital stay, he also showed signs of ataxia – a lack of coordination – and myoclonus – a spastic muscle twitch."

Testing found the presence of a telltale protein, and other conditions, that led to a diagnosis of CJD. Researchers noted that the patient's hearing never improved and he died a month after seeking treatment. Ramadan said the researchers' findings were only the fourth time, based on available literature, that hearing loss such as that found in their patient was recognized as the first symptom of CJD.

This "sensorineural hearing loss," also called "nerve deafness," is the most common cause of permanent impairment; it is hearing loss which results from involvement of the inner ear, auditory nerve, or central auditory pathways in the brain/brainstem.

As the first, or presenting, symptom of their patient, the researchers concluded that testing for CJD in those with fast-progressing hearing impairment should be considered by treating physicians.

Immune finding aids quest for vaccines to beat tropical infections

Scientists are a step closer to developing vaccines for a range of diseases that affect 200 million people, mainly in tropical south-east Asia, Africa and Central America.

Researchers studying infections caused by parasitic worms – which can lead to diseases such as elephantiasis and river blindness – have shown how these can shut down a part of the immune system that might otherwise fight sickness. Preventing this immune reaction enables the infection to persist, causing chronic illness.

Scientists have also shown how this immune response can be re-activated to fight invading parasites, and enable the immune system to develop natural resistance to infection.

Their findings could help inform the development of vaccines for these types of infections. They also point towards potential treatments for allergies, which occur when the same part of the immune system over-reacts to irritants.

Researchers looked at a part of the immune system that responds to parasite infections, in a study of mice. They found that when infection begins, cells that would normally launch a counter-attack on the invading parasite – and in so doing, help develop immunity – become dormant.

They found that blocking the action of a tiny molecule attached to the surface of the cell reactivates the cell, and enables a fresh attack on the infection. Scientists hope to investigate the reaction further to determine whether it applies to people and animals such as livestock.

The study, published in PLOS Pathogens, was funded by the Medical Research Council and the Wellcome Trust.

Dr Matt Taylor of the University of Edinburgh’s School of Biological Sciences, who led the study, said: "Understanding the intricacies of the immune system is a major goal in being able to control disease. This discovery brings us a step closer to explaining how long-term infections occur – and how we might, in time, be able to tackle them."

Current HIV Screening Guidelines Are Too Conservative

Mar. 12, 2013 — Early HIV treatment can save lives as well as have profound prevention benefits. But those infected with the virus first must be identified before they can be helped.

In a new study, two Northwestern University researchers report that current Centers for Disease Control and Prevention (CDC) HIV screening guidelines are too conservative and that more frequent testing would be cost-effective in the long run for both high- and low-risk groups.

The Northwestern team performed a mathematical modeling study to assess "optimal testing frequencies" for HIV screening in different risk groups. They concluded screening should be done up to every three months for the highest-risk individuals and low-risk groups should be tested every three years.

The CDC currently recommends annual testing for high-risk groups, such as people with HIV-positive sexual partners, people with multiple sexual partners, injection drug users and sex workers, and once-in-a-lifetime testing for low-risk groups (whose annual risk of acquiring HIV is only one-hundredth of one percent).

"Our results should encourage policymakers and medical professionals to reconsider how often adolescents and adults should be tested for HIV," said Benjamin Armbruster, an assistant professor of industrial engineering and management sciences at Northwestern’s McCormick School of Engineering and Applied Science.
He and Aaron Lucas, a doctoral student in Armbruster's group, modeled various scenarios in an attempt to "optimize the tradeoff" between the societal costs of testing versus the benefits of earlier HIV diagnosis over a patient's lifetime.

Their study is published in the March 2013 issue of AIDS, the official journal of the International AIDS Society.

Frequent testing has been shown to be an effective method for identifying new HIV infections. In the past, people with new HIV infections weren't treated until they had significant declines in immune functioning, as measured by the CD4 cell count. But there is a growing consensus that antiretroviral treatment is beneficial for all HIV-infected patients, regardless of CD4 count. Starting treatment immediately after diagnosis also reduces the risk of transmitting HIV.

Within its limitations, the Northwestern study suggests that current recommendations for HIV testing are "too conservative, especially for low-risk groups who would benefit from more frequent testing."

**Journal Reference:**

### New Sensor Developed for Methylated DNA

Mar. 14, 2013 — Collaborators from Mayo-Illinois Alliance for Technology Based Healthcare have developed a new, single molecule test for detecting methylated DNA. Methylation—the addition of a methyl group of molecules to a DNA strand—is one of the ways gene expression is regulated.

The findings appear in the current issue of Scientific Reports (Nature Publishing Group).

"While nanopores have been studied for genomic sequencing and screening analysis, this new assay can potentially circumvent the need for some of the current processes in evaluating epigenetics-related diseases," says George Vasmatzis, Ph.D., co-leader of Mayo's Biomarker Discovery Program in the Center for Individualized Medicine and co-lead author on the article. He says the assay could eliminate the need for bisulfite conversion of DNA, fluorescent labeling, and polymerase chain reaction (PCR).

"Next steps include increasing the spatial resolution by incorporating thinner membranes and by integrating the same preparation steps," says Rashid Bashir, Ph.D., professor of bioengineering, director of the Micro and Nanotechnology Laboratory, and co-lead author of the study at the University of Illinois at Urbana-Champaign.

A nanopore, in this case, is a very small hole in an artificial membrane, that allows only a single molecule to be located and identified. Researchers say this is useful as methylation in promoter sequences can indicate tumor development in most major types of cancer and may be a better biomarker than many genetic markers. Scientists are now able to differentiate methylated from non-methylated DNA by attaching a protein on the methylated nucleotides measuring ionic electrical current via a solid-state nanopore.

**Journal Reference:**
Jiwook Shim, Gwendolyn I. Humphreys, Bala Murali Venkatesan, Jan Marie Munz, Xueqing Zou, Chaitanya Sathe, Klaus Schulten, Farhad Kosari, Ann M. Nardulli, George Vasmatzis, Rashid Bashir. Detection and Quantification of Methylation in DNA using Solid-State Nanopores. Scientific Reports, 2013; 3 DOI: 10.1038/srep01389

### Post-Treatment HIV-1 Controllers with a Long-Term Virological Remission after the Interruption of Early Initiated Antiretroviral Therapy ANRS VISCONTI Study

Asier Sáez-Cirión mail, Charline Bacchus, Laurent Hoequellox, Véronique Avettand-Fenoel, Isabelle Girault, Camille Lecroux, Valerie Potard, Pierre Versmisse, Adeline Melard, Thierry Prazuck, Benjamin Descours, Julien Guergnon, Jean-Paul Viard. [...], the ANRS VISCONTI Study Group

**Abstract**
Combination antiretroviral therapy (cART) reduces HIV-associated morbidities and mortalities but cannot cure the infection. Given the difficulty of eradicating HIV-1, a functional cure for HIV-infected patients appears to be a more reachable short-term goal. We identified 14 HIV patients (post-treatment controllers [PTCs]) whose viremia remained controlled for several years after the interruption of prolonged cART initiated during the primary infection. Most PTCs lacked the protective HLA B alleles that are overrepresented in spontaneous HIV controllers (HICs); instead, they carried risk-associated HLA alleles that were largely absent among the HICs. Accordingly, the PTCs had poorer CD8+ T cell responses and more severe primary infections than the HICs did. Moreover, the incidence of viral control after the interruption of early antiretroviral therapy was higher among the PTCs than has been reported for spontaneous control. Off therapy, the PTCs were able to maintain and, in some cases, further reduce an extremely low viral reservoir. We found that long-lived HIV-infected CD4+ T cells contributed poorly
to the total resting HIV reservoir in the PTCs because of a low rate of infection of naïve T cells and a skewed distribution of resting memory CD4+ T cell subsets. Our results show that early and prolonged cART may allow some individuals with a rather unfavorable background to achieve long-term infection control and may have important implications in the search for a functional HIV cure.

Author Summary
There is a renewed scientific interest in developing strategies allowing long-term remission in HIV-1-infected individuals. Very rare (<1%) patients are able to spontaneously control viremia to undetectable levels (HIV controllers, HICs). However, the possibility to translate their mechanisms of control to other patients is uncertain. Starting antiretroviral therapy during primary infection may provide significant benefits to HIV-infected patients (i.e. reduction of viral reservoirs, preservation of immune responses, protection from chronic immune activation). Indeed, we have observed that some HIV-infected patients interrupting a prolonged antiretroviral therapy initiated close to primary infection are able to control viremia afterwards. We present here 14 of such post-treatment controllers (PTCs). We show that PTCs have achieved control of infection through mechanisms that are, at least in part, different from those commonly observed in HICs and that their capacity to control is likely related to early therapeutic intervention. We found that PTCs were able, after therapy interruption, to keep, and in some cases further reduce, a weak viral reservoir. This might be related to the low contribution of long-lived cells to the HIV-reservoir in these patients. Finally, we estimated the probability of maintaining viral control at 24 months post-early treatment interruption to be ~15%, which is much higher than the one expected for spontaneous control.


Editor: Jeffrey Lifson, SAIC-Frederick, United States of America

Received: September 5, 2012; Accepted: January 9, 2013; Published: March 14, 2013

Copyright: © 2013 Saez-Cirion et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: The VISCONTI study (Viro-Immunological Sustained CONtrol after Treatment Interruption) was funded by the ANRS, the French National Agency for Research on AIDS and Viral Hepatitis (Grant ANRS EP47). CB and BD received predoctoral fellowships from UPMC and ANRS, respectively. CL received a postdoctoral fellowship from ANRS. GP was funded by INSERM. (http://www.anrs.fr, http://www.inserm.fr, http://www.upmc.fr). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Introduction
HIV-1 infection is normally characterized by sustained viral replication and a progressive loss of CD4+ T cells, leading to AIDS. Combined antiretroviral therapy (cART) suppresses viral replication and drastically reduces morbidity and mortality [1]. However, cART does not eradicate infected cells [2], and plasma viremia generally rebounds quickly after treatment is discontinued [3]. The existence of a few HIV-infected patients who spontaneously controlled HIV replication to undetectable levels for many years (HIV controllers [HICs]) suggests that a functional HIV cure or remission might be possible. However, how or whether other patients can achieve an HIC-like status is unclear.

Emerging evidence shows that early treatment has long-term benefits [4]. Treatment initiation during primary HIV-1 infection (PHI) rather than during chronic HIV-1 infection (CHI) may i) further reduce residual viral replication [5], ii) limit viral diversity [6] and viral reservoirs [7], iii) preserve innate immunity and T and B cell functions [8], [9], [10], and iv) accelerate immune restoration [11]. Most relevant studies show that CD4+ T cell counts are higher and that viral rebound occurs later (and at a lower level) after the discontinuation of treatment that began during PHI compared with treatment that began during CHI [12], [13]. Although in most cases, these advantages wane soon after treatment interruption [14], the existence of individuals in whom the viral load remains undetectable for several years after the interruption of prolonged therapy that was initiated very early after infection (post-treatment controllers [PTCs]) was reported by our group in 2010 [15]. These individuals hold important clues in the search for a functional HIV cure. Here, we have identified and characterized a group of 14 PTCs. We analyzed whether PTCs shared parameters that have been associated with spontaneous control of viremia in HICs, to explore whether the efficient control of infection in PTCs may indeed be derived
from early treatment. In addition, we explored the level and distribution of the PTCs’ latent viral reservoir in the blood. Indeed reaching functional cure will likely require reducing not only the size but also the distribution of the HIV reservoirs, particularly among the CD4 T cells with long lifespan or important clonogenic properties, as naïve and central-memory T cells (TCM).

Results

Study population
We studied 14 HIV-1-infected patients with durable viral control following the interruption of effective cART that was initiated during PHI (PTCs). The patients’ characteristics are reported in Table 1 and Figure 1. All 14 patients were diagnosed with PHI in the late 1990s or early 2000s. Twelve patients had a symptomatic primary infection. During PHI (1.6 ± 1.1 – 2.1 months estimated after initial exposure), the PTCs had higher viral loads (median 5.0 log HIV-1 RNA copies/ml) and lower CD4+ T cell counts (median 502 cells/µl; Table 1) compared with the 8 patients in the ANRS PRIMO cohort who subsequently exhibited spontaneous control of viremia (median 3.0 log HIV-1 RNA copies/ml of plasma and 794 CD4+ T cells/µl at PHI (2.2 ± 1.7 – 3.5) months estimated after exposure, p = 0.11 for the delay when compared to PTC [16] (Figure 2). In contrast, PTC values during PHI were similar to those of patients in the ANRS PRIMO cohort who did not control their infection afterwards (5.1 log HIV RNA copies/ml and 517 cells/µl; Figure 2).
CD4+ T cell counts (A) and plasma viral load (B) during the primary infection for the patients enrolled in the ANRS PRIMO cohort who later exhibited spontaneous control of infection (preHIC; n = 8) [16], for the PTCs included in our study (n = 14), and for the patients in the ANRS PRIMO cohort who did not control infection (n = 1,245). The median and the 10th and 90th percentiles are shown for each group. doi:10.1371/journal.ppat.1003211.g002, doi:10.1371/journal.ppat.1003211.t001

The PTCs received standard cART (Table 1) available at the time, and their viral load became undetectable within a median of 3 months (0.5 to <8 months) after treatment began (Figure 1). The median cART duration was 36.5 months, and the plasma viral load was no longer detectable after the first undetectable sample during treatment. During the treatment period, all PTCs except two (OR2, with high CD4+ T cell counts of 955 cells/mm³ at PHI, and OR3, who was infected through a blood exposure accident and for whom no available CD4+ T cell counts were available before therapy) experienced an increase in their CD4+ T cell counts between PHI and treatment interruption (median 502 and 927 cells/mm³, respectively, p<0.001, n = 13). Following the interruption of cART, viral control persisted for a median of 89 months, and the CD4+ T cell counts remained stable (the median final CD4+ T cell count was 837 cells/mm³, p = 0.58, n = 14). Eight PTCs had viral loads below the detection limit in all available samples after treatment interruption, whereas occasional increases were recorded for the other six patients (Figure 1 and Table 1).

![Figure 2. Patients to become post-treatment controllers have higher viral loads and lower CD4+ T cell counts than HIV controllers during primary HIV infection.](image)

<table>
<thead>
<tr>
<th>Code</th>
<th>Sex</th>
<th>Year of diagnosis</th>
<th>PHI</th>
<th>Histologic initiation</th>
<th>ART combination</th>
<th>Time on cART (months)</th>
<th>Time since interruption (months)</th>
<th>CD4 T cell counts (cells/µl)</th>
<th>Last HIV-1 RNA VL * (copies/ml)</th>
<th>HIV-1 RNA VL since treatment interruption</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR1</td>
<td>M</td>
<td>1996</td>
<td>Sympt</td>
<td>2 NRTI</td>
<td>51</td>
<td>81</td>
<td>82</td>
<td>416</td>
<td>1057</td>
<td>3.3 [100 600]</td>
</tr>
<tr>
<td>OR2</td>
<td>F</td>
<td>2001</td>
<td>Sympt</td>
<td>3 NRTI             + P−</td>
<td>3 NRTI</td>
<td>101</td>
<td>955</td>
<td>867</td>
<td>404</td>
<td>3.8 [100 600]</td>
</tr>
<tr>
<td>OR3</td>
<td>F</td>
<td>1996</td>
<td>Sympt</td>
<td>2 NRTI             + P−</td>
<td>NRTI</td>
<td>107</td>
<td>354</td>
<td>441</td>
<td>222</td>
<td>3.4 [100 600]</td>
</tr>
<tr>
<td>OR4</td>
<td>M</td>
<td>1990</td>
<td>Sympt</td>
<td>3 NRTI             + P−</td>
<td>NRTI</td>
<td>72</td>
<td>302</td>
<td>400</td>
<td>222</td>
<td>2.3 [100 600]</td>
</tr>
<tr>
<td>OR5</td>
<td>M</td>
<td>2001</td>
<td>Sympt</td>
<td>2 NRTI             + P−</td>
<td>NRTI</td>
<td>13</td>
<td>397</td>
<td>123</td>
<td>502</td>
<td>2.0 [100 600]</td>
</tr>
<tr>
<td>OR6</td>
<td>F</td>
<td>1998</td>
<td>Sympt</td>
<td>2 NRTI             + P−</td>
<td>NRTI</td>
<td>86</td>
<td>377</td>
<td>1656</td>
<td>1598</td>
<td>7.3 [100 600]</td>
</tr>
<tr>
<td>OR7</td>
<td>M</td>
<td>1999</td>
<td>Asympt</td>
<td>2 NRTI             + P−</td>
<td>NRTI</td>
<td>75</td>
<td>393</td>
<td>176</td>
<td>787</td>
<td>4.3 [100 600]</td>
</tr>
<tr>
<td>OR8</td>
<td>F</td>
<td>2002</td>
<td>Sympt</td>
<td>2 NRTI             + P−</td>
<td>NRTI</td>
<td>12</td>
<td>311</td>
<td>1428</td>
<td>1400</td>
<td>7.1 [100 600]</td>
</tr>
<tr>
<td>OR9</td>
<td>F</td>
<td>2002</td>
<td>Sympt</td>
<td>3 NRTI             + P−</td>
<td>NRTI</td>
<td>17</td>
<td>393</td>
<td>734</td>
<td>279</td>
<td>5.9 [100 600]</td>
</tr>
<tr>
<td>OR10</td>
<td>M</td>
<td>2002</td>
<td>Sympt</td>
<td>3 NRTI             + P−</td>
<td>NRTI</td>
<td>39</td>
<td>489</td>
<td>856</td>
<td>973</td>
<td>5.9 [100 600]</td>
</tr>
<tr>
<td>OR11</td>
<td>M</td>
<td>2001</td>
<td>Sympt</td>
<td>3 NRTI             + P−</td>
<td>NRTI</td>
<td>101</td>
<td>482</td>
<td>833</td>
<td>541</td>
<td>4.9 [100 600]</td>
</tr>
<tr>
<td>OR12</td>
<td>M</td>
<td>2000</td>
<td>Asympt</td>
<td>3 NRTI             + P−</td>
<td>NRTI</td>
<td>56</td>
<td>84</td>
<td>455</td>
<td>452</td>
<td>4.4 [100 600]</td>
</tr>
<tr>
<td>OR13</td>
<td>M</td>
<td>1999</td>
<td>Sympt</td>
<td>2 NRTI             + P−</td>
<td>NRTI</td>
<td>48</td>
<td>380</td>
<td>1044</td>
<td>1251</td>
<td>6.0 [100 600]</td>
</tr>
<tr>
<td>OR14</td>
<td>M</td>
<td>1998</td>
<td>Sympt</td>
<td>3 NRTI             + P−</td>
<td>NRTI</td>
<td>113</td>
<td>822</td>
<td>993</td>
<td>1299</td>
<td>3.1 [100 600]</td>
</tr>
<tr>
<td>MEDIAN</td>
<td></td>
<td>1999</td>
<td></td>
<td></td>
<td></td>
<td>36.5</td>
<td>89</td>
<td>102</td>
<td>827</td>
<td>11.5 [100 600]</td>
</tr>
</tbody>
</table>

1. Male, F: Female;
2. Primary HIV-1 infection, Symptomatic or Asymptomatic;
3. NRTI: Nucleoside Reverse Transcriptase Inhibitor; P−: Protease Inhibitor; NNRTI: Non-nucleoside Reverse Transcriptase Inhibitor;
4. VL: Viral load;
5. NA: not available;
6. First determination 4 days after initiating therapy;
7. Two transient treatments during pregnancies since first interruption.

Table 1: Characteristics of PTC Included in the study.
The HIV-specific CD8+ T cell response of post-treatment HIV-1 controllers differs from that associated with HIV control in spontaneous HIV-1 controllers

We then compared specific parameters among the PTCs, HICs, patients with uncontrolled viremia (viremics [VIRs]) and patients receiving cART ([ARTs]; see methods). Protective HLA class I alleles (HLA-B*27 and B*57) have been consistently found to be overrepresented in cohorts of HICs [17], [18], [19] who spontaneously control HIV-1 infection. One of the PTCs had one HLA-B*57 allele and two PTCs had one HLA-B*27 allele. However, in contrast to the HICs from the ANRS HIV controller cohort, we found no overrepresentation of HLA-B*27 or HLA-B*57 in our PTC group compared with the general French population [20] (www.allelefrequencies.net; Figure 3A, Tables S1 and S2). Furthermore, the risk alleles HLA-B*07 and HLA-B*35 [17] were highly prevalent in the PTC group (29% of all HLA-B alleles), but they were underrepresented in the HIC group (p<0.001). Three and five of the 14 PTCs carried one HLA-B*07 allele and one HLA-B*35 allele, respectively. Three PTCs carried HLA-B*3501 (OR1, OR2 and OCP), whereas the other two (KPV and MWP) carried the HLA-B*3503 allele, which is associated with a more rapid progression to AIDS [21].

Figure 3. Post-treatment controllers differ from HIV controllers in terms of HLA class I profile, frequency and quality of the CD8+ T cell response and activation levels of CD8+ T cells.

A. The frequencies of the protective alleles HLA-B*27 and B*57 and the risk alleles HLA-B*07 and B*35 in the general French population (n = 6094 alleles [20], www.allelefrequencies.net), HICs (n = 148 alleles) and PTCs (n = 28 alleles). The statistical analyses are shown in Table S2. B. The frequency of HIV-specific CD8+ T cells, estimated as the number of CD8+ T cells producing IFN-γ upon stimulation with optimal HIV-1 peptides (spot-forming cells, SFC) in untreated viremic patients (VIRs) (n = 57), treated patients (ARTs) (n = 60), HICs (n = 100) and PTCs (n = 12). C. The capacity of CD8+ T cells from VIRs (n = 22), ARTs (n = 14), HICs (n = 73) and PTCs (n = 14) to suppress the HIV-1 infection of autologous CD4+ T cells, as determined by the log-fold decrease in the level of secreted p24 (CD4 vs. CD4:CD8 1:1 cell cultures). D. The percentage of CD8+ T cells from ARTs (n = 5), HICs (n = 58) and PTCs (n = 8) that expressed CD38, HLA-DR or both CD38 and HLA-DR ex vivo. B, C and D. The mean and standard deviation for each group are shown. doi:10.1371/journal.ppat.1003211.g003

We and others have shown that most HICs have high frequencies of highly efficient HIV-1-specific CD8+ T cells [22], [23]. In fact, the elevated number of HIV-specific CD8+ T cells producing IFN-γ in the
HICs was comparable to that in viremic patients (VIR) (Figure 3B). In contrast, we found that the PTCs had very weak HIV-specific CD8+ T cell responses during the viral control period. On average, the level of these responses in the PTCs were similar to that found in treated patients (ART), during both PHI and CHI (not shown), and were much lower than in viremic patients and HICs (Figure 3B). Indeed, HIV-specific CD8+ T cell responses were even barely detectable in some PTCs (Table S1).

We then examined the capacity of CD8+ T cells from the PTCs to suppress ex vivo the HIV-1 infection of autologous CD4+ T cells, as we recently showed that this test distinguishes the effective CD8+ T-cell responses found in many HICs from the ineffective responses in other patients [22]. The HIV-suppressive capacity of CD8+ T cells from the PTCs was poor (median decrease in p24: 0.39 log, Table S1), comparable with the capacity of cells from viremic patients (0.55 [0.43–1.00], p = 0.28) and treated patients (0.28 [0.12–0.86], p = 0.88) and far weaker than that observed in the HICs (1.63 [0.62–3.22], p<0.001) (Figure 3C). Of note, the capacity of CD8+ T cells from the PTCs to suppress HIV-1 infection was still weaker than that of the subset of HICs that did not bear the HLA-B*27 or B*57 alleles (1.55 [0.71–3.28], p = 0.002, n = 29).

PTCs have a low T cell activation status
We then examined the activation status of CD4+ and CD8+ T cells from the PTCs by evaluating the expression of HLA-DR and CD38. Because of the low or undetectable frequency of HIV-specific cells that were detected using tetramers in these individuals, the analyses were limited to the total cell population (Figures 3D and S1). HLA-DR and CD38 expression, both separately and in combination, were very weak in the PTCs during the period of viral control without therapy and similar to that observed in patients on cART, as expected within the context of very low viremia [24]. These results contrasted with the strong HLA-DR expression observed on CD8+ T cells from the spontaneous HIV controllers (Figure 3D) [22], which has also been reported by others [25].

PTCs have very low HIV reservoir levels that, in some cases, continue to decline for years after treatment interruption
Overall, the PBMC-associated HIV-1 DNA levels in the PTCs during the infection control (median 1.71 log copies/10^6 PBMC, Table 1) were similar to those in the HICs and much lower than those in patients with uncontrolled PHI or CHI or patients who started treatment during CHI [26], [27]. Sequential PBMC-associated HIV-1 DNA levels since PHI were available for 6 of the PTCs. In these PTCs, the HIV-1 DNA levels had declined strongly at or just before the treatment interruption (median 2,389 and 116 HIV-1 DNA copies/10^6 cells at PHI and before treatment interruption, respectively; p = 0.031; Figure 4A). The last available value, at a median of 6 years after the cART interruption, tended to be even lower (39 HIV-1 DNA copies/10^6 cells, p = 0.063; Figure 4A). Sequential PBMC-associated HIV-1 DNA levels were measured after treatment interruption for 8 PTCs (Figure 4B). The HIV DNA levels remained stable after cART discontinuation in two PTCs and a positive slope was observed for OR3, which is likely related to detectable viral replication at low levels in the last few years for this patient. In contrast, HIV DNA levels continued to progressively decline over the years in the five other PTCs in the absence of treatment. Thus, the PTCs had an extremely small viral blood reservoir, which in some cases continued to decline after long-term treatment interruption.
Figure 4. Post-treatment controllers have very low levels of cell-associated HIV DNA which keep decreasing after treatment interruption for some patients.

A. Levels of cell-associated HIV-1 DNA (median and IQR) in 6 PTCs at PHI, just before or at treatment interruption (TI), and the last available value obtained at a median of 6 years after cART discontinuation (Last).

B. The evolution of cell-associated HIV DNA after treatment interruption in PBMCs from 8 PTCs. The slope of the evolution of HIV-DNA levels after treatment interruption was calculated by linear regression (lines) of the available sequential measures (symbols). Five PTCs experienced a decline of their cell-associated HIV-DNA levels (left); two PTCs maintained stable levels and a positive slope was calculated for OR3 (right).

C. Infection levels in various cell populations from 11 PTCs: PBMCs, CD4+ T cells and monocytes; activated and resting CD4+ T cells; resting naïve (TN), central memory (TCM), transitional memory (TTM) and effector memory (TEM) CD4+ T cell subsets (see Figure S2 for the sorting strategy). The open symbols represent values below the threshold of detection. The medians are represented.

A, B, C. The results are expressed as the log10 HIV DNA copy numbers per million cells.

doi:10.1371/journal.ppat.1003211.g004

PTCs have a low and inducible HIV reservoir distributed in the resting memory CD4 T cell subsets

We quantified the distribution of the HIV reservoir among various sorted lymphocyte populations of live peripheral cells available from 11 PTCs (Figure 4C). The HIV DNA was detectable in the PBMCs from 7 out of 11 PTCs and in total purified CD4+ T cells from 6 out of 10 PTCs (from whom enough cells were recovered). The results were either reported as the actual HIV DNA copy numbers/million cells or as an estimated value calculated as 50% of the detection threshold value when HIV DNA was not detected. As expected, the HIV DNA was 9-fold higher in the CD3+CD4+ T cells than in the total PBMCs (median 2.3 versus 1.3 log HIV DNA copies/million cells). Among the CD4+ T cells, activated CD25+69+HLA-DR+ CD4+ T cells were significantly more infected than resting CD4+ T cells (2.8 versus 2 log HIV DNA copies/million cells, p = 0.01). In contrast, the CD3−CD4+ monocytes were minimally infected, with the total cell-associated HIV DNA level detectable in only 2 out of 10 samples (estimated median 2.3 log HIV DNA copies/million cells).
DNA copies/million monocytes). We also analyzed the reservoir distribution among the resting naïve (TN), central memory (TCM), transitional memory (TTM) and effector memory (TEM) CD4+ T cell subsets from 11 PTCs (Figure 4C). Cell-associated HIV DNA was detected in only 2 out of 11 samples in the resting naïve CD4 T cells (TN) (median 1.6 log HIV DNA copies/million TN, p = 0.001) and was lower than in resting memory CD4+ T cell subpopulations. In contrast, all resting memory CD4 T cells contained comparable levels of cell-associated HIV DNA (2.5, 2.4 and 2.3 median log HIV DNA copies/million in TCM, TTM and TEM cells, respectively).

To assess the presence of an inducible virus and the true nature of this HIV reservoir, we used anti-CD3 and anti-CD28 in the presence of IL-2 and IL-7 to stimulate the sorted resting CD4+ T cell subpopulations of 7 PTCs from whom an adequate number of cells was recovered (Figure 5). We observed a time-dependent virus product upon in vitro stimulation in 5 of the 6 sorted resting TCM, TTM and TEM subsets that were analyzed. We detected virus production from at least one T cell subset from each of the 7 tested patients. The failure to detect HIV production reflected the low number of HIV-infected cells added at baseline (a median of 6.5 HIV DNA copies in non-producing samples versus 97 HIV DNA copies when HIV RNA production could be detected). In line with the TN cells’ extremely low infection levels, virus production in these cells was observed in only 2 out of 5 PTCs samples tested. The stimulation of a higher number of resting TN cells with IL-7 alone triggered virus production in 3 TN samples, despite undetected TN-associated HIV DNA in 2 cases (Figure 5).

**Figure 5. HIV replication is inducible from the resting memory CD4 T cell subsets from post-treatment controllers.**

The cell capacity to replicate HIV was evaluated in 7 PTCs (each symbol represents one PTC) by stimulating sorted CD4 T cell subsets with an anti-CD3/anti-CD28 co-stimulation plus IL-2 and IL-7 (filled symbols and continuous lines) or IL-7 alone (empty symbols and dashed lines). HIV RNA was quantified in the supernatants of resting TN, TCM, TTM and TEM cells during a 13-day long culture. Results are expressed as the log10 of the ratio between the HIV RNA copy numbers quantified at a given day of culture and the level of cell-associated HIV DNA in the subset measured at D0 of culture. Kinetics
of HIV production in a patient has been represented with connecting lines. HIV RNA values that were under the detection threshold of the technique were arbitrarily placed at 0. ND is not done.
doi:10.1371/journal.ppat.1003211.g005

**Long-lived resting CD4+ T cells contributed minimally to the HIV reservoir in the PTCs**

We then compared the HIV reservoir distribution among the PTCs' resting CD4 T cell subsets to those of the HICs, whose total blood cells had similar low levels of HIV DNA. No differences were observed between the PTCs' resting CD4 T cell subsets' infection levels and those of the HICs (1.6, 2.7, 2.6 and 2.2 median log HIV DNA copies/million in the TN, TCM, TTM and TEM cells from HIC, respectively), except that the HIV DNA was undetectable in the TN cells from 9 out of 11 PTCs but only 4 out of 8 HICs (Figure 6A). To calculate each subset's contribution to the HIV reservoir, we evaluated the frequency of the resting CD4 T cell subsets in the blood (Figure S3). The predominance of the TTM subset in the PTCs drove the major contribution of this subset to the PTCs' resting CD4 T cell HIV reservoir (median 54%). This contribution of the TTM subset was significantly higher than that of the TCM which contributed to only 22%, the TEM (13%), and the TN subset which contributed very minimally to the resting HIV reservoir (6%; Figure 6B). In contrast, both TCM and TTM subsets contributed equally to the HIV reservoir in the HICs, as has been reported for other HIV-infected patients [28], [29]. Overall, such long-lived cells as the TN and TCM cells contributed very minimally to the PTCs' total HIV reservoir in resting CD4 T cells, which might have contributed to the gradual shrinking of the reservoir in some PTCs for whom the TTM subset was also the main contributor to the HIV reservoir (Figure S4).

![Figure 6](http://example.com/figure6.png)

**Figure 6. Weak contribution of long-lived resting CD4+ T cells to the HIV reservoir in the post-treatment controllers.**

A. HIV infection levels in the resting TN, TCM, TTM and TEM cells of 11 PTCs and 8 HICs. The results are expressed as the log10 HIV DNA copy numbers per million cells, and the medians are represented. The open symbols are values below the threshold of detection. 'ns' are non significant p values. B. CD4+ T cell subsets contribution to the resting HIV reservoir, considering both infection levels and frequency. The results are expressed as the median percentage of the resting CD4 HIV reservoir, with interquartile range [25%–75%] and minimum and maximum values. Statistical analyses were applied between all subsets from a single group as well as between each subset from the two groups.
doi:10.1371/journal.ppat.1003211.g006

**Higher-than-expected frequency of infection control after the interruption of long-term treatment initiated at PHI**

PTCs may represent between 5 and 15% of patients with early cART interruption [15], [30], [31]. To better understand this phenomenon, we estimated its frequency of occurrence within the French Hospital Database on HIV (FHDH ANRS CO4) (http://www.ccdes.fr/main.php?main_file=fl-1309272043-794.html). Between 1997 and 2011, 3,538 patients were included in the FHDH within 6 months of PHI. Among those, 1,013 patients were treated within 6 months post-infection, and 756 patients continued treatment for at least one year. Of those, only 70 patients with a viral load >50 copies/mL prior to treatment interrupted cART while their viral load was <50 copies/mL and with at least one viral load measurement recorded after treatment interruption. The mean number of viral load in the first three years post treatment interruption was 8 with a median delay of 3 months between 2 measurements. To
estimate the probability of maintaining virological control, we used Kaplan-Meier estimates and defined loss of control as either 2 consecutive viral loads >50 copies/mL or 1 viral load >50 copies/ml, followed by cART resumption (Figure 7). The probability of maintaining viral control at 12 months was estimated as 15.3% [4.4–26.3], and it was identical at 24 months post-cART interruption.

Kaplan-Meier curve of the probability for patients included in the FHDH between 1997 and 2011 to lose control of viremia after interruption of a, at least, one year-long cART initiated within 6 months of HIV infection, and who had at least one viral load determination 12 months after treatment interruption (n = 74). Loss of control was defined by 2 or more viral loads above 50 RNA copies/mL or one viral load above 50 RNA copies/mL followed by resumption of cART.

Discussion

Numerous efforts have been aimed to achieve a functional cure for HIV infection that would allow treatment to be stopped altogether. We studied 14 patients in whom viral replication was controlled to undetectable levels for several years after the discontinuation of cART. These PTCs with long-term virological remission may hold important clues about a possible functional cure for HIV.

The 14 PTCs presented in this study maintained lasting control of viremia after the interruption of prolonged therapy that began early during PHI. We found that most PTCs were readily distinguishable from spontaneous HICs in many respects. In many cases, spontaneous control seems to start very soon after HIV infection [16], [32], and most HICs have lower-than-normal viral loads during PHI [16]. In contrast, the PTCs had a more severe primary infection with higher viral loads and were frequently symptomatic, which may have prompted the early treatment in some cases. These observations are consistent with the generally unfavorable HLA genotypes of the PTCs. In particular, the risk alleles HLA B*35 and HLA-B*07, rarely observed in the HICs [17], were highly prevalent among the PTCs. Furthermore, two PTCs carried the HLA-B*3503 allele, which is associated with accelerated disease progression and impaired HIV-specific T cell function [33]. We cannot rule out the possibility that spontaneous control may have been masked in some cases by early therapy initiation. In particular, it might be possible that some potential HICs who lacked protective HLA alleles were more prone to have higher viral loads in primary infection and, hence, more likely to initiate therapy. However, other differences were observed between the PTCs and the HICs during the chronic phase of infection. In particular, the PTCs had a low frequency and quality of HIV-specific CD8+ T cell responses. Although some HICs do not exhibit strong HIV-specific CD8+ T cell responses [34], [35], the overall differences between the HICs and PTCs in our study were striking, even when the HICs carrying the protective HLA B*27 and B*57 alleles were excluded from the analyses. Finally, the PTCs were characterized by a lower CD8+ T cell activation status compared with the HICs.

The 5 to 15% of PTCs observed among the patients in the FHDH ANRS CO4 study and in other studies [15], [30], [31] appears higher than the proportion of HICs with spontaneous viral control in patients followed from primary infection [16], [36]. Therefore, our results strongly suggest that the infection control in the PTCs was not achieved spontaneously and was favored by the early onset of therapy. Because the interruption of long-term cART initiated early during PHI is not recommended, only a very small proportion (~2%) of the patients in the FHDH experienced such an interruption, which may explain the rarity of PTCs worldwide. It is also important to consider that the 14 PTCs studied here had exhibited infection control without therapy for a very long period, and they may differ from PTCs with a shorter period of control [31].

The control of viremia following treatment interruption was associated with very low HIV blood reservoirs in the PTCs. This observation, together with similar observations in the HICs [26], suggests that limiting the pool of infected cells is crucial for the successful control of viral replication in the absence
of therapy. In PTCs, the early cART initiation and the lengthy treatment period likely played an important role in reducing the reservoirs [7, 37]. Interestingly, five PTCs experienced a progressive decline in their viral reservoir after treatment interruption, which is one of the goals in the search for an HIV cure. However, very small HIV reservoirs do not guarantee infection control off therapy [38]. A key additional element might be a low reservoir distribution in cell subsets with long lifespan as naïve and central-memory T cells. Indeed we found that the cell subsets of all the PTCs analyzed ex vivo carried very low levels of HIV DNA. In particular, long-lived resting CD4+ T cells from the PTCs provided a minor contribution to the total HIV reservoir. Naïve CD4+ T cells were poorly infected, and overall the presence of the virus in these cells could not be detected (via DNA or viral replication) in 40% of the samples. This extremely low reservoir in PTCs’ naïve cells contrasts with the massive infection detected at the end of the first month after initial infection with a median of 3 log copies HIV-DNA/million naïve cells (C. Bacchus and A. Cheret, personal communication), as also reported a year after initial infection in the absence of treatment, although the naïve cells contained a log lower level of cell-associated HIV DNA than other memory subsets [39]. These discrepancies suggest that early therapeutic intervention is extremely efficient at decreasing those very long-lived reservoirs.

Central memory CD4+ T cells also contributed very weakly to the HIV reservoir because of a skewed resting CD4+ T cell subset distribution with a large proportion of shorter-lived transitional memory cells. The skewed distribution of the resting CD4+ T cells observed in the PTCs is also found in uncontrolled early infection (our own unpublished results), further indicating that early therapeutic intervention strongly contributed to the nature of the viral reservoir in these individuals. The TCM cells have been shown to be heavily infected a year after infection, and the main contributor to the total HIV reservoir in patients treated during chronic infection [28]. Similarly weakly differentiated memory CD4 T cells were shown to contain the majority of the HIV reservoirs in untreated chronically infected patients [40]. In contrast, we recently reported a protection of TCMs that contributed less to the total HIV reservoir in long-term non progressors carrying HLA-B*27 or B*57 alleles [29], and TCM protection has also been observed in the nonpathogenic SIV infection of sooty mangabeys [41]. Altogether our results suggest that a functional cure would most likely require reducing both the size and the distribution of the HIV reservoirs, particularly among those resting CD4 T cells with a long lifespan or important clonogenic properties, such as naïve and central memory T cells.

Early therapy may also limit viral diversity and offer protection of innate and specific immunity from the deleterious effect of chronic immune activation. However, it remains unclear why only a limited fraction of patients is able to control the infection after therapy interruption, and a study of the effectors of control in PTCs is underway. In addition, mechanisms that diminish the susceptibility of host cells to HIV-1 infection [26] and protect long-lived cell types [42] have been implicated in the control of HIV/SIV infection and pathogenicity in humans and nonhuman primates and may favor infection control after treatment interruption in some individuals. Finally, it is also possible that properties of the viruses infecting the PTCs studied, along with potential limitation of viral diversity by early institution of cART may play a role in the phenotype reported. We are currently addressing these questions.

Arguments against cART initiation during PHI include the potential for long-term toxicity, the development of resistant viruses and the cost. However, new antiretroviral drugs are well tolerated, highly effective and associated with excellent compliance, strongly reducing the risk of resistance [43]. In addition, early treatment initiation improves survival [4] and reduces the risk of HIV-1 transmission [44]. Here, we show that in some HIV-infected individuals with symptomatic primary infection and no favorable genetic background, off-therapy viral control for several years may be associated with a very early and prolonged antiretroviral treatment. These findings argue in favor of early cART initiation and open up new therapeutic perspectives for HIV-1-infected patients.

Methods

Ethics statement

All of the subjects provided their informed written consent to participate in the study. The CO6 PRIMO, CO15 ALT and CO18 HIV controller cohorts are funded and sponsored by ANRS and were approved by the ethics review committees of Ile de France III, VI and VII, respectively. The institutional review board of Institut Pasteur and Pitié-Salpêtrière Hospital (Paris, France) also approved the study protocol. The VISCONTI study was funded by ANRS (EP47), sponsored by Orléans Regional Hospital and approved by the Tours ethics review committee.

Subjects

The post-treatment controllers (PTCs) were defined as patients who initiated cART within 10 weeks of PHI and whose plasma HIV RNA levels remained less than 400 copies/mL for at least 24 months after
cART interruption. Primary infection was defined as symptoms associated with an incomplete HIV-1 Western blot and a positive p24 antigen test or detectable plasma HIV RNA, and/or seroconversion documented by a positive HIV antibody test that was preceded by a negative test less than 3 months before. Fourteen PTCs were included in this study. Four had been identified in a previous study [15], six were recruited from the ANRS CO6 PRIMO cohort of patients diagnosed during PHI [31], and four were recruited from patient follow-up at Hôpital de la Croix Rousse in Lyon, CHRU Gui de Chauliac in Montpellier, and CHU de Saint Louis in Paris, France.

The HIV controllers (HICs) were patients from the ANRS CO15 and CO18 cohorts who had been infected for more than 5 years, were naïve of antiretroviral treatment and whose last 4 consecutive plasma HIV RNA values were less than 400 copies/ml. Viremic (VIR) patients were defined as patients who were HIV-1-infected for more than 6 months, were not receiving antiretroviral therapy and had HIV-1 plasma viral loads greater than 7500 RNA copies/ml. cART-treated individuals (ARTs) were HIV-1-infected patients whose viral load had been less than 50 RNA copies/ml of plasma for at least 6 months on cART initiated either on PHI or CHI.

**HLA typing**
The subjects were serologically HLA-typed using complement-mediated lymphocytotoxicity testing (InGen One Lambda, Chilly Mazarin). High-definition genotyping of the HLA-B*35 alleles was conducted by direct exon sequencing.

**HIV-specific CD8+ T cell response**
Interferon (IFN)-γ secretion by HIV-specific CD8+ T cells was quantified ex vivo with an ELISpot assay [22]. For each subject, the optimal peptides (2 µg/mL) corresponding to known optimal CTL epitopes derived from the HIV-1 Env, Gag, Pol and Nef proteins were tested, depending on the results of the HLA typing.

The method used to assess the CD8+ T cells' capacity to suppress an ex vivo HIV-1 infection of autologous CD4+ T cells has been thoroughly previously described [45].

**Activation phenotyping**
The following antibodies were used: CD8-APC-H7 or -PerCP-Cy5.5 (SK1), CD3-APC or -APC-H7 (SK7), HLA-DR-PE-Cy7 (L243) and CD38-PerCP-Cy5.5 (HIT2) (BD Biosciences). The cells were fixed and analyzed with a FACSCanto I flow cytometer (BD Bioscience).

**Sorting of the PBMC subpopulations**
PBMCs that were cryopreserved and stored in liquid nitrogen and had more than 80% viability after thawing were sorted as live monocytes (CD3−CD4+) and activated and resting CD3+CD4+ T cells on a 5-laser FACS ARIA II cell sorter (Becton Dickinson) on the CyPS platform (UPMC), after staining with the following combination: Live-Dead Fixable Aqua (Life Technologies), CD3-Pacific Blue, CD4-AlexaFluor700, CCR7-PE Cyanine7 (3D12), CD27-APC, CD69-FITC and HLA DR-FITC (BD Pharmingen), CD45RA-ECD and CD25-FITC (Beckman Coulter). The resting CD4 T cells (CD25−CD69−HLADR−) were further sorted into the following categories: naïve (TN, CD45RA+CCR7+CD27+), central memory (TCM, CD45RA−CCR7+CD27+), transitional memory (TM, CD45RA−CCR7−CD27+), and effector memory (TEM, CD45RA−CCR7−CD27−) cells (Supplementary Figure S2). The collected cell numbers varied from 0.01 to 2 million cells among subsets and patients, and the purity of the sorted subsets was greater than 90%. The data were analyzed using Flowjo software (Treestar).

**HIV DNA quantification**
The total cell-associated HIV DNA was quantified using ultrasensitive real-time PCR (Biocentric, Bantol, France) in the PBMC, monocyte and CD4 T cell subsets, as previously described (ANRS assay [46]). The entire HIV DNA extract was tested in two to four PCRs. The results are reported as either the actual HIV DNA copy numbers/million cells or as an estimated value calculated as 50% of the detection threshold value when the cell HIV DNA was lower than the threshold. The thresholds varied according to the available cell numbers and were calculated for each assay [47].

**Detection and amplification of HIV-1 from peripheral blood CD4+ T cell subsets**
A first fraction of sorted resting CD4+ TN, TCM, TTM and TEM subsets from 7 PTCs was tested for the total cell-associated HIV DNA level (see above). A second fraction of the same samples was cultured in variable numbers in 10% FCS supplemented RPMI 1640 medium for 13 days after stimulation at Day 0 with anti-CD3/anti-CD28 plus IL-2 (Roche, 5 µg/ml) and human recombinant IL-7 (Cytheris, 1 ng/ml) or with human recombinant IL-7 alone. At Days 3, 6, 8, and 10, half of the supernatants were removed to quantify the HIV RNA, and IL-2 and IL-7 were added. The viral production kinetic in the supernatants was measured using real-time PCR HIV RNA quantification (Biocentric, Bandol, France). The viral production capacity of each subset was expressed as the ratio between the HIV RNA copies in the
supernatants at a given day of culture and the level of cell-associated HIV DNA of each subset measured at Day 0 of culture.

**Statistical analyses**
The Kruskal-Wallis nonparametric test was used to compare continuous variables between groups. A Wilcoxon matched-pairs signed rank sum test was used to compare variations in values (CD4+ T cell counts, HIV DNA levels) over time or to compare cell subsets in the sorting experiments. The allele frequencies in the different groups of patients were compared using Fisher’s exact test. A Kaplan-Meier estimate was used to assess the probability of post-treatment control in patients who discontinued early cART. All values given in the text are medians and (range) or [IQR]. The SigmaStat 3.5 software (Systat Software Inc.-SSI, CA) or SAS software package, Version 9.2 (SAS Institute, Cary, NC, USA) was used for all analyses.

**Supporting Information**

**Figure S1**

![Graph A: Percentage of CD4+ T cells expressing CD38, HLA-DR or both ex vivo.](image)

**Figure S2.**

Cell sorting scheme. Resting CD4 T cell subsets were selected out of a singlet lymphocyte population as assessed by cell size and structure. Live (Aqua-) resting CD3+CD4+ T cells (CD25−CD69−HLA-DR−) were further sorted as Naïve (TN, CD45RA+CCR7+CD27+), Central-Memory (TCM, CD45RA−CCR7+CD27+), Transitional-Memory (TM, CD45RA−CCR7−CD27+), and Effector-Memory (TEM, CD45RA−CCR7−CD27−) cells.

**Figure S3.**

Different frequency of resting CD4+ T cell subsets in post-treatment controllers and HIV controllers. The frequency of TN, TCM, TTM and TEM cells among circulating resting CD4+ T cells in the PTCs and HICs.
**Figure S4.**  
Weak contribution of long-lived resting CD4+ T cells to the HIV reservoir in the post-treatment controllers with declining levels of cell associated HIV-DNA. CD4+ T cell subset contribution to the resting HIV reservoir for 4 PTC for whom we observed a diminution overtime on their HIV blood reservoir levels and HIC, taking into consideration both the cell infection levels and their frequency. Results are expressed as the median percentage of the resting CD4 HIV reservoir with interquartile range [25%–75%] and minimum and maximum values.

(PDF)

**Table S1.**  
HLA-class I alleles and characteristics of the CD8+ T cell response in the post-treatment controllers.

(PDF)

**Table S2.**  
Comparisons of relevant HLA allele frequencies in post-treatment controllers, HIV controllers and the reference French population.

(PDF)

**Text S1.**  
List of scientists and clinicians who are associated to the VISCONTI study.

---

**Are we underestimating the proportion of virally-suppressed patients in the US?**

Gus Cairns  
Published: 15 March 2013

Several presentations at the recent 20th Conference on Retroviruses and Opportunistic Infections in Atlanta suggest that previous estimates of the proportion of people with HIV in the USA who are on antiretroviral therapy (ART) and with an undetectable viral load may have been too low and may be closer to the proportion virally suppressed in Europe.

**Background: a walk through the cascades**

The “HIV care cascade” is a way of calculating what proportion of HIV in a country or community is on ART and virally suppressed. Having a high proportion of people with HIV with undetectable viral loads is generally seen as critical to the success of ART to prevent HIV transmission and as an important component of programmes to reduce HIV incidence.

The care cascade calculation takes into account that having a high proportion of people with HIV essentially non-infectious is dependent on a chain of events happening, all of them at high frequency:

- A high proportion of people with HIV need to be diagnosed, which implies frequent testing among high-risk groups;
- A high proportion of the diagnosed need to be engaged in care, which suggests an easily-accessible healthcare system for all;
- A high proportion of those in care have to be on ART, which suggests guidelines with high CD4 count thresholds for care (or none), and few financial or availability barriers to ART;
- A high proportion of those on ART have to be virally suppressed, which suggests high adherence rates, good monitoring, and appropriate prescribing.

Reports in the last two years appear to show a large gap between the US and Europe in terms of the proportion virally suppressed. Last year at the IAPAC summit on antiretroviral-based prevention Dr Valerie Delpetch of the UK's Health Protection Agency showed that in the UK 53% of gay men with HIV have an undetectable viral load. Preliminary data suggest that the figure will be very similar for HIV-positive people in general, and indeed another study at the International AIDS Conference suggested that because incidence in gay men is higher than in other populations, the proportion of the HIV-positive population in general that is virally suppressed may be quite a lot higher than this, despite their tendency to be diagnosed later.

In contrast, two similar calculations for the US by the Centers for Disease Control have suggested that only 28%, or even 25%, of people with HIV are virally suppressed and even fewer of the most vulnerable groups, such as black gay men, heterosexual men and young people.

**France, the UK and the US**

A clue as to why, so far, ‘cascade’ calculations for the US have come out with such lower figures came from a study of the care cascade in France (Supervie).

This study, based on a large cohort of patients with HIV, calculated that 52% of people with HIV in France are on ART and with an undetectable viral load, and 56% of gay men, a little higher than the UK.
It estimated that 81% of people with HIV in the country are diagnosed (better than the UK estimate of 76%); found that 92% of those are in care; that 81% of those have been taking ART for more than six months; and that of them, 86% have a viral load below 50 copies/ml.

Counter-intuitively, the group with the highest rate of viral suppression is people who got HIV through injecting drug use, 66% of whom have an undetectable viral load; but this is because, as in the UK, France has done a good job of bringing down HIV infections in injecting drug users to a few per cent of the total, so most IDUs form an ageing cohort who are already in care. However IDUs also formed a disproportionately large part of the small number of people who are diagnosed but are not in care.

The French researchers cited the country’s health system for its relatively high number of virally-suppressed people. The French system is not free at point of demand and is insurance based; but unlike the USA, with some residency exceptions, people with HIV are entitled to recoup the cost of all their medical care.

Why are the US results so different? In most respects, the US health system actually performs just as well. For instance, in both countries, 81% of people with HIV are diagnosed; and though France performs better when it comes to the proportion with a viral load below 50 copies/ml (86% in France versus 77% in the US), in the US, more people with HIV who are in care are on ART (89% in the US versus 81% in France).

The big difference is the number who are linked to, and stay in, care after diagnosis. In France 92% of the diagnosed are linked to care (and an even higher proportion in the UK); in the US study cited, only 51% of the diagnosed thereafter attend clinics regularly.

**Are the US calculations wrong?**

Why? The assumption has been that inequalities built into the US healthcare system are to blame.

In the US, health care for HIV is covered by a complex system of healthcare benefits and entitlements. About a quarter of people with HIV are classified as uninsured, and people with HIV in general are 56% more likely to be uninsured than the general population.

The uninsured can still get HIV treatment via state-run AIDS Drugs Assistance Programs, which are funded by federal money under the Ryan White Care Act. In the past, a number of state ADAP programmes have run short of money and have sometimes placed people in need of HIV therapy on waiting lists.

The 2010 Affordable Care Act (‘Obamacare’) mandates that employer and private insurance schemes must cover long-term medical conditions and provides for the creation of insurance markets (state- or federally-administered) which will start in October, with a bridging insurance plan already in place. It also requires states to expand Medicaid, the main provider for people with disabilities, but the Supreme Court struck down federal powers to fine states that refused to comply.

What this means is that while the vast majority of US citizens can access HIV treatment, getting it is complex, can require satisfying stringent criteria, varies hugely by location, may be covered by several different schemes, often requires co-pays, and is currently in a state of flux. It also enshrines socioeconomic inequalities, with black people with HIV twice as likely as white people to be on ADAPs. There has been an assumption that this resulted in actual gaps in HIV care coverage.

However several studies from the US presented at the recent CROI conference suggested that instead it forced people to move from one provider to another or to space out medical visits.

**Defining ‘retention in care’**

The definition of ‘retention in care’, as used by the Centers for Disease Control paper that came out with the figure that 25% were virally suppressed, was the proportion of adults with HIV who received at least one medical care visit between January and April 2009.

When this finding was presented at the International AIDS Conference last year, it was suggested that many people on stable ART might attend appointments less often.

A study presented at the recent CROI conference (Horberg) by Kaiser Permanente (KP), the US’s largest private not-for-profit provider of HIV healthcare, suggested that the CDC ‘cascade’ calculation may considerably underestimate the proportion of people who are virally suppressed.

In particular, the CDC assumption had been that people who were not ‘retained in care’ could not be taking ART, but this might not be the case.

KP used its own database of 16,816 patients, which, because it provides coverage in general to the less-deprived populations, was largely male (87%) and older (average age 48 and 29% over 55). No data on ethnicity or sexuality were given.
KP used a broader definition of ‘retained in care’ (at least two visits in a year) and below 200 copies/ml as its definition of viral suppression. It also used a single measurement of viral load in any one year as its definition of viral undetectability rather than two consecutive ones.

Using these more liberal criteria, its estimate for the total number of diagnosed patients virally suppressed, at least in KP patients, was 60.2%. If the CDC estimate of 19% for the proportion of people with HIV who are undiagnosed is added, this would become 51% of all people with HIV – quite similar to France and the UK.

However the actual Kaiser figures for filled prescriptions and for viral undetectability showed that more people were prescribed ART and were virally suppressed than were defined as being ‘retained in care’. Using the proportion of all patients with a viral load under 200 copies/ml at the last test as its criterion for viral suppression rate, rather than the proportion counted as ‘being in care’, the result was that 80% of diagnosed KP clients with HIV were virally suppressed. Extending that to the whole population and adding in 19% undiagnosed, that would mean two-thirds of the HIV positive population had an undetectable viral load – or would do if they were all like KP clients.

**Seattle: adding in the lost-to-care**
The KP paper may overestimate the proportion of people in care and virally-suppressed as much as the CDC underestimates it, but a study using real figures from Seattle (Dombrowski) supported its findings to some extent.

This found that diagnosis rates were similar or higher than the CDC estimate. But it also did something the CDC did not, which was that by using data from real-life case investigation of people who apparently dropped out of care, it determined that about 10% of people classed as not receiving care had in fact moved out of area or away from the providers included in the study, and were in fact in care. It also found by investigation that another 10% of people who were listed as not being ‘retained in care’ because they did not have a CD4 or viral load test result recorded, were also in care: the issue in these cases was to do with medical note recording or of physicians deciding to monitor less often, not actual attendance.

Using these figures the Seattle team calculated that 79% of all people with HIV living in King County, Washington state (Seattle’s county) were linked to care as opposed to 66% in the CDC calculations, and 71% retained in care as opposed to 35%. This meant that the proportion of people with HIV who were virally suppressed was 57%, as opposed to 25% in the CDC figures. Again, very similar to the European figures.

**New York: increases in viral suppression**
Similar figures were obtained by a study from New York city (Stadelmann), although once again, these local figures may not be representative of all areas.

This paper used as its definition of viral suppression two successive viral load results under 400 copies/ml in a year, not dissimilar to the CDC studies.

However it did not assess linkage to or retention in care, and thus made no assumptions about whether only people classed as being retained in care could be assessed for viral load suppression.

It found that 52% of diagnosed HIV-positive people in New York were virally suppressed. If the estimated 19% of undiagnosed people is added in, this becomes 44%—lower than in Europe but a lot higher than the CDC estimate.

These figures are from 2010-2011 and represent a considerable increase from 2006-2007, when 38% of diagnosed, or 31% of all, people with HIV had an undetectable viral load.

The New York study also assessed the proportion of diagnosed people with persistently high viral loads (two successive measures of over 100,000 copies/ml), and who would therefore be very infectious.

It found that this proportion had declined from 7.4% of diagnosed people with HIV in 2006-2007 to 4.6% in 2010-2011. This does not imply a proportional decrease in very high viral loads in the whole HIV positive population, though, as high viral loads in the undiagnosed would be unaffected by ART.

Health inequalities had their effects on viral load undetectability: whereas 20% of the HIV positive population was white, white people formed only 9% of those with a persistently high viral load; conversely, though 45% of the patient population was black, they formed 54% of those with a persistently high viral load.

**Conclusion**
What these papers show in general is that the complexities of the US healthcare system make it very difficult to measure the true proportion of people with HIV in the country who are taking ART and are virally undetectable. The proportion may be much higher in some areas and for some populations, and the criteria used by the CDC may be too strict, especially as we move to less-frequent monitoring. But it also shows that even in areas with good coverage, health inequalities remain.
Plan to Fight Deadly TB Strain Advances in India

Wall Street Journal, (03.17.2013) Geeta Anand

To assist India in its fight against TB, the nonprofit Clinton Health Access Initiative and a McGill University professor brokered a deal with several makers of diagnostic equipment to give private Indian laboratories the same discounts on equipment to detect multidrug-resistant TB as is offered to the government of India and other developing countries. The laboratories agreed to a price that is about half of the current market price in India. According to Madhukar Pai, an associate professor at McGill University in Canada and one of the world’s top TB experts, they were able to convince the manufacturers that if the diagnostic equipment were more affordable, a greater number of patients would use them, which would be good for the manufacturers and for TB control.

The Indian Government, with the support of the World Health Organization (WHO), runs a national program that tests and treats patients for free. However, about half of India’s TB patients do not use the government program because they assume that they will receive substandard medical care in the public sector. Instead, they use private medical providers, who may offer cheap, inaccurate tests and inadequate treatments, hence increasing drug-resistant strains.

India’s Central TB Division has not endorsed the new initiative. As a result, it is difficult to determine its impact. It is important for New Delhi’s support of the initiative to persuade private physicians and patient to use the tests. Dr. Navin Dang, a New Delhi laboratory owner and an organizer of the initiative, said that WHO has endorsed all of the discounted diagnostics, and they are widely used globally. Indian TB officials argue that they have not validated some of the tests and even after about a year of pilot tests, the TB division has not adopted GeneXpert, a rapid test to detect TB and drug resistance in two hours.

Dr. Pai and Dr. Dang recently canceled plans to announce the initiative because of the TB Division’s refusal to participate. Pai noted that to get a huge uptake, government’s participation was needed to get the word to private-sector doctors and patients about the tests and the discounted prices. Even a personal visit by Dan and other organizers to the TB Division could not move the officials. The Joint Secretary of Health for India Anshu Prakash, who oversees TB, commented that he was not told why the TB Division did not endorse the initiative, but he suggested that it may have been because a written proposal was not submitted. Prakash also stated that he needed to review a proposal in writing before giving his endorsement.

Bill Gates Says Capitalism Funnels More Resources Into Minor Ailments Than Infectious Diseases

Bill Gates, co-chair of the Bill & Melinda Gates Foundation, "has declared capitalism 'flawed' because it channels more resources to curing minor ailments such as male baldness than to addressing the diseases that destroy millions of lives every year," The Independent reports. "The billionaire founder of the software giant Microsoft, who is now one of the world's most prominent philanthropists, told [the Royal Academy of Engineering’s Global Grand Challenges Summit] in London last week that it was an indictment of the economic system that dominates most of the planet that more money is spent on research to reverse hair loss than on tackling scourges of the developing world such as malaria," the newspaper adds (Chu, 3/16).

"Our priorities are tilted by marketplace imperatives,' he said. The malaria vaccine in humanist terms is the biggest need. But it gets virtually no funding. But if you are working on male baldness or other things you get an order of magnitude more research funding because of the voice in the marketplace than something like malaria," Gates added, according to Wired. "As a result, governments and philanthropic organizations have to step in to offset this 'flaw in the pure capitalistic approach,’" Gates said, the magazine writes (Solon, 3/14). The Independent adds, "His comments will be interpreted as another blast at the large pharmaceutical companies, which have long been criticized for plowing money
into developing 'lifestyle drugs' and neglecting research that could save the lives of the world's poorest" (3/16).

**Nigerian Polio Vaccinators Face Challenges But Aim For Eradication By 2018**

*The Independent* reports on the challenges polio vaccinators in Nigeria face, including "the daily gauntlet of militant gangs, kidnappers, and unforgiving waters, on a mission to immunize children." Polio remains endemic in Nigeria, as well as Pakistan and Afghanistan, "[a]nd it's why the vaccinators—determined to cut the deaths of children from preventable diseases and wipe out polio altogether by 2018—are prepared to endure all the country throws at them in a battle to succeed," the newspaper writes. "But while the risks are high, the consequences of doing nothing are worse," the newspaper continues, adding, "Each year 861,000 under-fives die here in Africa's third richest nation." According to *The Independent*, "the International Vaccine Access Center (IVAC) at the Johns Hopkins Bloomberg School of Public Health is spearheading the nation's vaccination program," and the program is beginning to see results (McNamara/Basnett, 3/15).

**More parents say they won't vaccinate daughters against HPV, researchers find**

*Parents increasingly concerned about potential side effects, study shows*  
ROCHESTER, Minn.—A rising percentage of parents say they won't have their teen daughters vaccinated to protect against the human papilloma virus, even though physicians are increasingly recommending adolescent vaccinations, a study by Mayo Clinic and others shows. More than 2 in 5 parents surveyed believe the HPV vaccine is unnecessary, and a growing number worry about potential side effects, researchers found. The findings are published in the new issue of the journal *Pediatrics*.

In all, researchers looked at three vaccines routinely recommended for U.S. teens: a vaccine to protect against the sexually transmitted HPV; Tdap, for tetanus, diphtheria and acellular pertussis; and the meningococcal conjugate vaccine, or MCV4 vaccine. While the up-to-date immunization rates rose for all three vaccines, the proportion of girls fully immunized against HPV (three doses over six months) was substantially lower than the proportion for the other two vaccines.

Five years ago, 40 percent of parents surveyed said they wouldn't vaccinate their girls against HPV. In 2009, that rose to 41 percent, and in 2010, to 44 percent.

"That's the opposite direction that rate should be going," says senior researcher Robert Jacobson, M.D., a pediatrician with the Mayo Clinic Children's Center. Parents concerned about HPV vaccine safety rose from 5 percent in 2008 to 16 percent in 2010, while less than 1 percent worried about the safety of the Tdap and MCV4 vaccines, the study found.

During the same years, more and more studies showed how safe and effective the HPV vaccine is in this age group, says Dr. Jacobson, who has taken part in the safety review committees for two such studies. The vaccine prevents cervical cancer and other genital cancers by preventing the HPV infections that lead to those cancers, he says.

Researchers analyzed vaccination data for teens ages 13 to 17 in the 2008-10 National Immunization Survey of Teens. They found that as of 2010, 8 of 10 teens had the Tdap vaccine and roughly 63 percent had the MCV4 vaccine. Only about one-third of girls were immunized against HPV.

The HPV vaccination rate did rise; it was only 16 percent in 2008. But at the same time, more parents reported that they did not intend to have their daughters vaccinated for HPV. Among the reasons they gave: the vaccine was not recommended; lack of knowledge; it is unnecessary; the vaccine is inappropriate for the child's age; worry about safety/side effects; and the child isn't sexually active.

According to parents surveyed, more clinicians are recommending the HPV vaccine, but still, they are advising it only about half the time. The facts show the vaccine is necessary, Dr. Jacobson says.

"HPV causes essentially 100 percent of cervical cancer and 50 percent of all Americans get infected at least once with HPV. It's a silent infection. You cannot tell when you've been exposed or when you have it," he says. "While most HPV infections clear, a percentage linger and start the process of cancerous changes. The HPV vaccine is an anti-cancer vaccine."

Dr. Jacobson says the vaccine is more effective in younger adolescents than older teens. Mayo Clinic routinely starts the series at age 9.

"The vaccine works better the younger the child is, and it doesn't work after the child is grown up and is exposed to the virus, so our message should be: 'Give this vaccine now to your child while your child is young and responsive to it,'" says Dr. Jacobson, medical director of the Employee and Community Health Immunization Program at Mayo Clinic.
Probiotics Reduce Stress-Induced Intestinal Flare-Ups, Study Finds

Mar. 14, 2013 — For those with irritable bowel syndrome who wonder if stress aggravates their intestinal disorder, a new University of Michigan Health System study shows it's not all in their head.

Researchers revealed that while stress does not cause IBS, it does alter brain-gut interactions and induces the intestinal inflammation that often leads to severe or chronic belly pain, loss of appetite and diarrhea.

Stress has a way of suppressing an important component called an inflammasome which is needed to maintain normal gut microbiota, but probiotics reversed the effect in animal models, according to findings published online ahead of print in Gastroenterology.

"The effect of stress could be protected with probiotics which reversed the inhibition of the inflammasome," says senior study author and gastroenterologist John Y. Kao, M.D., associate professor of internal medicine at the University of Michigan. "This study reveals an important mechanism for explaining why treating IBS patients with probiotics makes sense."

Probiotics are live bacteria that help grow the gut-dwelling "good" bacteria that keep pathogens in check, aid digestion and nutrient absorption and contribute to immune function.

U-M researchers including Chung Owyang, M.D., chief of the U-M Division of Gastroenterology, Gary Huffnagle, Ph.D., professor of pulmonary and critical care, and infectious disease expert Vincent Young, M.D., Ph.D., were able to identify the way stress significantly altered the composition of gut bacteria and the role of probiotics.

Maintaining healthy microbiota requires action by nucleotide-binding oligomerization domain protein-like receptors, pyrin-domain containing (NLRP)-6 inflammasomes. But when stressed, mice produced corticotropin-releasing hormone (CRH) that prevented inflammasomes from doing their job.

Inhibiting inflammasomes alters the composition of the gut, leading to intestinal inflammation. In the study, pretreatment with probiotic therapy reduced inflammation in mice with stress-induced small bowel inflammation.

"Additional clinical study is required to determine the optimal probiotic therapy," says Kao. "Patients can start living healthier lifestyles to improve their gut microbiota such as adding more fruits and vegetables to their diet, and looking for ways to keep stress in check."

Journal Reference:
Yundong Sun, Min Zhang, Chun-Chia Chen, Merritt Gilliland, Xia Sun, Mohamad El-Zaatari, Gary B. Huffnagle, Vincent B. Young, Jiajie Zhang, Soo-Cheol Hong, Yu-Ming Chang, Deborah L. Gumucio, Chung Owyang, John Y. Kao. Stress-Induced Corticotropin-Releasing Hormone-Mediated NLRP6 Inflammasome Inhibition and Transmissible Enteritis in Mice. Gastroenterology, 2013; DOI: 10.1053/j.gastro.2013.02.038

Swarm Intelligence: New Collective Properties of Swarm Dynamics Uncovered

Mar. 15, 2013 — A new study of animal swarms uncovers some new features of their collective behaviour when overcrowding sets in.

Swarming is the spontaneous organised motion of a large number of individuals. It is observed at all scales, from bacterial colonies, slime moulds and groups of insects to shoals of fish, flocks of birds and animal herds. Now physicists Maksym Romenskyy and Vladimir Lobaskin from University College Dublin, Ireland, have uncovered new collective properties of swarm dynamics in a study just published in EPJ B. Ultimately, this could be used to control swarms of animals, robots, or human crowds by applying signals capable of emulating the underlying interaction of individuals within the swarm, which could lead to predicted motion patterns elucidated through modelling.

The authors were inspired by condensed matter models, used for example in the study of magnetism, which were subsequently adapted to be biologically relevant to animal swarms. In their model, in addition to the ability to align with its neighbours, each model animal is endowed with two new features: one for collision avoidance and another preventing direction change at every step to ensure persistence of motion. The team performed computer simulations of up to 100,000 self-propelled particles, each mimicking an individual animal and moving at a constant speed on a plane surface.

They found that when the swarm becomes overcrowded, the globally ordered motion breaks down. At high density and when the nearest neighbours are within one step of each other, each animal can no longer decide on the safe direction of motion. Instead, it is busy correcting its motion to avoid collisions.

They also described, for the first time, a power law that quantifies the average degree of alignment in the direction of motion for animals within the swarm. The law describes how the alignment decays from
the centre of the swarm, where animals can best judge the swarm motion due to their maximum number of neighbours, to the periphery.

**Journal Reference:**

---

**Synthetic Peptide Fools Immune System**

Researchers have created a molecule that helps nanoparticles evade immune attack and could improve drug delivery

A synthetic molecule attached to nanoparticles acts like a passport, convincing immune cells to let the particles pass unimpeded through the body, according to a study published today (February 21) in Science. The computationally designed “self”-peptide could be used to better target drugs to tumors, to ensure pacemakers are not rejected, and to enhance medical imaging technologies.

“It’s the first molecule that can be attached to anything to attenuate the innate immune system, which is currently limiting us from delivering therapeutic particles and implanting devices,” said Dennis Discher, a professor of biophysical engineering at the University of Pennsylvania and a coauthor of the study.

“This is really interesting work,” said Joseph DeSimone, a chemical engineer at the University of North Carolina, Chapel Hill, who was not involved in the research, in an e-mail to The Scientist. “[It] strongly validates the idea of using biological evasion strategies.”

Macrophages recognize, engulf, and clear out foreign invaders, whether they’re microbes entering through a wound or a drug-loaded nanoparticle injected to target disease. Previously, researchers have attempted to escape this response by coating nanoparticles with polymer “brushes” to physically block the adhesion of blood proteins that alert macrophages to the particles’ presence. But these brushes can only delay the macrophage-signaling proteins for so long, and they can hinder uptake by the diseased cells being targeted.

With that in mind, Discher and colleagues tried instead to find a way to convince macrophages that nanoparticles are part of the body. Their previous research had shown that a membrane protein called CD47, which binds to macrophages in humans, signals “self” to the immune system, so that particles with this protein are not attacked.

Examining the architecture of the bond between CD47 and its macrophage receptor, SIRPα, the researchers were able to design a synthetic self-peptide with a similarly snug fit. “This is the key, literally, to unlocking innate immune pacification,” said Discher.

When they chemically synthesized the 21-amino-acid self-peptide and attached it to nanobeads as small as viruses in mice genetically engineered to have human-like SIRPα receptors, the researchers showed that beads with the self-peptide stayed in the blood of for longer than beads with no peptide: 30 minutes after being injected with equal numbers of each type, there were 4 times as many beads with the peptide attached than without. The results demonstrate that the synthetic molecule can reduce the rate at which phagocytes clear the beads from the body, said Discher.

Then, in mice with human lung cancer, the researchers injected fluorescently dyed beads with and without the peptide, and saw that the “self”-beads got through the macrophage-filled spleen and liver and accumulated in greater numbers in the tumor, providing a brighter signal under when imaged. In fact, the self-beads provided a signal from the tumor as strong as beads coated with human CD47.

Finally, to see whether the biological evasion strategy can be successfully combined with targeting, the researchers loaded an anticancer drug into self-beads also coated with antibodies that target cancer cells. Sure enough, these antibody-coated self-beads consistently shrank tumors more than antibody-coated beads lacking the peptide. This confirmed that when antibodies draw the attention of the macrophage, the self-peptides inhibit the macrophage’s response, acting as a “don’t-eat-me” signal, said Discher.

The results demonstrate that the synthetic peptide can provide therapeutic nanoparticles with extra time in the body—time that improves drug delivery. Furthermore, the relative simplicity of the peptide means it can be easily synthesized, making it an attractive component for use in a variety of future applications.

“The findings are “compelling” and “the technology merits moving forward,” Omid Farokhzad, director of the Laboratory of Nanomedicine and Biomaterials at Brigham and Women’s Hospital, part of Harvard Medical School, said in an e-mail to The Scientist.
A crucial next step is to test the efficacy of synthetic self-peptides in humans, Farokhzad added. “The truly relevant test is looking at human pharmacokinetics to see circulating half-life advantages of nanoparticles and their effect on therapeutic outcome.”


Circular RNA Surprise
Previously enigmatic circular RNAs have been found to influence gene expression by binding to and blocking another class of regulatory RNA, the microRNAs.

By Dan Cossins | February 28, 2013

Some circular RNA molecules serve as molecular “sponges,” binding to and deactivating gene modulators called microRNAs to influence gene expression, according to two papers published this week (February 27) in Nature. The findings reveal a hidden world of previously unexplored RNA molecules, and act as a reminder that there is more to RNA than simply being a messenger between DNA and the proteins it encodes.

Over the past 20 years, scientists have discovered a series of unexpected forms of RNA, from the unusually short and long to those that blocked other RNA strands from being translated into proteins. But because typical RNA sequencing approaches work by detecting molecules with tails, circular RNAs, whose ends are joined together, went unnoticed. Now, however, new sequencing methods are revealing the existence and function of circular RNA in nematodes, mice, and humans.

“It’s yet another terrific example of an important RNA that has flown under the radar,” Erik Sontheimer, a molecular biologist at Northwestern University in Evanston, Illinois, told Nature. “You just wonder when these surprises are going to stop.”

Looking at a circular RNA expressed in the brains of humans and mice, researchers at Aarhus University in Denmark, found that the molecules blocked a microRNA called miR-7 that usually inhibits gene expression of certain messenger RNAs. So the circular RNA was suppressing the activity of the blocker, resulting in an increase in expression of miR-7’s target genes.

Circular RNAs could also act as sponges for microRNA originating outside the cell, as some have possible binding sites for viral microRNAs. “They are so abundant, there are probably a multitude of functional roles,” Julia Salzman, a molecular biologist at Stanford University School of Medicine in California who was not involved in the study, told Nature.

Natural STD Protection for Women?
An interferon found in the female reproductive tract may help guard against sexually transmitted diseases such as herpes.

By Kate Yandell | February 28, 2013

The cells lining the human female reproductive tract continually express an immune protein that may help protect against infection, according to a study published today (February 28) in Science. The protein, called interferon-ε, improved symptoms of genital herpes and chlamydia in mice.

Other type 1 interferons—cytokines that activate the immune system—are only expressed when a pathogen is present. But interferon-ε, which was first characterized in 2004, appears to be an exception. “We think it’s there all the time, priming the system so you always have some degree of protection,” said Paul Hertzog, an immunologist at the Monash Institute of Medical Research in Australia, one of the discoverers of interferon-ε, and an author of the new paper.

Hertzog and colleagues tested whether the pathways that typically activate type 1 interferons had any influence on interferon-ε in mouse cells and found that they did not alter the protein’s expression. Interferon-ε was constantly present in the mouse reproductive tract in some amount in the absence of key activators, though its levels varied 30-fold depending on where the mice were in their estrous cycle.

The researchers also measured interferon-ε in women and found that, like in the mice, protein levels varied with the menstrual cycle. After menopause, the cytokine almost entirely disappeared. The patterns of its fluctuation indicated that estrogen was promoting its expression, while progesterone and progesterone derivatives were suppressing it, Hertzog said. Sure enough, giving estrogen to mice who’d had their ovaries removed induced interferon-ε expression, supporting this idea.

To explore the clinical implications of their finding, the team created mice lacking the gene for interferon-ε and exposed them to herpes virus and chlamydia. They found that signs of infection were more severe in the interferon-ε knock-out mice. Knock-out mice exposed to herpes had more genital sores than their wild-type counterparts and higher viral levels in the spinal cord, brainstem, and vagina. Knock-
out mice exposed to chlamydia showed more visual signs of infection, and the researchers found more bacteria in their reproductive tracts.

“Their work is very interesting,” said Charani Ranasinghe, an immunologist at Australian National University, whose recent research suggested that interferon-ε could be fighting local infections in the gut, lungs, and reproductive tract. “According to our work, [interferon]-ε is involved in mucosal immunity, and we too believe it could be used as a treatment against several mucosal infections,” said Ranasinghe, who responded to The Scientist by e-mail.

“The data [on herpes] looks pretty good to me, and of course the discovery of this new interferon-ε is very exciting,” said Uma Nagarajan, a professor of pediatrics not involved in the study who studies immune responses to chlamydia at the Children’s Hospital of Pittsburgh.

But the data on chlamydia were incomplete, she said. She would have liked to see how the interferon-ε affected inflammation of the oviducts, an important chlamydia symptom that can cause infertility. Only a small percentage of women who contract a chlamydia infection develop the disease, she said, and so it would be important to further understand role of interferon-ε in the infection’s progression. “They have done very preliminary work on the infection model.”

Going forward, Hertzog wants to also look at interferon-ε’s influence on other sexually transmitted diseases, fungal infections, and even cancers. He added that it could potentially be used as a therapy to provide women with better protection against pathogens, and that understanding the vaginal immune system could lead to a better understanding of how to formulate vaccines for sexually transmitted diseases.


**Prion-like Proteins Cause Disease**

Normal proteins with regions resembling disease-causing prions are responsible for an inherited disorder that affects the brain, muscle, and bone.

By Ed Yong | March 3, 2013

Individuals with a rare inherited syndrome called multisystem proteinopathy (MSP) harbor misbehaving proteins that fold incorrectly, change the shape of surrounding proteins, and clump together—much the way disease-causing prions do. The results, published today in *Nature*, suggest that the 250 or so human proteins with similar prion-like domains may also be involved in diseases of the brain or other organs.

“It is a strong paper,” said Lary Walker from Emory University, who studies the role of misfolded proteins in Alzheimer’s disease and was not involved in the work. “They make a compelling case for the involvement of these mutant proteins in disease.”

“It is likely that seeded protein aggregation will turn up in many more diseases of the brain and elsewhere,” he added. “RNA-binding proteins, and proteins with prion-like domains in general, are a good place to start the search.”

People with MSP suffer from a steady loss of brain, muscle, and bone tissue, as well as motor neurons. They experience the joint symptoms of several diseases, such as Lou Gehrig’s disease (ALS), Paget’s disease of bone, and frontotemporal dementia.

The causes of MSP are largely mysterious. Earlier studies have shown that mutations in a gene called *VCP* could lead to MSP, but J. Paul Taylor of St. Jude Children’s Research Hospital came across two families with many affected members and no signs of VCP mutations. His team, co-led by James Shorter from the University of Pennsylvania, sequenced their exomes and identified new variants that were found only in the affected individuals—one in the *hnRNPA2B1* gene and another in *hnRNPA1*. When introduced into mice and flies, they caused the same type of muscle loss seen in human MSP patients.

Both mutations converted the amino acid valine to another amino acid called aspartate, and both affect parts of the proteins that are similar to domains in prions—infectious misfolded proteins that also
clump together and cause brain diseases. These “prion-like domains” are found in some 250 human proteins, and this study is the clearest indication yet that they might play a role in disease.

Prion-like domains typically take on a loose and unfolded shape, but key mutations allow them to act as molecular zippers. When they find a partner, they zip up, transforming from a floppy, disordered structure into a rigid molecule, turning otherwise free-floating proteins into large clumps. Similarly, the mutant proteins identified in the new study can also force normal versions of hnRNPA2B1 and hnRNPA1 proteins into a more ordered structure and seed fresh clusters.

In normal physiology, proteins with prion-like domains assemble together to create temporary structures called RNA granules, which are necessary for controlling RNA. For example, during stressful conditions, RNA granules shut down the use of unnecessary genes by trapping the relevant RNAs. When conditions are better, the granules disassemble and free their trapped payload. Shorter and Taylor suspect that the two mutations they discovered stop the granules from disassembling, “which ultimately leads to disease,” the duo said in an e-mail to The Scientist.

Shorter and Taylor think that studying more families with MSP may help to reveal the role of other proteins with prion-like domains. “We have now identified about 2 dozen similar families, mostly referred by clinical colleagues, and we are sequencing them right now,” they said.


Bedeviled by Dengue
The global spread of dengue virus has immunologists and public-health experts debating the best way to curb infection.
By Beth Marie Mole | March 1, 2013

In 1961, during the first dengue outbreak physician Scott Halstead ever witnessed, children poured into Bangkok’s hospitals, passing and vomiting blood, faint from blisteringly high fevers. Twenty percent of the children would die within a few days as doctors scrambled to find treatments, with some in nearby Vietnam even plunging children into ice baths in an attempt to hold down their soaring temperatures.

The deadly illness caused by dengue virus wasn’t yet known as dengue in Thailand; doctors there referred to it as “Chinese medicine poisoning” based on a demographic quirk. Although half the city’s population was Chinese, the only time Thai doctors—who practiced Western medicine—treated Chinese children was when the children had been stricken with this mysterious, deadly illness. Thus, doctors imagined that a horrific poisoning caused by Eastern remedies was responsible for the influx of Chinese patients. Instead, Halstead explains, Chinese parents had quickly learned that the hospital, rather than traditional medicine, was the best bet—however slim the chances—for defeating dengue.

Halstead who was drafted into the US Army after World War II and originally sent to Japan in 1957 to study encephalitis, had just settled into a lab across the street from the Children’s Hospital in Bangkok.

“Everything I’ve ever done,” he says in retrospect, “is related to the treatment of dengue.” In the years that followed, he and his colleagues identified dengue as the cause of the outbreak and began tracking the four different versions of the virus, each transmitted by mosquitoes. Among their seminal discoveries, the researchers learned that the hemorrhagic disease that they saw in 1961 was most common when a child is infected with a second type of dengue—a finding that would prove pivotal in the decades-long search for a vaccine that continues today.

The World Health Organization estimates that more than 2.5–3 billion people, or more than 40 percent of the world’s population, are now at risk of being infected with dengue—including some in the developed world.

But as research efforts have evolved, so has the reach of dengue infections. The disease has now become prevalent in more than 100 countries, causing as many as 100 million infections per year. And it’s still spreading. The World Health Organization (WHO) estimates that more than 2.5–3 billion people, or more than 40 percent of the world’s population, are now at risk of being infected with dengue—including some in the developed world. In the past few years, cases of dengue have popped up in Texas, Florida, France, and Croatia. Of the 500,000 cases of severe dengue requiring hospitalization and the roughly 24,000 deaths they cause each year, most are in children, according to the WHO. And control measures have been met with challenges. One high-profile vaccine trial conducted by the French vaccine company Sanofi Pasteur partially failed last year, leaving investigators scratching their heads. The defeat comes amid fears of some researchers that vaccines have the potential to exacerbate a dengue infection rather than protect against it. “We’re in a mess,” Halstead says bluntly.
Going viral

When Halstead started digging into dengue in the 1960s, only a handful of countries were home to all four types of the virus, which represent four separate viral jumps from monkeys to humans between 100 to 800 years ago, according to the US Centers for Disease Control and Prevention (CDC). But in the decades since, the four types—simply called dengue 1, 2, 3, and 4—have independently made their way around the globe. Poor urban planning and warming climates are partly to blame, having opened new territories to the mosquitoes that carry the virus, and global travel and trade have provided the necessary transit.

Tires may be the best example of mosquito and dengue transport: shipped across the globe on barges, tires collect water rings that offer mosquitoes first-class tickets to new locations. Consequently, dengue’s vectors, Aedes aegypti (the yellow fever mosquito) and A. albopictus (the tiger mosquito), have surged in new locations in Central and South America, Australia, and even in the lower United States. The yellow fever mosquito is particularly insidious, biting during the day, and able to spawn in water-filled crannies no larger than a small cup. Its eggs can withstand drought conditions, allowing the population to quickly bounce back after a dry spell. It is now a common pest of dense urban areas—bringing disease with it.

After a cluster of dengue infections struck continental Europe, the European Union (EU) provided funding in 2011 for researchers to assess where dengue would strike next, amid fear that dengue would continue to spread in the developed world. The disease has traveled so quickly that “we only have estimates of the global burden of dengue, which is astonishing,” says epidemiologist Simon Hay of Oxford University, who is part of a consortium that’s developing risk maps of future dengue spread. The group is currently working on a map of where the disease exists now; only later will it work on how warming climates and city sprawl might change the map, says Jane Messina, the head medical geographer on the project.

Concerns have flared over the possibility that dengue could easily become endemic in Europe and in the United States, which had its own cluster of infections in 2012 in Texas and Florida. A. aegypti is now found in 23 states and A. albopictus in 26. “Dengue can occur anywhere the mosquito vectors occur,” says Ronald Rosenberg, the associate director of the CDC’s division of vector-borne diseases and a member of the WHO’s committee on neglected tropical diseases.

Concerns have flared over the possibility that dengue could easily become endemic in Europe and in the United States.

If experience from abroad is any indication, the economic impact of a dengue epidemic could be huge. Though severe disease is usually rare, it creates a big burden on health-care systems, says microbiologist and dengue expert Aravinda de Silva, of the University of North Carolina at Chapel Hill, who works in Sri Lanka where dengue has long been endemic. “Hemorrhagic fever is a massive concern. Every parent is terrified of their child getting it,” he explains. Parents often bring their children into the hospital at the first sign of dengue infection, which leads to many unnecessary hospitalizations—and the costs add up. With precautionary care as well as treatments for those who do get severe disease, dengue outbreaks weigh heavily on local economies—an outbreak in Thailand in 1994, for instance, cost an estimated $51 million, not including dengue prevention programs. Such an economic strain has brought the infection to the top of priority lists in health ministries around the world.

The global spread of dengue over the past 3 decades, however, has driven more resources to research on how the virus spreads and how it can be defeated.

Catching a virus

Before the name “dengue” caught on in the Americas, it was often referred to as “breakbone fever,” or la quebradora in Spanish. The nickname refers to the crippling aches that come with infection. Once delivered by a mosquito, dengue virus hijacks skin and some immune cells and hitchhikes through the lymphatic system, infecting other tissues and organs and loading the bloodstream with viral replicates. The body’s immune system counters with blazing inflammation, causing sharp pains in muscles.

Eighteenth-century reports of dengue infection describe the wretched gaits of patients as they staggered on swollen limbs, often in a stupor from fever.

For the lucky, those will be the worst of their symptoms. Others, however, can develop life-threatening disease. If the inflammation firestorm gets out of hand, it can damage the lining of capillaries—the smallest blood vessels—causing gaps through which blood plasma can seep. Systemic internal leaking quickly leads to a drop in blood pressure, followed by shock, organ failure, and massive bleeding.

Between 2005 and 2007, Managua, Nicaragua, saw a mysterious spike in the number of children entering hospitals with dengue hemorrhagic fever and shock syndrome. Since the 1980s, the
neighborhoods around the city had been riddled with *Ae. aegypti* as well as the four dengue types they carry, but no one knew why so many more children were suddenly developing severe disease. Infectious disease researcher Eva Harris and her team from the University of California, Berkeley, and the Nicaraguan Ministry of Health had been working on infectious disease there for decades, and noticed severe dengue disease occurred in children reinfected with a second type of the virus, similar to the pattern Halstead saw in the 1960s with second infections.

Simona Zompi, an immunologist working on Harris’s team, was dissecting the immune system’s response to dengue. When a person is initially infected, the dengue viral particles attack skin and immune cells by latching onto receptors on the cell surface. The cells enclose the viruses in a sac—a process called receptor-mediated endocytosis—that would normally digest the viral captives with enzymes in an acidic milieu, like a piece of food in the stomach. But dengue virus particles escape digestion by rearranging their envelope proteins to fuse with the sac’s membrane, opening a channel through which the capsid-encoated viral genome is released into the infected cell’s cytoplasm. Once free, the virus usurps the cell’s machinery to create a viral factory, triggering a full-blown dengue infection.

Zompi and the team focused on B cells, which react to the infection by creating antibodies against the infecting virus. Once the virus is defeated, some of these cells go into hibernation as memory B cells, which become quickly reactivated upon reinfection with dengue for the rest of the person’s life. Those antibodies normally foil infection by blanketing the viral particle, which prevents the virus from binding to cell receptors and entering cells. The antibody-coated virus is then taken up by monocytes or macrophages, which, after endocytosis, can digest the invader because the coated particle is unable to escape the digestive sac. (See illustration below.)

However, this protective immune response depends on whether antibodies can bind strongly enough to coat and disable—or neutralize—the virus, Zompi explains. Some antibodies bind poorly to the viral particle and, as a result, only a few manage to cling to the virus, which is just enough to entice macrophages and monocytes to engulf them. However, without complete antibody coverage of the viral coat, the virus can still escape from the endocytic sacs of the macrophages or monocytes and take over the very cells that have engulfed it. (See illustration below.) Now, the virus has an additional pathway to enter cells: all of the immune cells recruited and activated by the antibodies could potentially become virus-producing factories. Thus, antibodies with poor binding affinity and neutralizing potential effectively boost the infection, and the inflammatory response can crank to inferno levels, causing hemorrhagic fever and shock syndromes. Scientists refer to this explanation for severe disease as “antibody-dependent enhancement” (ADE) of the immune response.

Most antibodies generated against a specific dengue type, dengue 1 for example, will bind well and be able to thwart future infections of that type. But when a new type of dengue invades—dengue 2, for instance—the reactivated dengue 1 antibodies may only partially recognize the virus and thus lead to ADE. Indeed, Harris and Zompi found that as the predominant circulating dengue virus type switched in Managua, patients hospitalized with dengue made the strongest antibody response to a dengue type from a previous infection, not the current infecting dengue type. And most countries are infested with the four types of dengue that can fluctuate in prevalence.

Some researchers worry that vaccines designed to spur antibody production might instead trigger ADE if the B cells generate low-affinity antibodies rather than those that completely blanket the virus. If that were the case, being vaccinated could predispose children to ADE.
Vaccine development dissension
But not all dengue researchers are convinced that severe disease can be explained by the ADE response, says de Silva. There are two competing hypotheses about how severe hemorrhagic and shock diseases might unfurl: other immune cells, such as T cells, may present different fragments of the virus to the immune system, stimulating a greater inflammatory response; or, more virulent subtypes of each of the dengue types may trigger the more severe immune reaction characteristic of ADE. But the antibody hypothesis is certainly the front-runner, de Silva says—especially after his recent discovery of how dengue-neutralizing antibodies work.

A system makes a range of antibodies with different affinities to the virus. The antibodies capable of neutralizing the virus apparently latch onto a wedge between two adjacent molecules of an envelope protein on the coat of the intact virion. The new finding is significant because it casts doubt on past immunology and vaccine-development studies that have focused on generating an antibody response against only a small fragment of the envelope protein in isolation. Antibodies that don’t neutralize the virus by binding to the wedge may be candidate ADE generators, says de Silva.

Acknowledge the new complication, says Halstead. “A lot of people don’t like to have to address [the possibility of ADE] head on,” he says.

Indeed, current recommendations by the WHO on conducting clinical trials of candidate dengue vaccines don’t include specific guidelines for ADE. In fact, ADE and its potential causes are considered a hypothetical concern that should not interfere with development of a vaccine, according to the WHO.

“ADE may not be a concern, in my own opinion,” says infectious-disease researcher Claire Huang, of the CDC’s division of vector-borne diseases, echoing the WHO’s stance. Along with a team of researchers at the CDC, Huang has been toiling for years to develop an effective vaccine, and is working with the commercial vaccine developer Inviragen on a vaccine now in Phase 2 clinical trials. It may be more complicated, she adds, but most antibody responses are protective and robust.

The vaccine she’s been working on is tetravalent, meaning it’s designed to generate antibodies against all four types of dengue. Huang and her team borrowed the genetic backbone of an earlier successful vaccine against the type 2 virus, and dressed that genome, with its attenuating mutations, in the other three viral coats, creating four recombinant viruses. “From the outside, they all look like either dengue 1, dengue 2, dengue 3, or dengue 4, but inside they’re all the same attenuated virus,” she explains. The team hopes that the vaccine will produce a suite of neutralizing antibodies against all types of dengue.

So far, the results look promising. The vaccine sailed through Phase 1 trials, proving safe in healthy children and adults in Colombia and the U.S. who had no prior exposure to dengue. But the earlier tetravalent vaccine made by Sanofi Pasteur also had great early results. After buzzing through initial safety tests and Phase 2 trials, that vaccine moved to a Phase 2b trial, which enlisted 4,000 school-age children in Thailand’s Ratchaburi Province. The vaccine required three shots over the course of a year, and in September the company revealed that only 30 percent might be protected, although none of the results were statistically significant.
“I looked at the vaccine results and wondered if they had given people water,” Halstead says. “It was very surprising,” echoes de Silva. It’s still unclear what went wrong, he says. The vaccine showed 80 to 90 percent protection against dengue types 3 and 4, and around 60 percent protection against dengue 1. But it failed at protecting against dengue 2—the dengue that was circulating that year at high levels. The question we’re left with, de Silva says, is whether that virus was just a mismatch with the vaccine, or if the dengue 2 portion of the vaccine simply didn’t generate a good antibody response in the vaccinated children.

The only triumph of the trial was that it didn’t show an increase in severe disease. Of the 4,000 children vaccinated, only five developed severe disease. “The vaccine was still very safe,” says Dan Stinchcomb, cofounder and CEO of Inviragen. “[The Sanofi] vaccine is somewhat similar to ours, and the trial laid to rest one of the biggest concerns,” he says. The study demonstrated “that if you’re not fully protected against all four viruses, then you’re not more susceptible to severe disease,” or ADE.

But Halstead points out that the trial only followed children for a year—not long enough for them to become infected a second time. In that time frame, it’s impossible to know if the risks of ADE are actually diminished, he says.

A different angle
In the meantime, others are looking to non-vaccine strategies to contain the disease. Despite the potential for spread in the developed world, most global health experts are focused on developing countries. With advanced water-management systems, responsive public-health programs, and effective disease monitoring, dengue outbreaks in the U.S. or Europe have a good chance of being quashed quickly, says Rosenberg. “The risk pales in comparison to the daily risk of getting dengue in the tropics,” he adds. Because of logistical barriers in developing countries, some experts doubt that vaccines are the best answer to the dengue problem. “Implementing a vaccine is difficult,” says Rosenberg. For example, if the vaccine requires multiple boosters, most people in low-income communities will have difficulty receiving and/or affording all of the doses. “It’s unlikely that the vaccine is going to be the panacea for controlling dengue,” he says.

In the absence of an effective vaccine or a halt to mosquito breeding, clinicians have honed the art of treating severe disease. With no infection-specific protocol, doctors treat severe disease by compensating for lost fluids, which is akin to constantly pumping up a punctured tire. The challenge is to maintain a patient’s blood pressure at a high enough level to circulate blood without going too far and “popping” the system, thus causing life-threatening edemas that saturate the lungs or brain. This is tricky to do in adults, but even trickier in infants and children, who are more likely to develop the disease.

However, more and more doctors are beginning to learn best practices for controlling the disease. In Bangkok, where Thai clinicians are at the forefront of clinical case management, a 20 percent mortality rate of dengue-infected patients in the 1960s is now down to just 0.1 percent. Doctors and nurses convene after each death to discuss in painstaking detail what went wrong. Clinicians now have such a fine understanding of the physiology of the disease—and how to track and control it by monitoring vital signs and urine output, and administering delicate fluid therapies while monitoring plasma volume—that death is avoidable, says de Silva.

In Nicaragua, Harris and UC Berkeley-based researcher Josefina Coloma are working with an international nonprofit group to spur grass-roots community projects to educate residents about the mosquito life cycle, and to motivate them to eliminate standing water that can be a breeding ground for Ae. aegypti. Their preliminary findings showed that community efforts were able to reduce dengue infection. Simply informing people of the link between standing water and disease transmission has had an important impact, Harris says.

Indeed, the inability to keep dengue from spreading is a shame, Halstead says. Despite advanced research, he argues, dengue is a disease of medieval sanitation and water systems, irresponsible urbanization, and a lack of basic education about disease spread. “If we stop dengue by immunizing,” without mosquito controls and other prevention methods, Halstead says, “then I would say that human beings have copped out. If dengue [only] ends because of a dengue vaccine, then we’ve failed in our public-health efforts.”

**Immune to Failure**
With dogged persistence and an unwillingness to entertain defeat, Bruce Beutler discovered a receptor that powers the innate immune response to infections—and earned his share of a Nobel Prize.

By Karen Hopkin | February 1, 2013
Bruce Beutler published his first scientific paper at the age of 16. As an apprentice in his father’s lab at the City of Hope National Medical Center in Duarte, California, Beutler learned how to purify proteins and assay their activity—work that led to a pair of publications on the enzyme glutathione peroxidase, including that first one in the *Annals of Human Genetics* in 1974.

“I don’t think there were any other students in my high school who were spending their afternoons and weekends that way,” laughs Beutler. “But in those days I was very ambitious.” Graduating from the University of California, San Diego, when he was 18, Beutler went on to obtain a medical degree from the University of Chicago. “That was something my father advised me to do. He said, ‘If you go to medical school, you’ll learn all about the workings of one specific organism.’” This knowledge, Ernest Beutler told his son, “will be extremely useful to you, no matter what you decide to do in biology.”

“He also said, ‘If you don’t do well, you’ll have something to fall back on. You can always see patients.’” But for the young Beutler, that possibility seemed remote. “It never occurred to me that I might fail. Such thinking seemed counterproductive. If you allow for the possibility of failure, I think you’re less likely to succeed.”

That indefatigable zeal kept Beutler on track as he labored to unravel the mechanisms by which mammals detect and eliminate infection—work that would earn him a share of the 2011 Nobel Prize in Physiology or Medicine. Here he talks about admiring a mutation, constructing a catwalk, and making his family proud.

“I think it’s foolish for people to believe that all the easy things have been discovered.” Beutler breaks out *The call of the lab.* After completing his internship and residency at The University of Texas (UT) Southwestern Medical Center, Beutler “missed having the opportunity to make real discoveries.” So in 1983, he became a postdoc in the laboratory of Anthony Cerami at Rockefeller University. Cerami was studying cachexia, a form of wasting that often accompanies cancer or serious infections. In the latter case, the presence of a microbial molecule—such as the lipopolysaccharide (LPS) that coats the outer membrane of many bacteria—was thought to trigger the release of some yet-to-be-discovered factor that prevents host tissues from storing energy. “I was chomping at the bit to purify proteins again, so I energetically set about finding the molecule responsible.”

**Double duty.** The protein Beutler wound up isolating from mice, dubbed cachectin, shut down fat cells’ ability to take up triglycerides. But that wasn’t all it could do. “Cachectin turned out to be the mouse ortholog of human tumor necrosis factor [TNF], which had itself been recently isolated,” he says. So cachectin could also kill tumor cells. “For the first day or so I was actually disappointed, because some of the novelty was compromised. But then I realized, we now knew a great deal about the activities of TNF that no one else knew”—including the fact that it was an extremely toxic protein. “Those who had been working with TNF had been treating it as innocuous, and as a potential chemotherapy agent.” But Beutler found that mice injected with cachectin, now known as TNF, would die of shock within hours—and that blocking this protein with antibodies protected them from this form of toxic shock. The papers outlining these discoveries—which included a handful in *Science* and *Nature* throughout 1985 and 1986—“changed the whole thinking on TNF.”

**Thwarting TNF.** Beutler continued to pursue TNF when he left Rockefeller to start his own lab at UT Southwestern in 1986. His first order of business was devising a way to block the molecule’s actions. The plan was to sop up circulating TNF using its receptor as the sponge. The receptor had been cloned in another laboratory, and when the paper came out, Beutler says, “we more or less pounced on it.” He and his trainees, graduate student David Crawford and postdoc Karsten Peppel, attached the TNF-binding domain of the receptor to a hunk of antibody molecule, generating a large fusion protein that would be difficult for the body to eliminate. “It worked like a charm,” says Beutler. “The hybrid had a long half-life in vivo and was effective at neutralizing TNF.” That chimeric receptor, described in the *Journal of Experimental Medicine* in 1991, is now used to treat autoimmune disorders that stem from an inappropriate generation of TNF, such as rheumatoid arthritis and psoriasis.

**Swimming upstream.** Why TNF is produced in autoimmunity, Beutler says, “nobody really knows. That’s one of the big mysteries.” Could it be that the presence of a microbial component like LPS triggers an inflammatory response that then goes awry? “I thought if we could find the LPS receptor, we’d have a handle on the problem—and we’d learn how the body ‘knows’ it’s infected. It was that work that led to the Nobel Prize.” But it didn’t lead there quickly. “First I tried biochemistry. It was known that there were mice that were resistant to LPS”—animals that did not mount an immune response when exposed to this bacterial product. “It was speculated that these animals must have something wrong with their LPS receptor. So I tried to look for the receptor by comparing the membrane proteins of those mice to...
control strain that was sensitive to LPS. But that got me absolutely nowhere.” Three years and several different approaches later, Beutler was no closer.

“Then I decided to try positional cloning”—an approach that narrows down the location of a gene by determining which DNA markers tend to be inherited along with a given trait. At the time, the gene was “believed to be within an area that constituted about an eighth of chromosome 4.” With their first round of matings—which produced about 500 mice—they whittled that down to one-sixteenth of chromosome 4. “So we didn’t make a huge amount of progress.” Another series of matings—and another 2,000 mice—brought it down to about 2.6 million base pairs. “That was still an absolutely enormous area. But we couldn’t seem to reduce it any more than that.” So they started sequencing. “Our days consisted of setting up mini preps in the afternoon and then looking at ladders of bases on X-ray films the next morning. My assistant Betsy Layton would read the sequence to me and I would type. Then we would exchange duties. For 3 years, that was how it went.”

**Red herrings & the Big Kahuna.** Occasionally, those sequences would yield something that looked promising. “We might find a gene that appeared to exist in many different splice forms and think, ‘Could this be the basis of receptor diversity in the innate immune system, and maybe it’s the splice form that codes for the LPS receptor that is defective in these mice?’” Then they would sequence the corresponding cDNAs from both resistant and sensitive mice and find no difference. “And our hopes would be dashed. It wasn’t until we’d covered more than 90 percent of the region that we found what we were looking for”—thanks to the rise of genomics.

About midway through the project, geneticists had begun to catalog expressed sequence tags (ESTs)—short fragments of cDNAs that correspond to active genes. Every afternoon, Beutler’s crew would search the EST databases to see if any of their chromosome 4 sequences matched those of an expressed gene. “One night I was home, going over the results, and I saw a very strong hit.” One of the team’s sequences matched a gene that encoded a receptor called TLR4. But was this really the gene? TLR4 appeared to have some homology with another receptor involved in inflammation—one that binds IL-1—as well as with a protein called Toll that Jules Hoffmann had linked to immunity in *Drosophila*. “That encouraged me,” says Beutler. “But perhaps not as much as the fact that we had covered 90 percent of the area and we were running out of DNA. I thought: this must be the gene because we’re almost out of places to look.” It wasn’t until they saw a mutation—a single base-pair change in the gene from the LPS-resistant mice—that they knew they’d found the LPS receptor. “I kept coming back to look at the computer, thinking it might just be a mistake or that it was my imagination,” says Beutler. Postdoc Alexander Poltorak, the lead author on the resulting 1998 *Science* paper, had a similar reaction. “Both of us would come back to admire the mutation two or three times a day.”

**The screen team.** “Since then,” Beutler says, “I have been absolutely in love with genetics!” Now back at UT Southwestern, after spending 11 years at the Scripps Research Institute, Beutler continues to make mutant mice and confer with colleagues to devise new screens for identifying intriguing phenotypes, especially those that pertain to an ability to respond to infection. “It’s really wonderful to know that when you come to the lab in the morning, it’s likely that somebody is going to have uncovered a bizarre new phenotype—and that it’s going to be relatively easy to figure out what gene has been hit.” In about a third of the cases, he says, they discover something exciting. “This is a very effective way to find the unexpected, to see things that have maybe never been seen before.”

**Beutler believes California dreamin’.** “I remember having dreams about the [LPS receptor] gene. One time, I was in a hotel in the mountains in San Bernardino and I woke up in middle of the night suddenly convinced that I knew exactly what the gene was. I wrote it down on some paper at the bedside and fell back into a very satisfied sleep. When I woke up in the morning, I found it was absolute gibberish—some nonsensical enzyme that gave no insight at all. Of course, in the dream it made perfect sense.”

**Catching the fever.** Through all the ups and downs, Beutler stuck with the search for the LPS receptor. “It was pure obsession—a deep, visceral desire to find the gene. It was a kind of addiction. You might compare it to gold mining. People became addicted to that. They caught the fever and couldn’t stop. Because every day might bring the mother lode. I had this one goal and I always felt like success was just around the corner. It was so tantalizing. Although it was stressful and a lot of work, that was the most intense and exciting time of my career.”

**If we could do then . . .** “If we had to solve the problem of why this particular mouse strain does not detect LPS starting today, we would know the answer in about a week. We would just sequence the whole genome of the resistant strain and compare it to the reference sequence or to the sensitive strain. Massively parallel sequencing has changed the game completely.”
**Don’t sweat the big things.** “I tell trainees to try not to be deterred by the difficult funding situation. Because it’s always been difficult. And even though it seems like there’s so much more competition now, with advances in technology there really are more things to work on. I think it’s foolish for people to believe that all the easy things have been discovered, or that we’ve reached the end of what we can know. I see science as providing more opportunities than ever before. And I think it will only continue to accelerate.”

**Capitol connection.** In Stockholm, Beutler addressed a group of parliamentarians that meets with invited investigators to discuss issues of scientific importance. On his return, Beutler happened to mention the meeting to Texas Senator Kay Bailey Hutchison, who then organized a similar session for a handful of senators and Nobelists. Such high-powered powwows might be one way to influence policy. “If scientists remain mute, then we ought not to complain when legislation doesn’t go our way.”

**R-E-S-P-E-C-T.** “I went through some rough times with my sons in their adolescence. In those days, they thought I was sort of a nerd and they couldn’t understand why I was doing what I was doing. But when I won the Nobel Prize, they were so proud. I think that’s the greatest thing about the Nobel Prize. It’s sort of like a prize for them, too.”

**Beutler finds balance**

**Home sweet home.** Beutler worked with a builder to design his dream house in Dallas. “It’s kind of Tuscan, with a Southwestern feel”—and a catwalk that looks down over the living room. “I spend a good deal of time up there just musing.”

**For the birds.** “I used to be an avid birder. When I was in San Diego, I liked to go to the San Gabriel Mountains or sometimes to the desert or the seashore. But I’m happy to look for birds anywhere. I like being close to nature and seeing things I haven’t seen before.”

**Embracing Bach.** Beutler fell for Bach at the age of 15. “I went to a performance of the St. Matthew Passion, and I remember getting chills. I’d never heard a great choral masterpiece performed live before. I believe we evolved an ability to recognize the emotional quality of the human voice, and that explains much of our appreciation of music.” He even tried his hand at writing his own fugues. “I wanted to feel what was so special about creating music in the style of Bach. Natural scientists discover things—but Bach invented them. It’s good to strive to be as inventive as one can be—particularly in science.”

**Greatest Hits**

- Discovered that tumor necrosis factor (TNF) mediates endotoxic shock, and turned its receptor into a therapeutic agent that eradicates TNF activity.
- Using a classical genetic approach, identified the receptor that recognizes the microbial molecule lipopolysaccharide (LPS). Demonstrated that this LPS receptor, called TLR4, is a key player in innate immunity, as it alerts the body to a broad range of bacterial infections. These efforts were recognized by a 2011 Nobel Prize.
- Launched a program to generate and study hundreds of informative mouse mutants, many of which show defects in immune function. These mice are fostering the discovery of molecules involved in immune signaling.

**Salt at Fault?**

**Two groups of researchers independently showed that high salt exposure stimulates cells implicated in multiple sclerosis and other autoimmune diseases.**

By Kate Yandell | March 6, 2013

Salt may play an important role in autoimmune diseases, according to two new papers published today (March 6) in *Nature*. Exposure to high levels of salt was found to make both cultured mouse and human T cells more pathogenic, and high-salt diets worsened autoimmune disease in mice.

“I thought the papers were very exciting and provocative,” said John O’Shea, a doctor at the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS), who wrote a *Nature* commentary accompanying the new findings and was not involved in the study.

Lawrence Steinman, a neurologist and immunologist at Stanford School of Medicine, who was also not involved in the work, said, “I think it’s beautiful research looking at the pathways that feed to one of the major types of autoimmune cells.”

The first research team, based at Harvard University, the Massachusetts Institute of Technology (MIT), and the Broad Institute, came to investigate salt in a roundabout way. Some forms of T helper cells, called T helper 17 (TH17) cells, have been implicated in a variety of autoimmune diseases, and the researchers wanted to understand what makes naive, immature T cells differentiate into pathogenic ones.
Under ordinary circumstances, T helper cells protect the body from pathogens, and each differentiated T helper cell type specializes in a different type of invader. The TH17 cells target bacteria and fungi. But a certain flavor of TH17 cells, which the researchers call “highly pathogenic,” appears to be involved in attacking the body’s own cells.

To understand how TH17 cells come to be, Aviv Regev, a computational biologist at the Broad Institute and MIT, worked with colleagues to take snapshots of regulatory circuits as T helper cells developed into TH17 cells. Collaborating with the lab of Vijay Kuchroo of Brigham and Women’s Hospital, the researchers organized the proteins into a hierarchy of importance as nodes in regulatory pathways that influenced the development of TH17 cells, which they published in a third paper in Nature today. The enzyme SGK1 was at the top of their list.

Knowing that SGK1 is involved in mediating salt uptake in the gut and salt reabsorption in the kidneys, the researchers decided to see what happened if they added extra salt to the cells. Not only were the salt-cultured mouse T helper cells more likely to develop into TH17 cells, but the cells that developed were more pathogenic. When the researchers fed mice a high-salt diet and induced EAE, a mouse model for multiple sclerosis, the mice showed worse symptoms than usual. When they knocked out SGK1, salt’s ill effects went away. “Salt has got this unique ability to convert the non-pathogenic cells to the pathogenic ones,” Kuchroo said.

Meanwhile, David Hafler’s lab at Yale University was coming to similar conclusions from the opposite direction. The group had completed a study where they measured TH17 cells in the blood of healthy human subjects, sequenced the people’s microbiomes, and had them fill out questionnaires about their diets. While the study was supposed to be focused on the influence of the microbiome, the researchers noticed that participants who frequently ate in fast food restaurants had elevated levels of pathogenic TH17 cells. They hypothesized that the saltiness of the food could be part of the explanation.

“That led to a whole series of experiments trying to figure out the role of salt,” Hafler said. Unlike Regev and Kuchroo’s labs, which looked at TH17 differentiation in mouse cells, Hafler’s lab added salt to human cell cultures. They also found that it was associated with more pathogenic TH17 cells. “Salt just seems to trigger all the genes associated with bad autoimmune T cells,” Hafler said.

He and colleagues looked at the genes expressed in the cells during development using a microarray chip. A variety of genes involved in salt-related inflammation, including SGK1, were upregulated, and a series of tests showed that several of these genes were necessary for salt to have its deleterious effect. Experiments in mice with EAE also supported the findings, and Hafler is now seeking grants to study the effects of a low-salt diet on autoimmune disease in humans.

“It suggests a very interesting hypothesis about an environmental factor that we all consume in our diet and a set of immune disease we see greatly increasing in Westernized cultures,” said Regev.

But O’Shea cautioned that salt has not been explicitly shown to have an effect on human autoimmune diseases. “This is an artificial model of autoimmunity,” he said. The scientists induced autoimmunity in the mice, and the salt only exacerbated their condition. It remains to be seen, he said, whether salt can induce or worsen disease in humans.


**Novel Virus Entry Portal Found**

Researchers identify the target protein of a recently discovered human coronavirus, shedding light on infection and possible interspecies spread.

By Sabrina Richards | March 13, 2013

Dutch researchers have identified the host cell protein that allows a recently discovered coronavirus to enter its target cells, according to a study published today (March 13) in Nature. The structure of the protein, called DPP4, appears to be conserved between bats and humans, suggesting that the new findings will help shed light on zoonotic transmission of the virus, as well as provide a target for potential vaccines.

“It’s a huge study,” said Ralph Baric, a virologist at the University of North Carolina, Chapel Hill, who was not involved in the research. “Any time you identify a receptor that a virus uses, it tells you a whole lot about its biology—where it targets, how it causes disease. And it tells you a lot about trans-species movement and the frequency of that event.” The fact that the new coronavirus can use both bat and
human receptors for infection without drastically mutating raises the unsettling possibility that some coronaviruses are “pre-primed” to jump species, Baric added.

Until the deadly SARS virus was identified as a coronavirus in 2003, only two coronaviruses were known to infect humans, generally causing mild colds. Since then, three more have been identified, though none as virulent as SARS. The latest virus, discovered in a Qatari man in June 2012 and dubbed human coronavirus-EMC (hCoV-EMC) by researchers at the Erasmus Medical Center (EMC) in The Netherlands, is associated with severe respiratory disease and kidney failure, and, like SARS, appears related to bat coronaviruses. Unlike SARS, however, the new virus doesn’t easily transmit between humans. Of the 13 people who’ve been infected, eight have died, though it’s not clear whether the virus was the direct cause of death, noted EMC virologist Bart Haagmans.

Coronaviruses get their name from structures called spike proteins that stud their capsids and determine which host cells a particular coronavirus can bind and infect. In order to understand which species and which cells hCoV-EMC targets, Haagmans collaborated with researchers at Utrecht University to create a recombinant EMC spike protein and screen for cells that bound to it, identifying a human liver cell line. The researchers then used a detergent to release the liver cells’ membrane-bound proteins, employing the recombinant spike protein to fish out the specific receptor to which the spike protein bound. Finally, a mass spectrometry analysis of the protein identified it as dipeptidyl peptidase 4 (DPP4)—a novel receptor for coronaviruses.

Sure enough, antibodies against DPP4 block virus infection, while expressing DPP4 in virus-resistant cells rendered them easily infected by hCoV-EMC. The researchers also found that DPP4 is expressed on a variety of cells, including kidney cells and cells deep in the lung. In contrast to viruses like influenza, which infect upper respiratory cells and transmit easily between people, hCoV-EMC’s deep lung infection may explain the virus’s propensity for causing severe respiratory disease but being difficult to transmit between humans, said Haagmans.

“Now [that] we know that EMC spike and human DPP4 are likely the most important [proteins in hCoV-EMC infection], we can start looking at ways to interfere with that interaction to protect people,” noted virologist Rachel Roper of East Carolina University, who was not involved in the research.

This could include using recombinant molecules to screen for drug targets that block spike protein binding to DPP4 or creating vaccines focused on the EMC spike protein to raise antibodies that prevent viral binding to target cells, Roper noted. Indeed, a recent Journal of Virology paper demonstrated that serum from hCoV-EMC-infected patients could block virus binding to target cells, suggesting that neutralizing antibodies can be produced.

The researchers also found that the virus can infect bat cells using bat DPP4. Though hCoV-EMC is related to bat coronaviruses, so far no infections have been linked to bats, suggesting an intermediate host carried the virus from bats to humans, said Gary Whittaker, a virologist at Cornell University who was not involved in the research.

DPP4’s role in hCoV-EMC’s pathogenesis should also be investigated more fully, noted Sonia Navas-Martin, a molecular virologist at Drexel University who did not participate in the study. DPP4 plays important roles in hormone regulation and it may be that hCoV-EMC affects the protein’s function after infection, she explained. But for now, the findings are a huge step forward. “To my knowledge, DPP4 was not previously known as a receptor for any other virus.”


**High Maternal Viral Load Key to Vertical Transmission of Hepatitis B**

Mar 15, 2013

By Megan Brooks

NEW YORK (Reuters Health) Mar 15—High maternal viral load is the most important factor causing maternally transmitted hepatitis B virus (HBV) infection and is significantly correlated with e antigen (HBeAg) positivity, according to a prospective study from Taiwan.

"Additional interventions should be considered in these mothers," Dr. Huey-Ling Chen from the Hepatitis Research Center, Hospital and College of Medicine, National Taiwan University in Taipei and colleagues conclude in a report online now in the Journal of Hepatology.

They say their data also provide "important information for the rational design of future screening and intervention strategies to further reduce maternally transmitted HBV infection."
Despite effective immunoprophylaxis, breakthrough HBV infection does occur and may result in mother-to-infant transmission. Transmission from highly viremic mothers remains a major challenge in eradicating HBV-related diseases, the investigators say.

Some studies have suggested that antiviral therapy in highly viremic HBV-infected pregnant women can reduce maternal viral load and transmission risk. Yet the optimal cutoff level of maternal viral load for antiviral therapy in pregnancy remains a topic of debate, they note.

Dr. Chen’s team designed their prospective study to assess the rate and risk factors for maternally transmitted HBV infection despite immunoprophylaxis.

Mothers who were positive for hepatitis B surface antigen (HBsAg) were invited to join the study at the time of prenatal visits or delivery. All were HIV-negative. Maternal viral load was determined by a real-time PCR-based assay. Children were tested for HBsAg between four and eight months and/or one to three years of age. The study included a total of 303 mother-infant pairs.

The researchers report that 81 mothers (26.7%) were HBeAg-positive; all of their infants as well as most infants with HBeAg-negative mothers received hepatitis B immune globulin.

HBeAg-positive mothers had significantly higher viral loads than HBeAg-negative mothers (7.4 vs 2.7 log10 copies/mL, p<0.0001).

The 10 children born to HBeAg-positive mothers with high viral load (median, 8.4 log10 copies/mL) were chronically infected.

Maternal viral load was significantly associated with risk of infection in analyses adjusted for maternal age, birth type, factors related to maternal-fetal hemorrhage, gestational age, infant gender, birth weight, timeliness of vaccination, and feeding practice. The adjusted odds ratio for each log10 copy/mL increase was 3.49 (95% CI 1.63 to 7.48; p=0.001).

High maternal viral load was “the most important factor in maternally transmitted HBV,” the investigators say. The rate of neonatal infection at maternal viral load 7 log10 copies/mL was 6.6%, and jumped to 14.6% and 27.7% at 8 and 9 log10 copies/mL, respectively.

The investigators think additional strategies to further reduce mother-to-child transmission should be considered in mothers with a viral load above 7-8 log10 copies/mL.

While there is no consensus on the optimal cutoff value of maternal viral load for antiviral treatment, the investigators say their findings support antiviral therapy to reduce transmission in HBV-infected pregnant women with a viral load above 7 log10 copies/mL—and especially in those with a viral load above 8 log10 copies/mL.

Dr. Chen and colleagues emphasize, however, that the advantages of antiviral therapy need to be balanced against the risks. “Although current reports show no significant increase in birth defects and pregnancy complications,” they say, “more long-term safety data of antiviral therapy, continued epidemiological surveillance on HBsAg-positive mothers and their children, and cost-effectiveness analyses are needed to develop a safe and cost-effective preventive intervention strategy.”

Dr. Chen did not respond to request for comment.

Dr. Ameeta Singh, clinical professor in the division of infectious diseases at the University of Alberta, Edmonton, who reviewed the study for Reuters Health, said the findings are "definitely significant and support previous similar reports from the published literature. They also report increasing rates of transmission at increasing levels of viral load which makes sense but it is always helpful information to both patients and providers when deciding on treatment."

Dr. Singh also noted that the exact viral load cut-off at which treatment should be offered is unclear "and varies between specialists."

Information from Industry
Rebif® (interferon beta-1a): Update your knowledge
Explore efficacy data
"Very few places," she added, "offer routine services and recommend referral to a specialist of all HBsAg infected mothers during pregnancy—many providers are either not aware that anything other than routine immunoprophylaxis can be offered in some situations. In response to similar work that we undertook a few years ago, the province of Alberta has made some changes to provincial programming to encourage providers to refer all HBsAg infected mothers to specialists."

"I think raising awareness among clinicians—particularly primary care providers and OB/GYN—that more can be done to reduce mother-to-child HBV in certain situations would be a good thing," Dr. Singh concluded. The authors and Dr. Singh have no conflicts of interest. The study was funded by the Center for Disease Control, Department of Health, Taiwan.

J Hepatol 2013.
Mozambique Completes First HIV Vaccine Clinical Trial

Last week, "researchers at Mozambique’s Polana Cancio Centre for Research and Public Health completed a trial evaluating the safety of an HIV vaccine candidate," the country's "first HIV vaccine trial," and it "is set to embark on a second, a demonstration of the country's increased HIV research capacity," PlusNews reports. The Phase I study "was conducted through the U.K. HIV Vaccine Consortium’s Tanzania and Mozambique HIV Vaccine Programme (TaMoVac)," the news service writes, noting the second study of a Phase II HIV vaccine candidate also will be conducted by TaMoVac. "According to Ilesh Jani, director general of Mozambique's National Institute of Health, the studies, while small, mark important first steps towards bolstering clinical trial and research capacity for diseases such as HIV and malaria," PlusNews states, adding, "These diseases, along with malnutrition, continue to drive death rates in the country" (3/15).

HIV Treatment Should Be Started Earlier For Most, But Challenges Remain

Noting "French researchers have identified 14 adults" who appear to have been functionally cured of HIV after beginning treatment "within a couple of months of infection" and continuing treatment "for one to seven and a half years" before stopping, a New York Times editorial writes, "The researchers estimate that as many as 15 percent of adults who start treatment early and continue for at least a year may then be able to stop their drug regimen and live healthily without the drugs." The newspaper continues, "Although the study was small, the findings suggest that treatment should be started earlier for most people, a difficult feat without a lot more testing to identify who is infected and prompt treatment of those who test positive." The editorial writes, "The findings also suggest that many people taking antiviral drugs may be able to stop safely, provided doctors can find some sure way to identify them" (3/18).

Philippines Government Must Support Reproductive Health Law

"Women in the Philippines have lacked autonomy over their bodies for decades. The overwhelming political power of the country's Catholic church leaders—and the government's acquiescence to many of their demands—has resulted in reproductive health care restrictions so severe they amount to human rights violations," Melissa Upreti, regional director for Asia at the Center for Reproductive Rights, writes in The Guardian's Poverty Matters Blog. However, "[w]e hope this is beginning to change at last," with the Responsible Parenthood and Reproductive Health Act of 2012, which was "signed into law by President Benigno Aquino in December and [is] in the process of implementation," she notes, adding that the law "has the potential to bring modern contraception to all Filipina women, and with it hope that thousands of maternal deaths will be prevented, and families and communities will rise out of devastating poverty."

"The law represents significant progress, but it is far from ideal," Upreti continues, adding, "It fails to legalize all contraceptives, sanctions ideological bias in hospitals and reinforces the legal status of human embryos." Because of opposition to the law from the "Catholic hierarchy and their allies," "[i]t crucial for the government to defend it ferociously on behalf of the countless women whose ability to plan their families would be devastated should opponents prevail," she writes. "Ultimately, the true potential of the act rests with the president and the policymakers who brought about its passage. Together, they must exert the political will necessary to overcome religious opposition to women's fundamental human rights and fully implement the act," she states (3/19).

Sex between monogamous heterosexuals rarely source of hepatitis C infection

Individuals infected by the hepatitis C virus (HCV) have nothing to fear from sex in a monogamous, heterosexual relationship. Transmission of HCV from an infected partner during sex is rare according to new research published in the March issue of Hepatology, a journal published by Wiley on behalf of the American Association for the Study of Liver Diseases (AASLD).

Experts estimate that HCV affects up to 4 million Americans, most of whom are sexually active. Medical evidence shows HCV is primarily transmitted by exposure to infectious blood, typically through intravenous (IV) drug use. However, there are conflicting reports regarding sexual activity and HCV transmission with some studies suggesting that exposure to infected blood during sex—through bodily fluids such as vaginal secretions, semen or saliva—may carry a minimal infection risk.

"Generally the risk for transmitting HCV to sex partners is very low," explains lead study author Dr. Norah Terrault with the University of California, San Francisco. "Yet, lack of quantitative data about the
risk of HCV transmission with sexual activity remains a limitation for doctors counseling their patients on safe sex practices.”

To specifically quantify the risk HCV transmission from a chronically infected individual to their sex partner, researchers recruited 500 anti-HCV-positive individuals, who were negative for the human immunodeficiency (HIV), and their long-term heterosexual partners. Couples were surveyed about lifetime risk factors for HCV infection, sexual practices of the couple, and sharing of personal items. The team analyzed blood samples to determine the presence or absence of active virus in the blood and compared the HCV strains in those couples with HCV present.

The majority of HCV infected individuals who participated in the study were non-Hispanic whites, had a median age of 49 years, and sexual activity with their partners ranging from 2 to 52 years. HCV prevalence among partners was 4%, with 9 couples having similar viral strains and viral samples from 3 couples were highly related which is consistent with HCV transmission between the partners.

The maximum incidence rate of HCV transmission by sex was 0.07% per year or roughly 1 per 190,000 sexual contacts that researchers based upon 8377 person-years of follow-up. The team did not identify any specific sexual practices linked to HCV infections among the couples. "Our study provides clinicians with important information for counseling chronic HCV patients in long-term sexual relationships, supporting the current recommendations that couples not change their sexual practices if they are in a monogamous heterosexual relationship," concludes Dr. Terrault.


URL: http://doi.wiley.com/10.1002/hep.26164

Drug-Resistant MRSA Bacteria: Here to Stay in Both Hospital and Community

Mar. 15, 2013 — The drug-resistant bacteria known as MRSA, once confined to hospitals but now widespread in communities, will likely continue to exist in both settings as separate strains, according to a new study.

The prediction that both strains will coexist is reassuring because previous projections indicated that the more invasive and fast-growing community strains would overtake and eliminate hospital strains, possibly posing a threat to public health.

Researchers at Princeton University used mathematical models to explore what will happen to community and hospital MRSA strains, which differ genetically. Originally MRSA, which is short for methicillin-resistant Staphylococcus aureus, was confined to hospitals. However, community-associated strains emerged in the past decade and can spread widely from person to person in schools, athletic facilities and homes.

Both community and hospital strains cause diseases ranging from skin and soft-tissue infections to pneumonia and septicemia. Hospital MRSA is resistant to numerous antibiotics and is very difficult to treat, while community MRSA is resistant to fewer antibiotics.

The new study found that these differences in antibiotic resistance, combined with more aggressive antibiotic usage patterns in hospitals versus the community setting, over time will permit hospital strains to survive despite the competition from community strains. Hospital-based antibiotic usage is likely to successfully treat patients infected with community strains, preventing the newcomer strains from spreading to new patients and gaining the foothold they need to out-compete the hospital strains.

The researchers made their predictions by using mathematical models of MRSA transmission that take into account data on drug-usage, resistance profiles, person-to-person contact, and patient age.

Published February 28 in the journal PLOS Pathogens, the study was conducted by postdoctoral researcher Roger Kouyos, now a scholar at the University of Zurich, and Eili Klein, a graduate student who is now an assistant professor in the Johns Hopkins School of Medicine. They conducted the work under the advisement of Bryan Grenfell, Princeton’s Kathryn Briger and Sarah Fenton Professor of Ecology and Evolutionary Biology and Public Affairs at Princeton’s Woodrow Wilson School of International and Public Affairs.

Journal Reference:
We Are Still at Risk of the Plague, New Study Says; Historical Review Provides Lessons for the Control of the 'Black Death'

Mar. 15, 2013 — Today archaeologists unearthed a 'Black Death' grave in London, containing more than a dozen skeletons of people suspected to have died from the plague. The victims are thought to have died during the 14th century and archaeologists anticipate finding many more as they excavate the site. The Plague is by definition a re-emerging infectious disease which affects the lungs and is highly contagious, leading to mass outbreaks across populations. History shows us that population levels suffered globally due to the plague, with around 75 million people globally perishing during the 14th century Black Death.

This study, published in *Infection, Genetics and Evolution*, analysed the Great Plague of Marseille, which caused 100,000 deaths between 1720 and 1723. The researchers aimed to highlight issues we are facing with infectious diseases today, to identify the best ways to respond to epidemics and whether we are still at risk of the plague re-emerging again.

Results show that a number of factors show we are still at risk of plague today. This is largely due to transport trade and novel threats in developing countries where multi-drug resistant pathogens are currently emerging and spreading rapidly. This genetic change has also contributed to a development in the way the bacteria infect new hosts meaning they can now live in mammalian blood.

The study also highlighted the need for effective management of epidemics in future. Fear of an infection can have a negative impact on a population’s economic situation due to a significant loss of tourism, and widespread panic. History has shown us that providing the necessary information about diseases and improving the management of epidemics are vital steps for avoiding panic and containing diseases.

**Journal Reference:**

### Tuberculosis: Europe’s Ticking Timebomb

by bruce on Mar 19, 2013 • 12:31 pm

**The TB Europe Coalition calls on the European Commission to increase public health funding to fight resurgence of tuberculosis in Europe and eradicate deadly strains.**

1. Drug-resistant forms on the rise in Europe; treatment costs to increase 50 times if epidemic left unchecked
2. MEPs get involved in the fight against deadly strains of the disease
3. Photo exhibition held in European Parliament to call attention to the issue

Brussels, 20th March 2013 — On World Tuberculosis Day the TB Europe Coalition called on the European Commission to substantially increase funding to fight tuberculosis in Europe. Tuberculosis causes not only illness and death, according to estimates, it costs **EU Member States €15 million every week and €750 million every year.**

Most people consider TB a disease of the past, yet this ancient disease is posing new challenges for countries across the globe as TB becomes more and more resistant to existing drugs. Europe is no exception – the World Health Organisation reported an estimated 76,000 cases of multi-drug resistant TB in the WHO European Region in 2011, accounting for a **quarter of the global burden**[1].

While TB can be treated, multi-drug resistant TB (MDR-TB) and extensively-resistant TB (XDR-TB) are much more costly and difficult to treat and present an emerging health threat to the general population. Indeed Eastern Europe and Central Asia now have the world’s highest rate of new TB patients with MDR- and XDR-TB, as a third of all TB cases are resistant to at least one first-line drug[2].

**Fifteen of the 27 MDR-TB high-burden countries worldwide are located in WHO European region and include five EU Member States:** Romania, Bulgaria, Estonia, Lithuania and Latvia[3]. Even in terms of treatment success rates Europe is lagging behind in its response to TB: some European countries rank much lower than African countries. The treatment success rate for MDR-TB in Romania is 20%, compared to 50% in the Democratic Republic of Congo[4]. The greatest reason for this is a failure to provide patients with an adequate level of MDR-TB treatment, mainly due to drug stock-outs.

Curing a “normal” case of TB in the EU costs €1,000 on average, compared to €50,000 for MDR-TB. “**Domestic governments often do not have the resources or the political will to adequately fight MDR-TB. The current health, development and research policies of the European Commission must help countries to address this re-emerging cross-border health threat**” says Fanny Voitzwinkler, Coordinator of the TB Europe Coalition.
“Unless we urgently scale up our efforts, the MDR-TB epidemic could grow out of control and if that happens, the consequences for Europe would be very serious indeed” says Lucica Ditiu, Executive Secretary of the Stop TB Partnership. In 2011, WHO Europe launched a $5-billion Action Plan to Combat MDR-TB in the years 2011-2015 in the Region, which if not implemented will cost the Region $12 billion in lost productivity and treatment of new cases.

An end to TB is possible, progress has already been made: the TB death rate worldwide dropped 41% between 1990 and 2011[5]. With adequate political and financial commitment for research and development into new drugs, diagnostics, and vaccines but also for fully-funded national TB programmes, the fight against TB could be an easy win in global health. WHO and partners estimate that there is a €1.2 billion annual gap in international support to TB on top of a €1 billion annual gap for TB research and development for the period 2014-2016. The next EU Research Framework Programme “Horizon 2020” should prominently feature tuberculosis as a priority in its societal challenges pillar.

The Coalition welcomed the growing engagement of Members of the European Parliament to solve this issue. Fourteen MEPs recently agreed to sponsor a Written Declaration on Multi-Drug Resistant TB in Europe, which will be launched and open for signatures in the spring in the European Parliament.

European Parliament Member from Romania Claudiu Tănăsescu says he will not cease to draw attention to this issue at a policy level:

“Some people think TB only affects people with ‘poor lifestyles’ – by which they often mean Roma, or prisoners or migrants. But TB can affect any of us. TB anywhere is TB everywhere, and I will continue to raise the issue in the European Parliament”.


Nearly one in ten S.African soldiers has HIV/AIDS: army

The South African army Tuesday said 8.5 percent of the country’s 79,200 soldiers have HIV/AIDS, angrily dismissing claims of a higher prevalence rate.

The surgeon general of the South African National Defence Force, Vejay Ramlakan said a study conducted in 2012 "showed a prevalence rate of 8.5 percent compared to the national prevalence rate of about 19 percent".

“This is in stark contrast to claims by some academics the last few years that the HIV and AIDS infection rate among soldiers is as high as 28 percent,” said a statement.

South Africa has no law barring infected people from serving in the army, in a country where some six million people are living with the HIV virus.

As many as 1.7 million people are on the state-funded anti-retroviral programme, the largest in the world.


Long-term Effects Similar for Two-, Three-dose Regimens of Hepatitis A Vaccine

Healio, (03.19.2013)

Researchers report that a two-dose regimen of hepatitis A vaccine and a three-dose regimen were equally effective after more than 14 years in protecting Alaska Native study participants ages 12 to 24 years.

The study compared levels of hepatitis A antibody (anti-HAV) results in two groups. The first group of 101 Alaska Natives between the ages of 12 and 24 initiated two-dose vaccination (720 ELU) for hepatitis A more than five years earlier; a 24-person subgroup received vaccination between the ages of three and six. Researchers compared results for this subgroup with a similar second group who received a three-dose vaccination (360 ELU).

Researchers found no significant difference between the groups in “anti-HAV geometric mean concentration (GMC)” according to when immunization was started (ages 1, 2, 3 to 6, and 7). Those who received vaccination at one to two years consistently had the lowest GMC levels. Eleven years after the second dose, GMC levels for 5 percent of the two-dose group were not seroprotective.
The two- and three-dose participants also had similar GMC levels 10, 12, and 14 years after vaccination. All two-dose participants and almost all three-dose participants who could be evaluated, continued to have protection from hepatitis A 10, 12, 14, and 15 years after the second dose. The study also found that the three-dose participants were protected after 17 years.

Study authors recommended continuing evaluation to determine if and when hepatitis A boosters will be needed. The full report, “Duration of Protection Against Hepatitis A for the Current Two-dose Vaccine Compared to a Three-dose Vaccine Schedule in Children,” was published online in the journal Vaccine (2013; doi:10.1016/j.vaccine.2013.02.048).

**Philippines Supreme Court Temporarily Halts Implementation Of Reproductive Health Law**

"The Philippines Supreme Court temporarily halted the implementation of a law that provides state funding for contraceptives, legislation opposed by the dominant Roman Catholic Church but supported by reproductive health activists," the Associated Press reports, adding, "The Responsible Parenthood Law was passed by lawmakers late last year despite the church’s opposition but petitioners questioned its legality on several grounds, saying it offends religious beliefs and fosters abortion, which remains illegal in the country" (3/19). "In a 10-to-5 ruling, the court froze for 120 days when the law could take effect," the New York Times writes, adding, "Supporters and opponents of the legislation will argue their cases before the Supreme Court on June 18, said a court spokesman, Theodore Te" (Whaley, 3/19). The law "was expected to take effect by the end of March," IRIN notes. The news service discusses maternal mortality in the country and details arguments for and against the law (3/20).

**Some Public Health Experts Concerned Over Saudi Arabia's Response To SARS-Like Virus**

"A SARS-like virus has infected 15 people, nine of whom have died, mostly in Saudi Arabia, worrying some Western scientists who question whether the kingdom is sharing enough critical data on the outbreak," "[b]ut a top Saudi Arabia health official rejected those complaints on Tuesday and said the virus posed a low risk of pandemic," the Wall Street Journal reports. "Of the deaths confirmed in humans since April, six have occurred in Saudi Arabia, including three since February, said the World Health Organization, which has issued a global alert," according to the newspaper. "In the SARS outbreak a decade ago, China drew international criticism for issuing slow and contradictory accounts of the first cases," and "[s]ome European and American scientists who played central roles in the SARS outbreak have expressed concern that Saudi Arabia isn’t sharing critical information on the new coronavirus, which has been known to cause everything from the common cold to SARS," the Wall Street Journal writes, noting that Saudi Deputy Health Minister Ziad Memish said, "We don't take it lightly, we're watching very closely, and we think the whole scientific community should be doing the same" (Knickmeyer/McKay, 3/19).

**New hope to beat malaria once and for all**

*The discovery of a molecule which could lead to powerful new anti-malaria drugs*

"The 4(1H)-quinolone-3- diarylethers are selective potent inhibitors of the parasite mitochondrial cytochrome bc1 complex," Professor Avery said. "These compounds are highly active against the types of malaria parasites which infect humans, Plasmodium falciparum and Plasmodium vivax," she said. "What is really exciting about this study is that a new class of drugs based on the 4(1H)-quinolone-3-diarylethers would target the malaria parasite at different stages of its lifecycle." This provides the potential to not only kill the parasite in people who are infected, thus treating the clinical symptoms of the disease, but also to reduce transmission rates.

"Just one of these properties would be of great benefit but to achieve both would really make a difference in reducing the disease burden on developing nations," Professor Avery said.

"There is also the real possibility that we could begin to impact on the incidence and spread of malaria, bringing us closer to the ultimate goal of wiping out malaria altogether."

The selected preclinical candidate compound, ELQ-300, has been demonstrated to be very effective at blocking transmission in the mouse models. There is a further benefit in that the predicted dosage in patients would be very low and it’s expected that ELQ-300, which has a long half-life, would provide significant protection.
The development of a new chemical class of anti-malarial drugs is very timely as the parasite is becoming increasing resistance to treatments currently available

Explaining how extra virgin olive oil protects against Alzheimer's disease
The mystery of exactly how consumption of extra virgin olive oil helps reduce the risk of Alzheimer's disease (AD) may lie in one component of olive oil that helps shuttle the abnormal AD proteins out of the brain, scientists are reporting in a new study. It appears in the journal ACS Chemical Neuroscience.

Amal Kaddoumi and colleagues note that AD affects about 30 million people worldwide, but the prevalence is lower in Mediterranean countries. Scientists once attributed it to the high concentration of healthful monounsaturated fats in olive oil — consumed in large amounts in the Mediterranean diet. Never research suggested that the actual protective agent might be a substance called oleocanthal, which has effects that protect nerve cells from the kind of damage that occurs in AD. Kaddoumi's team sought evidence on whether oleocanthal helps decrease the accumulation of beta-amyloid (Aβ) in the brain, believed to be the culprit in AD.

They describe tracking the effects of oleocanthal in the brains and cultured brain cells of laboratory mice used as stand-ins for humans in such research. In both instances, oleocanthal showed a consistent pattern in which it boosted production of two proteins and key enzymes believed to be critical in removing Aβ from the brain. "Extra-virgin olive oil-derived oleocanthal associated with the consumption of Mediterranean diet has the potential to reduce the risk of AD or related neurodegenerative dementias," the report concludes.

Insights into the immune system, from the fates of individual T cells
Findings could enable better modeling and manipulation of immune response
By charting the differing fates of individual T cells, researchers have shown that previously unpredictable aspects of the adaptive immune response can be effectively modeled. The crucial question: What determines which of the immune system's millions of cells will mobilize to fight an acute infection and which will be held back to survive long-term, forming the basis of the immunological memory? The scientists' findings, published in the journal Science, could have implications for improved immunotherapy and vaccination strategies.

The scientists found that the immediate immune response to an infection or tumor is mounted by a relatively tiny fraction of the so-called CD8+ T cells that are capable of recognizing the associated antigen. These few rapidly expand into giant populations of short-lived T cells targeted at killing infected cells or cancer cells. Meanwhile the vast majority remain in smaller populations geared toward longevity, to help ensure that the immune system will remember the antigen when it appears again in the future.

"Up to now, it was only possible to observe groups of immune cells during the response to an infection," says Prof. Dirk Busch of the Technische Universitaet Muenchen (TUM). "We have developed technology that enables us to observe individual T cells." Together with innovative cell processing technology, the researchers brought theoretical systems biology and clinical expertise to bear on this investigation, a collaboration of TUM, the University of Heidelberg, the Helmholtz Center Munich, the German Cancer Research Center (DKFZ), and the National Center for Infection Research (DZIF).

Marking the threshold of predictability
A single T cell is theoretically capable of generating an adaptive immune response by developing into diverse and expanding populations, fighting the acute infection as well as providing lasting memory for the future. But a fundamental question – whether an effective response is predetermined on the level of an individual T cell or emerges from the commingled fates of multiple cells – had never been put to the test. Another unresolved question concerned the order in which populations of short-lived killer cells and long-lived memory cells develop.
To address these questions, researchers at TUM began by introducing specially marked T cells into mice and then triggering a specific immune reaction. Around seven days later, they were able to determine how many descendant cells, and what kinds, had been generated by individual T cells. Biomathematical modeling, using an approach co-developed with the group of Prof. Thomas Hoefer at Heidelberg, helped to explain what the data showed. "One can't predict which 'career paths' the descendants of an individual killer T cell will take," says first author Veit Buchholz, a medical resident at TUM. "This is a matter of chance, like a single roll of the dice. To generate a predictable immune response, we have found that a sample of at least 50 individual cells is needed."

From analysis of many of the huge populations of short-lived killer cells and the relatively tiny populations of long-lived memory cells, the researchers were able to reconstruct the T cells' development program and predict their behavior: All of the cells proceed along the same path of development, but they don't go the same distance. That is, the few cells that generate giant populations of short-lived infection fighters have gone through the same stage as those fated to produce memory cells – but they have left that stage behind to provide immediate protection.

Beyond the results themselves, another important outcome of this study is increased confidence in the combined power of the in vivo and in silico approaches. "The fact that the experimental results confirmed our predictions in detail has strongly supported our theory," says Prof. Hoefer, leader of the Heidelberg group.

There are several ways these findings could become important in the setting of human health, the researchers explain – in improving the effectiveness of immunotherapy against cancer, for example, or in optimizing treatment for older people, who tend to have significantly fewer copies of a given type of immune cell. "The future memory cell stands at the beginning of an expansion process with two extreme forms of differentiation," Buchholz says, "and ideally there should be a balance, so that the memory pool is not depleted. So we can think about how to tweak vaccination schemes to first allow expansion and not let differentiation kick in too early."

**Baffling Blood Problem Explained**

**60-year-old health mystery solved by UVM and French research team**

In the early 1950’s, a 66-year-old woman, sick with colon cancer, received a blood transfusion. Then, unexpectedly, she suffered a severe rejection of the transfused blood. Reporting on her case, the French medical journal *Revue D'Hématologie* identified her as, simply, "Patient Vel."

After a previous transfusion, it turns out, Mrs. Vel had developed a potent antibody against some unknown molecule found on the red blood cells of most people in the world—but not found on her own red blood cells.

But what was this molecule? Nobody could find it. A blood mystery began, and, from her case, a new blood type, "Vel-negative," was described in 1952.
Soon it was discovered that Mrs. Vel was not alone. Though rare, it is estimated now that over 200,000 people in Europe and a similar number in North America are Vel-negative, about 1 in 2,500.

For these people, successive blood transfusions could easily turn to kidney failure and death. So, for sixty years, doctors and researchers have hunted—unsuccessfully—for the underlying cause of this blood type.

But now a team of scientists from the University of Vermont and France has found the missing molecule—a tiny protein called SMIM1—and the mystery is solved.

Reporting in the journal *EMBO Molecular Medicine*, UVM’s Bryan Ballif, Lionel Arnaud of the French National Institute of Blood Transfusion, and their colleagues explain how they uncovered the biochemical and genetic basis of Vel-negative blood.

“Our findings promise to provide immediate assistance to health-care professionals should they encounter this rare but vexing blood type,” says Ballif.

The pre-publication results were presented online, March 18, 2013, and the finalized report will be published, as an open-access article, in the next edition of the journal.

(Last year, Ballif and Arnaud identified the proteins responsible for two other rare blood types, Junior and Langeris, moving the global count of understood blood types or systems from 30 to 32. Now, with Vel, the number rises to 33.)

**New DNA tests**

Before this new research, the only way to determine if someone was Vel-negative or positive was with tests using antibodies made by the few people previously identified as Vel-negative following their rejection of transfused blood. Not surprisingly, these antibodies are vanishingly rare and, therefore, many hospitals and blood banks don’t have the capacity to test for this blood type.

“Vel– blood is one of the most difficult blood types to supply in many countries,” the scientists write, "This is partly due to the rarity of the Vel– blood type, but also to the lack of systematic screening for the Vel–type in blood donors.”

In response, the UVM and Paris researchers developed two fast DNA-based tests for identifying Vel-negative blood and people. These tests can be easily integrated into existing blood testing procedures—and can be completed in a few hours or less.

“It’s usually a crisis when you need a transfusion” says Ballif. “For those rare Vel-negative individuals in need of a blood transfusion, this is a potentially life-saving time frame.”

**Protein hunters**

To make their discovery, Arnaud and coworkers in Paris used some of the rare Vel-negative antibody to biochemically purify the mystery protein from the surface of human red blood cells. Then they shipped them to Ballif in Vermont.

The little protein didn’t reveal its identity easily. “I had to fish through thousands of proteins,” Ballif says. And several experiments failed to find the culprit because of its unusual biochemistry—and pipsqueak size. But he eventually nabbed it using a high-resolution mass spectrometer funded by the Vermont Genetics Network. And what he found was new to science. “It was only a predicted protein based on the human genome,” says Ballif, but hadn’t yet been observed. It has since been named: Small Integral Membrane Protein 1, or SMIM1.

Next, Arnaud’s team in France tested seventy people known to be Vel-negative. In every case, they found a deletion—a tiny missing chunk of DNA—in the gene that instructs cells on how to manufacture SMIM1. This was the final proof the scientists needed to show that the Vel-negative blood type is caused by a lack of the SMIM1 protein on a patient’s red blood cells.

**Your blood**

Today, personalized medicine—where doctors treat us based on our unique biological makeup—is a hot trend. “The science of blood transfusion has been attempting personalized medicine since its inception,” Ballif notes, “given that its goal is to personalize a transfusion by making the best match possible between donor and recipient.”

"Identifying and making available rare blood types such as Vel-negative blood brings us closer to a goal of personalized medicine,” he says. “Even if you are that rare one person out of 2,500 that is Vel-negative, we now know how to rapidly type your blood and find blood for you—should you need a transfusion.”
Experts sound worldwide alert over deadly bat virus
By Agence France-Presse
Thursday, March 21, 2013 6:07 EDT
Experts on infectious diseases Thursday warned people to stay away from bats worldwide after the recent death of an eight-year-old boy bitten in Australia.

The boy last month became the third person in the country to die of Australian bat lyssavirus (ABLV), for which there is no effective treatment.

Doctors Joshua Francis and Clare Nourse of Brisbane’s Mater Children’s Hospital warned an infectious diseases conference that human-to-human transmission of the virus may be possible.

Francis said the boy was bitten during a family holiday to Queensland in December 2012, but did not tell his parents.

Three weeks later he began to suffer convulsions, abdominal pain and fever, followed by progressive brain problems.

Doctors frantically tried to establish what was wrong and on day 10 of his admission the lyssavirus was detected.

He fell into a coma and died on February 22.

Francis told the Canberra conference the warning to avoid bats around the world was issued not just because of the danger posed by the animals themselves, but due to the risk, however remote, that the virus could be spread between humans.

“Human to human transmission of lyssaviruses has not been well documented, but it is theoretically possible,” he said.

International guidelines recommend post-exposure prophylaxis for anyone who has been exposed to the saliva or neural tissue of an infected person through broken skin or mucous membrane contact.

“ABLV has proved fatal in all cases reported to date. There is a need for increased public awareness of the risk associated with bat contact,” Francis said. “In short, people should stay away from bats.”

ABLV was first identified in Australian bats and flying foxes and is common in both, though human infection is extremely rare.

Two adult cases were confirmed in 1996 and 1998. One was a woman bitten by a flying fox after wrestling it off a child, the other a carer who looked after the animals.

Other lyssavirus strains circulate in bats in the United States and Europe and the experts said their warning applies to wherever bat or flying fox populations exist.

Reinventing The Condom
Papa Salif Sow, Stephen Ward
March 18, 2013
Condoms: Modernizing a life-saving tool
What is one of the oldest medical devices in existence? What is the most effective method of preventing sexual transmission of HIV? What medical product is so simple that it can easily be manufactured by the millions and costs just pennies? The answer to all three is the same – the condom. The fact that such a modest device, nothing more than an inert sheath of latex, is one of the most effective tools in our armamentarium against HIV infection, and additionally prevents unintended pregnancy, is frankly astounding. When used consistently and correctly, condoms are extremely effective at preventing HIV infection and unplanned pregnancy.

Quite simply, condoms save lives. But if condoms are so marvelous, why are we seeking ideas for the Next Generation of Condoms in our current Round of Grand Challenges Explorations?

It may seem obvious, but the success and impact of any public health tool hinges on that tool being used consistently and correctly by those who need it. Vaccines sitting on shelves don’t prevent disease. New tuberculosis drug regimens won’t help if patients stop taking them halfway through the necessary days. Likewise, the potential value of condoms is limited by inconsistent use.

Women, particularly those in high risk groups such as commercial sex workers, often face difficulties negotiating condom use; the fact that the term “condom negotiation” even exists and is so common in discussions about HIV prevention or reproductive health speaks to the central shortcoming of our current generation of condoms. The undeniable, and unsurprising, truth is that most men prefer sex without a condom, while the risks related to HIV infection and complications of unplanned pregnancy are disproportionately borne by their partners.
It may seem obvious, but the success and impact of any public health tool hinges on that tool being used consistently and correctly by those who need it.

Ultimately, the field is moving toward new classes of products, referred to as Multi-purpose Prevention Technologies (MPTs) that will meet multiple of the sexual and reproductive health needs of men and women, including HIV prevention and contraception. These might include combination vaginal rings, co-administered or co-formulated injectable products, or new “on demand” products like fast-dissolving vaginal films. A number of concepts are already being actively pursued by product development organizations (please see the CAMI database). While potentially transformational, most of these products are high risk, years away from being available, and their path through development, regulatory approval, and delivery remains unclear.

In the meantime, we have a product that is safe and effective, but underutilized. What if we could develop a condom that would provide all the benefit of our current versions, without the drawbacks? Even better, what if we could develop one that was preferred to no condom?

While there have been few modification to condom design that have had substantial impact on the condom market, there are opportunities for taking a radically different approach to condoms being pursued currently. Researchers at the University of Washington are developing a condom using a technique known as electrospinning, which creates tightly woven fabric out of nanometer-sized polymer strands and which could be used to deliver spermicidal or microbicidal agents in addition to providing a barrier.

Origami Condoms provides an excellent example of a private enterprise focused on new condom design to promote consistent use by emphasizing the sexual experience.

The idea of a condom that men would prefer to no condom is a revolutionary idea, but we know more today about sexual function than at any time in the past, and advances in relevant disciplines such as neuroscience, vascular biology, urology, reproductive biology, materials science, and other fields can contribute to new and unconventional approaches. We hope this GCE call will provide a thought-provoking challenge to innovators from many areas who may never have thought about how they could build a better condom.

**Study offers new way to discover HIV vaccine targets**

*Ragon Institute researchers develop a method to identify weak points in viral proteins that could be exploited for vaccine development*

Decades of research and three large-scale clinical trials have so far failed to yield an effective HIV vaccine, in large part because the virus evolves so rapidly that it can evade any vaccine-induced immune response.

Researchers from the Ragon Institute of MGH, MIT and Harvard University have now developed a new approach to vaccine design that may allow them to cut off those evolutionary escape routes. The researchers have developed and experimentally validated a computational method that can analyze viral protein sequences to determine how well different viral strains can reproduce in the body. That knowledge gives researchers an unprecedented guide for identifying viral vulnerabilities that could be exploited to design successful vaccine targets.

The team, led by Arup Chakraborty, the Robert T. Haslam Professor of Chemical Engineering, Chemistry, Physics and Biological Engineering at MIT, has designed protein fragments (peptides) that would target these weaknesses. Ragon Institute researchers are now developing ways to deliver the peptides so they can be tested in animals.

"We think that, if it continues to be validated against laboratory and clinical data, this method could be quite useful for rational design of the active component of a vaccine for diverse viruses. Furthermore, if delivered properly, the peptides we have designed may be able to mount potent responses against HIV across a population," says Chakraborty, who is also the director of MIT's Institute for Medical Engineering and Science.

Chakraborty and his colleagues describe their findings in the March 21 issue of the journal *Immunity*. Lead author of the paper is Andrew Ferguson, a former postdoc in Chakraborty's lab who is now an assistant professor at the University of Illinois at Urbana-Champaign. Other authors are Bruce Walker, director of the Ragon Institute and a professor at Harvard Medical School; Thumbi Ndung'u of the Ragon Institute and the Doris Duke Medical Research Institute in South Africa; and Jaclyn Mann and Saleha Omarjee of the Doris Duke Medical Research Institute.
“This work stems from the novel approach to science that is the central mission of the Ragon Institute: to draw researchers from diverse scientific disciplines to catalyze new advances, the ultimate mission being to harness the immune system to prevent and cure human diseases,” Walker says.

**Rapid evolution**

Typically when a vaccine for a disease such as smallpox or polio is given, exposure to viral fragments primes the body’s immune system to respond powerfully if it encounters the real virus. With HIV, it appears that when immune cells in a vaccinated person attack viral peptides that they recognize, the virus quickly mutates its protein sequences so immune cells no longer recognize them.

To overcome this, scientists have tried analyzing viral proteins to find amino acids that don’t often mutate, which would suggest that they are critical to the virus’s survival. However, this approach ignores the fact that mutations elsewhere in the protein can compensate when those seemingly critical amino acids are forced to evolve, Chakraborty says.

The Ragon Institute team focused on defining how the virus’s ability to survive depends on the sequences of its proteins, if they have multiple mutations. This knowledge could enable identification of combinations of amino acid mutations that are harmful to the virus. Vaccines that target those amino acids would force the virus to make mutations that weaken it.

With existing HIV protein sequence data as input, the researchers created a computer model that can predict the fitness of any possible sequence, enabling prediction of how specific mutations would affect the virus.

In this paper, the researchers focused on an HIV polyprotein called Gag, which is made up of several proteins that together are 500 amino acids long. The proteins derived from Gag are important structural elements of the virus. For example, a protein called p24 makes up the capsid that surrounds the virus’s genetic material.

Each position in HIV proteins can be occupied by one of 20 possible amino acids. Sequence data from thousands of different HIV strains contain information on the likelihood of mutations at each position and each pair of positions, as well as for triplets and larger groups. The researchers then developed a computer model based on spin glass models, originally developed in physics, to translate this information into predictions for the prevalence of any mutant.

Using this model, the researchers can enter any possible sequence of Gag proteins and determine how prevalent it will be. That prevalence correlates with the fitness of a virus carrying that particular protein sequence, a relationship that the researchers demonstrated by using the model to predict the fitness of a few dozen Gag protein sequences, and verified by engineering those sequences into HIV viruses and testing their ability to replicate in cells grown in the lab. They also tested their predictions against human clinical data.

**Visualizing fitness**

The model also allows the researchers to visualize viral fitness using "fitness landscapes" — topographical maps that show how fit the virus is for different possible amino-acid sequences for the Gag proteins. In these landscapes, each hill represents sequences that are very fit; valleys represent sequences that are not.

Ideally, vaccine-induced immune responses would target viral proteins in such a way that mutant strains that escape the immune response correspond to the fitness valleys. Thus, the virus would either be destroyed by the immune response or forced to mutate to strains that cannot replicate well and are less able to infect more cells.

This would mimic the immune response mounted by people known as "elite controllers," who are exposed to the virus but able to control it without medication. Immune cells in those people target the same peptide sequences that the model predicted would produce the biggest loss of fitness when mutated.

This general approach could also be used to identify vaccine targets for other viruses, Chakraborty says.

"The reason we are excited about this is that we now have a method that combines two technologies that are getting cheaper all the time: sequencing and computation," he says. "We think that if this continues to be validated, it could become a general method of obtaining the fitness landscapes of viruses, allowing you to do rational design of the active components of vaccines."

"This work is a great example of how integrating expertise from different scientific disciplines — in this case physics, computational biology, virology and immunology — can accelerate progress toward an HIV vaccine," Walker says.
Lawyer fights 'widow sex' tradition in Malawi
From Robyn Curnow and Jenni Watts, CNN
March 21, 2013 – Updated 10:47 GMT (18:47 HKT)

(CNN) — Lawyer and human rights activist Seodi White has long been an outspoken campaigner for gender justice in Malawi, a country where half its women are married before the age of 18.

As the head of the Malawian chapter of Women in Law in Southern Africa (WLSA), White is at the forefront of the battle against inequality, traveling around the country to promote education and to stop young girls from giving up on school and marrying in their early teens.

But the prominent activist, who is herself the mother of a young daughter, is not only concerned with the rights of teenage girls. She is also targeting cultural practices that harm older, vulnerable women in Malawi.

'Widow cleansing'
One such custom, prevalent in the southern tip of the landlocked country, is "widow cleansing," a traditional practice in which a widow is expected to have sexual relations, "in order to cleanse her," explains White.

"There is a belief that if she does not sleep with someone, the spirit of her dead husband will come and visit upon her and her family will be cursed," she adds.

White says that the practice is not forced upon widows. Instead, she says, the tradition has become so much part of the culture that widows themselves call for it.

"It's a mindset issue," says White. "Even the widows, they've told me, 'I don't want to die, I don't want a curse to come to my husband.' They cry to be cleansed."

White says the tradition, which involves unprotected sex, thus increasing the chances of HIV infection, has been turned into a business.

"There are professional cleansers in villages," says White. She says these men charge widows up to $50 for their services, in a country where the minimum wage is less than $1 per day.

In this country, to get ahead in life, to beat poverty, you need education.

In recent times, there have been several initiatives by White's NGO, as well as other groups, to try and change the situation. One effort is to target the "professional cleansers" in attempt to get them to change their ways.

"Some have actually come out in the open and said: 'I used to be a commercial cleanser, I'm HIV positive, I've stopped, it's not fine and I go village by village telling other commercial cleansers to stop this, it's a risky taboo behavior.'"

The power of education
A daughter of a professor of English, White grew up in Malawi in a relatively privileged family. She received her law degree in Botswana before moving to the UK to focus on gender and development studies.

White saw first-hand the difference that education can make to a woman's life, and that's why all her efforts to promote gender equality—from campaigning against child marriage and domestic abuse to protecting widows' rights through her work as a lawyer—have been shaped by the transformative power of education.

"In this country, to get ahead in life, to beat poverty, you need education," she says.

"I know the difference between an uneducated woman in Malawi and a person of education, as I am, and I decided to use my position to uplift others," adds White.

"I decided I'm going to dedicate my life to dealing with injustice, just because I don't like it when a structure or system puts others in poverty, puts others in a position of inequality," adds White.

'The dispossession of widows'
Another campaign spearheaded by White is the fight against the prevalent culture of property grabbing, one of the most deep-rooted forms of discrimination suffered by widows in Malawi.

White says that all across the country widows are at risk of having their matrimonial property taken by their late husband's relatives, often leaving them and their children homeless.

"The way our family structures are done is that when a man and a woman get married they are not considered related," says White. "A man is still looked at by his family as he is theirs and the woman is looked at by her family as she is theirs."

White says this entrenched culture, coupled with a prevalent assumption that women do not have an earning capacity, has condemned many widows to acute poverty.
"When the husband dies," says White, "his people, they come in and say, 'what did our son buy in this house? Where is the stuff?' They don't look at the stuff as belonging to the family."

WSLA fought hard for more than 10 years to advance women's rights to keep their marital estate, calling for reform in Malawi's inheritance laws. Its campaign, which met strong resistance, finally succeeded in 2011 when the country's parliament voted to make property grabbing an offense and protect the spouse's and children's share in the deceased's assets.

"A law is a law—it might not be like it's working immediately, but it's got staying power," says White, adding that more needs to be done to raise awareness about the reforms and to inform widows of their rights.

White says that despite all the difficulties, it is victories like this that make her decision to commit her life in the fight against gender injustice worthwhile.

"I've been working on this business for 15 years; They've been moments of hope," she says.

Vaginal Products Linked to Infections

Researchers have found that products inserted vaginally can damage vaginal tissue and increase the user's susceptibility to STDs such as herpes, chlamydia, and HIV. The most commonly used products were for washing, douching, or as commercial lubricants. Joelle Brown of the University of California, Los Angeles, and colleagues recruited 141 women in Los Angeles who completed questionnaires about product use and were administered lab tests for vaginal infections at entry into the study and one year later.

The researchers found that 66 percent of participants reported washing, douching, or inserting commercial lubricants and over-the-counter products other than tampons in the previous months. Approximately 45 percent of the study participants reported using washes, 70 percent used commercial lubricants, 17 percent used petroleum jelly, and 13 percent used oils. Lab tests showed that 40 percent of the women who used petroleum jelly had bacterial vaginosis, compared to 18 percent who did not use petroleum jelly. Also, 44 percent of participants who used intravaginal oils tested positive for Candida, the fungus that causes yeast infections, compared to 5 percent of those who did not use oils. The researchers suggest that the infection may have resulted from the products upsetting the women's internal pH and beneficial microbe communities, allowing harmful organisms to multiply.

Since the study did not aim to identify the causes of the infections, it did not prove products were to blame. Brown noted that commercial sexual lubricants that were designed for internal use were not associated with increased risk of infection in the study, but that they require further evaluation. She commented that women were exposed to a great number of products on the market that were targeted to modifying the vaginal environment. Brown explained that the Food and Drug Administration "strongly urges" cosmetic manufacturers to test their products for safety but does not require it.


Certain bacteria suppress production of toxic shock toxin: Probiotic potential looms

Certain Streptococci increase their production of toxic shock syndrome toxin 1, sometimes to potentially dangerous levels, when aerobic bacteria are present in the vagina. But scientists from the University of Western Ontario have discovered certain strains of lactobacillus bacteria are capable of dampening production of that toxin according to research published in the journal Applied and Environmental Microbiology.

"The risk of potentially fatal toxic shock syndrome appears to be influenced by the types of bacteria present in the vagina," says principal investigator Gregor Reid.

In planning the study, "I figured that the Staphylococcus aureus strains with the ability to produce toxic shock syndrome toxin might only do this under certain environmental conditions," says Reid. "In the vagina, that means depending on pH and the other bacteria living there."

The researchers took swabs from women with clinically healthy vaginal status, with intermediate status, and from those diagnosed with bacterial vaginosis. They then identified the bacterial species, and assayed for toxic shock syndrome toxin 1. "In particular, Streptococcus agalactiae, often referred to as Group B streptococci, an organism of particular concern when giving birth, increased toxin production 3.7-fold," says Reid. But various species of lactobacillus repressed toxin production, one by 72 percent.

"These experiments emphasize that for proper clinical care of women, we need to know all bacterial types present in the vagina," says Reid. "Culturing is inadequate, and while some microscopy is feasible if
the viewer develops the expertise to assess the vaginal smears, rapid 16s sequencing systems are needed as a diagnostic tool," because many species are "very difficult to culture," or have never been cultured.

"We need to vastly improve how we diagnose infections and determine the risk of infection of women," says Reid. He also recommends "improving our ability to manipulate microbiota [with probiotics] in lieu of using broad spectrum antibiotics that were developed 40 years ago, and are not very effective in the vagina, and certainly not designed to neutralize toxins."

**Quirky Lyme Disease Bacteria: Unlike Most Organisms, They Don't Need Iron, but Crave Manganese**

Saito collaborated with biomedical researchers at Johns Hopkins University, applying his proteomic techniques to explore proteins in a terrestrial organism, the bacteria that cause Lyme Disease. Unlike all other known organisms, Borrelia burgdorferi need manganese (blue dot), rather than iron, to serve as linchpins bonded into key enzymes. The scientists found that to cause disease, Borrelia require unusually high levels of manganese. The findings open new avenues to search for ways to attack the bacteria. (Credit: Illustration by P. John Hart, University of Texas)

Mar. 21, 2013 — Scientists have confirmed that the pathogen that causes Lyme Disease—unlike any other known organism—can exist without iron, a metal that all other life needs to make proteins and enzymes. Instead of iron, the bacteria substitute manganese to make an essential enzyme, thus eluding immune system defenses that protect the body by starving pathogens of iron.

To cause disease, *Borrelia burgdorferi* requires unusually high levels of manganese, scientists at Johns Hopkins University (JHU), Woods Hole Oceanographic Institution (WHOI), and the University of Texas reported. Their study, published March 22, 2013, in *The Journal of Biological Chemistry*, may explain some mysteries about why Lyme Disease is slow-growing and hard to detect and treat. The findings also open the door to search for new therapies to thwart the bacterium by targeting manganese.

"When we become infected with pathogens, from tuberculosis to yeast infections, the body has natural immunological responses," said Valeria Culotta, a molecular biologist at the JHU Bloomberg School of Public Health. The liver produces hepcidin, a hormone that inhibits iron from being absorbed in the gut and also prevents it from getting into the bloodstream. "We become anemic, which is one reason we feel terrible, but it effectively starves pathogens of iron they need to grow and survive," she said.

*Borrelia*, with no need for iron, has evolved to evade that defense mechanism. In 2000, groundbreaking research on *Borrelia*’s genome by James Posey and Frank Gherardini at the University of
Georgia showed that the bacterium has no genes that code to make iron-containing proteins and typically do not accumulate any detectable iron.

Culotta’s lab at JHU investigates what she called "metal-trafficking" in organisms—the biochemical mechanisms that cells and pathogens such as Borrelia use to acquire and manipulate metal ions for their biological purposes.

"If Borrelia doesn't use iron, what does it use?" Culotta asked.

To find out, Culotta’s lab joined forces with Mak Saito, a marine chemist at WHOI, who had developed techniques to explore how marine life uses metals. Saito was particularly intrigued because of the high incidence of Lyme Disease on Cape Cod, where WHOI is located, and because he specializes in metalloproteins, which contain iron, zinc, cobalt, and other elements often seen in vitamin supplements. The metals serve as linchpins, binding to enzymes. They help determine the enzymes’ distinctive three-dimensional shapes and the specific chemical reactions they catalyze.

It’s difficult to identify what metals are within proteins because typical analyses break apart proteins, often separating metal from protein. Saito used a liquid chromatography mass spectrometer to distinguish and measure separate individual Borrelia proteins according to their chemical properties and infinitesimal differences in their masses. Then he used an inductively coupled plasma mass spectrometer to detect and measure metals down to parts per trillion. Together, the combined analyses not only measured the amounts of metals and proteins, they showed that the metals are components of the proteins.

"The tools he has are fantastic," Culotta said. "Not too many people have this set of tools to detect metalloproteins."

The experiments revealed that instead of iron, Borrelia uses that element’s next-door neighbor on the periodic chart, manganese, in certain Borrelia enzymes. These include an amino peptidase and an important antioxidant enzyme called superoxide dismutase.

Superoxide dismutase protects the pathogens against a second defense mechanism that the body throws against them. The body bombards pathogens with superoxide radicals, highly reactive molecules that cause damage within the pathogens. Superoxide dismutase is like an antioxidant that neutralizes the superoxides so that the pathogens can continue to grow.

The discoveries open new possibilities for therapies, Culotta said. "The only therapy for Lyme Disease right now are antibiotics like penicillin, which are effective if the disease is detected early enough. It works by attacking the bacteria’s cell walls. But certain forms of Borrelia, such as the L-form, can be resistant because they are deficient in cell walls."

"So we’d like to find targets inside pathogenic cell that could thwart their growth," she continued. "The best targets are enzymes that the pathogens have, but people do not, so they would kill the pathogens but not harm people." Borrelia’s distinctive manganese-containing enzymes such as superoxide dismutase may have such attributes.

In search of new avenues of attack, the groups are planning to expand their collaborative efforts by mapping out all the metal-binding proteins that Borrelia uses and investigating biochemical mechanisms that the bacteria use to acquire manganese and directs it into essential enzymes. Knowing details of how that happens offers ways to disrupt the process and deter Lyme Disease.

Journal Reference:

Meningitis Outbreak: New York City Advises Vaccines For All Gay Men
A deadly meningitis outbreak among gay men in New York City just got more serious, officials said today. Two men died of meningitis during the first two months of the year, raising the total number of cases to 22, with 3 of the last 5 cases resulting in death. The city's Department of Health upgraded its response to recommend vaccinations for all gay men throughout the city, regardless of HIV status and geographical location
By Matthew Mientka | Mar 23, 2013 03:20 PM EDT
A deadly meningitis outbreak among gay men in New York City just got more serious, officials said today.

The New York City Department of Health upgraded its advisory on the meningitis outbreak to recommend vaccines for all gay throughout the city, regardless of HIV status. Two men died of the disease earlier this year in an outbreak that has infected 22 and killed 5 since September.
Two men died of meningitis during the first two months of the year, raising the total number of cases to 22, with 3 of the last 5 cases resulting in death.

The city’s Department of Health upgraded its response to recommend vaccinations for all gay men throughout the city, regardless of HIV status and geographical location. Previously, the city had advised recommendations for HIV-positive gay men living in certain neighborhoods of Brooklyn.

In September, the city recommended the vaccines specifically for gay men who had engaged in sex at any bar or party after Sept. 1 or who had looked for sex through gay social media sites such as "Grindr" or "Adam4Adam." They also issued recommendations based on geographical location: the Brooklyn neighborhoods of Bedford-Stuyvesant, Brownsville, Bushwick, Clinton Hill, Crown Heights, Downtown Brooklyn, Dumbo, East New York, Prospect Heights and Williamsburg.

City health officials said they’ve become increasingly worried about this strain of meningitis, which involves an inflammation of the lining of the brain and spinal cord and is highly dangerous. The outbreak could potentially widen and claim many more lives before stopped.

The disease is spread by contact with bodily fluids such as spit, mucus and other fluids from the nose or mouth, according to the Centers for Disease Control and Prevention. Transmissions of meningococcal disease typically occur through kissing, sex, sharing of cigarettes or utensils, and close contact in general. Symptoms include high fever, headache, stiff neck and rash. The CDC said a single injection of the vaccine may protect most people, though some—including those with HIV—may require a follow-up injection two months later. The vaccine is 80 to 90 percent effective.

The Huffington Post said today that news outlets in New York City had lagged in coverage of the outbreak and criticized the New York Times in particular for covering the story two weeks following the March 6 government warning. By contrast, the San Francisco Chronicle covered the story immediately.

**Early treatment may hold key to HIV 'functional cure'**

Monday March 18 2013

Antiretroviral drugs can help stop the HIV virus from replicating.

BBC News reports that one in 10 people diagnosed with HIV who receive early treatment could be ‘functionally cured’. The news is based on a French study that found 14 people achieved a functional cure three months after they started treatment for HIV. Once treatment stopped, the researchers found that the patients’ viral levels were controlled and their immune systems remained stable for just over seven years.

The researchers compared the 14 patients with other people infected with HIV, including patients who also started early treatment but did not respond as positively. They identified various differences among the patients, including important differences in their immune systems.

The news follows a recent story about a baby born with HIV who achieved a functional cure following aggressive early treatment.

A functional cure means that the HIV virus is still present in the body but at such low levels that it can no longer be detected by standard blood tests. HIV treatment aims to achieve this, as the disease is less likely to progress and the long-term outlook for patients is improved.

More research is needed to understand how and why a functional cure can be achieved in some people, and whether it can be extended to more people with the disease.

**Do you need a HIV test?**

If you’re worried you could have HIV, get tested now. The sooner you are diagnosed, the better your chances of staying healthy and having a normal life expectancy.

People who have a high risk of catching HIV include:

- men who have had unprotected sex with men
- women who have had unprotected sex with men who have sex with men
- people who have lived in or travelled extensively in Africa
- people who have had unprotected sex with a person who has lived in or travelled in Africa
- people who inject illegal drugs
- people who have had unprotected sex with somebody who has injected illegal drugs
- people who have caught another sexually transmitted infection

**Where did the story come from?**

The study was carried out by researchers from Unité de Régulation des Infections Rétrovirales, Paris, and other institutions in France, and was funded by the ANRS and the French National Agency for Research on AIDS and Viral Hepatitis.
It was published in the peer-reviewed scientific journal PLOS Pathogens, which is published on an open access basis (freely available for download).

The media coverage of the study must be read in the correct context: the researchers have reported the experiences of a select sample of 14 people who managed to achieve a functional cure with early treatment.

This does not represent a new treatment or cure for HIV. Rather, it is an examination of the characteristics of a select sample of people who achieved the optimal response to existing HIV treatment. Reports that "one in 10 people could be functionally cured" are slightly misleading. It comes from the researchers’ estimate that between 5 and 15% of people who successfully respond to antiretroviral therapy and come off treatment would be able to maintain control of their viral levels (a functional cure) for about two years, like the people in this study. It does not mean that one in 10 people with HIV can be functionally cured.

**What kind of research was this?**
This was a case series reporting on 14 individuals infected with HIV whose virus levels remained controlled at low levels for several years, even after stopping their long-term antiretroviral treatment.

HIV is treated with a combination of antiretroviral drugs. Antiretroviral therapy (ART) is not considered to be a cure for HIV, but aims to stop the virus replicating and reduce its levels so that they cause less harm to the body's immune system.

The overall aim of ART is to reduce the viral load (the number of particles of HIV present in each millilitre of blood) to levels that can no longer be detected by standard blood tests (undetectable levels). If this is achieved and the virus can no longer be detected on standard tests, this is known as a functional cure.

It is called a functional cure because the virus has not completely gone from the body and can still be detected at very low levels on extremely sensitive tests. However, a person with a functional cure should have a good life expectancy and a reduced risk of the disease progressing or developing associated complications. A person with HIV normally has to continue on ART long-term to prevent viral levels from increasing again (viral rebound).

One of the factors that researchers say can have an influence on the success of treatment is how soon people start treatment after they acquire the infection.

This study reports on a small number of people with HIV who started ART very early and were later able to come off it, with the virus continuing to be controlled at undetectable levels for several years, even without treatment. The researchers say that these people may "hold important clues in the search for a functional HIV cure".

**What did the research involve?**
The researchers identified 14 people who started ART early. These people were able to come off treatment as they had achieved a functional cure. The people were all diagnosed in the late 1990s or early 2000s. The researchers looked at the characteristics of these 14 'responders', including when their levels became undetectable, how long they were treated for, and how long their levels remained undetectable off treatment.

They compared them with three other groups:
- people who also received early treatment but did not respond
- people who started treatment later, which is representative of many people affected by HIV
- eight people whose bodies naturally spontaneously controlled their HIV levels (likely due to some factor in their own biology rather than due to early treatment – these people are thought to be rare)

**What were the basic results?**
In the first two months after being infected with HIV, the 14 responders had similar amounts of the HIV virus in their blood (viral load) compared with those who did not respond to early treatment. However, they had higher viral levels than those whose bodies managed to spontaneously control their HIV levels.

The 14 people received the standard combination ART that was available at the time, and their viral levels became undetectable an average of three months after treatment began. The average duration of ART was 36.5 months, and during this time almost all but two of these people demonstrated an increase in the levels of a particular immune cell HIV normally targets (CD4 cells).

After stopping treatment, their viral levels remained controlled and CD4 levels remained stable for around 89 months. During this time, eight of the people had levels that remained undetectable on all tested blood samples, while for six people there were occasional increases.
They found several other differences between the 14 responders, the spontaneous controllers, and people who did not respond to early treatment or who started treatment late. For example, the functioning of certain immune cells in the responders differed from that of the spontaneous controllers.

They also found that, like spontaneous controllers, the responders had lower levels of HIV genetic material in their blood during treatment compared with those who did not respond to early treatment or who were late starting treatment.

The differences observed between the 14 responders and the spontaneous controllers suggested that the way these two groups achieve HIV control is through at least partly different ways.

The researchers estimated that about 15% of people who achieve undetectable HIV levels with ART and come off treatment would be able to maintain control of their viral levels (a functional cure) for about two years.

**How did the researchers interpret the results?**

The researchers say that their results show that early and prolonged combined ART may allow some individuals to achieve undetectable HIV levels that can be controlled for several years without treatment. They say that these people "may hold important clues about a functional cure for HIV".

**Conclusion**

While such a case series suggests that it is possible that some people can have a functional cure from early HIV treatment, only a very small sample of people with HIV experienced this. Although maintaining HIV at undetectable levels in the body even without treatment is the ultimate goal for all people with HIV, it may not be possible in all people.

Whether an individual is able to achieve a functional cure from HIV may be influenced by various things, such as:

- how soon after infection they started treatment
- which drug regimen they received (in the developing world obtaining access to the most effective combinations may be more difficult)
- compliance with drug regimens
- the person’s individual biology and how they respond to treatment

A small number of people infected with HIV (less than 1%) are somehow able to spontaneously control their HIV levels at undetectable levels. This is likely to be due to some factor in their biological make-up. As the researchers say, this makes it difficult to translate their mechanisms of control to other people.

The group of 14 individuals who are the focus of the current study achieved long-term viral control, which appears to at least partly be a result of early ART treatment. This may offer useful information that could help researchers translate their success to other people. For example, if achieving a functional cure depends on how soon people start treatment after becoming infected, it could be a finding that could have a huge impact on HIV treatment.

However, even though starting treatment early seems achievable, this may not always be possible as it relies upon knowing that infection has occurred. This is likely to rely on factors such as the person developing symptoms when they first contract the infection (this can often be a mild flu-like illness), or the person being aware that they could have been exposed to the virus.

For many people with HIV, early treatment is not possible, as they only find out they have the condition when HIV has already damaged their immune system to the point where they start becoming ill. This may be many years, and even up to a decade, after they first contracted HIV.

This is why it is important to get regular HIV tests if you are in a high-risk group for HIV. Read more about HIV testing.

This study unfortunately does not present a complete cure for HIV, but a functional cure where early antiretroviral treatment was able to reduce the viral load of HIV to undetectable levels. This is the ultimate aim of all antiretroviral treatment: preventing the disease from progressing and giving people a positive outlook and a good life expectancy.

**Could that cold sore increase your risk of memory problems?**

MINNEAPOLIS – The virus that causes cold sores, along with other viral or bacterial infections, may be associated with cognitive problems, according to a new study published in the March 26, 2013, print issue of Neurology®, the medical journal of the American Academy of Neurology.

The study found that people who have had higher levels of infection in their blood (measured by antibody levels), meaning they had been exposed over the years to various pathogens such as the herpes
simplex type 1 virus that causes cold sores, were more likely to have cognitive problems than people with lower levels of infection in the blood.

"We found the link was greater among women, those with lower levels of education and Medicaid or no health insurance, and most prominently, in people who do not exercise," said author Mira Katan, MD, with the Northern Manhattan Study at Columbia University Medical Center in New York and a member of the American Academy of Neurology. The study was performed in collaboration with the Miller School of Medicine at the University of Miami in Miami, FL.

For the study, researchers tested thinking and memory in 1,625 people with an average age of 69 from northern Manhattan in New York. Participants gave blood samples that were tested for five common low grade infections: three viruses (herpes simplex type 1 (oral) and type 2 (genital), and cytomegalovirus), chlamydia pneumoniae (a common respiratory infection) and Helicobacter pylori (a bacteria found in the stomach).

The results showed that the people who had higher levels of infection had a 25 percent increase in the risk of a low score on a common test of cognition called the Mini-Mental State Examination.

The memory and thinking skills were tested every year for an average of eight years. But infection was not associated with changes in memory and thinking abilities over time.

"While this association needs to be further studied, the results could lead to ways to identify people at risk of cognitive impairment and eventually lower that risk," said Katan. "For example, exercise and childhood vaccinations against viruses could decrease the risk for memory problems later in life."

**Sequencing tracks animal-to-human transmission of bacterial pathogens**

**HEIDELBERG, 25 March 2013** – Researchers have used whole genome sequencing to reveal if drug-resistant bacteria are transmitted from animals to humans in two disease outbreaks that occurred on different farms in Denmark. The results, which are published today in *EMBO Molecular Medicine*, confirm animal-to-human transmission of methicillin-resistant *Staphylococcus aureus* (MRSA), a disease-causing bacterium that carries the recently described mecC gene. The mecC gene is responsible for resistance to the penicillin-like antibiotic methicillin.

Drug-resistant bacterial infections pose a significant challenge to public health and may have severe and sometimes fatal consequences. As the costs of whole genome sequencing methods continue to plummet and the speed of analysis increases, it becomes increasingly attractive for scientists to use whole genome sequencing to answer disease-related questions.

"We used whole genome sequencing to see if we could determine if the two disease outbreaks were caused by the same bacterium and to investigate if the pathogens were transmitted from animal to humans or the other way around," remarked Mark Holmes, from the University of Cambridge and the senior author on the paper. "At first glance, it seems reasonable to expect the same pathogen to be the source of the two outbreaks at the two geographically close locations. By looking at the single differences in nucleotides or SNPs in the DNA sequences of each isolate, it became obvious that two different strains of bacteria were responsible for the two disease outbreaks. In one case, the results also clearly showed that the most likely direction of transmission was from animal to human."

Methicillin-resistant *S. aureus* can lead to debilitating skin and soft tissue infections, bacteremia, pneumonia and endocarditis. The researchers used an Illumina HiSeq sequencing system to take a close look at the nucleotide sequence of each pathogen. By comparing single difference in nucleotides in the two sequences (single nucleotide polymorphisms) they were able to reach conclusions about the identity of the pathogens and the routes of infection.

The researchers emphasize that while whole genome sequencing cannot replace other more traditional types of diseases analysis it can greatly increase the ability of scientists to distinguish between different pathogens as the cause of disease.

"Our findings demonstrate that the MRSA strains we studied are capable of transmission between animals and humans, which highlights the role of livestock as a potential reservoir of antibiotic-resistant bacteria," remarked Ewan Harrison, one of the lead authors of the study.
New urgency in battle against 'bound legs' disease

Konzo's harm goes beyond its devastating physical effects

The harm done by konzo – a disease overshadowed by the war and drought it tends to accompany – goes beyond its devastating physical effects to impair children's memory, problem solving and other cognitive functions.

Even children without physical symptoms of konzo appear to lose cognitive ability when exposed to the toxin that causes the disease, researchers report in the journal Pediatrics.

"That's what's especially alarming," said lead author Michael Boivin, a Michigan State University associate professor of psychiatry and of neurology and ophthalmology. "We found subtle effects that haven't been picked up before. These kids aren't out of the woods, even if they don't have the disease."

Konzo means "bound legs" in the African Yaka language, a reference to how its victims walk with feet bent inward after the disease strips away motor control in their lower limbs. Its onset is rapid, and the damage is permanent.

People contract konzo by consuming poorly processed bitter cassava, a drought-resistant staple food in much of sub-Saharan Africa. Typically, the plant's tuber is soaked for a few days, then dried in the sun and ground into flour – a process that degrades naturally occurring cyanide.

"As long as they do that, the food's pretty safe," said Boivin, who began studying konzo in 1990 as a Fulbright researcher in the Democratic Republic of Congo. "But in times of war, famine, displacement and hardship, people take shortcuts. If they're subsisting on poorly processed cassava and they don't have other sources of protein, it can cause permanent damage to the nervous system.

"Konzo doesn't make many headlines because it usually follows other geopolitical aspects of human suffering," he added. "Still, there are potentially tens of millions of kids at risk throughout central and western Africa. The public health scope is huge."

To find out if the disease affects cognitive function, Boivin and colleagues from Oregon Health and Science University turned to the war-torn Congo. They randomly selected 123 children with konzo and 87 neighboring children who showed no signs of the disease but whose blood and urine samples indicated elevated levels of the toxin.

Using cognitive tests, the researchers found that children with konzo had a much harder time using working memory to solve problems and organize visual and spatial information.

They also found that konzo and non-konzo children from the outbreak area showed poor working memory and impaired fine-motor skills when compared to a reference group of children from a part of the region unaffected by the disease.

Konzo's subtler impacts might seem minor compared to its striking physical symptoms, but Boivin noted that the cognitive damage is similar to that caused by chronic low-grade exposures to other toxic substances such as lead.

Scientists eventually may be able to prevent such damage by creating nontoxic cassava varieties and introducing other resilient crops to affected regions, Boivin said. Meanwhile, public health education programs are under way to help stop outbreaks.

"For now," he said, "if we could just avoid the worst of it – the full-blown konzo disease that has such devastating effects for children and families – that's a good start."

Cleverly designed vaccine blocks H5 avian influenza in models

WASHINGTON, DC – March 25, 2013 – Until now most experimental vaccines against the highly lethal H5N1 avian influenza virus have lacked effectiveness. But a new vaccine has proven highly effective against the virus when tested in both mice and ferrets. It is also effective against the H9 subtype of avian influenza. The research is published online ahead of print in the Journal of Virology.
The strength of the new vaccine is that it uses attenuated, rather than "killed" virus. (Killed viruses are broken apart with chemicals or heat, and they are used because they are safer than attenuated viruses.) Killed virus vaccines against avian influenza are injected into the bloodstream, whereas this vaccine is given via nasal spray, thus mimicking the natural infection process, stimulating a stronger immune response.

The danger of current attenuated virus vaccines is that they might exchange dangerous genetic material with garden variety influenza viruses of the sort that strike annually, potentially rendering a lethal but hard to transmit influenza virus, such as H5, easily transmissible among humans. To mitigate those dangers, the study authors, led by Daniel Perez of the University of Maryland, came up with an ingenious design. Influenza viruses carry their genetic material in eight "segments," explains coauthor and University of Maryland colleague Troy Sutton. When viruses reassort, they exchange segments. But each segment is unique, all eight are needed, and the viruses are unfit if they contain more than eight segments.

The vaccine is based on an attenuated version of the H9 virus, with an H5 gene added into one of the H9 virus' segments, to confer immunity to the H5 virus. Segment 8, which is composed of the so-called NS1 and NS2 genes, was split apart, and the NS2 gene was moved into segment 2, adjacent to the polymerase gene, which copies the virus' genetic material during replication. Placing NS2 next to the polymerase gene slowed its function, interfering with the virus' replication. That makes the vaccine safer.

The next step was to engineer the H5 gene into the vaccine. It was inserted into segment 8, where the NS2 gene had been.

Another aspect of the new vaccine's design makes it safer still, by rendering successful reassortment less likely. Both NS1 and NS2 are needed for viral replication. Since the two genes are now separated into different segments, any reassortment will have to include both segments, instead of just segment 8, in order for a reassortant virus to be viable. This greatly reduced the probability of successful reassortment.

The World Health Organization (WHO) recognizes avian influenza subtypes H5, H7, and H9 as potential pandemic viruses, because they all have in rare instances infected humans, and because they circulate in wild birds. Single reassortants could be sufficient to breach the species barrier, and since they do not circulate among us, we lack any immunity. Moreover, H5 is unusually lethal, having killed roughly half of those few it is confirmed to have infected.


March 26, 2013

HIV Tests Are Needed Much More Frequently Than the CDC Advises

Significantly raising the frequency of HIV tests would benefit both high- and low-risk groups and prove cost-effective as well, according to research from Northwestern University. Publishing their findings in the journal AIDS, investigators conducted mathematical modeling to determining what the best frequencies for HIV screens would be among high-risk (those with an annual incidence of 1.0 percent or more), moderate-risk (0.1 percent incidence) and low-risk (0.01 percent incidence) groups. They based their calculations on the assumption that newly diagnosed people would enter treatment immediately—otherwise known as “test-and-treat.”

Currently, the Centers for Disease Control and Prevention (CDC) advises that high-risk groups, including those with HIV-positive or multiple partners, injection drug users and sex workers, undergo annual HIV tests. Low-risk groups are advised to test once in a lifetime. The CDC does not distinguish moderate-risk groups in their recommendations.

The investigators at Northwestern deduced that the optimal frequency for testing among high-risk groups is every three months, for moderate-risk every nine months, and low-risk about every two and a half years. They found that the extra tests would come at “a relatively low cost to society.”

The paper concludes that “[t]he current CDC guidelines for HIV testing are too conservative, and more frequent testing is cost-effective for all risk groups.”

HIV sufferers need hepatitis safeguards

Contact(s): Andy McGlashen
Stronger protections are needed to prevent people with HIV from also becoming infected with hepatitis, researchers argue in a new study led by Michigan State University.
Behaviors that put people at higher risk of contracting HIV – sharing needles, having unprotected sex or getting blood transfusions, for instance – also raise their risk of getting hepatitis B or C, diseases that attack the liver and, if untreated, can be deadly.

The study, which included all registered cases of HIV in Michigan, found about four percent of HIV-positive people also had hepatitis. That’s less than some previous studies have found elsewhere, but it still represents a significant public health concern, said Zahid Butt, who led the research as a doctoral student in MSU’s Department of Epidemiology and Biostatistics.

“Ultimately, because of the fact that they’re suffering from two diseases, they’re more likely to die than if they only have one,” said Butt, who now runs the epidemiology division of a public health institute in Islamabad, Pakistan.

For example, having HIV more than triples the risk of liver disease, liver failure and liver-related death among individuals who also have hepatitis C, according to the Centers for Disease Control and Prevention.

The researchers found the highest rate of co-infection among males, particularly those who marked their race as “other,” which included anything other than white, black or Hispanic. Butt said that was surprising, since previous studies have found African American men were at highest risk for co-infection.

“It could be that we’re getting a cohort of people who were not vaccinated in childhood because they’re coming from countries that don’t require vaccination,” he said. “It also may be that some marginalized groups might not get vaccinated because they don’t trust the health care system.”

Butt said all states should require children to be vaccinated against hepatitis B when they go to school, as most states already do (there is no hepatitis C vaccine). He also said HIV-positive people should protect themselves from hepatitis B by getting vaccinated.

Published in the journal Epidemiology and Infection, the study also found that people who had received transfusions or other blood products were at the highest risk for co-infection. Butt said that raises concerns about whether current safeguards are sufficient to protect people who need transfusions.

“There’s a real need for proper screening of blood products,” he said. “Even with the screening process we have in place, there was a high risk of infection through blood products. We still have a four percent co-infection prevalence, which shouldn’t be the case.”

### Potential Chagas Vaccine Candidate Shows Unprecedented Efficacy

Mar. 26, 2013 — Scientists are getting closer to a Chagas disease vaccine, something many believed impossible only 10 years ago. Research from the Sealy Center for Vaccine Development at the University of Texas Medical Branch at Galveston has resulted in a safe vaccine candidate that is simple to produce and shows a greater than 90 percent protection rate against chronic infection in mice.

In a paper published online in PLOS ONE, the researchers describe how they identified and tested potential Trypanosoma cruzi (also known as T. cruzi or Chagas disease) antigen candidates and delivery models to establish the safety and efficacy of a vaccine formulation known asTeVac3. This potential vaccine could halt the irreversible heart and organ damage that afflicts approximately 30 percent of those infected with Chagas.

"This signals a scientific breakthrough—unprecedented vaccine efficacy for a common parasitic disease with no cure for chronic sufferers," said lead author Nisha Garg, PhD, professor of microbiology, immunology and pathology at UTMB. "If this vaccine proves practical, it could be approved in as few as five years for use in canines, which are reservoir hosts of the disease. As many as 20 percent of dogs may be infected in Texas alone, developing the same heart conditions as humans but mistaken by vets for heartworm."

The study also provides further evidence that a human Chagas vaccine is possible, a topic of debate among some who still believe that Chagas heart disease is the result of an autoimmune disorder, she added.

T. cruzi, transmitted by the triatomine insect, or "kissing bug," is prevalent in almost all Latin American countries and is becoming more common in the U.S. The World Health Organization estimates that approximately 10 million people—mostly children—are infected worldwide. Approximately 13,000 die each year from the complications of Chagas-induced heart disease—a result of the chronic infection Garg and her team aim to vaccinate against. It is estimated that the global economic burden of Chagas is about $7 billion a year.
TcVac3: The Path of Discovery
TcVac3 is the result of rigorous computational/bioinformatics analysis and screening of the *T. cruzi* genome for potential candidate antigens over several years by Garg and her team. These analyses led the researchers to three potential antigens (TcG1, TcG2 and TcG4) for further investigation.

Next, they began testing these antigens and potential vaccine delivery models—how the components are arranged in the actual vaccine—to determine the best approaches.

Early experiments proved that delivery of the candidate antigens by a DNA-prime/protein boost approach, along with co-delivery of IL-12 and GM-CSF cytokine adjuvants meant to enhance the immune response, was effective in generating antibody and T cell responses capable of providing more than 90 percent control of acute infection and parasite burden in infected mice.

Recognizing, however, that this vaccine delivery model was quite complex, the scientists sought to simplify the vaccine using a DNA-prime/Modified Vaccinia Ankara (MVA)-boost approach—a delivery model that offers many advantages: it can accommodate multiple foreign genes in its genome; may be administered by a variety of routes; has an excellent safety record; and has been shown to generate immune responses to a variety of foreign antigens. MVA itself can act as an adjuvant since it provides a signal to the innate immune system and can boost T cells.

Based on preliminary studies by the researchers that showed this delivery model to be potent, the scientists next tested the protective efficacy of TcVac3, constituted of just the TcG2 and TcG4 candidates and lacking the adjuvants, delivered by the DNA/MVA approach.

With two doses of the vaccine, the mice with TcVac3-induced antibodies exhibited 92 to 96 percent protection against chronic infection. They found that the DNA/MVA approach increased the vaccine efficacy enough to omit one of the antigens and the adjuvants, making it a much simpler but still highly effective vaccine.

"Because Chagas is most prevalent in developing countries, it is essential that a potential vaccine be inexpensive to develop and easy to deliver," said Garg. "TcVac3 accomplishes this goal, making it not just an effective candidate, but an ideal one."

Future research will determine if the vaccine composition can be simplified even further. In addition, the scientists are already conducting related trials in canines. Garg and her team are also working on preclinical trials of human patient samples, testing for immune response in patients that are already infected but not showing signs of chronic disease. Results of both studies are anticipated later this year.

Journal Reference:

Innate immune system can kill HIV when a viral gene is deactivated
March 28, 2013 in HIV & AIDS
Human cells have an intrinsic capacity to destroy HIV. However, the virus has evolved to contain a gene that blocks this ability. When this gene is removed from the virus, the innate human immune system destroys HIV by mutating it to the point where it can no longer survive.

A New Drug Shows Promise of Hepatitis C Cure
March 27, 2013 in Hepatitis
Researchers report success in blocking the hepatitis C virus’s ability to colonize liver cells in 18 hepatitis C-infected patients with five weeks of treatment using the antisense oligonucleotide miravirsen. Fourteen weeks after miravirsen injections ended, hepatitis C viral loads were still undetectable in 5 of 18 patients.

Miravirsen binds tightly to messenger proteins of liver cells and blocks the hepatitis C virus from colonizing the proteins, which the virus needs to survive and replicate. Without a foothold in liver cells, the hepatitis C virus does not have the chance to develop resistance to protease inhibitors such as teleprevir and boceprevir. As a result, hepatitis C patients could be able to stop taking interferon and ribavirin, which cause side effects like fatigue, anxiety, depression, flu-like symptoms, nausea, and diarrhea.

Harvard University physician Dr. Judy Lieberman and Stanford University professor Dr. Peter Sarnow cautioned that long-term miravirsen use could be unsafe because it also targets “genetic material that helps suppress the development of fatty liver, liver fibrosis, and liver tumors”—side effects of hepatitis C. Miravirsen does offer the side benefit of lowering cholesterol; hepatitis C patients taking protease inhibitors cannot take statins that lower cholesterol.
Although miravirsen might not present a safe cure for hepatitis C, Sarnow and Lieberman stated that miravirsen could become part of a drug regimen that can keep hepatitis under control. Worldwide, 170 million people are infected with hepatitis C.


**Diarrhea Incidence Higher In Dry Seasons, Study Shows**

"Diarrhea, killer of 1.5 million children annually, is likely to become more prevalent in many developing countries as the climate changes, a report says," the Daily Climate/Scientific American reports. However, unlike conventional beliefs that diarrhea incidence increases during rainy and wet seasons, the researchers, led by Kathleen Alexander, an associate professor of wildlife at Virginia Tech's College of Natural Resources and Environment, "found an unexpected peak of diarrhea during the hottest and driest part of the year, when there were the most flies," according to the news service.

The study, published this week in the International Journal of Environmental Research and Public Health, "is based on three decades of historical data" from Botswana "and has implications for arid countries worldwide," the news service notes. "Diarrheal case incidence peaks in both seasons in Botswana, with cases 20 percent more frequent on average in the dry than the wet season," according to the study, the Daily Climate states. Alexander said, "Our findings suggest that climate change will increase the occurrence of diarrhea and the burden of disease among vulnerable populations in Botswana and similarly affected regions," the news service adds (Kirby, 3/28).

**Declaring a truce with our microbial frienemies**

Managing bacteria and other microorganisms in the body, rather than just fighting them, may be lead to better health and a stronger immune system, according to a Penn State biologist.

Researchers have historically focused on microbes in the body as primarily pathogens that must be fought, said Eric Harvill, professor of microbiology and infectious disease. However, he said that recent evidence of the complex interaction of the body with microbes suggests a new interpretation of the relationship.

"Now we are beginning to understand that the immune system interacts with far more beneficial bacteria than pathogens," said Harvill. "We need to re-envision what the true immune system really is."

Harvill said that this reinterpretation leads to a more flexible approach to understanding how the immune system interacts with microbes. This approach should balance between defending against pathogens and enlisting the help of beneficial microbes.

While the role that some bacteria play in aiding digestion is better known, microbes assist in improving body functions, including strengthening the immune system and responding to injuries.

In some cases, attacking pathogens can harm the beneficial effects microbes have on immune system, according to Harvill. For example, patients on antibiotics have an increased risk of contracting yeast infections and MRSA.

"Viewing everything currently considered immunity, including both resistance and tolerance, as aspects of a complex microbiome management system that mediates interactions with the sea of microbes that surround us, many of which are beneficial, can provide a much more positive outlook and different valuable perspectives," Harvill said.

The system that includes bacteria and other microbes in the human body, or the microbiome, is much larger and more integrated into human health than most people suspect, according to Harvill.

"The human body has ten times more bacterial cells than human cells," said Harvill. "Adding to the complexity is the adaptive capacity of the human immune system. The immune system can develop antibodies against certain pathogens, which it can reuse when threatened by future attacks from the same pathogen.

Harvill, who described his alternative viewpoint in the latest issue of mBio, said that some researchers have not yet accepted this broader approach to the immune system.

"Among immunologists or microbiologists this is an alien concept," said Harvill. "It's not part of how we have historically looked at the immune system, but it's a useful viewpoint."

Other researchers who study plant and nonhuman biology are already starting to embrace the concept. For example, plant biologists are beginning to recognize that viruses can help plants resist drought and heat.
"Within nonhuman immunology, this is not an alien concept because they have seen many examples of beneficial relationships between the host and its microbial commensals," Harvill said. Harvill said adopting this new perspective could be the first step toward new medical treatments.

"This new viewpoint suggests new experiments and results will be published," said Harvill. "And, hopefully, the concept becomes more and more mainstream as supporting evidence accumulates."

New Insights Into How Genes Turn On and Off
Mar. 27, 2013 — Researchers at UC Davis and the University of British Columbia have shed new light on methylation, a critical process that helps control how genes are expressed. Working with placentas, the team discovered that 37 percent of the placental genome has regions of lower methylation, called partially methylated domains (PMDs), in which gene expression is turned off. This differs from most human tissues, in which 70 percent of the genome is highly methylated.

While PMDs have been identified in cell lines, this is the first time they have been found in regular human tissue. In addition to enhancing our understanding of epigenetics, this work could influence cancer research and help illuminate how environmental toxins affect fetal development. The paper was published online this week in the Proceedings of the National Academy of Sciences (PNAS).

Since it was unraveled more than ten years ago, the human genome has been the focus of both popular interest and intense scientific focus. But the genome doesn't act alone; there are many factors that influence whether genes are turned on or off. One of these is an epigenetic process called methylation, in which a group of carbon and hydrogen atoms (a methyl group) attaches to DNA, adjusting how genes are expressed.

"I like to think of epigenetics as a layer on top of your genetic code," said senior author Janine LaSalle, professor of medical microbiology and immunology. "It's not the DNA sequence but it layers on top of that—and methylation is the first layer. Those layers provide a lot of information to the cells on where and when to turn on the genes."

How and when genes are activated (or inactivated) can have a profound impact on human development, cancer and the biological legacy of environmental toxins. Prior to this research, PMDs had only been found in cultured cell lines, which led some scientists to wonder if they existed outside the test tube. This study confirms they exist in placental tissue, a critically important window into fetal development.

"The placenta is the interface between mother and fetus," said LaSalle, who is a researcher affiliated with the UC Davis MIND Institute. "It's a time capsule from when a lot of important methylation events occurred."

In addition, placental tissue was interesting to study because it has a number of invasive characteristics often associated with cancer. In fact, a number of cancers, such as breast and colon, have widespread PMDs. LaSalle notes that anti-cancer epigenetic therapies that adjust methylation could be refined based on this improved understanding of PMDs.

This work could also enhance our ability to detect genetic defects. Methylation, and other epigenetic data, provides information that cannot be found in the genome alone. For example, the vast majority of cells in the body contain identical genetic code. However, the added information provided by methylation allows scientists to determine where specific DNA came from.

"Methylation patterns are like fingerprints, showing which tissue that DNA is derived from," LaSalle said. "You can't get that information from just the DNA sequence. As a result, methylation studies could be a very rich source for biomarkers."

In the study, PMDs encompassed 37 percent of the placental genome, including 3,815 genes, around 17 percent of all genes. When found in low-methylation regions, these genes were less likely to be transcribed into proteins. Researchers also found that PMDs also contain more highly methylated CpG islands (genomic areas with large numbers of cytosine-guanine pairs), which are often associated with gene transcriptional silencing of promoters.

Because the placental PMDs contained many genes associated with neuronal development, and specifically autism, LaSalle notes that future research could investigate how epigenetics impacts autism genes at birth.

"We are looking for biomarkers that predict neurodevelopmental outcomes," LaSalle said. "Now we have a series of snap shots from a critical period where we think environmental factors are playing a role in the developing brain."
Journal Reference:
The human placenta methylome. Proceedings of the National Academy of Sciences, 2013; DOI: 10.1073/pnas.1215145110