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32. Immune avoidance mechanism could lead to treatments for deadly mosquito-borne viruses
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46. Gut Bacteria Vary with Diet
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48. Prove Antibacterials are Safe: FDA
49. Epigenetics enigma resolved
50. New drug candidates show promise for cure for Chagas disease
51. Toys, books, cribs harbor bacteria for long periods, study finds
52. Genetic Clue to Fighting New Strains of Flu
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**Hepatitis C virus dried on inanimate surfaces can remain infectious for up to six weeks**

Michael Carter  
Published: 03 December 2013

Dried spots of blood contaminated with hepatitis C virus (HCV) can remain infectious for up to six weeks at normal room temperatures, research published in the online edition of the *Journal of Infectious Diseases* shows. Commercially available antiseptics reduced the infectivity of the blood spots, but only when used at recommended concentrations.

“We observed that HCVcc [cell culture] could maintain infectivity for up to 6 weeks at 4°C and 22°C,” write the authors. “Commercially available antiseptics reduced the infectivity of HCV on surfaces only when used at the recommended concentrations, but not when further diluted.”

The investigators believe their findings could explain hospital-acquired HCV infections in individuals who have not undergone surgery or received blood products, and also the ongoing HCV epidemic among injecting drug users.

HCV is a blood-borne virus.Injecting drug use is a well-known risk factor, and a large number of individuals were infected with HCV after receiving blood or blood products. But research suggests that hospital-acquired infections are occurring among patients who did not receive blood/blood products or undergo an invasive procedure. Investigators from Yale University hypothesised that this was due to contact with infectious quantities of HCV in minute dried blood spots on inanimate surfaces and objects.

They therefore performed a series of experiments to establish the circumstances in which healthcare workers or patients could come into contact with infectious HCV dried on surfaces. The investigators believe theirs is the first study to “closely simulate” the conditions leading to hospital-acquired HCV infection.

Blood spots with potential infectious HCV titres were dried onto plates and stored at temperatures of 4°C, 22°C and 37°C for up to six weeks. The authors also examined the effect of three commercially available antiseptics – bleach, cavicide and ethanol – on the infectivity of the HCV-contaminated dried blood spots.

Using a testing assay with a detection limit of 1000 RLA, potentially infectious HCV was recovered from dried blood spots stored at 37°C for up to seven days. At temperatures of 4°C and 22°C, replicating HCV was recovered for up to six weeks of storage. The infectivity of the dried spots declined sharply during the first two weeks of storage at these temperatures. Nevertheless, potentially infectious quantities of HCV – albeit at low levels – continued to be recovered for up to six weeks.

Blood spots with higher HCV titres (10^6 infectious units/ml) were also tested. Almost all spots stored at 4°C and 22°C remained potentially infectious after three weeks of storage. After ten days of storage, 100% of spots stored at 37°C also contained replicating HCV.

But commercially available antiseptics were highly effective against the HCV-contaminated blood spots. One minute of exposure to bleach (diluted to a ratio of 1:10) was 100% effective, whereas cavicide at a similar concentration was 94% effective and ethanol (70%) eliminated HCV in 87% of blood spots. The effectiveness of these disinfectants was significantly reduced when their concentrations were reduced below recommended levels.

“There are several commercially available antiseptics that are effective against HCV,” write the authors.

They conclude that HCV can remain infectious at room temperatures for up to six weeks, “a biological basis for recent observational studies reporting increasing incidence of nosocomial [hospital-acquired] HCV infections and continued high incidence among people who inject drugs.”

**Reference**


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**Big insurers cover people with HIV**

December 3 2013 at 08:00am  
By Londiwe Buthelezi  
Johannesburg—The availability of life expectancy data for people living with HIV in South Africa was likely to prompt more life assurers to offer policies for HIV positive people, insurers said yesterday.
On Sunday, Liberty’s flagship life product, Lifestyle Protector, became available to people living with HIV, with educational benefits for children of HIV-positive parents.

The company said it had taken it more than a decade to follow smaller insurers in offering insurance products for HIV-positive people because the industry did not have sufficient data to correctly price and underwrite risks associated with HIV.

“The latest research suggesting that HIV-positive individuals have life expectancies similar to HIV-negative individuals (in some instances) was a defining moment for the industry as a whole,” Liberty Retail spokesman Nicholas van der Nest said.

Sanlam, which was the first major local life insurer to offer standard life cover to people living with HIV in July, said yesterday that it had long wanted to provide cover to HIV-positive individuals but had no means of assessing its underwriting risk before the life expectancy data became available.

The two insurers said the underwriting process for HIV-positive people was similar to any other policy, where policies were priced according to risk.

But, in the case of Sanlam, a unique questionnaire is filled in by both the applicant and their doctor indicating the date of diagnosis, CD4 count and past complications, and people with a CD4 count of less than 500 must have been on antiretroviral treatment for at least six months.

Liberty also requires additional information on current and historical CD4 counts, viral load test results, as well as other information from the treating doctor.

Liberty said it believed cover for HIV-positive individuals was becoming a focus area for South African insurers.

As more life insurers started underwriting normal rather than specialised insurance policies for HIV-positive people, Marx said they had seen the cost of cover decline.

Altrisk, the first South African insurer to cover HIV infected individuals in 1999, said yesterday that it had reviewed its pricing because HIV-positive people were living longer.

“The reality is that in 14 years, we have only ever had two HIV claims – a trend that echoes the research coming from the reinsurers,” Dalene Allen, a specialist risk consultant and co-founder of Altrisk, said during its underwriting workshop yesterday.

Allen said the company’s underwriting statistics showed that people who had heart disease or diabetes as pre-existing conditions when they took out life cover sometimes paid higher premiums than many HIV-positive people who managed their condition well.

Apart from life expectancy data, increased competition in this area did not come as a surprise since national antiretroviral programmes had reduced the number of Aids deaths a year by about 25 percent to date.

In the past, only the small insurers, the likes of Altrisk and All Life, offered risk cover to HIV-positive individuals.

According to Des Martin, the president of the HIV Clinicians Society of SA, recent research shows that an HIV-positive person aged 30 years, in a developed country with access to proper health care and antiretrovirals and a CD4 count of 432, will have a life expectancy of 75 years. The same individual with a lower CD4 count of 140 on ARVs has a life expectancy of 71.5 years.—Business Report

**PM takes public HIV test, check-up**

03 December, 2013 07:00:00 By Noxolo Nkabinde
Prime Minister Sibusiso Dlamini arrived at the Mbabane government hospital without his undisclosed fiancée for blood test yesterday.

This was after the premier was challenged by HIV/AIDS activist Hannie Dlamini to go for an HIV test and urged him to do so with his fiancée.

Dlamini and his fiancée have been making news since he disclosed in public that he was now in love and it was expected that he would take her along with him to do the public HIV test, as couples usually do.

As if he knew what was expected by media representatives who were present to witness him take the test, Dlamini playfully asked the media what more they were expecting from his visit to the hospital. “Benibhakeni kani,?” he asked.

Dlamini said he had not just gone to have an HIV test but to do a full medical check-up which he usually takes every six months.

**Meeting**

He said he had already scheduled a meeting for the check-up but due to engagements he could not keep the appointment and it had to be rescheduled to yesterday. Dlamini (Hannie) had urged the premier and
his fiancée to take the test during his address at King Sobhuza Memorial at a World Aids Day commemoration on Sunday.

The premier announced his new found love a Sunday ago at Bhekinkhosi Church of the Nazarene but he has not identified her yet. He said the public would know about his fiancée when the time was right.

He has, however, hinted to the media on different occasions that he would in fact be getting married to the unidentified woman.

Our sister publication, the Sunday Observer, tracked down a woman believed to be the lover who is currently employed at one of the government offices in Lobamba but has since taken leave from work.

It was then that an employee confirmed knowledge of her colleague’s relationship with the premier.

Minister of Health Sibongile Ndelala-Simelane applauded the step taken by the premier as she said he had truly led by example. She said it was important for one to know their HIV status and do regular check-ups.

She also applauded that Dlamini went to a government hospital to do his routine medical check-up.

**Minister wants all cabinet colleagues to take HIV tests**

MINISTER of Health Sibongile Ndelala-Simelane has challenged all ministers to go for HIV testing as the world commemorates AIDS awareness month.

She said Prime Minister Sibusiso Dlamini had already led by example as he took his HIV test along with a full body check-up yesterday.

**Lead**

“Leaders should be in the lead for everything.”

“The prime minister has shown us here today that he is responsible for his health by taking the tests and doing the check-up.

**Important**

“It is important that if a leader tells his people about something he is well versed himself, so I would love to encourage all the other cabinet ministers to take the tests too,” she said.

**Test**

The minister also took a public test with her husband Mabandla at the commemoration of the World AIDS Day that was held at King Sobhuza II Memorial on Sunday.

**NIH announces plan to increase funding toward a cure for HIV/AIDS**

At a White House event today to mark the 25th annual World AIDS Day, President Obama announced that the National Institutes of Health plans to redirect AIDS research funds to expand support for research directed toward a cure for HIV. NIH plans to invest an additional $100 million over the next three fiscal years on this increasingly promising area of HIV/AIDS research.

In the three decades since AIDS was first reported, the NIH has been the global leader in research to understand, prevent, diagnose, and treat HIV infection and its many associated conditions. NIH-funded researchers — in partnership with academia and the biotechnology and pharmaceutical industries — have helped develop more than 30 life-saving antiretroviral drugs and drug combinations for treating HIV infection. These antiretroviral drugs have transformed life with HIV infection for those who have access to and can tolerate the therapies. However, treatment requires lifelong access and adherence to these medications and management of treatment-related toxicities and clinical complications.

Important recent advances in basic and therapeutics research aimed at eliminating viral reservoirs in the body are spurring scientists to design and conduct research aimed at a cure or lifelong remission of HIV infection. Key stakeholders from academia, government, foundations, advocacy groups and industry have concluded that developing a cure for HIV is one of the most important biomedical challenges of the 21st century. This will require an extraordinary, collaborative global effort, including public-private partnerships and innovative alliances to share scientific expertise and accelerate the search for a cure.

In a presentation at the White House event today, Anthony S. Fauci, M.D., director of the National Institute of Allergy and Infectious Diseases, the component of NIH with the largest investment in HIV/AIDS research, discussed the public health and scientific rationale for expanded research in this area.

“Although the HIV/AIDS pandemic can theoretically be ended with a concerted and sustained scale-up of implementation of existing tools for HIV prevention and treatment, the development of a cure is critically important, as it may not be feasible for tens of millions of people living with HIV infection to access and adhere to a lifetime of antiretroviral therapy,” Dr. Fauci noted. “Our growing understanding of the cellular hiding places or ‘reservoirs’ of HIV, the development of new strategies to minimize or deplete
these reservoirs, and encouraging reports of a small number of patients who have little or no evidence of virus despite having halted antiretroviral therapy, all suggest that the time is ripe to pursue HIV cure research with vigor."

Funding for these new initiatives will come from existing resources and a redirection of funds from expiring AIDS research grants over the next three years. NIH Director Francis S. Collins, M.D., Ph.D., said, “Flat budgets and cuts from sequestration have had a profound and damaging impact on biomedical research, but we must continue to find ways to support cutting-edge science, even in this environment. AIDS research is an example of an area where hard-won progress over many years has resulted in new and exciting possibilities in basic and clinical science in AIDS that must be pursued.”

Jack Whitescarver, Ph.D., director of the Office of AIDS Research, a component of the Office of the Director of NIH, said, “We have listened very carefully to the scientific consensus of experts from within the NIH and around the world. We have been building the portfolio of HIV cure research over the past few years, and now is the time to accelerate our research focused specifically toward the goal of sustained or lifelong remission, in which patients control or even eliminate HIV without the need for lifelong antiretroviral therapy.”

It is anticipated that a significant portion of the new investment will support basic research, which will also benefit all other areas of AIDS research, as well as research on other diseases. These studies will include research on viral reservoirs, viral latency, and viral persistence, as well as studies of neutralizing antibodies. Research on animal models, drug development and preclinical testing of more potent antiretroviral compounds capable of diminishing viral reservoirs, and clinical research, including studies on therapeutic vaccines and other immune enhancers, will also be supported.

Other high-priority AIDS research will continue to be supported. These priorities include: prevention research, including vaccines, microbicides, and other biomedical and behavioral prevention strategies, such as the use of antiretroviral drugs as prevention; research to develop better, less toxic treatments and to investigate how genetic determinants, sex, gender, race, age, nutritional status, treatment during pregnancy, and other factors, including stigma and adherence, interact to affect treatment success or failure and/or disease progression; and studies to address the increased incidence of malignancies, cardiovascular, neurologic, and metabolic complications, and premature aging associated with long-term HIV disease and antiretroviral treatment. Through all of this research, NIH is committed to the ultimate goal of a world without AIDS.

**N.H. Hospital Worker Gets 39 Years in Hepatitis Case**

*Boston Globe* (12.02.2013) By Holly Ramer

The Boston Globe reported that on December 2, New Hampshire Judge Joseph LaPlante sentenced a traveling medical technician to 39 years in prison for infecting dozens of people with hepatitis C in hospitals across the nation. The technician, who admitted to stealing painkillers and replacing them with saline-filled syringes infected with his blood, had worked in 18 hospitals in seven states before coming to Exeter Hospital in 2011. At least four other hospitals had fired the technician for alleged drug use and theft, but he continued to find cardiac technologist positions in Maryland, Kansas, Pennsylvania, Michigan, New York, Arizona, Georgia, and New Hampshire hospitals. To date, 46 of the technician’s former patients have received diagnoses of his same strain of hepatitis C; the technician infected 32 of these patients at Exeter Hospital.

The technician, who pleaded guilty in August to 16 federal drug charges, expressed sorrow and attributed his crimes to alcohol and drug addiction. Prosecutors had asked for a 40-year prison sentence, but defense attorneys requested a 30-year sentence because of the technician’s “mental and emotional problems” and addictions that clouded his judgment. Authorities in Kansas stated that hepatitis C complications contributed to the death of one patient infected by the technician.

Several patients infected by the technician attended the sentencing hearing; they expressed their anger and pain, and urged the judge to hand down a harsh punishment. Hepatitis C could cause liver disease and chronic health problems and would prevent infected persons from donating blood or organs for life-saving medical procedures like stem cell transplants.
First real-time flu forecast successful
Researchers take a page from weather forecasting to predict seasonal influenza outbreaks in 108 cities across the country

Scientists were able to reliably predict the timing of the 2012-2013 influenza season up to nine weeks in advance of its peak. The first large-scale demonstration of the flu forecasting system by scientists at Columbia University's Mailman School of Public Health was carried out in 108 cities across the United States.

Results are published online in the journal *Nature Communications*.

The flu forecasting system adapts techniques used in modern weather prediction to turn real-time, Web-based estimates of influenza infection into local forecasts of the seasonal peak by locality. Influenza activity peaked in cities in the southeast as early as December 2012, but crested in most of the country in the first weeks of 2013.

Year to year, the flu season is highly variable. It can happen anywhere from December to April. But when it arrives, cities can go from practically no cases to thousands in a very short time. "Having greater advance warning of the timing and intensity of influenza outbreaks could prevent a portion of these influenza infections by providing actionable information to officials and the general public," says first author Jeffrey Shaman, PhD, assistant professor of Environmental Health Sciences at Columbia University's Mailman School of Public Health.

For the public, the flu forecast could promote greater vaccination, the exercise of care around people sneezing and coughing, and a better awareness of personal health. For health officials, it could inform decisions on how many vaccines and antiviral drugs to stockpile, and in the case of a virulent outbreak, whether other measures, like closing schools, are necessary.

Study Results
The new study builds on the researchers' 2012 study that used the system to retrospectively predict the peak of the flu in New York City for the years 2003-2008. That research was limited to one city and performed as a test of the system. The current study is the first to make predictions in actual real-time and for the whole country.

Beginning in late November of 2012, the researchers used the flu forecasting system to perform weekly estimates for 108 cities. They shared the results with the CDC and posted them online in an academic archive. Near the end of 2012, four weeks into the flu season, the system had predicted 63% of cities accurately. As the season progressed, the accuracy increased. By week four, it successfully predicted the seasonal peak in 70% of the country. It was able to give accurate lead-times up to nine weeks in advance of the peak; most lead-times were two to four weeks.

The flu forecasts were also much more reliable than those made using alternate, approaches that rely on historical data. "Our method greatly outperformed these alternate schemes," says Dr. Shaman.

The researchers saw regional differences in the accuracy of the system, but they were likely within normal variation. "As an example, retrospectively, we've been able to predict the flu in Chicago very well; this year we did a terrible job in that city. For other cities, the opposite held. It averages out. On the whole the system performed very well," Dr. Shaman says. However, there were hints of geographical differences. "We were able make better predictions in smaller cities. Population density may also be important. It suggests that in a city like New York, we may need to predict at a finer granularity, perhaps at the borough level. In a big sprawling city like Los Angeles, we may need to predict influenza at the level of individual neighborhoods."

Google Flu Trends Goes "Off the Rails"
The researchers designed the flu forecasting system to use combined data from 1) Google Flu Trends, which makes estimates of outbreaks based on the number of flu-related search queries, and 2) region-specific reports from the Centers for Disease Control on verified cases of flu. The system approach is analogous to weather forecasting, which employs real-time observational data to reduce model forecasts error. In the last year, the researchers slightly modified the system to be more representative of flu rather than flu and other respiratory problems. Nevertheless, there was unusual level of "noise" in the data related to problems with Google Flu Trends.

How did this happen? One explanation is the high number of media stories about the flu, including some about the flu forecasting system itself. The result was a spike in people using Google to research the flu, which could have overloaded the Flu Trends algorithm. It's an irony not lost on Dr. Shaman. "There was a tremendous amount of media attention accorded to the flu last year. I was part of the problem myself," he says. Another factor may have been the particular strain of flu in circulation. "The flu was very
virulent and was making people very sick, more so than previous seasons," says Dr. Shaman. Again this could have led to spike in flu-related Google search queries. (In October, Google announced that it has revised the Flu Trends, which Dr. Shaman hopes will make flu forecasting more accurate.)

The system will be put back in action as soon as the flu season begins again. "Right now there are few cases of the flu, but as soon as the needle starts to move, we will start making predictions," says Dr. Shaman. This season the forecasts will be more readily available to the public on a website hosted by Columbia's Mailman School of Public Health expected to launch in the coming weeks.

Worldwide, influenza kills an estimated 250,000 to 500,000 people each year, according to the World Health Organization. In the U.S. 3,000-49,000 die from the flu every year, and about 45% of Americans were vaccinated for the flu, according to the CDC.

**Tuberculosis: Nature has a double-duty antibiotic up her sleeve**

A natural antibiotic turns out to be a lethal weapon in the fight against tuberculosis. Scientists have discovered it has an unexpected dual action that dramatically reduces the probability that TB bacteria will become resistant

Technology has made it possible to synthesize increasingly targeted drugs. But scientists still have much to learn from Mother Nature. Pyridomycin, a substance produced by non-pathogenic soil bacteria, has been found to be a potent antibiotic against a related strain of bacteria that cause tuberculosis. The EPFL scientists who discovered this unexpected property now have a better understanding of how the molecule functions. Its complex three-dimensional structure allows it to act simultaneously on two parts of a key enzyme in the tuberculosis bacillus, and in doing so, dramatically reduce the risk that the bacteria will develop multiple resistances. The researchers, along with their colleagues at ETH Zurich, have published their results in the journal *Nature Chemical Biology*.

Stewart Cole, director of EPFL's Global Health Institute, led a team that discovered the anti-tuberculosis effect of pyridomycin in 2012. By inhibiting the action of the "InhA" enzyme, pyridomycin literally caused the thick lipid membrane of the bacterium to burst. Now the scientists understand how the molecule does this job.

**Dual anti-mutation ability**

The tuberculosis bacillus needs the InhA enzyme along with what scientists refer to as a "co-factor," which activates the enzyme, in order to manufacture its membrane. The scientists discovered that pyridomycin binds with the co-factor, neutralizing it.

But pyridomycin doesn't stop there. It also blocks another element needed for making the membrane, the InhA binding site. "Researchers in the pharmaceutical industry have been looking for this weakness in the TB bacillus for decades," explains Ruben Hartkoorn, first author on the article.

By binding simultaneously onto these two elements and neutralizing them, pyridomycin prevents the bacterium from generating its membrane, and it ends up bursting like a balloon. Better still, this dual action drastically reduces the risk that the bacteria will become resistant, because in order to develop resistance, two different specific mutations must exist at the same time. This is increasingly important because cases of multi-resistant TB are on the rise.

**Nature's twisting paths – a lesson in efficiency**

"It's a powerful lesson from nature with respect to drug design," explains Cole, co-author and EPFL professor. "The three-dimensional structures of naturally occurring molecules are often more complex, more twisted, than synthetic molecules, and that's precisely what allows pyridomycin to bind onto these two sites simultaneously."

In fact, it binds so effectively that the molecule is not yet ready to be used therapeutically: it doesn't last long enough in the patient's body. This is the point at which bioengineering needs to take over from Mother Nature – to develop a more robust version of the molecule. This is what the ETH team led by Karl-Heinz Altmann is working on. "Eventually we could multiply the molecule's binding sites, so that it could inhibit critical functions of other pathogenic bacteria," says Cole.

**Have researchers found a new treatment for sepsis?**

University of Leicester academics discover new receptor that may be instrumental in the body's response to devastating disease

Sepsis, or septicaemia, is a devastating disease that is difficult to diagnose early and for which treatment options are limited. The number of deaths from sepsis exceeds those from lung cancer, and from breast and bowel cancer combined.
Sepsis can affect any age group and is the leading cause of death in Intensive Care: it is estimated that 37,000 people die from severe sepsis in the UK each year with annual NHS costs exceeding £1.5billion.

Sepsis has until recently been under-recognised and despite advances in understanding the biological processes involved, there is still no effective treatment beyond supportive therapy.

Professor David Lambert and Dr Jonathan Thompson of the Department of Cardiovascular Sciences at the University of Leicester have published two collaborative research papers indicating that a newly discovered receptor in the body — similar to the receptors for endorphins or for morphine— might be important in the body's response to sepsis, which could be the key to unlocking a new treatment in the future.

This new receptor is called the 'nociceptin receptor' and the natural substance that activates it is called **nociceptin**.

The body's initial response to sepsis is to produce an intense reaction from the immune system to fight the infection. This first involves activation of white blood cells, stress hormones and other substances, known as 'inflammatory mediators', which cause inflammation.

It has already been found that nociceptin is involved in inflammation; it affects how white blood cells work. This suggests strongly that nociceptin has an important role in the body's response to inflammation and sepsis. Their theory, which they have explored in both research papers, is that **nociceptin makes inflammation or sepsis worse; by blocking the nociceptin system, the symptoms of sepsis could be reduced, which could lead to new treatments.**

In the first of the two papers Professor Lambert, as part of a collaboration with Dr Zoë Brookes at the University of Sheffield and Dr Girolamo Calo and Dr Remo Guerrini at the University of Ferrara, has shown for the first time using fluorescent chemistry—which was designed in Ferrara—that **nociceptin receptors are found on blood vessels with no nerve supply** and that in a laboratory model of sepsis, blocking these receptors is protective. This work was funded by British Journal of Anaesthetia / Royal College of Anaesthetists and Anaesthetic Research Society.

In the second paper, funded by the Association of Anaesthetists of Great Britain and Ireland and British Journal of Anaesthesia / Royal College of Anaesthetists, Dr Thompson and Professor Lambert have discovered that nociceptin levels in the bloodstream are elevated in patients with sepsis in Intensive Care, demonstrating that nociceptin activation might be important in critically ill patients suffering from sepsis.

Sepsis remains a leading cause of admission to Intensive Care Units, with high mortality, costs, and long-term morbidity in those who survive. The incidence of severe sepsis has increased over the last decade, making the discovery of new treatments highly desirable.

Dr Jonathan Thompson said: "Sepsis is a major health problem for the NHS that has often been under-recognised. It can be rapidly fatal, especially if not diagnosed and treated early, because inflammation can spread and affect many different organs in the body.

"Clinicians are making progress in the early recognition and treatment of sepsis, but we have no specific drugs that effectively stop the spread of inflammation, or the biological processes involved. We have found that nociceptin, a chemical similar to endorphins produced in the body, is increased in inflammation and sepsis.

"This suggests that drugs which block the nociceptin receptor could dampen the widespread inflammation that occurs in sepsis, and improve outcome. More work is needed, but these drugs are being developed. If they are effective then we could potentially save many lives."

Professor David Lambert added: "I am particularly excited by these findings as they translate many years of laboratory work into a possible target for this disease."

The first paper, 'The Nociceptin/Orphanin FQ Receptor Antagonist UFP-101 Reduces Microvascular Inflammation to Lipopolysaccharide In Vivo', can be accessed at the following link: http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0074943

The second paper, 'The Nociceptin/Orphanin FQ System Is Modulated in Patients Admitted to ICU with Sepsis and after Cardiopulmonary Bypass', can be accessed at the following link: http://HIV-1 movement across genital tract cells surprisingly enhanced by usurping antibody response

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**UCI-led study results have important implications for HIV vaccine development**

Irvine, Calif., Dec. 3, 2013 — Infectious disease researchers have identified a novel mechanism wherein HIV-1 may facilitate its own transmission by usurping the antibody response directed against itself. These
results have important implications for HIV vaccine development and for understanding the earliest events in HIV transmission.

In a study appearing in the November issue of *PLoS Pathogens*, Dr. Donald Forthal of UC Irvine and colleagues studied the mechanisms employed by the virus to cross genital tract tissue and establish infection. Since cervicovaginal fluid is acidic and HIV-1 in cervicovaginal fluid is likely coated with antibodies, they explored the effect of low pH and HIV-1-specific antibodies on transcytosis, the movement of HIV-1 across tight-junctioned epithelial cells.

The researchers found that the combination of HIV-1-specific antibodies and low pH enhanced transcytosis as much as 20-fold.

Virus that underwent transcytosis under these conditions was infectious, and infectivity was highly influenced by whether or not the antibody neutralized the virus. They observed enhanced transcytosis using antibody from cervicovaginal and seminal fluids and using transmitted/founder strains of HIV-1. Enhanced transcytosis was due to the Fc neonatal receptor (FcRn), which binds immune complexes at acidic pH and releases them at neutral pH. Finally, staining of human tissue revealed abundant FcRn expression on columnar epithelial cells of penile urethra and endocervix.

The study is accessible at *PLoS Pathogens* at:
http://www.plospathogens.org/article/info%3Adoi%2F10.1371%2Fjournal.ppat.1003776
www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0076682

**Toxigenic C. difficile resides harmlessly in infants, poses risk to adults**

Infants and toddlers frequently carry toxigenic *Clostridium difficile*, usually with no harm to themselves, but can serve as a reservoir and spread the bacteria to adults in whom it can cause severe disease, according to a study by a team of Swedish researchers published ahead of print in the *Journal of Clinical Microbiology*.

In the study, which involved following 42 children from birth to 1 ½ or 3 years, the investigators found that *C. difficile* strains persisted for more than six months in roughly one third of such infants. A majority of the persistent colonizations belonged to either of two toxigenic ribotypes which both have commonly been isolated from adult and elderly people with *C. difficile* toxin-mediated disease in Sweden and in other countries.

Previous studies from the 1980s found that the rate of colonization peaked during the first six months of life, and then declined, as the gut microbiota became more complex. A 2000 study by the current study author found that colonization by *C. difficile* kept rising until about a year of age.

"We think that this is the result of an impoverishment of the gut flora, that infants have fewer types of bacteria in their gut, compared to 30 years ago," says first author Ingegerd Adlerberth, of the University of Gothenberg, Sweden. "It is known that gut microbiota of high complexity suppresses *C. difficile* growth and toxin production. That is why treatment with broad-spectrum antibiotics is a risk factor for *C. difficile* disease."

The paper concludes with a warning that the prevalence of toxigenic *C. difficile* bacteria in the gut of infants and young children "provides ample opportunity for spread to individuals at risk for *C. difficile* disease."

*C. difficile* disease has been notoriously hard to treat in the elderly, who often undergo numerous courses of antibiotics without eliminating the disease. Recently, a still highly experimental treatment, fecal transplant, has proven far more successful. That treatment involves taking fecal material from a healthy person, and inserting it into the diseased patient’s colon.

A copy of the manuscript can be found online at [http://bit.ly/asmtip1213a](http://bit.ly/asmtip1213a). The article is scheduled for formal publication in the January 2014 issue of the *Journal of Clinical Microbiology*. 
New Research Shows Promise for Possible HIV Cure

Dec. 3, 2013 — Researchers have used radioimmunotherapy (RIT) to destroy remaining human immunodeficiency virus (HIV)-infected cells in the blood samples of patients treated with antiretroviral therapy, offering the promise of a strategy for curing HIV infection.

Results of the study were presented today at the annual meeting of the Radiological Society of North America (RSNA).

Highly active antiretroviral therapy (HAART) has transformed the outlook for patients infected with HIV by suppressing the replication of the virus in the body. However, despite the success of HAART in effectively reducing the burden of HIV, scientists believe reservoirs of latently infected cells persist in the body, preventing the possibility of a permanent cure.

"In an HIV patient on HAART, drugs suppress viral replication, which means they keep the number of viral particles in a patient's bloodstream very low. However, HAART cannot kill the HIV-infected cells," said the study's lead author, Ekaterina Dadachova, Ph.D., professor of radiology, microbiology and immunology at Albert Einstein College of Medicine in the Bronx, N.Y. "Any strategy for curing HIV infection must include a method to eliminate viral-infected cells."

In her study, Dr. Dadachova and a team of researchers administered RIT to blood samples from 15 HIV patients treated with HAART at the Einstein-Montefiore Center for AIDS Research.

RIT, which has historically been employed to treat cancer, uses monoclonal antibodies—cloned cells that are recruited by the immune system to identify and neutralize antigens. Antigens are foreign objects like bacteria and viruses that stimulate an immune response in the body. The antibody, designed to recognize and bind to a specific cell antigen, is paired with a radioactive isotope. When injected into the patient's bloodstream, the laboratory-developed antibody travels to the target cell where the radiation is then delivered.

"In RIT, the antibodies bind to the infected cells and kill them by radiation," Dr. Dadachova said. "When HAART and RIT are used together, they kill the virus and the infected cells, respectively."

For the study, Dr. Dadachova's team paired the monoclonal antibody (mAb2556) designed to target a protein expressed on the surface of HIV-infected cells with the radionuclide Bismuth-213.

The researchers found that RIT was able to kill HIV-infected lymphocytes previously treated with HAART, reducing the HIV infection in the blood samples to undetectable levels.

"The elimination of HIV-infected cells with RIT was profound and specific," Dr. Dadachova said. "The radionuclide we used delivered radiation only to HIV-infected cells without damaging nearby cells."

An important part of the study tested the ability of the radiolabeled antibody to reach HIV-infected cells in the brain and central nervous system. Using an in vitro human blood brain barrier model, the researchers demonstrated that radiolabeled mAb2556 could cross the blood brain barrier and kill HIV-infected cells without any overt damage to the barrier itself.

"Antiretroviral treatment only partially penetrates the blood brain barrier, which means that even if a patient is free of HIV systemically, the virus is still able to rage on in the brain, causing cognitive disorders and mental decline," Dr. Dadachova said. "Our study showed that RIT is able to kill HIV-infected cells both systemically and within the central nervous system."

According to Dr. Dadachova, clinical trials in HIV patients are the next step for the RIT treatment.

1950s Pandemic Influenza Virus Remains a Health Threat, Particularly to Those Under 50

Dec. 3, 2013 — St. Jude Children’s Research Hospital scientists have evidence that descendants of the H2N2 avian influenza A virus that killed millions worldwide in the 1950s still pose a threat to human health, particularly to those under 50. The research has been published in an advance online edition of the Journal of Virology.

The study included 22 H2N2 avian viruses collected from domestic poultry and wild aquatic birds between 1961 and 2008, making it the most comprehensive analysis yet of avian H2N2 viruses.
Researchers reported the viruses could infect human respiratory cells. Several strains also infected and spread among ferrets, which are susceptible to the same flu viruses as humans. Based on those and other indicators, one virus was classified as possessing a high risk for triggering a pandemic.

Researchers found evidence the viruses were susceptible to current antiviral medications and could likely be controlled with an available prototype vaccine.

Such protection was unavailable in 1957 when an H2N2 virus that included genes from avian flu viruses emerged. Federal health officials estimate the 1957-58 pandemic killed 1 to 2 million people worldwide. While the H2N2 strain disappeared from flu viruses circulating in humans in 1968, it has persisted in the world’s bird population.

"This study suggests H2N2 has the characteristics necessary to re-emerge as a significant threat to human health in part because most individuals under the age of 50 lack immunity to the virus," said corresponding author Robert Webster, Ph.D., a member of the St. Jude Department of Infectious Diseases. "This highlights the importance of continued surveillance of viruses circulating in animals and additional research to enhance our ability to identify viruses that are emerging health threats."

The research stems from the institution’s role as a National Institute of Allergy and Infectious Diseases Center of Excellence for Influenza Research and Surveillance. St. Jude is also home to the only World Health Organization Collaborating Center focused on the spread of animal flu viruses to humans.

Historically, pandemic flu viruses arise when bird and human flu viruses swap genes. The mixing can result in novel viruses capable of spreading efficiently in humans and against which the human immune system is unprepared. "One school of thought regarding emerging flu viruses is that in more than 100 years, only three of the 18 subtypes of influenza A have caused pandemics. The H2 subtype is one," Webster said. The H2N2 viruses in this study remained genetically similar to the 1957 pandemic strain.

Along with being able to infect human trachea and other mammalian cells growing in the laboratory, five viruses also infected ferrets, according to researchers. Ferrets are a reliable model for studying flu’s spread in humans. The five strains were among the nine H2N2 viruses that researchers tested in ferrets.

Three of the strains demonstrated a surprising ability to spread among ferrets housed in the same cage. The strains included the Dk/HK319/79 virus, which researchers classified as having high pandemic potential. The virus was isolated in 1979 from a duck in Hong Kong. The other viruses were classified as having low to intermediate pandemic potential. None of the viruses studied in ferrets spread via airborne transmission.

In addition, none of the viruses showed changes in the two viral proteins viewed as indicators of avian flu virus adaptation to human infection and transmission. Those markers are the hemagglutinin (HA) protein that the virus uses to infect cells and the PB2 protein, which is required for viral replication. The viruses in this study had HA and PB2 proteins with a preference for infecting avian, rather than human cells.

"While these viruses genetically look very avian, this study shows they can behave like mammalian viruses and replicate in multiple mammalian models of flu," said the study's first author, Jeremy Jones, Ph.D., a postdoctoral fellow in Webster’s laboratory. "That is troubling because some of the original H2N2 pandemic viruses looked avian when the pandemic began in 1957, but in a few short months, all of the isolated viruses had picked up the genetic signatures of adaptation to humans. Our results suggest the same could happen if the H2N2 viruses again crossed from birds into humans."

Work is underway at St. Jude to identify other changes that are critical to the ability of avian flu viruses to infect and replicate in mammalian cells, Jones said.

Journal Reference:

New Weapon in War Against Superbugs
Dec. 2, 2013 — In the arms race between bacteria and modern medicine, bacteria have gained an edge. In recent decades, bacterial resistance to antibiotics has developed faster than the production of new antibiotics, making bacterial infections increasingly difficult to treat. Scientists worry that a particularly virulent and deadly “superbug” could one day join the ranks of existing untreatable bacteria, causing a public health catastrophe comparable with the Black Death.

Now research led by Dr. Udi Qimron of Tel Aviv University's Department of Clinical Microbiology and Immunology at the Sackler Faculty of Medicine has discovered a protein that kills bacteria. The isolation of this protein, produced by a virus that attacks bacteria, is a major step toward developing a substitute for conventional antibiotics. "To stay ahead of bacterial resistance, we have to keep developing new
antibiotics," said Dr. Qimron. "What we found is a small protein that could serve as a powerful antibiotic in the future."

Dr. Ido Yosef, Ruth Kiro, and Shahar Molshanski-Mor of TAU's Sackler Faculty of Medicine and Dr. Sara Milam and Prof. Harold Erickson of Duke University contributed to the research, published in the Proceedings of the National Academy of Sciences.

Teaming up with a killer
Bacterial resistance is a natural process. But over the past sixty years or so, the misuse and overuse of antibiotics has pushed more and more bacteria to become more and more resistant, undermining one of the pillars of modern health care. Recently, the World Health Organization named growing antibiotic resistance one of the three greatest threats to public health.

Bacteriophages, often referred to as "phages," are viruses that infect and replicate in bacteria. Because they coevolved with bacteria, they are optimized to kill them. As proof of their endurance, phages are the most common life form on earth, outnumbering bacteria 10 to one. In places like the former Soviet Union, phages have been used to treat bacterial infections for the past hundred years. Harmless to humans, they inject their DNA into bacteria and rapidly replicate, killing their hosts.

"Ever since the discovery of bacteriophages in the early 20th century, scientists have understood that, on the principle of the 'enemy of my enemy is my friend,' medical use could be made of phages to fight viruses," said Dr. Qimron.

Breaking out the little guns
Dr. Qimron and his colleagues set out to understand how all 56 proteins found in T7, a particularly virulent phage that infects Escherichia coli bacteria, contribute to its functioning. They discovered that one of the proteins, called 0.4, impedes cell division in E. coli, causing the cells of the bacteria to elongate and then die. The protein is common to many bacteria and a similar process occurs in all bacteria, so the finding may have wide application.

No bacteriophage preparation has been approved in Western medicine for treating systemic bacterial infections. One reason is their inability to penetrate body tissues effectively. They are filtered effectively from the bloodstream by the spleen and liver, and occasionally neutralized by antibodies. But the 0.4 protein is much smaller than a whole phage, and so should be able to penetrate tissue better, getting to the bacteria to do its deadly work.

The major challenge for pharmaceutical companies will be figuring out how exactly to deliver the protein as a drug, said Dr. Qimron. In the meantime, he continues to hunt for other proteins that kill bacteria.

Journal Reference:

Key Found to Restoring 'Exhausted' HIV-Fighting Immune Cells
Dec. 2, 2013 — Researchers have identified a protein that causes loss of function in immune cells combatting HIV. The scientists report in a paper appearing online Dec. 2 in the Journal of Clinical Investigation that the protein, Sprouty-2, is a promising target for future HIV drug development, since disabling it could help restore the cells' ability to combat the virus that causes AIDS.

"A large part of the reason we lose wars against viruses that cause chronic infection is that immune cells called T cells get turned off," says Jonathan Schneck, M.D., Ph.D., a professor of pathology in the Johns Hopkins Institute for Cell Engineering, who led the study. "We've been trying for some time to find out why that is, and in our study we were able to identify a family of proteins called Sprouty, specifically Sprouty-2, as a culprit."

T cells, a type of white blood cell, are programmed to recognize and kill cells infected with a specific virus or other disease-causing agent. Doing so effectively requires a T cell to perform multiple functions—par for the course when the cells are fighting a fresh infection. But when infection drags on,
as HIV does, T cells often become "exhausted," losing two or more functions, except among so-called "elite suppressors"—rare HIV-infected patients whose T cells never seem to tire.

To investigate the source of exhaustion, the Johns Hopkins team first sought a way to recreate that phenomenon in the laboratory. Yen-Ling Chiu, M.D., a graduate student in Schneck's laboratory, was able to simulate the effects of long-term chronic infection by growing influenza-fighting T cells and dosing them with large amounts of antigen, a type of molecule that, like a red flag waved at a bull, signals immune cells to attack. Doing this "made the T cells dysfunctional—they looked like exhausted T cells in HIV," Chiu says.

Chiu next looked for differences in proteins made in the dysfunctional cells compared to those in fresh T cells. It turned out, he says, that many of the proteins whose quantities were different between the two groups of cells were involved in a biochemical chain of events called the MAPK/ERK pathway. That pathway controls a variety of important processes, such as cell division. One of the proteins that was more abundant in the exhausted T cells than in the fresh T cells was Sprouty-2, which, other studies had shown, slows down the MAPK/ERK pathway. Suspecting that Sprouty-2 could be the culprit in T cell exhaustion, Chiu used a specially engineered virus to disable the Sprouty-2 gene in some T cells and found that they were more likely to retain all of their functions than were the cells with working Sprouty-2.

"It was a surprise to find T cell exhaustion controlled by such a central pathway, and especially that it worked in such a highly fine-tuned way, keeping some T cell functions going but not others," says Schneck.

Though the finding was intriguing, Schneck says, "the real test was whether it held up in blood samples from HIV patients." In one experiment, reducing the amount of Sprouty-2 in exhausted HIV-fighting T cells partially restored them, the researchers found. Drawing on the work of other groups, which had shown that reducing the amount of another protein, called PD-1, modestly improved the function of exhausted T cells, the research team next tried disabling both Sprouty-2 and PD-1 in the HIV-fighting cells. "That reversed the exhaustion completely," Schneck says.

"By identifying Sprouty-2 and related proteins as potential targets, these results support the idea that immunotherapy may someday lead to a functional cure for HIV-1 infection," says Joel Blankson, M.D., Ph.D., an associate professor in the Department of Medicine's Division of Infectious Diseases, who also took part in the study.

There are no known chemical compounds that can block Sprouty-2 in living animals, so Schneck's group plans to begin looking for one—a first step toward potentially developing new drugs based on the discovery. Schneck notes that they will also investigate whether Sprouty protein causes exhaustion in T cells fighting other chronic infections, such as tuberculosis and hepatitis C.

Journal Reference:

'Nanosponge Vaccine' Fights MRSA Toxins

The glowing yellow specks in the image show uptake of the nanosponge vaccine by a mouse dendritic cell—an immune-system cell. The detained alpha-haemolysin toxins were labeled with a fluorescent dye which glows yellow. The nanosponge vaccine with detained toxins and can be seen glowing yellow after uptake by the dendritic cell. The cell is membrane stained red and the nucleus stained blue.

Nanospinges that soak up a dangerous pore-forming toxin produced by MRSA.
Dec. 1, 2013 — Nanosponges that soak up a dangerous pore-forming toxin produced by MRSA (methicillin-resistant *Staphylococcus aureus*) could serve as a safe and effective vaccine against this toxin. This "nanosponge vaccine" enabled the immune systems of mice to block the adverse effects of the alpha-haemolysin toxin from MRSA—both within the bloodstream and on the skin. Nanoengineers from the University of California, San Diego described the safety and efficacy of this nanosponge vaccine in the December 1 issue of *Nature Nanotechnology*.

The nanosponges at the foundation of the experimental "toxoid vaccine" platform are bio-compatible particles made of a polymer core wrapped in a red-blood-cell membrane. Each nanosponge's red-blood-cell membrane seizes and detains the *Staphylococcus aureus* (staph) toxin alpha-haemolysin without compromising the toxin's structural integrity through heating or chemical processing. These toxin-studded nanosponges served as vaccines capable of triggering neutralizing antibodies and fighting off otherwise lethal doses of the toxin in mice.

"With our toxoid vaccine, we don't have to worry about antibiotic resistance. We directly target the alpha-haemolysin toxin," said Liangfang Zhang, a nanoengineering professor at UC San Diego Jacobs School of Engineering and the senior author on the paper. Targeting the alpha-haemolysin toxin directly has another perk. "These toxins create a toxic environment that serves as a defense mechanism which makes it harder for the immune system to fight Staph bacteria," explained Zhang.

Beyond MRSA and other staph infections, the nanosponge vaccine approach could be used to create vaccines that protect against a wide range of toxins, including those produced by *E. coli* and *H. pylori*.

This work from Zhang's Nanomaterials and Nanomedicine Laboratory at the UC San Diego included nanoengineering post-doctoral researcher Che-Ming "Jack" Hu, nanoengineering graduate student Ronnie Fang, and bioengineering graduate student Brian Luk.

The researchers found that their nanosponge vaccine was safe and more effective than toxoid vaccines made from heat-treated staph toxin. After one injection, just 10 percent of staph-infected mice treated with the heated version survived, compared to 50 percent for those who received the nanosponge vaccine. With two more booster shots, survival rates with the nanosponge vaccine were up to 100 percent, compared to 90 percent with the heat-treated toxin.

"The nanosponge vaccine was also able to completely prevent the toxin's damages in the skin, where MRSA infections frequently take place," said Zhang, who is also affiliated with the Moores Cancer Center at UC San Diego.

**Fighting Pore-Forming Toxins**

This work is a twist on a project the UC San Diego nanoengineers presented earlier this year: a nanospone that can sop up a variety of pore-forming toxins—from bacterial proteins to snake venom—in the body.

Pore-forming toxins work by punching holes in a cell's membrane and letting the cell essentially leak to death. But when toxins attack the red blood cell membrane draped over the nanoparticle, "nothing will happen. It just locks the toxin there," Zhang explained.

The nanoengineers wondered what would happen if they loaded one of their nanosponges with staph toxin in this way, and presented the whole package to an essential part of the immune system called dendritic cells. Could the loaded particles trigger an immune response and work as a toxoid vaccine?

Staph toxin is so powerful that it kills immune cells in its unaltered form. Most vaccine candidates, therefore, use a heat or chemically processed version of the toxin that unravels some of its proteins and makes it a little weaker. But this process also makes the immune response to the toxin a little weaker.

"The more you heat it, the safer the toxin is, but the more you heat it, the more you damage the structure of the protein," Zhang explained. "And this structure is what the immune cell recognizes, and builds its antibodies against."

The nanosponge toxoid vaccine gets around this problem by detaining—but not changing—the staph toxin. Like a dangerous but handcuffed prisoner, the staph toxin can be led to the dendritic cells of the immune system without causing any harm.

Before this, "there was no way you could deliver a native toxin to the immune cells without damaging the cells," Zhang said. "But this technology allows us to do this."
Each vaccine particle is approximately 85 nanometers in diameter; for comparison, about 1000 of them would fit across the width of a single human hair. They are cleared from the body after injection in about two weeks, the researchers found.

**Staphylococcus aureus**
Staph bacteria are one of the most common causes of skin infections, and can cause blood poisoning and surgical infections as well as pneumonia. According to the Centers for Disease Control and Prevention, about 80,000 Americans suffer from invasive MRSA infections each year, and over 11,000 of those individuals die. At the moment, there are no vaccines approved to protect humans against the toxins associated with staph infections, including those caused by MRSA strains.

The idea for a staph vaccine came about when the researchers considered the success of their nanosponge. If the particle was so good at collecting toxins, they wondered, what were the potential uses of a particle full of toxin? "To be honest, we never thought about the vaccine use from the beginning," Zhang noted. "But when we do research, we always want to look at a problem in reverse."

In a way, the toxoid vaccine hearkens back to their first use for the particles, as a cancer drug delivery device, Zhang noted.

The particles "work so beautifully," Zhang said, that it might be possible to detain several toxins at once on them, creating "one vaccine against many types of pore-forming toxins," from staph to snake venom.

**Journal Reference:**

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**Exonic Transcription Factor Binding Directs Codon Choice and Affects Protein Evolution**
Andrew B. Stergachis1, Eric Haugen1, Anthony Shafer1, Wenching Fu, Benjamin Vernot1, Alex Reynolds1, Anthony Raubitschek2,4, Steven Ziegler3, Emily M. LeProust4,*, Joshua M. Akey1, John A. Stamatoyannopoulos

**Abstract**
Genomes contain both a genetic code specifying amino acids and a regulatory code specifying transcription factor (TF) recognition sequences. We used genomic deoxyribonuclease I footprinting to map nucleotide resolution TF occupancy across the human exome in 81 diverse cell types. We found that ~15% of human codons are dual-use codons ("duons") that simultaneously specify both amino acids and TF recognition sites. Duons are highly conserved and have shaped protein evolution, and TF-imposed constraint appears to be a major driver of codon usage bias. Conversely, the regulatory code has been selectively depleted of TFs that recognize stop codons. More than 17% of single-nucleotide variants within duons directly alter TF binding. Pervasive dual encoding of amino acid and regulatory information appears to be a fundamental feature of genome evolution.

**Editor's Summary**
Transcription Factor Binding Sites
Transcription factors (TFs) are proteins that bind to DNA to control gene transcription. Stergachis et al. (p. 1367; see the Perspective by Weatheritt and Babu) examined TF binding within the human genome in more than 80 cell types. Nearly 15% of coding regions simultaneously specify both amino acid sequence and TF recognition sites. The distribution of the TF binding sites evolutionarily constrains how codons within these regions can change, independent of encoded protein function. Thus, TF binding may represent a widespread and strong evolutionary force in coding regions.

**Duons: Researchers Find Second Code Hiding within DNA**
Dec 13, 2013 by Sci-News.com
A large team of scientists led by Washington University geneticist Dr John Stamatoyannopoulos has found a ‘secret’ second code hiding within human DNA. Since the genetic code was deciphered in the 1960s, researchers have assumed that it was used exclusively to write information about proteins.

The genetic code uses a 4³=64-letter alphabet called codons.

Dr Stamatoyannopoulos with co-authors were stunned to discover that some codons, which they called duons, can have two meanings. One describes how proteins are made, and the other instructs the cell on how genes are controlled.
These two meanings seem to have evolved in concert with each other. The gene control instructions appear to help stabilize certain beneficial features of proteins and how they are made.

“For over 40 years we have assumed that DNA changes affecting the genetic code solely impact how proteins are made. Now we know that this basic assumption about reading the human genome missed half of the picture,” said Dr Stamatoyannopoulos, who is the senior author of the study published in the journal Science.

The study highlights that DNA is an incredibly powerful information storage device, which nature has fully exploited in unexpected ways.

The discovery of duons has major implications for how scientists and physicians interpret a patient’s genome and will open new doors to the diagnosis and treatment of disease.

“The fact that the genetic code can simultaneously write two kinds of information means that many DNA changes that appear to alter protein sequences may actually cause disease by disrupting gene control programs or even both mechanisms simultaneously,” Dr Stamatoyannopoulos said.

12/13/2013 @ 12:35PM
Human DNA Is Not A Document, It's An App

Yesterday, scientists at the University of Washington announced what they characterized as an important breakthrough in our understanding of the nature of DNA. Led by genome scientist Dr. John Stamatoyannopolous, the team discovered evidence of a “second code” written into DNA. This code controls what are called transcription factors (TF) which regulate the flow (or transcription) of genetic information from DNA to messenger RNA that manage the synthesis of proteins described in the DNA.

Transcription factors are nothing new, they have been an object of study for more than 20 years. What they are claiming is new here, according to the paper published in the journal Science yesterday, is that “~15% of human codons are dual-use codons (“duons”) that simultaneously specify both amino acids and TF recognition sites.” Codons are he nucleotide triplets (the “N” in DNA) that specify which amino acid to add next in the process of synthesizing a protein. Of particular significance is that the experiments were carried out on cell lines from the human exome, the 1% the genome that remains inside mature RNA and whose mutations are thought to harbor 85% of disease-causing mutations.
As my colleague Emily Willingham makes clear (see link above), this may not be all that it is cracked up to be. The salient fact is that “the genome contains more of these dual-use DNA sequences than previously thought.” Let’s accept the fact that this is not a discovery that schools Francis Crick and James Watson, but does it change the way people will think about DNA? Changing the way people think is the coin of the realm in technology because, even more than science, it trades on the new. With anything truly new, we need analogies as prosthetics before a new paradigm is accepted. When Crick and Watson announced their discoveries about the structure and function of DNA in 1953, it took years before the general public had any idea what the double helix really meant.

Half a century later we think about code more as computer code, and the publicity around the U. Washington research may be a good time to bring popular notions of how DNA works up to date. Willingham also explains that it is a commonplace that “the DNA sequence both contains code for proteins and serves a regulatory purpose.” Rather than being a “second code,” as the University’s article states, it is really “a different (but already recognized) use of the existing code, now identified as occurring at a greater frequency in areas that use the same code for proteins.”

Accepting all that, the idea of a code within a code is a powerful cultural meme that once applied to DNA may not go away so fast. And perhaps should not. The fact that this discovery may be overstated doesn’t mean that it doesn’t capture something interesting about the way the world works. A lot of great abstract art in the early 20th century was the result of misunderstanding contemporary physics! With that in mind, I have prepared seven metaphors to help us understand the implications of this concept. I’m thinking of the classic elevator pitch where you say that the new thing is like one or more things we already know, but with a crucial difference.

**DNA As An App**

For so long we have considered the genetic code to be something like a book to be read, a recipe for making proteins. This new discovery makes me think that DNA is actually less like a document and more like an app. These transcription factors bind to two specific sequences of DNA right next to the genes that they regulate. So we can think of these TFs as kinds of functions that employ certain logic to turn the transcription of genetic material on and off and to regulate its speed. This reminds me of the reactive data bindings in the JavaScript app framework called Meteor. In effect, the TF binds to all areas producing a certain type of protein in a certain cell line (the scope of the function) and keeps them all coordinated in real time.

**DNA Contains Puns**

One of the most startling things about the dual nature of the duons is that it makes us wonder what else could be hiding within those double helixes? The duons are like words or phrases within the “text” of our DNA that mean two different things, depending on context. And yet, as in human language, not all words have this double meaning. The Science paper suggests that these duons are “highly conserved” through evolution, which means that they are the Darwinian keepers. But as with puns and other figures of speech, the duons power also contains the danger of miscommunication since mutations within them are highly likely to lead to disease.

**DNA As Zappos**

Another concept of DNA has been that it is a factory for making proteins. But in the present context DNA appears to be more like Zappos or Amazon’s distribution hubs or the UPS Sort. DNA contains the instructions for making all of the proteins that a body needs, yes, but it also choreographs the elaborate logistical dance that delivers the right proteins to the right location at just the right time.

**DNA As Playing Battleship**

The dual-functioning nature of some DNA code puts us in a curious position when trying to determine, for instance, genetic factors of disease (think of the troubles of 23andMe.) In the present scenario, it is as if we have been playing a game of Battleship looking at the known position of our pieces (the genes), but not
of the positions of the duons that contain the code of the TFs. And it turns out that the correlation between genes associated with a given disease and co-located TFs will be likely spots for aiming the big guns at.

**Genome 2.0**
We have just gotten through the human genome and perhaps we need to start again! Hidden within what scientists have already decoded is at least one more layer of code. It is interesting that at the time that Wilson and Crick came out with their discovery, another DNA researcher, Rosalind Franklin, argued that other structures could satisfy the x-ray data for DNA and wondered if their’s was "the solution or a solution?" More than 50 years later it seems that perhaps they were all part right.

**DNA As Language**
Intriguing for those with a familiarity with quantitative linguistics and current work on natural language processing is how the instance of a dual-functioning code in DNA begins to make genetics seem more like a language with all the messiness that entails and less like a pure instruction set. If certain sequences have an ambiguous identity and shift modes between description and process, there is room for many errors and chaotic recursions to arise, just like in human language.

**DNA As Scaling Mechanism**
Finally, DNA is a model of natural economy. There are a limited number of sequences that can be generated from the base amino acids and a limited number of transcription factors that can be deployed in combinations to yield all of the complexity of a living breathing human being. Nature, I think, is simplicity scaled. And what the inclusion of the TFs within the DNA code looks like to me is that code contains both the description of the proteins it can create and the methods to scale those proteins up from a zygote all the way up to an adult and through an entire life cycle. These two processes, specifying and scaling, integrated together into the same nucleotide sequences is a conceptually elegant way of tying together DNA’s dual nature. I wonder how that would work with software?

**Don't Be Duped By 'Duon' DNA Hype**
With today’s headlines hyping “Second Code Uncovered Inside the DNA,” you might think that scientists are running around in circles in their labs, tearing out their hair, and screaming, “Crick, you loser!” But the real reaction of scientists to these headlines is more along the lines of this Twitter conversation among several scientific experts and science writers. They have good reason to be snarky.

The hype began with the way hype often begins: an institutional news release offering us the holy grail/huge breakthrough/game-changing finding of the day. This kind of exaggeration is the big reason any science consumer should look well beyond the news release in considering new findings. A news release is a marketing tool. You're reading an advertisement when you read a news release. In this case, the advertisement/news release not only goes off the rails with the hype, it’s also scientifically garbled and open to all kinds of misinterpretation, as the comments at the link to the release make clear.

Here’s some of the hype with a soupçon of garble.

Since the genetic code was deciphered in the 1960s, scientists have assumed that it was used exclusively to write information about proteins. UW scientists were stunned to discover that genomes use the genetic code to write two separate languages.

Scientists have not assumed that the genetic code “was used exclusively to write information about proteins,” or even ever assumed that it “writes information about proteins,” whatever that means. A quick primer: Proteins are molecules that do the work of an organism, and that includes the work of copying DNA for protein production and cell division. Even nonmajors biology textbooks cover the fact that the DNA sequence both contains code for proteins and serves a regulatory purpose, making it possible to copy that code into a form the cell can read, recipe-like, to build the proper protein. We even have names for these regulatory DNA sequences: promoters, enhancers, termination sequences. (edited) I’d be stunned if UW scientists were genuinely “stunned” to discover this dual use of DNA sequences to “write” “two separate languages” because what they really describe is the use of a single language, the language of nucleotides, for two known purposes. They themselves noted that “the potential for some coding exons to accommodate transcriptional enhancers or splicing signals has long been recognized.”

The release quotes study author John Stamatoyannopoulos as saying that

For over 40 years we have assumed that DNA changes affecting the genetic code solely impact how proteins are made,” said Stamatoyannopoulos. “Now we know that this basic assumption about reading
the human genome missed half of the picture. These new findings highlight that DNA is an incredibly powerful information storage device, which nature has fully exploited in unexpected ways.”

I can only hope that Stamatoyannopoulos didn’t really say that. The authors report that changes in a single DNA sequence can influence both the protein it encodes and the place where other proteins bind to initiate copying. So evolutionarily, a single change could influence two endpoints—copying the sequence and what gets made using the same sequence. That’s cool, but not actually new. How it goes beyond “solely impact(ing) how proteins are made” goes beyond me, and it certainly doesn’t miss half the picture. Indeed, based on the study itself (paywalled), it could possibly have missed up to a fifth of the picture.

The release also contains gems such as “The genetic code uses a 64-letter alphabet called codons.” This sentence makes me sad. Codons consist of three nucleotides—which we designate with the letters A, C, G, and T/U—and there are 64 of these triplets, 61 of which serve as molecular code words for 20 amino acids (here is a DNA nucleotide codon table, too; these are the codons the authors address). Some amino acids get more than one word to designate them. The cell “reads” these code words and uses the amino acids they designate to build proteins. There.

Finding new ways to make viral load testing cheaper
CAPE TOWN, 13 December 2013 (IRIN)—Are you taking antiretroviral (ARV) drugs and want to know how well you’re doing? If you live in a wealthy country, chances are that your progress is regularly checked using the "gold standard"—a viral load test. If you’re in a developing country, where more people are on ARV medication and the need is greater, the expensive and complex test is hard to find, making it even more difficult to monitor whether your treatment is failing and you need to change your medication.

But as the number of people receiving HIV treatment rises, and more people become eligible for treatment, the prohibitive cost of viral load tests will have to come down, and donors should use their purchasing power to push for better prices, said medical charity Médecins Sans Frontières (MSF) in a report released at the 17th International AIDS Conference on AIDS and STIs in Africa. Almost 10 million people are on ARVs in developing countries, and an estimated 18 million more need the medication.

The World Health Organization (WHO) recommends routine viral load monitoring six months after starting the drugs, and then once a year to make sure the treatment is working, and to find treatment failures that must be switched to a different regimen, or to identify recipients who need support to stick to their medication. However, an MSF survey of 23 resource-limited countries found that the test was available in only four of them, while virtually all countries included viral load monitoring in their treatment guidelines.

The test measures how well the virus is being suppressed by the ARVs. An increased viral load—the amount of virus in the blood—indicates that a patient has developed resistance to one or more ARV drugs. The goal of taking HIV treatment is to strengthen your immune system to the point where there are undetectable levels of virus in your blood, and the risk of transmitting HIV is very low. Viral load testing is much more accurate and can identify problems sooner than CD4 testing, which is currently used by countries without viral load tests.

There are growing fears that national treatment programmes are missing a large number of treatment failures, which could lead to the development of widespread resistance. At the conference MSF presented study findings from Zimbabwe, Malawi and Kenya, where 10 percent of patients had high, viral loads despite most of them showing no signs clinically or when their CD4 counts were tested.

"The treatment wasn’t working for them, and we didn’t know it. We would have had to wait till they get sick, or their immune system drops, to help them," Sharonann Lynch, HIV Policy Advisor for MSF’s
Access Campaign, told IRIN. The study also found that patients were being switched to pricier second-line regimens unnecessarily.

"This is a life-long commitment, it helps greatly to have proof that treatment is working and the virus is suppressed"

When one looks at the overall cost of implementing a viral load test, including human resources, laboratories and transporting samples, the reagents—substances that produce a chemical reaction—used to run each test accounted for as much as 75 percent of the outlay. Most countries are paying more than US$20 for reagents per test, but the price could be lower than $10. In South Africa, the government is rethinking the use of CD4-count tests for monitoring patients on treatment, and now pays less than $15 per test for reagents.

"There is no reason to be paying more than $10 [for each test] in 2014," Lynch noted. There were opportunities for countries and donors such as the Global Fund to fight AIDS, Tuberculosis and Malaria, and the US Presidents Emergency Plan for AIDS Relief (PEPFAR), to slash prices by pooling their resources and buying the test materials in bigger quantities.

The Global Fund has no "ideological objections" to teaming up to buy commodities at a lower price if it makes sense, Mark Edington, head of grant management at the Global Fund, told IRIN, but the proposal would have to be looked at by the Fund's technical teams before any decisions would be taken.

The Fund has already worked with the UK Department for International Development (DFID) and the US government's President's Malaria Initiative (PMI) to purchase insecticide-treated bednets at a lower price; it is currently looking to work with PEPFAR on getting male circumcision devices cheaply.

Cost can longer be viewed as a barrier to implementing viral load testing. MSF has suggested innovative strategies such as the use of dried blood spot samples and the pooling of viral load samples to cut prices even further. At their HIV treatment sites in Malawi, for instance, MSF would test five different samples at the same time and were able to cut costs by a third, Lynch said.

She believes the benefits of viral load testing are best summed up by the "undetectable club". The members are women who have started a support group in South Africa's KwaZulu-Natal province to help each other keep their viral loads undetectable. "We're entering a new era of HIV treatment... People are [becoming] proud that they are on treatment," Lynch says. "It also helps for them to know that they are responsible for suppressing the virus...This is a life-long commitment, it helps greatly to have proof that treatment is working and the virus is suppressed."

**Breakthrough could lead to protection from fatal infections**

**Research shows that deletion of the Epac1 gene protects from fatal rickettsiosis**

Researchers at the University of Texas Medical Branch at Galveston have discovered a way to block a disease pathway that could be a breakthrough in defeating some of the world's most devastating human infections.

Rickettsioses are a group of insect-borne diseases caused by bacteria. One type, typhus fever, has been cited as a high-level threat by the National Institutes of Health because the bacteria can spread and multiply very easily, and the untreated infection can lead to death.

What researchers at UTMB have found is a way to protect against what can be a fatal rickettsial infection. Their findings appear in the *Proceedings of the National Academy of Sciences*.

"Even more exciting, there is preliminary evidence that the experimental drug we have identified as being effective against rickettsiae may also be effective against viruses," said Dr. David Walker, chairman of the department of pathology at UTMB and executive director for the Center for Biodefense and Emerging Infectious Diseases.

Many scientists are concerned that temperature increases due to global climate change will lead to more widespread cases of rickettsioses, since the bacteria are spread by ticks, lice, fleas and chiggers that thrive in warmer climates. In addition, because the bacteria are easily transmitted, they could pose a bioterrorism threat, Walker said.

The diseases, which include Rocky Mountain spotted fever, can lead to death. In fact, a fatality rate as high as 32 percent has been reported in hospitalized patients with Mediterranean spotted fever.

"We believe that it is imperative that we find a way to control this disease," Walker said.

In their study at UTMB, scientists know signals to cells can be controlled by a molecular messenger known as cyclic AMP, which plays crucial roles in the development of many human diseases, including those caused by bacteria and viruses.
In humans and animals, the effects of this messenger are controlled by two types of receptors, one known as protein kinase A, or PKA, and a newly identified protein known as Epac. PKA and Epac can act in concert or in opposition to control many cell functions.

Two leading scientists of this multidisciplinary, cutting-edge research collaboration at UTMB, Dr. Bin Gong and Dr. Xiaodong Cheng, used mice in which the gene for the Epac receptor was inactivated. They found that mice infected with deadly Rickettsia bacteria are resistant to fatal infection.

The mechanism by which this happens is being identified, and now a new candidate drug that inhibits Epac, known as ESI for Epac-specific inhibitor, has also been shown to protect normal mice from a fatal rickettsial infection. The researchers are currently designing a second-generation ESI that is more potent and is not toxic even at high doses. There are also indications from preliminary experiments that ESI protects animals against some lethal viral infections.

"This is an exciting development, given that our arsenal of treatments for these bacteria is quite limited," Walker said.

Spontaneous fusion with macrophages empowers cancer cells to spread

Cancer cells fused with macrophages exhibit enhanced adhesive strength, formed tumors more rapidly than unfused cancer cells and flourished under conditions that dramatically inhibited growth of unfused cancer cells

Cancer cells that spontaneously fuse with macrophages, the immune system's healthy scavenger cells, play a key role in the metastasis, or spread of the cancer to other areas of the body, according to research to be presented Sunday, Dec. 15, at the American Society for Cell Biology annual meeting in New Orleans.

The researchers, Alain Silk, Ph.D., Melissa Wong, Ph.D., and colleagues at Oregon Health & Science University (OHSU) in Portland followed the work of German pathologist Otto Aichel, who suggested in 1911 that a cancer cell under attack by a white blood cell might spontaneously fuse with that cell to produce a hybrid cell with chromosomal abnormalities that could lead to cancer.

Although Aichel's theory was dismissed by his contemporaries, recent discoveries about the broader role of cell fusion in tissue homeostasis and regeneration have revived scientific interest in his ideas. Today there is strong evidence of fusion between cancer and normal cells in human cancer, but it has not been apparent whether cell fusion could provide cancer cells with a selective advantage that enhances cancer progression.

The OSHU researchers began by confirming that cells from various types of cancer could readily and spontaneously fuse with macrophages. By intensively studying the fusion-derived cancer cells, the researchers determined that these cells exhibited enhanced adhesive strength, formed tumors more rapidly than unfused cancer cells and flourished under conditions that dramatically inhibited growth of unfused cells.

"Overall, our findings demonstrate that spontaneous fusion of cancer cells with macrophages can profoundly and significantly impact the phenotype of tumorigenic cells, with implications for our basic understanding of cancer cell biology and the process of tumor evolution," the researchers said.

As cancer progresses, tumor cells acquire new capabilities, or phenotypes. They must grow in an uncontrolled manner, leave their site of origin and become resistant to anti-cancer drugs. Previous studies on the biology of cancer have revealed that cancer progression are determined by changes to the cancer genome, epigenetics, influences from the microenvironment, exosomes and the interplay with the immune system. The OSHU research implicates the fusion of cancer cells with macrophages as a new potentiator of cancer progression.

Glucose: Potential new target for combating annual seasonal influenza

Reducing viruses' glucose supply weakens the microbes' ability to infect mammalian cells in lab cultures

Reducing glucose metabolism dials down influenza viral infection in laboratory cell cultures, providing an entirely new approach for combating seasonal flu, according to research that will be presented on Sunday, Dec. 15, at the American Society for Cell Biology (ASCB) annual meeting in New Orleans.

While annual flu shots are based on the U.S. Centers for Disease Control (CDC)'s predictions of the viruses that will be in widest circulation each flu season, the new approach targets one metabolic requirement of all influenza viruses: glucose.

Reducing viruses' glucose supply weakens the microbes' ability to infect host cells, said Amy Adamson, Ph.D., and Hinissas Pascaline Kohio of the University of North Carolina, Greensboro.
Fever, ache, and the other miseries of influenza viral infection afflict 5 to 20 percent of the U.S. population each year. While the flu is usually not life-threatening to the majority of its victims, the Spanish flu pandemic of 1918 demonstrated that flu viruses can evolve into lethal agents that spread worldwide. Because flu viruses change continually through mutation and genetic swaps, the CDC reformulates the flu vaccine each year.

Yet to infect cells, the influenza virus is dependent upon the actions of the cell’s own proteins, and so another strategy for slowing viral infection would be to target essential viral needs, for example, their dependence on cellular glucose. Dr. Adamson and Kohio showed that influenza A infection can be controlled in laboratory cultures of mammalian cells by altering glucose metabolism.

When the influenza virus initially infects a cell, and the virus is confined in an endocytic vesicle, the viral proteins HA and M2 use the acidic environment inside the vesicle to fuse the viral lipid envelope with that of the vesicle, and then release the viral genome into the cytosol. The acidic pH that mediates these important viral processes is established and maintained by the cell’s vacuolar-type H+ ATPase (V-ATPase) proton pump. The researchers found that this dependence could be used to manipulate the infection’s success.

Specifically, they were able to suppress viral infection of cells by dismantling the V-ATPase through the lowering of glucose levels. In addition, they inhibited infection by treating cells with chemical inhibitors of glycolysis, the initial pathway of glucose catabolism. Conversely, influenza viral infection of cells could be increased by giving cells more glucose than normal, the researchers report in the journal *Virology*, [http://www.ncbi.nlm.nih.gov/pubmed/23876457](http://www.ncbi.nlm.nih.gov/pubmed/23876457).

The ease with which the researchers could dial viral infection down by controlling glucose levels and thus V-ATPase activity suggested a new strategy for throttling influenza viral infection. "Taken together, we propose that altering glucose metabolism may be a potential new approach to inhibit influenza viral infection," said Dr. Adamson and Kohio.

Reference: "Glycolytic Control of Vacuolar-Type ATPase Activity: A Mechanism to Regulate Influenza Viral Infection," on Sunday, Dec. 15, in the 1:30 to 3 p.m. poster session, "Host-pathogens/Host-commensal Interactions II."

Silencing signals sent by parasite could aid sleeping sickness fight

A new discovery by scientists could help combat the spread of sleeping sickness.

Insights into how the parasites that cause the disease are able to communicate with one another could help limit the spread of the infection.

The findings suggest that new drugs could be designed to disrupt the flow of messages sent between these infectious microorganisms.

Sleeping sickness – so named because it disrupts sleep patterns – is transmitted by the bite of the tsetse fly, and more than 69 million people in Africa are at risk of infection. Untreated, it can damage the nervous system, leading to coma, organ failure and death.

During infection, the parasites – known as African trypanosomes – multiply in the bloodstream and communicate with each other by releasing a small
molecule. When levels of this molecule become sufficiently high, this acts as a signal for the parasites to stop replicating and to change into a form that can be picked up by biting flies and spread.

A team led by researchers at the University of Edinburgh were able to uncover key components of the parasites’ messaging system. They used a technique known as gene silencing, to identify those genes that are used to respond to the communication signals and the mechanisms involved.

Professor Keith Matthews, of the University of Edinburgh’s School of Biological Sciences, who led the research, said: "Parasites are adept at communicating with one another to promote their survival in our bodies and ensure their spread – but by manipulating their messages, new ways to combat these infections are likely to emerge."

The research, carried out in collaboration with the University of Dundee, was published in the journal Nature, and funded by the Wellcome Trust.

**New Strain of Bird Flu Packs a Punch Even After Becoming Drug-Resistant**

Dec. 11, 2013 — Researchers at the Icahn School of Medicine at Mount Sinai reported that a virulent new strain of influenza – the virus that causes the flu – appears to retain its ability to cause serious disease in humans even after it develops resistance to antiviral medications. The finding was included in a study that was published today in the journal *Nature Communications.*

To develop genetic mutations that make them less susceptible to anti-flu drugs. However, these mutations usually come at a cost to the virus, weakening its ability to replicate and to spread from one person to another.

An avian strain of influenza A that emerged in China last spring, could rapidly develop a mutation that made it resistant to treatment with the antiviral medication Tamiflu (oseltamivir). However, patients in whom drug resistance developed often had prolonged, severe infections and poor clinical outcomes. No vaccine is currently available to prevent H7N9, which infected at least 135 people and caused 44 deaths during the outbreak. In the absence of a vaccine, antiviral drugs are the only means of defense for patients who are infected with new strains of the flu.

"In this outbreak, we saw some differences in the behavior of H7N9 and other avian influenza strains that can infect humans, beginning with the rapid development of antiviral resistance in some people who were treated with oseltamivir and the persistence of high viral loads in those patients," said lead investigator Nicole Bouvier, MD, Assistant Professor of Medicine, Infectious Diseases at the Icahn School of Medicine at Mount Sinai.

Specifically, the investigators found that a drug-resistant H7N9 virus retained its ability to replicate in human respiratory cells and was comparable to a non-resistant form of the virus in producing severe illness in animal models. And although H7N9 appears to have a limited ability to spread readily from human to human, transmissibility in animal models was comparable between drug-susceptible and drug-resistant strains. "Transmission was inefficient for both of the H7N9 viruses that we tested in our experiments," said Dr. Bouvier. "But surprisingly, transmission of the drug-resistant virus was no less efficient than that of the drug-sensitive version."

"Many of the people infected with H7N9 during the outbreak in China were elderly or had other conditions that predisposed them to severe influenza illness," observed Dr. Bouvier. "Nevertheless, our
study suggests that flu viruses can indeed develop drug-resistant mutations without suffering a penalty in terms of their own fitness.”

Older antiviral drugs such as amantadine are no longer effective in treating most strains of the flu that infect humans. Newer antiviral drugs called neuraminidase inhibitors block an enzyme that helps the virus replicate. These drugs include Tamiflu, a pill, and Relenza (zanamivir), a powder that is inhaled. Both medications have drawbacks: flu viruses can develop resistance to the medications in people who take them, and, in many parts of the world, neither drug is available in an intravenous form to treat those with severe infections.

“Our study underscores the need to develop a bigger arsenal of antiviral drugs and vaccines, which will allow us to outsmart the influenza virus,” said Dr. Bouvier. “Researchers at Mount Sinai are actively engaged in identifying new targets for drug therapy and are working to develop a universal vaccine that will prevent multiple strains of influenza.”

Journal Reference:

From Friend to Foe: How Benign Bacteria Evolve Into Virulent Pathogens
Dec. 12, 2013 — Bacteria can evolve rapidly to adapt to environmental change. When the "environment" is the immune response of an infected host, this evolution can turn harmless bacteria into life-threatening pathogens. A study published on December 12 in PLOS Pathogens provides insight into how this happens.

Isabel Gordo and colleagues from the Instituto Gulbenkian de Ciencia in Oeira, Portugal, have for the first time devised an experimental system to observe and study the evolution of bacteria in response to encounters with cells of the mammalian immune system. They found that in less than 500 bacterial generations (or 30 days), the bacteria became more resistant to being killed by immune cells and acquired the ability to cause disease in mice.

"Escherichia coli bacteria show an extraordinary amount of diversity: Many are benign commensal bacteria, but some are deadly pathogens," says Isabel Gordo. "It is thought that many strains of E. coli that cause disease in humans evolved from commensal strains. We thought that experimental evolution would be a powerful tool to directly observe some of the steps E. coli may take in the transition from commensalism to pathogenesis.”

For their study, the scientists studied initially benign E. coli bacteria that were continuously confronted with macrophages, which are part of our immune system and can swallow and digest bacteria. They grew a mix of bacteria and macrophages in a liquid culture (a glass bottle that contains a nutritious broth). Once a day, they diluted the mix, and every other day they took a sample of the bacteria for further analysis. As a control, they grew, diluted, and analyzed bacteria from the same ancestral strain but grown without macrophages.

From day four on, bacteria that had been exposed to macrophages started to show changes in their phenotype (their appearance), whereas such changes were never observed in the controls. The selective pressure imposed by the presence of the macrophages prompted changes in the bacteria that were consistently observed in six independent experimental series. The changes affected the phenotype of the
bacteria (with new variants forming either "small colonies" or "mucoid colonies"), their fitness, and their genetic make-up.

When the scientists looked at the interaction between new variant bacteria and macrophages more closely, they found that the small colony variants were more resistant to being digested by macrophages than the ancestral strain, and the mucoid variant was less likely to be gobbled up. When they infected mice with mucoid variant bacteria, they also found that the variants have increased ability to cause disease in mice.

"We demonstrate," the scientists say, "that E. coli can adapt to better resist macrophages within a few hundred generations, and that clones with morphologies and traits similar to those of pathogenic bacteria rapidly emerge."

Journal Reference:

HIV-1 Transmission during Early Infection in Men Who Have Sex with Men: A Phylodynamic Analysis
Erik M. Volz mail, Edward Ionides, Ethan O. Romero-Severson, Mary-Grace Brandt, Eve Mokotoff, James S. Koopman Published: December 10, 2013 DOI: 10.1371/journal.pmed.1001568

Abstract

Background
Conventional epidemiological surveillance of infectious diseases is focused on characterization of incident infections and estimation of the number of prevalent infections. Advances in methods for the analysis of the population-level genetic variation of viruses can potentially provide information about donors, not just recipients, of infection. Genetic sequences from many viruses are increasingly abundant, especially HIV, which is routinely sequenced for surveillance of drug resistance mutations. We conducted a phylodynamic analysis of HIV genetic sequence data and surveillance data from a US population of men who have sex with men (MSM) and estimated incidence and transmission rates by stage of infection.

Methods and Findings
We analyzed 662 HIV-1 subtype B sequences collected between October 14, 2004, and February 24, 2012, from MSM in the Detroit metropolitan area, Michigan. These sequences were cross-referenced with a database of 30,200 patients diagnosed with HIV infection in the state of Michigan, which includes clinical information that is informative about the recency of infection at the time of diagnosis. These data were analyzed using recently developed population genetic methods that have enabled the estimation of transmission rates from the population-level genetic diversity of the virus. We found that genetic data are highly informative about HIV donors in ways that standard surveillance data are not. Genetic data are especially informative about the stage of infection of donors at the point of transmission. We estimate that 44.7% (95% CI, 42.2%-46.4%) of transmissions occur during the first year of infection.

Conclusions
In this study, almost half of transmissions occurred within the first year of HIV infection in MSM. Our conclusions may be sensitive to un-modeled intra-host evolutionary dynamics, un-modeled sexual risk behavior, and uncertainty in the stage of infected hosts at the time of sampling. The intensity of transmission during early infection may have significance for public health interventions based on early treatment of newly diagnosed individuals.

Editors' Summary

Background
Since the first recorded case of AIDS in 1981, the number of people infected with HIV, the virus that causes AIDS, has risen steadily. About 34 million people are currently HIV-positive, and about 2.5 million people become newly infected with HIV every year. Because HIV is usually transmitted through unprotected sex with an infected partner, individuals can reduce their risk of infection by abstaining from sex, by having only one or a few partners, and by always using condoms. Most people do not become ill immediately after infection with HIV, although some develop a short flu-like illness. The next stage of HIV infection, which may last more than ten years, also has no major symptoms, but during this stage, HIV slowly destroys immune system cells. Eventually, the immune system can no longer fight off infections by other disease-causing organisms, and HIV-positive people then develop one or more life-
threatening AIDS-defining conditions, including unusual infections and specific types of cancer. HIV infection can be controlled, but not cured, by taking a daily cocktail of antiretroviral drugs.

**Why Was This Study Done?**
The design of effective programs to prevent the spread of HIV/AIDS depends on knowing how HIV transmissibility varies over the course of HIV infection. Consider, for example, a prevention strategy that focuses on increasing treatment rates: antiretroviral drugs, in addition to reducing illness and death among HIV-positive people, reduce HIV transmission from HIV-positive individuals. “Treatment as prevention” can only block transmissions that occur after diagnosis and entry into care. However, the transmissibility of HIV per sexual contact depends on a person’s viral load, which peaks during early HIV infection, when people are often unaware of their HIV status and may still be following the high-risk patterns of sexual behavior that caused their own infection. Epidemiological surveillance data (information on HIV infections within populations) can be used to estimate how many new HIV infections occur within a population annually (HIV incidence) and the proportion of the population that is HIV-positive (HIV prevalence), but cannot be used to estimate the timing of transmission events. In this study, the researchers use “phylodynamic analysis” to estimate HIV incidence and prevalence and the timing of HIV transmission during infection. HIV, like many other viruses, rapidly accumulates genetic changes. The timing of transmission influences the pattern of these changes. Viral phylodynamic analysis—the quantitative study of how epidemiological, immunological, and evolutionary processes shape viral phylogenies (evolutionary trees)—can therefore provide estimates of transmission dynamics.

**What Did the Researchers Do and Find?**
The researchers obtained HIV sequence data (collected for routine surveillance of antiretroviral resistance mutations) and epidemiological surveillance data (including information on the stage of infection at diagnosis) for 662 HIV-positive men who have sex with men living in the Detroit metropolitan area of Michigan. They constructed a phylogenetic tree from the sequences using a “relaxed clock” approach and then fitted an epidemiological model (a mathematical model that represents the progress of individual patients through various stages of HIV infection) to the sequence data. Their approach, which integrates surveillance data and genetic data, yielded estimates of HIV incidence and prevalence among the study population similar to those obtained from surveillance data alone. However, it also provided information about HIV transmission that could not be obtained from surveillance data alone. In particular, it allowed the researchers to estimate that, in the current HIV epidemic among men who have sex with men in Detroit, 44.7% of HIV transmissions occur during the first year of infection.

**What Do These Findings Mean?**
The robustness of these findings depends on the validity of the assumptions included in the researchers’ population genetic model and on the accuracy of the data fed into the model, and may not be generalizable to other cities or to other risk groups. Nevertheless, the findings of this analysis, which can be repeated in any setting where HIV sequence data for individual patients can be linked to patient-specific clinical and behavioral information, have important implications for HIV control strategies based on the early treatment of newly diagnosed individuals. Because relatively few infected individuals are diagnosed during early HIV infection, when the HIV transmission rate is high, it is unlikely, suggest the researchers, that the “treatment as prevention” strategy will effectively control the spread of HIV unless there are very high rates of HIV testing and treatment.
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<th>DMA MSM in Phylogeny</th>
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*HIV diagnosis concurrent with AIDS diagnosis.

*HIV diagnosis concurrent with low sequence ambiguity.

doi:10.1171/journal.pmed.1001568.t001
Figure 1. HIV transmission model and phylogeny.
Figure 2. Estimated transmission patterns through time.
Early HIV Infection in the United States: A Virus’s Eye View
Timothy B. Hallett, Published: December 10, 2013, DOI: 10.1371/journal.pmed.1001569

Early after infection with HIV, the concentration of virus in the body increases rapidly before an immune system response begins to hold it under temporary control [1]. During that short time of elevated viral concentration, the infected individual may be much more infectious than at other times [2], [3]. At advanced infection, viral concentration can increase again, potentially leading to a late surge in infectiousness. However, the actual influence of each stage of infection on the onward spread of HIV does not depend just on the biology of the infection but also on the patterns of sexual partnership formation through which transmission can occur. Understanding the contributions of the different phases of infection to the onward spread of HIV is essential in planning effective interventions to control the epidemic. To estimate these contributions epidemiologists have previously resorted to mathematical models, but uncertainties in key parameters, as well as variability between populations, have contributed to a very wide range of model estimates of what proportion of transmissions occur during different stages—for early HIV infection (EHI), estimates range from less than 5% to more than 90% [4]. Therefore, new data on this topic are of substantial interest, and in this issue of *PLOS Medicine*, Erik Volz and colleagues [4] present a novel approach that opportunistically leverages genetic sequence data from a
population of men who have sex with men in Detroit, Michigan, to add significantly to our understanding of HIV epidemic dynamics.

The basic premise of using genetic sequence data is that within an individual early in infection, the population of virus is relatively homogenous and similar to that in the individual that infected him. Over time, however, greater diversity in the genetic make-up of the virus population develops, and this will be occurring independently in the “donor” and “recipient”. This process gives rise to stereotypical patterns of viral diversity across a population that depend on how much transmission happens early in infection. By constructing a model of that process and fitting it to the genetic sequence data and other demographic and epidemiological information, Volz et al. [4] can “back into” an estimate of how much transmission occurs during the first year of infection. The major theoretical development of this study has been in describing how to appropriately combine those diverse sources of data, which is essential for fitting a model in such a way that inferences can be drawn on model parameters, such as the rate of transmission from different phases of infection.

The analysis by Volz and colleagues finds that during the first year of HIV infection individuals are eight times as infectious as during chronic infection. These estimates appear similar to independent estimates from cohort studies (making allowance for the different definitions of EHI used) [3],[5]. This pattern of infectiousness means that, according to the Volz et al. model, 42%–46% of transmissions come from persons in EHI, which is also consistent with expectations from other estimates [1].

Recognising EHI’s large influence on the dynamics of HIV in the sort of epidemic seen in this population could have practical significance in the response to epidemics. First, it means that partner-notification approaches could be particularly useful, as they could lead to the discovery of undiagnosed infections at a time when they are most infectious [6]. A concentration of the transmission potential of the virus early in infection also modulates the types of sexual behaviours that are important for sustaining HIV transmission—in particular, short gap lengths between partners, or concurrent sexual partners, would be expected to be more important for the spread of HIV than if transmission rates did not change over the course of infection [7]. The timing of transmission of HIV also influences the selective pressures acting on the virus, which shapes how the virus evolves within the host and across the whole population [8]; it has been hypothesised that substantial early transmission of HIV could mean that drug-resistant strains can spread more easily [9].

It has also been argued that a large contribution of EHI to transmission implies a limited potential for treatment interventions to reduce transmission, because the virus will typically have been passed on before individuals can be initiated on treatment [10]. However, there is a counter-argument [11], as some models suggest that whatever the contribution of EHI to epidemic spread really is, it would not affect the impact of treatment on HIV incidence because, in order to match the observed epidemic trajectory, models with large EHI contributions tend to have epidemics with weaker overall potential for spread. It would be useful, therefore, to understand how model projections for the impact of treatment programmes—as well as the value of other forms of intervention—in Detroit would be updated if the new genetic phenotype data were introduced.

The increase in transmission during the last stage of infection (AIDS) appears to be another strong signal in these data. An increase in transmission at that time could mean that even ART programmes that initiate patients on treatment relatively late in their infection could materially contribute to reducing HIV incidence, as earlier mathematical models suggested [12]. However, the limitations of the “health-care cascade” in the US (at least in 2007) are also highlighted in the Volz et al. analysis, in their finding that half of all new infections are transmitted from persons already in care. Although the analysis also did not find evidence that persons diagnosed with HIV were any less likely to transmit HIV than those unaware of their infection status, it should be noted that the analysis was not designed to test this specifically. Finally, it is reassuring that this new way of analysing data produces overall estimates of HIV incidence that are very similar to those generated using standard surveillance and estimation methods.

The analysis is very complex and combines cutting-edge modelling approaches from several different fields. The authors have gone to lengths to test their method’s performance in order to check that the sorts of inferences that they draw can safely be made. However, many aspects of the systems are uncertain, and—as the authors note—some of these uncertainties, particularly in the structure of the model, are not fully reflected in the final results. For instance, the model does not examine how different forms of sexual partnership formation might interfere with the conclusions reached. Although an allowance is made for the possibility that the virus could have passed between two sampled individuals via another unsampled individual, questions may still arise about the possibility for a bias: if unsampled individuals (not in the
clinic) tend to have many partners, then it seems likely that the contribution of EHI would be greater than estimated here.

It is hard to know how these finding might or might not be extrapolated to other settings. Similar reports of “bursts” of HIV transmission, and a high contribution of EHI, have been reported before among populations of men who have sex with men in the UK [13], suggesting that Detroit could be a typical example. But other aspects of the Detroit sample, including the relatively high CD4 cell counts at HIV diagnosis, might make the setting less typical. However, as the data used in this study will be available in other settings too, it could be very useful to repeat the analysis across multiples cities and compare the results. The data required may not currently exist in southern Africa, where the same insights would be especially useful, but this may become possible in the future.

Another issue complicating comparisons is variable definitions of “EHI”, with earlier analyses not using a common duration post-infection [14]. Indeed, the definition of EHI of Volz et al. of one year after infection extends much beyond the period of “acute” infection and high viral load, so it is not possible to fully determine the extent to which transient viral dynamics versus, perhaps, transient behaviours underlie the findings.

By harnessing yet more data in a mathematical model, Volz et al. have successfully advanced our understanding of the epidemiological dynamics of HIV. They have confirmed that a large proportion of transmission can be traced back to a small sliver of time during an infected person’s life, and this dynamic should inform the interpretation of biological, behavioural, and programme data in HIV epidemics around the world.

**Disseminated Histoplasmosis in HIV-Infected Patients in South America: A Neglected Killer Continues on Its Rampage**

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HIV/AIDS is not a neglected disease. Histoplasmosis is not considered a neglected disease in North America. However, in South America, it should be. It often affects neglected populations and represents a lethal blind spot of the HIV/AIDS data collection systems. Counts of new AIDS cases and AIDS-related deaths are useful to follow the epidemic; however, they overlook the exact cause of death. In the context of the South American pathogen ecology, the systemic mycosis due to *Histoplasma capsulatum* var. *capsulatum* is probably on the top of the list of AIDS-defining illnesses and AIDS-related deaths [1], yet it is mostly undiagnosed and is not even on the diagnostic algorithm used by a significant proportion of clinicians facing a febrile, severely immunodepressed patient in the region.

**The Invisible Burden**

Studies performed in the 1950s and 1960s on the histoplasmin skin test positivity in South America showed positivity rates around 30% from Trinidad and Tobago in the North, to Uruguay and Argentina in the South. The pathogen is there [2]. Despite this, expertise and awareness of this disease is limited to mycologists and some clinical teams scattered throughout the South American continent [2]–[16]. But those with expertise are the exception rather than the rule. Imported cases in Europe occurring in HIV-infected residents or travellers from South America, notably in France, Spain, and Italy, are starting to be recognized, but often late in the course of the disease because clinicians are not familiar with this “endemic” disease [17]–[19].

For too long, the absence of a simple, reliable, and affordable diagnostic test has made it difficult to determine the burden of this disease in HIV-infected patients in much of South America. The gold standard for diagnosis relies, so far, on the culture of fluid and tissue samples [20]. This requires invasive investigations by clinicians (bone marrow aspirates; biopsies of the liver, lymph node, and intestine; etc.). From the lab perspective, direct examination may accelerate diagnosis, but culture may take weeks and require a BSL 2 laboratory. Detection of specific antigens in serum or urine samples is not available for diagnosis in most of South America, and galactomannan detection (cross reactivity during histoplasmosis) is not being used as a standard of care. Contact with clinicians from various countries suggests that, although severe histoplasmosis often kills in a few days, most clinicians are not very aggressive in their investigations. Moreover, presumptive antifungal therapy is rare. Biopsies are usually immersed in formalin by surgeons rather than sent for culture. Most often, clinicians do not take proper samples for mycological diagnosis, creating a vicious cycle that diminishes the capacity of mycological...
laboratories and perpetuates underfunding and the absence of diagnosis and, thus, the “nonexistence” of the very disease that is killing numerous patients.

In French Guiana, there has been a mycology laboratory since 1997 with a BSL 3 laboratory. The virtuous cycle between laboratory and clinicians has been fruitful: clinicians are well informed about histoplasmosis and are quite aggressive in looking for the infection, while the mycology laboratory has the capacity to appropriately process and identify Histoplasma in clinical specimens [21]. Although published maps [2] show no histoplasmosis in most of French Guiana, the recent figures that have emerged are striking. With 1.5 cases per 100 patient-years, histoplasmosis is the most common cause of AIDS-defining illness. Interestingly, despite awareness of this disease and availability of liposomal amphotericin B, it has also been the leading cause of AIDS-related death for decades. A recent 2-year study of all HIV patients admitted in Saint Laurent du Maroni hospital showed that 41% of admitted patients with CD4 counts below 200 had disseminated histoplasmosis, and 85% of admitted patients with CD4 counts below 50 and isolated fever had disseminated histoplasmosis. This is a clear message for physicians when admitting a severely immunodepressed HIV patient in the region: “Don’t miss histoplasmosis!”

**No Data = No Existence. Meanwhile, Patients Continue Dying from a Treatable Disease...**

The high prevalence of HIV–Histoplasma coinfections on the South American continent is not a trivial problem. The scarcity of the published research on this topic reflects the tragic fact that this problem is evolving under the radar of health care systems and is truly a neglected disease. Generations of young doctors will learn to look for tuberculosis, pneumocystosis, and bacterial pneumonia when confronted with a febrile patient with respiratory signs, but not for histoplasmosis. Similarly, important clinical clues, such as cytopenia (ascribed to bone marrow involvement with or without haemophagocytic syndrome) and liver enzyme abnormalities will often not lead to the suspicion of disseminated histoplasmosis. A big danger is that a smear negative, treatment resistant, “tuberculosis-like” syndrome may often be labelled drug-resistant tuberculosis, when it was never tuberculosis in the first place. It is thus of paramount importance to fill this knowledge gap and revise the diagnostic and therapeutic algorithms in the region.

The “Know your epidemic, know your response” UNAIDS slogan should also be applied to histoplasmosis.

**We Need Research and We Need to Act Now**

The HIV/AIDS epidemic is still active in countries in the Amazon basin. Guyana, Suriname, French Guiana, and the Brazilian state of Amapa all have HIV prevalence rates over 1% of the population. Although the AIDS incidence has steadily declined in the southern states of Brazil, the situation in the northern (Amazonas, Roraima, and Amapa) and the northeastern states of Brazil is still concerning. A very coarse calculation based on 600,000 HIV patients and an annual histoplasmosis incidence rate of 1.5% would estimate the annual number of cases to be in the thousands. Unfortunately, histoplasmosis thus still has a future in HIV patients. Although there is a need for epidemiologic research to measure the true burden of disease in various regions of South America, actions can, and should, be taken to diagnose and treat patients now. We need to develop standard mycological practices in the area that emphasize early and aggressive clinical diagnosis, and we need to develop new rapid diagnostic tools and advocate for affordable treatment. New affordable diagnostic assays (CDC, Immy) are presently being tested in Brazil, French Guiana, Suriname, and Colombia. We hope they will allow us to improve our knowledge of local epidemiologies and reduce patient mortality through early diagnosis and increased awareness.

Although amphotericin B is available, it has potential significant renal side effects. Liposomal amphotericin B is the treatment of choice of the most severe cases of HIV-associated histoplasmosis, but its cost exceeds 800 US dollars per day. Gilead Sciences, Inc. has committed to the procurement of HIV drugs at affordable prices. Extending this policy to the problem of treating HIV-associated histoplasmosis, a neglected disease, would be an important step in improving the health of HIV/AIDS patients in the region. In the near future, DNDi (Drugs for Neglected Diseases initiative) should provide a low-cost, heat-stable alternative to liposomal amphotericin B that could be valuable for histoplasmosis treatment. Our focus has been HIV-associated disseminated histoplasmosis; however, it should be emphasized that the problem of missing the diagnosis of histoplasmosis in South America also extends to some immunocompetent patients or patients with causes of immunodepression other than HIV [22].

We need tests; we need treatments; but first of all, we physicians need to integrate our South American epidemiology in our diagnostic algorithms. Looking for malaria in febrile patients in malaria-endemic areas is automatic; looking for histoplasmosis in febrile, immunosuppressed, HIV-infected patients in South America, Central America, and perhaps way beyond [23], [24], is not. The sooner we do it, the better for our patients.
How patent rules could keep new hepatitis C drug from saving 'millions of lives'

While affordable medicine advocates in India prepare to fight the patenting of a new hepatitis C drug, the US works to strengthen pharmaceutical patents through an international trade deal.

Doctors are calling Gilead Sciences’ new hepatitis C drug a “game changer.” The pill, called sofosbuvir, is more effective than comparable drugs, works more quickly and makes treatment less painful.

“Suddenly, it’s realistic to think we can cure most patients with hepatitis C,” said Dr. Greg Fitz, president of the American Association for the Study of Liver Diseases, in a news conference where Gilead presented research on sofosbuvir.

But patient rights advocates argue that the drug’s price tag — an estimated $84,000 per treatment — puts sofosbuvir out of reach for many.

The Food and Drug Administration gave the go-ahead on Dec. 6. Gilead will own the market in the United States for at least 20 years before generic drug producers can get in the game and bring prices down.

But in India, advocates for affordable medicine are fighting to block the pharmaceutical company’s patent. If they win, the country will be able to crank out low-cost copies of the drug for millions in India and other developing countries.

In the meantime, in Singapore last week, dignitaries from 12 countries met to hammer out a trade agreement that threatens to ban such lawsuits.

As part of the pact, called the Trans-Pacific Partnership, US negotiators are attempting to strengthen pharmaceutical patents, lengthen monopoly protection beyond the typical 20-year limit and dismantle international laws that enable poor countries, where high costs may bar sick people from getting medicine, to develop cheaper alternatives.

India was not represented at the negotiating table and isn’t slated to sign the deal, but experts say the outcomes of the talks could still have significant consequences for the country and the future of generic drugs.

Hepatitis treatment for all

Hepatitis C, an infectious disease that attacks the liver and often leads to cancer, is spread primarily by blood-to-blood contact. Most often associated with intravenous drug use, infection can also be traced back to poorly sterilized medical equipment or inadequately screened blood transfusions.

“You can pick it up at the dentist or during surgery,” said Leena Menghaney, the coordinator for Doctors Without Borders’ access to affordable medicine campaign in India. “The symptoms move slowly, so you may not know you are sick for a decade.”

Ninety percent of hepatitis C patients live in low- and middle-income countries. In India, the World Health Organization estimates as many as 12 million people may be chronically infected with hepatitis C. Nearly 96,000 Indians die from the infection each year.

Until now, doctors primarily have been treating the infection with two drugs: interferon alfa, which is given in weekly injections for 24 or 48 weeks, and daily tablets of ribavirin. The combination can cause debilitating side effects, including flu-like symptoms, anemia and depression, and it only cures about half of patients.

Gilead’s new drug, a daily pill, boasts a 90 percent cure rate and much fewer side effects, Menghaney said. Because the treatment is administered orally and not intravenously, it wouldn’t require a trained doctor to administer — a perk that’s especially important in poor countries where healthcare workers are in short supply.

“This drug could save millions of lives in developing countries,” Menghaney said. “It’s a complete game changer in public health.”

Even before sofosbuvir came along, treatment for hepatitis C was out of reach for many in India, which the World Bank reports had a per capita GDP of $1,489 in 2012. One vial of the injectable drug interferon costs $350.

“Patients sell their homes, their gold, everything they have to get treatment,” said Menghaney. “Some people even take out bank loans.”

The cost is prohibitive even for NGOs. Doctors Without Borders runs India’s only free-of-cost hepatitis C program. Menghaney said drug companies would not negotiate a deal with the NGO, so it has only been able to enroll three patients.

“The treatment just isn’t scalable,” she said. “We need generic competition to bring down the prices.”
The Initiative for Medicines, Access and Knowledge, a legal group based in New York, is challenging Gilead's patent on the grounds that sofosbuvir is based on an “old science.” India’s patent laws don’t consider new forms of a known substance to be innovative unless it improves efficacy.

“India’s patent law doesn’t give monopolies for old science or for compounds that are already in the public domain,” said I-MAK director Tahir Amin in a press release. “We believe this patent on sofosbuvir does not deserve to be granted in India and have the legal grounds to prove it.”

A recent study from Britain’s Liverpool University suggests a 12-week course of the drug could be produced for as little as $62 to $134.

“It only takes a few grams of these drugs to cure hepatitis C,” study author Andrew Hill told Forbes. “Companies have a choice: continue treating a very small number of people with hepatitis C at a very high cost, or expanding access to these treatments, lowering treatment costs significantly, and working towards eradication of this disease.”

In response to the patent opposition, Gilead told PharmaTimes that it is committed to making sure hepatitis C treatments reach as many patients as possible.

“As we did with our current HIV access program, we are working very closely with advocates in the communities that are affected by HCV to develop an appropriate access and pricing strategy,” Gilead stated.

A regional trade deal with global impacts

The Trans-Pacific Partnership, a free trade deal between the US, Canada and 10 countries in the Asia-Pacific region, is one of several regional and sub-regional trade negotiations that have sprung up in recent years as World Trade Organization talks have hit an impasse.

US leaders have called the TPP the first step in an attempt to import stricter rules into the World Trade Organization.

“Our goal is for high standards for the Trans-Pacific Partnership to enter the bloodstream of the global system and improve the rules and norms,” said Vice President Joe Biden in a speech in April. “What we’re talking about is shaping a new standard that then becomes the metric by which all future trade agreements are measured.”

As part of the deal, US negotiators have attempted to crack down on pre-patent oppositions, like the one that has been filed against Gilead. In a draft of the trade agreement published by Wikileaks in November, the TPP forbade challenges to patents until after they were granted.

Drug companies say stronger patent protection is necessary to allow them to recover investments and continue research. But activist groups like Doctors Without Borders argue that stricter intellectual property rules will delay the development of generic drugs and keep healthcare prices high.

Unless India joins the TPP (which isn’t an unheard-of proposal), advocates for affordable medicine will still be able to file pre-grant oppositions, said Amitendu Palit, a senior research fellow at the Institute of South Asian Studies at the National University of Singapore. India’s scientists can continue to develop generics and distribute them to Indians.

But, member or not, because India is the world’s biggest producer of generic medicine, the country’s economy would likely sustain a hefty hit if the trade agreement is passed as written, Palit said. TPP countries represent 40 percent of the world’s gross domestic product and a number of them import generic drugs from India. When the pact goes into effect, those countries will have to close their accounts.

“When the TPP kicks in with much stronger intellectual property rules, Indian producers will be feeling the heat,” he said.

The TPP also includes an anti-counterfeit provision, which empowers customs officials to seize shipments of drugs they suspect to be counterfeit without a formal complaint. Because generic drugs are more susceptible to counterfeit, Palit said, even valid shipments from India are likely to run into roadblocks.

“Say there’s a TPP country located between India and another country where generic shipments from India are travelling,” Palit said, “India’s trucks are going to become targets.”

The tension between India and US pharmaceutical companies is longstanding. India’s patent system is notorious for tripping up US patent holders. Each year, generic medicines companies based in India chip away a larger share of the US market.

In a landmark decision in April, India’s Supreme Court denied the pharmaceutical company Novartis AG patent protection for its cancer drug Glivec. A slew of hits to the pharmaceutical industry followed. Roche lost its patent for the hepatitis C drug Pagasys. Merck was denied a patent for asthma treatment Singulair. Gilead’s HIV drug Viread and Pfizer’s cancer drug Sutent both failed to win patents.
Members of the Senate finance committee and a group of 170 House legislators this summer prevailed upon Secretary of State John Kerry to address the issue, saying Indian patent policies were undermining US business innovation.

"Since early 2012, India’s policies and actions have undermined patent rights for at least nine innovative medicines," said Pfizer’s chief intellectual property officer, Roy Waldron, testifying before a subcommittee of the House Energy and Commerce Committee in July. “This is not only creating significant uncertainty in the market but it also undermines our ability to compete fairly in India, and our willingness to invest there."

Erasing Gay Men From AIDS History
Filed By Mark S. King | December 17, 2013 10:00 AM
Kenneth Cole's appearance on Chelsea Lately last week was meant to promote the documentary he produced about the history of the American Foundation for AIDS Research (The Battle of AmFAR). Instead, the shoe designer insulted gay activists everywhere.

When asked by Chelsea Handler how he became involved in AIDS research, the (straight) designer replied, "This was like 25 years ago and people weren’t talking about AIDS then because stigma was so devastating (and arguably stigma has killed more people than the virus itself has), and the gay community wasn’t speaking up, they were afraid to."

Let’s see. Twenty-five years ago gay people like myself were in our fifth year of AIDS activism, community service, and weekly memorials. We were screaming as loud as we could. And because of our voices, things changed, despite Cole’s inaccurate observations.

The first person to call out Cole’s ignorance was iconic activist Sean Strub, founder of POZ Magazine and author of the upcoming AIDS memoir, Body Counts. Strub’s posting, "Kenneth Cole Needs a History Lesson," has been gaining traction since it published on Friday:

I’ve got news for Ken Cole. Twenty-five years ago, it was almost solely members of the gay community who were speaking up about AIDS. In fact, in 1987, the executive directors of almost all the national lesbian and gay organizations protested government inaction in an act of civil disobedience and got arrested in front of the White House.

Cole’s remarks are part of a larger tendency of people re-framing AIDS history to suit their own purposes, in this case, promoting the amfAR documentary and canonizing two of its founders, Mathilde Krim and Elizabeth Taylor. And a storyline in which the straights come to rescue the diseased gays, I might add, may assuage heterosexual guilt for their own inaction.

For his part, Kenneth Cole responded to my Twitter tirade about the vital role of the gay community during early AIDS by tweeting, "@MyFabDisease agree, our film Battle of amfAR confirms your point. I was saying that because of stigma, many others were reluctant to speak."

But regrettably, the HBO documentary doesn't confirm the role of gay community at all. In fact, it minimizes it.

At least Phill Wilson, a leading black voice on HIV, represents those caught up in the maelstrom of human tragedy, as he explains nearly dying himself from AIDS before combination therapies were approved (thanks in no small part to gay treatment advocates). But early gay activists such as Richard Berkowitz and Michael Callen are given very short shrift, with Berkowitz used primarily as a gay mouthpiece to praise amfAR's Mathilde Krim—as if his own monumental contribution writing "How to have sex in an epidemic" weren’t enough.

Except for fleeting images of ACT UP, the documentary suggests that gay men struggled powerlessly until the straight cavalry arrived. This would come as some surprise to the gay men with AIDS who, in 1983, created The Denver Principles, the historic document of social empowerment that changed attitudes towards people with AIDS, and healthcare itself, forever.

The person with HIV whose story the documentary eventually tells in the most detail is HIV advocate Regan Hoffman, bless her, who also happens to be the straightest, whitest woman with AIDS who has ever lived.

Words and images matter. If the very significant role of gay men in AIDS activism and research is being downplayed now, what will history report in another 30 years? If we as gay community don’t stand up for our place in history, then I fear no one else will.

Some might argue that any documentary about AIDS and the current state of research is a positive thing, and that attitude is understandable. And I am grateful for the massive contributions of amfAR and the deep pockets of people like Kenneth Cole.
The trade-off, however, shouldn't be the truth.

Germans 'cut HIV out of infected cells'
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German scientists have managed to remove HIV from cells while leaving those cells alive—opening the door to potential cures for the disease.

Biomedicine researchers at Dresden's Technical University succeeded in curing several HIV-infected mice with the new method which uses an enzyme to cut the virus from the DNA of infected cells.

"There are various methods and similar approaches, but removing the virus from infected cells is unique," said Professor Joachim Hauber, head of the antiviral strategy section at partner research institute, Hamburg's Heinrich Pette Institute.

He said this approach was the only one so far which could actually reverse an HIV infection, leaving the treated cells healthy.

Whether this would function with people could only be established in clinical trials, he said, for which the money is not yet available.

Dresden team leader Professor Frank Buchholz said the 'molecular scissors' could be ready to use in ten years—as a somatic genetic therapy (using a patient's own genetically altered cells).

"Blood would be taken from patients and the stem cells which can form blood cells, removed," he said.

Laboratory work would introduce the crucial HIV-cutting enzyme into the stem cells, altering their DNA. They would then be put back into the patient.

The theory is that the genetically altered immune cells would reproduce, cut the HIV from infected cells—enabling them to function again.

This was the effect seen at least in part, among the mice.

"The amount of virus was clearly reduced, and even no longer to be found in the blood," said Hauber.

President of the German Aids Society, Professor Jürgen Rockstroh said he hoped funding could be found for further work on the approach.

"It is one of the most exciting things of all," he said. "There is a vague hope of cure, but that must first be proven."

The Dresden team have managed to create this enzyme—via mutation and selection—so that it identifies HIV.

"The HI-pathogen is a retrovirus which gets into the genetic substance in DNA," said Buchholz. Certain recombinase-class enzymes can cut up the DNA double helix and put it back together again in a different pattern.

The researchers have managed to manipulate the enzyme so that it can identify a particular sequence and remove it—and they say it is more than 90 percent effective in identifying the HI-virus in this way.

Hauber said the deciding phase, of bringing the approach to treating people in clinical studies, would be difficult in Germany.

"The potential is not being used," he said, claiming that pharmaceutical companies have until now shown little interest in investing in potential cures for Aids.

He and Buchholz said they would be looking for sponsors and public money for their future research.

Immune avoidance mechanism could lead to treatments for deadly mosquito-borne viruses

PITTSBURGH, Dec. 18, 2013 – A mosquito-borne virus that kills about half of the people it infects uses a never-before-documented mechanism to "hijack" one of the cellular regulatory systems of its hosts to suppress immunity, according to University of Pittsburgh Center for Vaccine Research scientists. The discovery, which will be published in the journal Nature and is funded by the National Institutes of Health (NIH), could aid in the development of vaccines and treatments for eastern equine encephalitis virus (EEEV), a rare but deadly disease that is found primarily in the Atlantic and Gulf States. It also may be useful in efforts to inhibit other diseases, such as West Nile virus, dengue, rhinovirus and SARS.

"Anytime you understand how a virus causes a disease, you can find ways to interrupt that process," said senior author William Klimstra, Ph.D., associate professor at Pitt's Center for Vaccine Research. "And this
discovery is particularly exciting because it is the first time that anyone has shown a virus using this particular strategy to evade its host’s immune system and exacerbate disease progression.” EEEV carries ribonucleic acid (RNA) as its genetic material. Dr. Klimstra and his colleagues discovered that EEEV evolved to have a binding site in its RNA that fits perfectly with a small piece of RNA, called microRNA, in the cells of the organism that the virus is invading. Typically, microRNAs are produced by the host to control its own cellular processes.

When the virus binds with the microRNA in certain cells involved in triggering an immune response in a human, it restricts its own replication. This allows the virus to evade an immune response because the viral replication in these cells is what would normally tip off the host’s immune system and induce it to mount an attack to rid the body of the virus.

Meanwhile, the virus is able to replicate and spread undetected in the cells of the host’s neurological system and cause overwhelming disease. EEEV causes inflammation of the brain that begins with the sudden onset of headache, high fever, chills and vomiting and can quickly progress to disorientation, seizures and coma.

There is no treatment for the disease, but it is rare, with about five to 30 cases reported in the U.S. annually, according to the U.S. Centers for Disease Control and Prevention. It has a 30 to 70 percent fatality rate, the highest of any North American mosquito-borne virus, with significant brain damage in most survivors.

It does not transmit easily to humans, and the mosquito species that typically carries it is usually found in swampy areas that aren’t highly populated, though it has been found in more common mosquitoes, spurring pesticide spraying, curfews and outdoor event cancellations in recent years in states such as Massachusetts, where EEEV is more frequently found.

In the laboratory, Dr. Klimstra and his colleagues created a mutant version of EEEV without the microRNA binding site, which allowed them to discover that the binding site is key to the virus evading detection. When this manufactured mutant version was tested in the laboratory, the researchers found that the host’s immune system was able to mount an effective response to the mutant virus. Dr. Klimstra added that the studies were mostly done in the Regional Biocontainment Laboratory at Pitt, a unique, high-security facility constructed with Pitt and NIH funds.

"Viruses are constantly evolving and changing," said Dr. Klimstra. "However, the genetic sequence that allows EEEV to bind to our microRNA has persisted. We find it in samples from the 1950s, which indicates tremendous evolutionary selection pressure to maintain this mechanism. Ultimately, these results suggest that the mutant virus could be used as an EEEV vaccine and that microRNA blockers could have potential for use as a therapeutic treatment for EEEV-infected patients who currently can be treated only with supportive care."

**Flusurvey: Preliminary findings released**

**Will we have a flu-free Christmas? UK reporting half the number of flu cases compared to this time last year**

Preliminary results from the first month of the Flusurvey run by scientists at the London School of Hygiene & Tropical Medicine indicate that flu is yet to take hold of the UK, with just 6,000 cases per 100,000 people reported, compared to 12,000 cases per 100,000 people for the same period in 2012.

Findings from flusurvey.org.uk show that where some cases of influenza-like illness have been reported, the highest rates were on the South East Coast, followed by Scotland and Wales.

More than 4,000 people have signed up to the UK’s biggest crowd-sourced study of influenza since it launched a month ago. Last week (week ending 15 December) the 0-18 age group was reporting the highest rates of flu across the UK, a trend which was also seen in previous years. The lowest rates of flu so far are among the over 65s.

This year sees a particular focus on measuring the progress of flu among young people. Schools across the UK are taking part in Flusurvey for the first time as researchers, working in partnership with the British Science Association, monitor the impact of the virus in classrooms.

Flusurvey researcher Dr Alma Adler, Research Fellow at the London School of Hygiene & Tropical Medicine, said: "Flu levels are still very low but where there are flu cases, we’re seeing most of them among under-18s. This is in line with what we already know from previous years about children being the ‘key spreaders’ of flu. Flu cases usually dip during the school holidays, so we may see even lower levels of people reporting influenza-like illness over the festive season."
The annual UK Flusurvey aims to collect data from men and women of all ages around the country, in order to map trends as seasonal flu takes hold, enabling researchers to analyse how the virus spreads and who it affects. Anyone can take part in Flusurvey and it only takes a couple of minutes each week. The online questionnaire at flusurvey.org.uk allows people to report their symptoms directly and the data are supplied to Public Health England’s national surveillance programmes.

School classes participating in Flusurvey will also gain access to scientific data during National Science & Engineering Week in March 2014 so they can analyse anonymised data showing the volume of flu cases and factors affecting its transmission. Teachers can sign up and access the resource pack online.

Commenting on the project, Imran Khan, CEO of the British Science Association, said: "UK school children will be at the forefront of science helping researchers understand more about flu in a landmark year for study of the virus. As well as being an important part of collating the data, they will also have the chance to examine the latest findings and trends, which may even relate to their local school or area. We hope this opportunity to engage with a live science project will show the important role that science has in many aspects of their lives.”

Animal vaccine study yields insights that may advance HIV vaccine research
A vaccine study in monkeys designed to identify measurable signs that the animals were protected from infection by SIV, the monkey version of HIV, as well as the mechanism of such protection has yielded numerous insights that may advance HIV vaccine research. Seven laboratories collaborated on the research led by Mario Roederer, Ph.D., and John R. Mascola, M.D., at the Vaccine Research Center of the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health.

By examining both viral amino-acid sequences and the animals' immune responses, the scientists could determine the mechanisms of protection from SIV infection. The study demonstrated that antibodies to the virus spikes that SIV uses to infect cells are necessary and sufficient to prevent SIV infection. The study also identified clear measures of immune responses in monkeys that predict protection from SIV infection.

Amid the genetically heterogeneous mix of SIV to which the vaccinated monkeys were exposed, vaccine-induced immune responses tended to block infection by those viruses sensitive to neutralization by SIV antibodies, while neutralization-resistant forms of SIV tended to cause infection. A two-amino-acid change to the spikes on SIV converted neutralization-sensitive SIV to neutralization-resistant SIV, and vice versa. A similar change to the spikes on HIV had a related effect. Thus, SIV and HIV escape the immune system in similar ways, the scientists discovered. They concluded that the reasons why future human HIV vaccine trials fail or succeed will become clearer if scientists integrate information on the amino-acid sequence and neutralization sensitivity or resistance of the infecting virus together with information about volunteers’ immune responses to the vaccine.

**Article:** M Roederer et al. Immunological and virological mechanisms of vaccine-mediated protection against SIV and HIV. *Nature* DOI: 10.1038/nature12893 (2013).
Scientists Discover How Immune Cells Die During HIV Infection; Identify Potential Drug to Block AIDS

Dec. 19, 2013 — Research led by scientists at the Gladstone Institutes has identified the precise chain of molecular events in the human body that drives the death of most of the immune system’s CD4 T cells as an HIV infection leads to AIDS. Further, they have identified an existing anti-inflammatory drug that in laboratory tests blocks the death of these cells—and now are planning a Phase 2 clinical trial to determine if this drug or a similar drug can prevent HIV-infected people from developing AIDS and related conditions.

Two separate journal articles, published simultaneously today in Nature and Science, detail the research from the laboratory of Warner C. Greene, MD, PhD, who directs virology and immunology research at Gladstone, an independent biomedical-research nonprofit. His lab’s Science paper reveals how, during an HIV infection, a protein known as IFI16 senses fragments of HIV DNA in abortively infected immune cells. This triggers the activation of the human enzyme caspase-1 and leads to pyroptosis, a fiery and highly inflammatory form of cell death. As revealed in the Nature paper, this repetitive cycle of abortive infection, cell death, inflammation and recruitment of additional CD4 T cells to the infection "hot zone" ultimately destroys the immune system and causes AIDS. The Nature paper further describes laboratory tests in which an existing anti-inflammatory inhibits caspase-1, thereby preventing pyroptosis and breaking the cycle of cell death and inflammation.

"Gladstone has made two important discoveries, first by showing how the body’s own immune response to HIV causes CD4 T cell death via a pathway triggering inflammation, and secondly by identifying the host DNA sensor that detects the viral DNA and triggers this death response," said Robert F. Siliciano, MD, PhD, a professor of medicine at Johns Hopkins University, and a Howard Hughes Medical Institute investigator. "This one-two punch of discoveries underscores the critical value of basic science—by uncovering the major cause of CD4 T cell depletion in AIDS, Dr. Greene’s lab has been able to identify a potential new therapy for blocking the disease’s progression and improving on current antiretroviral medications."

The research comes at a critical time, as so-called AIDS fatigue leads many to think that HIV/AIDS is solved. In fact, HIV infected an additional 2.3 million people last year, according to UNAIDS estimates, bringing the global total of HIV-positive people to 35.3 million. Antiretroviral medications (ARVs) can prevent HIV infections from causing AIDS, but they do not cure AIDS. Further, those taking ARVs risk both a latent version of the virus, which can rebound if ARVs are discontinued, and the premature onset of diseases that normally occur in aging populations. Plus, some 16 million people who carry the virus do not have access to ARVs, according to World Health Organization estimates.

Seeking solutions for all these challenges, the new Gladstone discovery builds on earlier research from Dr. Greene’s lab, published in Cell in 2010. This study showed how HIV attempts, but fails, to productively infect most of the immune system’s CD4 T cells. In an attempt to protect the body from the spreading virus, these immune cells then commit "cellular suicide," leading to the collapse of the immune system—and AIDS.
After that research, the Gladstone scientists began to look for ways to prevent this process by studying exactly how the suicidal response is initiated. Working in the laboratory with human spleen and tonsil tissue, as well as lymph-node tissue from HIV-infected patients, the researchers found that these so-called abortive infections leave fragments of HIV's DNA in the immune cells. As described in Nature, pyroptosis ensues as immune cells rupture and release inflammatory signals that attract still more cells to repeat the death cycle.

"Our studies have investigated and identified the root cause of AIDS—how CD4 T cells die," said Gladstone Staff Research Investigator Gilad Doitsh, PhD, who is the Nature paper's lead author, along with Nicole Galloway and Xin Geng, PhD. "Despite some 30 years of HIV research, this key HIV/AIDS process has remained pretty much a black box."

Once the scientists discovered this key process, as described in Nature, they began to investigate how the body senses the fragments of HIV's DNA in the first place, before alerting the enzyme caspase-1 to launch an immune response in the CD4 T cells. To identify the so-called DNA sensor, the scientists found a way to genetically manipulate CD4 T cells in spleen and tonsil tissue. In doing so, they discovered that reducing the activity of a protein known as IFI16 inhibited pyroptosis, explained Zhiyuan Yang, PhD, a Gladstone postdoctoral fellow who is one of the paper's two lead authors.

"This identified IFI16 as the DNA sensor, which then sends signals to caspase-1 and triggers pyroptosis," says Kathryn M. Monroe, PhD, the Science paper's other lead author, who completed the research while a postdoctoral fellow at Gladstone. "We can't block a process until we understand all of its steps—so this discovery is critical to devising ways to inhibit the body's own destructive response to HIV. We have high hopes for the upcoming clinical trial."

The Phase 2 trial—which will test an existing anti-inflammatory's ability to block inflammation and pyroptosis in HIV-infected people—promises to validate a variety of expected advantages to this therapy. For example, by targeting the human body, or host, instead of the virus, the drug is likely to avoid the rapid emergence of drug resistance that often plagues the use of ARVs. The anti-inflammatory may also provide a bridge therapy for the millions without access to ARVs, while also reducing persistent inflammation in HIV-infected people already on ARVs. Many suspect this inflammation drives the early onset of aging-related conditions such as dementia and cardiovascular disease. By reducing inflammation, the drug might also prevent expansion of a reservoir of latent virus that hides in the body where it thwarts a cure for HIV/AIDS.

"This has been an absolutely fascinating voyage of discovery," said Dr. Greene, who is also a professor of medicine, microbiology and immunology at the University of California, San Francisco, with which Gladstone is affiliated. "Every time we turned over an 'experimental rock' in the studies, a new surprise jumped out."

Journal References:

BCG Vaccine More Effective Than Previously Thought
Dec. 19, 2013 — The BCG vaccine has been found to be more effective against the most common form of tuberculosis than previously thought, according to a new study in Clinical Infectious Diseases.

Bacillus Calmette Guérin (BCG) vaccine is included in the childhood vaccination programme of many countries, and is the only licensed vaccine against tuberculosis (TB). However, it has previously been thought to only be effective against the less common forms of the disease that occur away from the lungs. Its efficacy against pulmonary TB, found in the lungs and by far the greatest burden of TB, has varied widely depending on location, ranging from 0% in South India to 80% in the UK.

In order to better understand the reason behind this variability, researchers led by the London School of Hygiene & Tropical Medicine conducted a systematic review of global literature on all reported BCG trials across 10 medical electronic databases, looking at the factors affecting its level of protection against pulmonary TB.

The research shows for the first time that the BCG vaccine is actually highly protective against pulmonary TB in all parts of the world, including significant protection when administered in the tropics.

The main reason for the apparent variation in protection against disease seen in previous studies was found to be due to prior infection reducing the efficacy of the vaccine. BCG vaccination for those with no
history of prior TB infection, including young infants, showed a much higher efficacy against pulmonary TB.

The study therefore highlights a new role for BCG in fighting pulmonary TB, a need for early vaccination, and further suggests that any new TB vaccine based on BCG will also need to be administered before infection has occurred.

Lead author Dr Punam Mangtani, Clinical Senior Lecturer in Epidemiology at the London School of Hygiene & Tropical Medicine, said: "This research corrects a longstanding misconception that BCG is ineffective against pulmonary disease, and confirms its importance in controlling the major burden from TB and main source of transmission in all settings. Now that we know previous infection can lower the protection provided by the vaccine, it is important that BCG is given as early as possible in a person's life, and ideally immediately after birth."

**Journal Reference:**


**Mathematical Perspective of Seasonal Variations in Lyme Disease Transmission**

Different stages of a tick lifecycle. (Credit: Centers for Disease Control Prevention Public Health Image Library (PHIL))

Dec. 19, 2013 — Lyme disease is a common tick-borne illness caused by a bacterium, which is transmitted to humans through the bite of infected ticks. The transmission dynamics of Lyme disease is dependent on a variety of factors, including the length of the tick’s life cycle, availability of hosts, climatic conditions and seasonal influences, which are important to understand for control strategies.
In a paper published last month in the SIAM Journal on Applied Mathematics, authors Yuxiang Zhang and Xiao-Qiang Zhao propose a reaction-diffusion model to study transmission dynamics of Lyme disease while taking into account seasonality.

Ticks live for roughly 2 years, and their life cycle includes three stages: larva, nymph and adult. Ticks climb on to host animals who brush against vegetation from the tips of grasses and shrubs. Once they attach themselves, they feed on blood by inserting their mouthparts into the skin of a host, thus transmitting the disease. After obtaining a blood meal—which can take anywhere between 3 and 5 days—ticks drop off their hosts and prepare for the next stage of the life cycle.

Adult ticks feed and mate on larger mammals such as deer. After obtaining their meal, adult females drop off their hosts and lay eggs on the ground. The eggs hatch into larvae, and larval ticks feed on smaller animals such as mice and birds. After obtaining a blood meal from these smaller hosts, larvae drop off and are inactive until they grow into nymphs. Nymphs feed on small rodents, birds and other small mammals, and then molt into adults. The cycle thus repeats itself.

For Lyme disease to exist in an area, the bacteria that cause it, the ticks that carry the bacteria, and mammals that provide food to ticks in their various life stages must be present. Seasonal variations in temperature, rainfall and resource availability also affect disease transmission and dynamics.

"Ticks develop slowly or become less active in colder temperatures, and rainfall is also critically important for their development, survival, and activities," explains author Xiao-Qiang Zhao. "According to a report from the Public Health Agency of Canada on Lyme disease cases in Ontario between 1999 and 2004, most occurred in late spring and summer, when the young ticks are most active and people are outdoors more often."

A previous model proposed a reaction and diffusion model to study global dynamics of Lyme disease. A reaction-diffusion model takes into account the interaction (reaction) of constituents within the system (in this case pathogens, susceptible hosts and infective hosts) and their change in density over time within their respective populations (diffusion).

The previously-proposed spatial model treats population densities in a continuous two-dimensional space, factoring in birth, death, infection and developmental advancement. However, the model does not account for seasonal patterns.

In this paper, the authors modify this previous model into a reaction and diffusion model in a periodic environment, which models seasonable variables (eg. temperature) as a periodic function. A periodic function is one that repeats its values at regular intervals.

Since seasonal variations are critical for tick development and their activities, which are strongly affected by temperatures, the authors assume that the development rates of ticks as well as their activity rates are time-dependent. The model is governed by a periodic reaction-diffusion system that factors in the densities of susceptible and pathogen-infected mice, densities of susceptible and infectious larvae and nymphs, and densities of uninfected and pathogen-infected adult ticks.

The authors introduce the basic reproduction number $R_0$, which is the number of infectious cases one case of disease can generate on average over the course of the infectious period in a susceptible population. The authors show that $R_0$ can serve as the threshold parameter for stability of either the disease-free or endemic steady state.

"We introduced the basic reproduction ratio $R_0$ and obtained the global dynamics of the disease in terms of $R_0$," says Zhao. The disease is expected to die out when $R_0 < 1$ and stabilize in a positive periodic state when $R_0 > 1$. Hence, the objective is to drive $R_0$ to less than 1. "In the case where $R_0 > 1$, we may get an approximate value of the infection level, and then alter some parameters to drive $R_0 < 1$ so that the disease can be eradicated ultimately," explains Zhao.

"We also established the existence and a computational formula of the spreading speed of infection when $R_0 > 1$," says Zhao. "To control the disease, we may use strategies to reduce the spreading speed. For example, we may use some chemical methods to reduce the infection susceptibilities or the total number of hosts."

Future research involves taking into account the time between various stages of the tick life cycle.

"One future direction is to incorporate time delays between tick life stages into our model," says Zhao. "Another challenging problem is to study the spreading speeds and traveling waves in the case where some parameters are spatially dependent."

**Journal Reference:**

Infectious diarrhea germs stick to healthcare worker hands
Study shows healthcare workers' hands contaminated with C. difficile after routine care
CHICAGO (December 23, 2013) – A new study finds nearly one in four healthcare workers' hands were contaminated with Clostridium difficile spores after routine care of patients infected with the bacteria. The study was published in the January issue of Infection Control and Hospital Epidemiology, the journal of the Society for Healthcare Epidemiology of America.

"This is the first known study focusing on the carriage of viable C. difficile spores on healthcare workers' hands," said Caroline Landelle, PharmD, PhD, lead author of the study. "Because C. difficile spores are so resistant and persistent to disinfection, glove use is not an absolute barrier against the contamination of healthcare workers' hands. Effective hand hygiene should be performed, even in non-outbreak settings."

Researchers compared hand contamination rates among healthcare workers caring for patients with C. difficile with healthcare workers caring for non-colonized patients after routine patient care and before hand hygiene. All patients with C. difficile were being treated with infection control measures that consisted of (1) placing patients into a single-bed room with dedicated equipment; (2) wearing disposable gowns with full-length sleeves and a pair of gloves on entering the room; (3) hand hygiene with alcohol-based hand rub before wearing gloves, before and after body fluid exposure, and hand washing with medicated soap and water followed by use of alcohol-based hand rub after glove removal; and (4) daily room cleaning with a hypochlorite-based disinfectant.

Contamination of healthcare workers' hands occurred with high-risk contact (e.g., patient washing, digital rectal exam, bed linen change, colonoscopy) or when workers didn't use gloves. Hand contamination was also associated with the duration of high-risk contact and was more common among nursing assistants (42 percent) than among other healthcare workers (19 percent for nurses and 23 percent for physicians), likely because nursing assistants had more high-risk contact.

Nipah Protein Structure Revealed
The structure of a key protein of the deadly virus could serve as a stepping stone to antiviral therapy.
By Jef Akst | November 18, 2013
The Nipah virus phosphoprotein, P, plays a key role in viral replication. Therefore, understanding its structure may shed light on ways to disrupt the viral life cycle. “If you can prevent the virus from making more RNA, then it can’t replicate, which is a good strategy for developing antiviral medications,” Jessica Bruhn, a graduate student in Erica Ollmann Saphire’s lab at Scripps Research Institute in La Jolla, California, said in a press release.

With this in mind, Bruhn, Saphire, and colleagues subjected the protein to X-ray crystallography, observing diffraction patterns that revealed its shape: “a long, parallel, tetrameric, coiled coil with a small, α-helical cap structure,” according the paper published in the December issue of the Journal of Virology. Interestingly, this shape is highly similar to the P protein structure of the sendai, measles, and mumps paramyxoviruses, all of which are long, tetrameric coils, despite the fact that these viruses share little sequence identity with one another or with Nipah.

“It was surprising to us that this structure is so similar to those from measles and mumps viruses, even though they are only 5 [percent] to 26 percent identical in sequence,” Bruhn said in the release. The results suggest “a common requirement for scaffolding or spatial organization of the functions of P in the virus life cycle,” the authors wrote in the paper.

The team is now focusing on what makes the Nipah virus so deadly. Having first emerged in the late 1990s, the virus has demonstrated lethality rates of 40 percent to 100 percent in humans. It is currently found in Southeast Asia, particularly Malaysia, Bangladesh, and India. Collaborating with the Centers for Disease Control and Prevention, Saphire’s group plans to more closely examine the role of the P protein in
infection, and understand why previous reports have suggested the protein was a trimer, with just three components, instead of four.

Tracking Fecal Transplants
A long-term study confirms transplants of stool microbes from healthy donors can successfully clear recurrent Clostridium difficile infections.

By Tracy Vence | November 26, 2013

Patients given fecal microbiota transplants (FMTs) to treat recurrent Clostridium difficile infections (RCDI) cleared the bacteria in just days, and their intestinal microbiota were restored nearly to a pre-C. diff state within a year, according to a longitudinal study published in PLOS ONE today (November 26).

The study “adds a puzzle piece to the overall picture of the importance of the stool microbiome in health as well as disease,” David Suskind from Seattle Children’s Hospital and the University of Washington School of Medicine, who was not involved in the work, told The Scientist in an e-mail. “This study also confirms the restorative powers of fecal transplant on microbial diversity and its positive effects on clinical outcomes.”

Researchers from the University of Maryland School of Medicine’s Institute for Genome Sciences and Sinai Hospital in Baltimore used 16S rRNA gene amplicon pyrosequencing to track microbial events associated with RCDI and FMT treatment over time in 14 RCDI patients receiving transplants, as well as their donors, for up to one year post-treatment.

Through this analysis, the team both confirmed that RCDI is associated with reduced diversity and compositional changes in the fecal microbiota and evaluated post-FMT microbiota dynamics. “When we compare sick patients and healthy individuals’ microbiota composition, we see that there is not just higher diversity, but also a lot of intra-individual variation in the rare members between healthy individuals, and it seems to be exactly that fraction [of diversity] that is lost in the sick patients,” Fricke said. “So [sick patients] kind of get reduced to a more common core of shared species, and the rest is filled up by a few, very dominant ‘bad bacteria’”—such as Streptococci and Gram-negative Enterobacteria or Enterococci—he added.

FMT swiftly remedied all 14 patients’ C. diff symptoms—within two to three days—and led to an eventual redistribution of the microbial composition to resemble healthier diversity. Specifically, the team pinpointed certain microbes that were generally reduced in RCDI patients but partially restored five or more months after FMT. But given their sample size, the researchers could not pinpoint any bacterial groups that were significantly associated with RCDI or successful FMT treatment.

RCDI patients have “significant reduction in [stool microbe] diversity compared with healthy individuals,” said study coauthor Florian Fricke, an assistant professor of microbiology and immunology at Maryland. “After successful [FMT] treatment, the diversity increases and we see a trend that they don’t quite reach the diversity of healthy individuals, although we would need more cases to statistically confirm that.”

Moreover, it’s unclear exactly how the introduction of donor microbes affects the composition in RCDI patients’ guts. “We cure the patient, but we don’t know what’s happening,” said Josbert Keller, a gastroenterologist at Hagaziekenhuis Hospital in The Netherlands, who was not involved in the work. “It’s a completely unstandardized treatment. . . . We need to understand the mechanism of action of donor feces to see what changes and, ideally, we should understand what changes are really needed to cure the patient.”

Indeed, among several unresolved questions is which donor strains are responsible for the experimental treatment’s success. And scientists caution that transplanted stool microbes could have unintended effects later in the recipient’s life.

“I’m not necessarily a wholesale proponent of this [FMT] because we are blaming microbiota for a lot of diseases,” said Vincent Young, an associate professor of microbiology and immunology at the University of Michigan, who was not involved in the work. “We’re making them better from the point of view of
recurrent *Clostridium difficile* infections, but are we also potentially setting them up for something more if we’re not careful?”

Fricke and his colleagues plan to continue following the patient-donor pairs in hopes of addressing some of these longer-term questions.

But while there are many unknowns, experts agree that FMT can be a life-saving option for RCDI patients who have run out of treatment options. “Over time, FMT will be more accepted as standard practice,” said Suskind. “The challenges are not truly challenges, but more opportunities to better study and further the science of the stool microbiome.”


**Next Generation: Bactericidal Surface**

*A synthetic material covered in nano-spikes resembling those found on insect wings is an effective killer of diverse microbes.*

By Jef Akst | November 26, 2013

*Diplacodes bipunctata* dragonfly wings are covered by nanoscale pillars, which exhibit strong antibacterial activity.

**The material:** Black silicon, a synthetic material studded with needle-shaped nanostructures that is used primarily for sensor applications, serves as a potent antibacterial agent, killing some 450,000 cells per minute in just one square centimeter, according to a study published today (November 26) in *Nature Communications*.

“If it’s manufacturable, if it’s transferable to other surfaces and fabrics, it could be a major breakthrough,” said Stephen Kelly, a nanoparticle researcher at the U.K.’s University of Hull who was not involved in the research. “It’s interesting in itself in that it clarifies that you can have mechanical effects to kill bacteria, but more importantly, it offers the potential for antibacterial surfaces which will kill a whole range of different kinds of bugs.”

**What’s new:** Nanoparticles with antimicrobial effects have long been used to coat materials in clinical settings. “Bedding in hospitals, nurses uniforms, or bandages, you can make them antibacterial, soaking them in silver nitrate,” Kelly explained. But it was unclear whether the nanoparticles worked by some sort of chemical effect, with ions diffusing from the nanoparticles to the bacteria, or by physically distorting the cell wall and breaking open the cell. “The actual mode of antibacterial action of nanoparticles has been disputed for a long time,” Kelly said.
Now, microbiologist Elena Ivanova of Swinburne University of Technology in Australia and her colleagues have shown that certain nanostructures can indeed kill based on texture alone. The group had previously demonstrated that cicada wings (Psaltoda claripennis), which are covered in dense nanopillar textures, were highly lethal to the opportunistic human pathogen Pseudomonas aeruginosa, and provided evidence to suggest that the wings’ biochemical properties were not responsible. “We showed that bactericidal nature of the wing is due to the mechanical rapture of bacterial cells,” Ivanova told The Scientist in an e-mail.

The sophisticated nanomorphology of dragonfly wings (top left) and that of a synthetic homologue, photovoltaic black silicon (bottom right), are capable of killing bacterial cells on contact. IVANOVA ET AL. Recognizing that dragonflies (Diplacodes bipunctata) also have similar nanopillars on their wings, and that black silicon was known for a similar nano-texture, Ivanova and her colleagues decided to explore the bactericidal potential of the two surfaces. Sure enough, both caused significant deformation of the cell walls of P. aeruginosa, Staphylococcus aureus, and Bacillus subtilis, and even killed B. subtilis spores—at rates more than sufficient to stave off the bacteria. “[They] provided potent bactericidal activity against not only Gram-negative bacteria but also against the more rigid and lysis-resistant Gram-positive bacteria and their spores,” Ivanova said.

“This [study] seems to state clearly that this is a mechanical effect and that you can have a very efficient antibacterial effect of a surface, which is just based on deformation of the cell wall, just stressing the cell wall,” Kelly said. “It’s really novel.”

**Importance:** In the face of ever-evolving multidrug-resistant microbes, and with an insufficient antibiotic pipeline, an antibacterial surface could be just what the doctor ordered. “This opens the avenue of developing surfaces which have very strong antibacterial effects to kill of bacteria which are becoming resistant to all the known antibacterial agents,” said Kelly, who suspects it would be difficult for bacteria to evolve structural resistance to black silicon. “They would have to develop much thicker cell walls, which are flexible and permeable. That would be a real challenge.”

Furthermore, Kelly added, a synthetic surface has the advantage over antimicrobial nanoparticles in that it will not result in the release of nanoparticles into the environment. “Because you’re changing the surface, then it’s not getting into the ecosystem in any way.”

**Needs improvement:** The critical limitation to black silicon’s bacteria-fighting power is cost. The ion-beam technology used to make the material is “fairly expensive,” Kelly said, “and not generally applicable to common, cheap surfaces.” In contrast, the demand for new antimicrobial products “is a fairly low-cost, high-volume market,” he noted. “So I think manufacturability and scalability are the key questions.”

In the meantime, Ivanova and her colleagues “plan to explore a range of other materials [whose] surfaces maybe suitable for fabrication similar structural nano-patterns to create surfaces free from bacteria,” she said.


**Bad Blood**

A rare bleeding disorder leads scientists to uncover an unusual blood component that might be common to us all.

By Kerry Grens | November 1, 2013

In the late 1990s, a hematologist in Texas approached Dianna Milewicz with a problem. One of his patients had a rare bleeding disorder that could not be explained, and a clinical assessment of the patient’s family members revealed that several of them also had it. The initial patient had required a blood transfusion as a toddler after falling down, and again at age 12 when he had a tooth removed. When his sister had lost baby teeth, her gums had to be packed with gauze to soak up the excessive bleeding. Childbirth was extremely dangerous, and doctors refused to perform elective surgery on some family members for fear of fatal bleeds. “They tried numerous treatments,” says Milewicz, a medical geneticist at the University of Texas Health Science Center at Houston. “But nothing seemed to help very much in this family.”

Milewicz agreed to investigate what came to be known as the East Texas bleeding disorder, and she obtained blood samples from 16 family members affected by the disorder and 13 who were unaffected. The family did not want to be interviewed or identified. Through a linkage analysis, Milewicz and her colleagues focused on a region of chromosome 1 that contained the gene for a coagulation factor called factor V (FV).

The variant they identified in the gene seemed like a great lead, given FV’s crucial role in developing blood clots. But when they took a closer look, they found that the resulting amino acid change was in a portion of the protein that gets cleaved off from a precursor of FV before it becomes active in coagulation.
Furthermore, activity assays showed FV was behaving properly in the patients. “Our conclusion was this wasn’t causing the disease,” Milewicz says.

For about a decade the project was set aside, until a graduate student in Milewicz’s lab decided to pick up where the others had left off. She went through the entire region of chromosome 1 that showed up in the linkage analysis and sequenced all the genes, but didn’t find any disease-causing mutations. “At that point we were stuck,” Milewicz says. They shifted gears and decided to take an old-school biochemical approach, collecting plasma from family members. Western blots of the samples showed a 250 kilodalton band of a particular protein that was prominent in the patients, but less so in the unaffected family members.

I think these rare diseases can tell us a lot about the biology of coagulation.—Dianna Milewicz, University of Texas Health Science Center

The protein they isolated turned out to be a splice variant of FV—a product of the very mutation they had picked up on years before. The variant produces a shorter transcript, which the team called FV-short. Further studies conducted by Björn Dahlbäck’s group at Lund University in Sweden showed that FV-short binds to an anticoagulant agent called tissue factor pathway inhibitor-α (TFPIα). When the two bind, the levels of TFPIα skyrocket and inhibit coagulation (J Clin Invest, 123:3777-87, 2013). Elisabetta Castoldi, a researcher at Maastricht University in the Netherlands, says the finding is exciting because it shows FV is not just the procoagulant protein everyone thought it was. “This highlights the anticoagulant side of factor V, even if it is indirect,” she says.

Most bleeding disorders are caused by a deficiency in coagulation factors, not an excess of anticoagulation proteins, making East Texas bleeding disorder especially intriguing. But Milewicz’s group found that family members without the disorder also have small amounts of FV-short, suggesting that it might be a normal part of blood in all people, its smaller anticoagulant effect masked by the coagulating power of full-length FV.

A new view of FV is now emerging, one in which the protein has both pro- and anticoagulant properties. Too much of one can lead to unhealthy blood clots, and too much of another can lead to hemorrhaging. The findings are “opening up a lot of things as far as how coagulation works,” says George Broze, a hematologist at Washington University School of Medicine in St. Louis, who was not involved in the research. “This is a major advance.”

Kenneth Mann, an FV expert at the University of Vermont, says that researchers will have to confirm that FV-short is present in healthy people by taking a large plasma pool and isolating the protein. Still, the idea that FV-short is present in unaffected individuals is exciting, Mann notes, “because it says that the factor V short form corresponds to a new regulatory element of the coagulation system which somehow preserves TFPIα in blood.” Although the mechanism is unclear, it appears that FV-short stabilizes TFPIα in circulation, whereas ordinarily it gets cleared from the blood quickly. “Why these levels of TFPIα are elevated in the presence of short-form factor V is basically the intriguing question,” says Mann.

For Castoldi, the results support evidence she previously published showing that TFPIα levels are reduced in people with FV deficiency, indicating that factor V binds TFPIα. “At the time we had a really hard time to get this finding accepted and published. People would not believe factor V binds to TFPIα,” she says. “Now this paper about East Texas bleeding disorder for us is a confirmation that there is an interaction between factor V and TFPIα, and this finding is extended with a physiological meaning for this interaction.”

Even with the scientific progress made regarding FV, there is still no cure for East Texas bleeding disorder. Milewicz says there might be some promise in TFPIα inhibitors that are in development. She adds that she’s grateful so many family members were willing to participate in the study. “I think these rare diseases can tell us a lot about the biology of coagulation.”

**Taking Shape**

The causes of a cell’s three-dimensional structure remain a fundamental mystery of cell biology.

By Wallace F. Marshall | December 1, 2013

Cells of different tissues and organisms exhibit an array of dramatically different shapes and sizes. Here, clockwise from top left: retinal rods and cones, stacked red blood cells, Purkinje cells in the cerebellum, sensory hair cells in ear, the unicellular protist *Stentor coeruleus*, and the corneal endothelium.

When we first learn about cells in grade school, we’re told to imagine them as “blobs.” And indeed, some cells really are blob-shaped, perhaps most famously the amoebas that ooze across the bottom of a
pond engulfing smaller organisms. Most of the tissue culture cells that serve as workhorses for cell biology research, such as HeLa cells, are also pretty bloblike. But if we stop looking at cells grown in a dish and start examining those found inside the human body, we are immediately struck by the wide range of beautiful and intricate shapes.

Cell interiors are also full of three-dimensional geometric complexity. Cells aren’t just watery bags of enzymes, but rather are compartmentalized into a large number of organelles, each of which carries out different biochemical pathways that together determine a particular cell’s physiology. These organelles divide up cells much as rooms do houses, with the remarkable feature that their size, shape, and number are all dynamically variable depending on the needs of the cell. For example, the endoplasmic reticulum, a network of membrane tubes and sacs specialized for processing secreted proteins, becomes massively enlarged in cells of the pancreas that specialize in secretion.

Examining cells found inside the human body, we are immediately struck by the wide range of beautiful and intricate shapes.

Another factor influencing cells’ geometry is their building materials. Cells are constructed of squishy materials such as lipid membranes and proteins, both of which can undergo drastic shape changes as they interact with other molecules in the cell. These building materials can move rapidly from one place to another, and their levels and distribution in the cell generally result from a balance of ongoing assembly and disassembly.

Biologists often refer to the genome as the internal blueprint of a cell or organism, but it is not a blueprint in the conventional sense: it does not specify the location of each part. Rather, the genome can only specify what parts to make and when to make them, but not where they go or how they fit together.

So what dictates a cell’s geometric shape? Despite decades of research since the internal structures of the cell were first visualized by electron microscopy, we still know next to nothing about how its molecular components give rise to a complex three-dimensional cell. As-yet-unidentified mechanisms are needed for the cell to assume its final form. And because structure and function are so intimately linked in biology, unveiling those mechanisms is an ongoing goal of cell biologists seeking to understand not just how cells look, but how they work.

How big is big enough?
Perhaps the most fundamental aspect of cellular geometry is size. A key feature of any cell, for example, is organelle size, which varies greatly across cell type and function. Such need-based differences in organelle size suggest that cells have regulatory pathways that adjust organelle size as necessary for a particular cell, but in most cases we don’t know what these pathways are. One outstanding question is whether or not such control systems need to be able to measure organelle size in order to make adjustments.

In the case of the endoplasmic reticulum (ER), where proteins destined for secretion are folded into their final shapes, inhibition of protein folding when the organelle’s capacity is exceeded triggers a gene expression program that drives the production of more ER membrane. Such a need-based feedback control system ensures that a cell makes ER of just the right size.

For other organelles, size appears to reflect a dynamic balance between growth and degradation. Cilia are linear organelles formed by bundles of microtubules that push out a protrusion of the cell membrane, and the length of each cilium is determined by the length of the microtubules it comprises. These microtubules undergo constant disassembly at the tip, and the only reason the cilium can maintain a constant length or even grow is by continual assembly of new tubulin protein into the proximal end of the microtubules. Whereas disassembly occurs at a constant rate throughout cilia development, assembly appears to be regulated as a function of length, so that as the cilium gets longer, its assembly rate slows down, until eventually the two rates, of assembly and disassembly, exactly balance each other.2

Determinants of cell shape
Several factors interact to determine the three-dimensional structure of cells and organelles.

See full infographic: JPG | PDF© LUCY READING-IKKANDA

When we see the intricacy of intracellular structures and the diversity of whole-cell shapes, we immediately ask what genes or proteins might sculpt these complex features. In some cases, proteins have indeed been found whose function seems to determine the shape of organelles. Membrane-bending proteins such as the BAR domain proteins and reticulons, for example, help to dictate the tubular shape of the ER. (See illustration.) They do this by having a high affinity for curved surfaces, so that they can selectively bind to tube-shaped areas of the organelle’s membranes. The binding energy of the proteins overcomes the energetic cost of bending a membrane that would prefer to be flat. Simply adding membrane-bending proteins to a round lipid vesicle is enough to cause the vesicle to turn into a network of narrow tubes, highlighting the ability of individual proteins to drive complex geometrical transitions.3
In addition to the importance of proteins in sculpting cellular structures, the physical properties of the structures themselves cannot be discounted. Membrane-enclosed organelles get much of their shape from the basic properties of membranes. A lipid bilayer in which the two layers are identical will be most stable as a flat sheet, but if the lipid composition of each layer is different, then the membrane will have an intrinsic curvature. (See illustration.) In membranes containing mixtures of lipids with different intrinsic curvatures, the lipids tend to separate into distinct domains with their own favored configurations, thus producing complex nonspherical shapes. Such lipid-based mechanisms have been proposed to underlie the curvature of bacterial cell poles, which differ from the shape of the rest of the cell. Membrane tension and elasticity also affect cell shape by determining how much bending or stretching results from localized forces applied to a membrane, such as those generated by the cytoskeletal filaments that move and flex the cell.

In these examples, shape is determined in part by the physical parameters of the components that make up the organelle: if the chemical composition were to change, the shape would be altered. In other cases, shape arises from mathematical necessity. Perhaps the simplest case is the influence of the surface area-to-volume ratio. In a spherical organelle, the surface area-to-volume ratio is a function of organelle size, and no matter what genes a cell might express, it can’t vary the surface area and volume independently without changing the shape or the surface area-to-volume ratio. Starting with a sphere of fixed volume, as the surface area is increased, the organelle becomes less and less spherical and starts to resemble a pancake or sac.

A recent study of the ER, which takes the shape of a stack of pancakes, illustrates how these different factors can work together. A substantial fraction of the ER consists of flat membranous sacs, and in order to fit a large surface area into a small cell, these sacs are stacked one on top of another. Specific proteins play a role in arranging the flat shape of the stacks. The authors found that these sacs are joined by curved tubular connections that have a helical shape similar to the ramps connecting one level of a parking garage to another. (See “Intracellular Spirals” here.) It takes a whole team of architects to design a parking garage, so surely making such a complicated structure inside the cell would require active regulation by a complex network of proteins. But in fact, a simple mathematical model showed that the spiral structure of the ER stack connectors was a direct consequence of the basic physics of lipid membranes: the resulting parking garage-like shape minimizes the elastic energy of the membranes.

Organelles also have to fit together inside the cell, and it is interesting to consider how much of the 3-D structure of a given organelle might be driven by the geometry of organelles packed near it. For example, the nucleus often has a dimple that appears to be caused by the centrosome pressing into it. In this case, the depression itself might have no functional consequences at all, but arises simply as a side effect of packing organelles together. In other cases, organelles appear to work together to sculpt one another, such as when the tubes of the ER help drive fission of mitochondria. (See “Give Me a Hug,” The Scientist, February 2012.) Clearly, understanding cellular geometry will require a systems-level approach to determine how the separate behaviors of multiple parts give rise to the function of the entire system.

**From structure to function**

While researchers continue to puzzle over the material properties that dictate organelle shape, there is the additional question of how that shape influences function. As described above, shape may coincidentally arise simply from the physics of the system, with no relation to organelle function. But often, specialized proteins have evolved that affect organelle shape, and organelle size and shape can be altered in coordination with specialization in cell function, suggesting that organelle geometry can indeed be functionally important.

For instance, organelle geometry is often perturbed in cancer: most tumor cells show numerous alterations in organelle size and shape, such as the presence of huge vacuoles full of mucus or fragmented nucleoli. It is these alterations that allow cytopathologists to diagnose cancer by looking at cells under the microscope, and although the molecular reasons for these changes are almost entirely unknown, the fact that they occur at all raises the possibility that these changes are part of the development of a malignant cell phenotype. But so far, the evidence is purely correlative. What the field needs most right now is a way to directly test whether changes in organelle geometry can play a causal role in cell behavior.

One case in which organelle shape has been shown to play a functional role is in the exchange of lipids between the endoplasmic reticulum and the mitochondrion. In 2012, Christiane Voss of the National Institute of Diabetes and Digestive and Kidney Diseases and colleagues found that defects in the reticulon proteins that shape the tubules of the ER can disrupt connections between the organelles and impair the ability of lipids to transfer from the ER to the mitochondria, a process vital for the synthesis of
mitochondrial membrane. As our knowledge of the mechanisms that determine geometry of other organelles increases, it should be possible to engineer systematic changes in the geometry of each organelle to probe the consequences for cell behavior and physiology.

Understanding cellular geometry will require a systems-level approach to determine how the separate behaviors of multiple parts give rise to the function of the entire system.

In general, we can imagine membrane-bound organelles to be like reaction vessels in a chemical factory, where the volume determines the quantity of biochemical reactants each organelle can hold, while the surface area determines the rate of exchange of reactants and products between the cytoplasm and the organelle interior. As with any chemical process, the exchange rate and volume can affect the concentrations of intermediates that accumulate, which in turn can dramatically alter the rate and even the outcome of the reactions that take place within.

If an organelle’s size and shape do indeed affect the function of its biochemical pathways, it raises the interesting possibility that we could retune cell physiology by manipulating organelle geometry. For instance, if a tumor cell has an enlarged nucleus, perhaps we could make the cell less malignant if we could drive its nucleus back to a normal size. Currently, however, we do not know enough about the molecules that regulate organelle geometry to even begin testing such a strategy.

**Geometry of the whole cell**

While much about cell geometry remains a mystery, researchers are starting to identify molecules that regulate specific geometrical features, such as length, volume, or curvature, of individual cellular components. But even if we understand how the geometries of intracellular organelles or various types of cellular protrusions are determined, we will still be faced with the question of overall cell shape and internal structure.

One specific aspect under investigation is cell polarity. In many cells, it is possible to define one or more axes of polarization, which serve as references to specify the position of intracellular structures. For example, in epithelial sheets, cells resemble hexagonal columns, with the basal end of the cells attached to the basement membrane and cilia sprouting from the opposite, apical end. This polarized shape is critical for organization of tissues based on epithelial sheets, such as the tubes found in the lung or kidney. Similarly, migrating cells, such as white blood cells, develop a clear anterior-posterior polarization axis in response to a chemoattractant gradient, with a branched actin network assembly at the anterior end to drive forward motion.

The cell polarity axes are conceptually similar to the body axes of a developing embryo, and indeed, cells face many of the same developmental challenges as embryos. Just as in embryos, different structures must form at defined relative positions, and this requires long-range coordination of morphogenetic activities. In embryos, this coordination is accomplished in part through the action of diffusible morphogen molecules, but we know far less about such morphogens that underlie the development of patterning in single cells, if they even operate at this level.

One challenge to studying the developmental biology of individual cells is that within an animal, cells can obtain positional cues from neighboring cells, which greatly complicates the study of cell shape. But studying individual cells growing in isolation has its own drawbacks. Regrettably, most animal cells grown in culture lose their normal morphology and become blob-like, although careful quantitative analysis has shown that cultured cells still have defined shapes in the sense that they only take on a subset of the total set of bloblike shapes. Growing cells in 3-D cultures might help to elicit more diverse cell shapes in culture. (See “Enter the Third Dimension,” *The Scientist*, September 2012.)
Free-living unicellular organisms provide an excellent platform for studying the development of overall cell shape. They tend to have highly reproducible shapes with well-defined geometrical forms, and because they often grow in isolation from other cells in nature, the cells themselves contain all the information necessary for generating these shapes. Yeast has long served as a model system for studying cell shape, and the power of classical yeast genetics and systematic knockout libraries has been productively applied to the problem of geometry. But since all yeast cells are more or less egg-shaped, the range of shapes that can be studied in such systems is limited.

Ciliated protists provide a unique opportunity for understanding the development of cell shape. The surface of these unicellular organisms is covered with orderly rows of cilia arranged in complex patterns that vary only slightly from one cell to the next. Cilia, at least visually, appear much more like man-made machines than do typical blob-like mammalian culture cells. One of the most intriguing features of ciliate patterning is that it is self-propagating. If a region of the cell surface is excised from a ciliate, rotated so that the cilia rows face in the other direction, and reimplanted, the progeny of that cell will inherit the rotated ciliary rows. Interestingly, careful experiments ruled out the possibility that this inheritance was mediated by the cell’s genome; rather, it appears to be dictated by the organelles. The ability of such structures to direct their own reproduction during cell division creates a whole new level of complexity in how cell geometry is determined, and we currently have almost no understanding, at a molecular level, of how this structural inheritance occurs.

The giant ciliated protist Stentor coeruleus, which can be more than 1 millimeter long, provides an even more extreme case of pattern formation in ciliates. (See photograph above.) These unicellular organisms have a highly complex pattern of cilia on their surfaces, and are remarkable not only for their size and internal complexity, but also for their ability to regenerate. If any part of Stentor is surgically removed, that specific part will regenerate in a few hours. The mechanism by which such a cell can sense the loss of one part of itself and direct that one part to regenerate is a complete mystery.

Cell geometry has many more unknowns for us to ponder. Given the fact that cell shape involves not only biochemistry and genetics, but also physics and mathematics, progress in this field is going to require a highly interdisciplinary approach, which should serve as a paradigm for combining the physical and biological sciences with applied mathematics.

Wallace F. Marshall is an associate professor of biochemistry and biophysics at the University of California, San Francisco. Originally trained as an electrical engineer, he became interested in cell biology out of a desire to understand how cells can solve complicated engineering problems, such as determining the size and shape of their sub-structures.

References

HIV Returns in Patients Thought Cured *****
The virus is back in two patients in Boston who received bone-marrow transplants and did not have detectable levels of virus for months.
By Abby Olena | December 10, 2013
Physician-scientists reported in July that two male patients had stopped anti-retroviral therapy and were HIV-free after bone-marrow transplants that occurred in 2008 in one man and 2010 in the second. Now, the scientists have announced that HIV is back in both patients.
“It’s disappointing and very sobering,” virologist Deborah Persaud of the Johns Hopkins Children’s Center in Baltimore, Maryland, told Nature News.
The men were given high-risk bone-marrow transplants to treat blood cancer and had no detectable levels of HIV in their blood eight months post-transplant. They each stopped taking anti-retroviral drugs earlier this year and did not test positive for HIV for months. Timothy Henrich of Brigham and Women's Hospital in Boston, one of the patients’ physicians, said today in a statement that one man’s HIV returned in August and the second man’s blood tested positive in November. Both men are now back on anti-retroviral drugs and doing fine, and in the statement, Henrich acknowledged “their commitment to research.” He added that the reappearance of the virus demonstrates that “there may be an important long-lived HIV reservoir outside the blood compartment.”

Anthony Fauci, the director of the National Institute of Allergy and Infectious Diseases, told The New York Times that the return of HIV in the men “doesn’t put an end to this avenue of research, but it certainly does put a damper on it.”

“The failure to cure these individuals will certainly influence the conduct of future clinical trials,” Steven Deeks, an HIV researcher and physician at the University of California, San Francisco, told Nature News. “It is now clear viral rebounds can happen at any time, even months after stopping therapy. People will have to followed very carefully for more prolonged periods than in the past.”

How Bacteria Evade the Immune System

*Escherichia coli* can quickly evolve to resist engulfment by macrophages, scientists have found.

By Laasya Samhita | December 12, 2013

Bacteria exposed to antibiotics rapidly acquire mutations that allow them to develop resistance to the drugs, and this process is fairly well understood. Scientists have now looked at the evolution of bacterial resistance toward live agents: cells of the immune system. In a report published in *PLOS Pathogens* today (December 12), a team led by Isabel Gordo from the Instituto Gulbenkian de Ciência in Oeiras, Portugal, challenged the common human intestinal bacterium *Escherichia coli* with mouse macrophages—immune system cells that engulf foreign elements like bacteria—and observed the rapid evolution of mutants capable of escaping capture. The same *E. coli* mutants could successfully establish infections in mice.

“This work on the development of *E. coli* macrophage resistance and virulence is important,” wrote James Shapiro, a professor of microbiology at the University of Chicago who was not involved in the work, in an e-mail to The Scientist. “It documents how encounters with mammalian host defense cells can stimulate rapid adaptation in bacteria.”

Although the authors only tested for *E. coli* virulence for one month, some mutants emerged in as few as four days.

Bacteria have evolved several defences to avoid being internalized by macrophages—sticky outer coverings, formation of filaments and biofilms among them. The evolution of such defences has been studied previously, but not through an evolutionary experiment such as this one performed by Gordo’s team. “I would have thought that the answer goes back 100 years or so, but apparently this is not the case,” Moselio Schaechter, a professor emeritus at the Tufts University School of Medicine in Boston who was not involved in the work told The Scientist in an e-mail. “It’s surprising that this finding has not emerged earlier,” he continued. “It seems like an obvious question to ask.”

The researchers followed the evolution of six *E.coli* strains, with or without mouse macrophages at a ratio of 1:1. Every 24 hours, the numbers of bacteria were adjusted so that they did not exceed a maximum of ~10^8. As early as four days into the month-long experiments, two new types of colonies were visible:
Gut Bacteria Vary with Diet

Extreme diets can alter the microbial makeup of the human GI tract, and change the behavior of those bacteria.

By Jef Akst | December 13, 2013

Ten volunteers who agreed to eat a diet entirely provided by microbiologist Harvard Peter Turnbaugh and his colleagues are living proof of the gut microbiome’s lability. After five days, those that ate only animal products had a similar suite of bacteria in their guts, which varied dramatically from the microbiome of those feasting on a high-fiber, plant-only diet. Specifically, while the types of bacteria present remained largely unchanged, the abundances of different types responded to the dietary restrictions, according to a study published this week (December 11) in Nature.

“It’s a landmark study,” Rob Knight, a microbial ecologist at the University of Colorado, Boulder, who was not involved with the work, told ScienceNOW. “It changes our view of how rapidly the microbiome can change.”

In 2009, Turnbaugh’s team had shown a similar effect in mice: a change in diet could affect the murine gut microbiome in a single day, in fact. To see if the results would hold up in people, Turnbaugh, along with Lawrence David from Duke University and their colleagues, fed five volunteers a high-protein diet, with meals of bacon and eggs for breakfast, spareribs and brisket for lunch, and salami and cheese for dinner. This group could also snack on pork rinds and string cheese. The high-fiber group, on the other hand, got fruits, vegetables, grains, and beans. Before, during, and after the diet change, the team

one that formed smaller colonies than the original strain—termed SCV, for small colony variants—and one that formed large mucoid colonies—dubbed MUC, or mucoid translucid colonies.

“What was amazing was how fast these variants appeared,” said Gordo.

In contrast, the control experiment where no macrophages were used did not show any colony morphology variation even after the full 30 days. The authors found that 30-day-old SCVs outcompeted the ancestral strain when introduced into macrophages and tested after two hours. However, the advantage was restricted to the early phase of its interaction.

The MUCs, on the other hand, steadily increased in number and eventually took over the entire population in five of the six strains tested. This suggested that the MUCs consisted of mutants that could avoid engulfment. Indeed, upon quantifying E. coli mutants within and outside macrophages in an infection experiment, the authors found significantly fewer MUCs inside macrophages as compared with the same experiment performed on the ancestral strain. The authors then tested the virulence of the MUC strains in vivo by mixing 30 day MUCs from all six evolved lines and introducing them into mice. As expected, mice exposed to MUCs showed significantly reduced survival compared with those exposed to ancestral strains or the control E.coli evolved without macrophages. Curiously, the control strains appeared less virulent than the ancestral ones. Gordo’s team is now pursuing this observation by sequencing the control set. “But the bottom line is that we do not know why [control] strains are less virulent,” she explained.

To understand the basis for the virulence of the MUCs, the authors sequenced sample genomes from each of the six lines, finding that they all had a common mutation—a transposon insertion in the promoter region of a gene called ypfF. Although this gene has not been characterized in E. coli, its homolog in the closely related bacterium, Salmonella, plays a role in the secretion of colonic acid, which could help explain the increased mucoid nature of the experimental mutant colonies.

Further, each of the clones sequenced showed a range of mutations including in the promoter of a protease called lon, which is known to enhance the overall frequency of mutations in the cell. “The role of transposable elements in this adaptive response fits with over four decades of research showing mobile DNA to be a major agent of genome change,” said Shapiro.

Ashley Franks, a senior lecturer in the department of microbiology at La Trobe University in Australia pointed out a different angle. Studies like this, she wrote in an email to The Scientist, demonstrate “a medical consequence of what is occurring with genetic rearrangements of the DNA sequence by the microbes themselves.”

Because the mechanism by which this leads to increased virulence in E. coli is not clear, further work is needed to make the leap to what happens in a real infection inside the human body. “Clearly, these experiments are greatly simplified from the real situation in the living organism. However, it is an important step towards a more realistic experimental environment,” Shapiro added.

collected stool samples from each participant to assess the bacterial composition of their guts, as well as the gene expression activity of those microbes.

Before the week was up, the researchers found noticeable differences in the gut bacteria of the two treatment groups. The meat-eaters harbored more bacteria that are able to tolerate high levels of bile acids, which are secreted by the body to help digest meat. The bacteria isolated from this group also appeared to increase expression of genes involved in breaking down proteins. Plant-eaters, on the other hand, had fewer bile-resisting bacteria and higher expression levels of gene associated with carbohydrate digestion.

“There are interesting evolutionary implications to such a rapid response,” David told *National Geographic’s Not Exactly Rocket Science.* “The ability for the gut microbiome to quickly react to changes in diet may have conferred an extra layer of nutritional buffering, and provided ancient humans with increased dietary flexibility.”

“The paper has made the next leap in the field,” Mayo Clinic gastroenterologist Purna Kashyap told *NPR.* Of course, he added, the results will need to be well verified and elaborated upon before any sort of health-care recommendation can be made. “With discovery comes responsibility. Once you make this big finding, it needs to be tested appropriately.”

**Fighting Flu**

**Researchers link host glucose metabolism with severity of influenza infection.**

By Abby Olena | December 15, 2013

Vast amounts of time and research dollars go into studying how the quickly mutating influenza virus works, anticipating which strains will be most active each year, and changing the flu vaccine accordingly. Researchers from the University of North Carolina, Greensboro, recently approached the problem from a different perspective by looking at the host’s response to the virus. Amy Adamson and Hinissan Kohio showed that flu infection is linked to glucose metabolism in mammalian cells. They presented their work today (December 15) in a poster session at the American Society for Cell Biology meeting in New Orleans, Louisiana, and in a paper published in September in *Virology.*

“Understanding host responses to infection will be important,” said Olivia Perwitasari, who is a postdoctoral fellow in Ralph Tripp’s lab at the University of Georgia and was not involved in the work. “The majority of the focus in antiviral therapeutics is still trying to target the virus itself,” she said, but because viruses change so rapidly, and host targets are more stable, “targeting the host is up and coming.”

Adamson first became interested in host glucose metabolism and its link with influenza based on a screen in fruit flies, where glycolysis—the process by which glucose is converted to energy—pathway members appeared as potential regulators of viral infection. “It’s really kind of unconventional because most virologists are not going to be using fruit flies,” said Adamson, but “we’ve found some interesting interactions.”

To closely examine the connection, Adamson and Kohio fed Madin-Darby Canine Kidney (MDCK) cells increasing concentrations of glucose in the culture media and then challenged them with influenza A H1N1. They observed a dose response: the higher the concentration of glucose, the higher the percentage of cells that were infected with virus. The researchers showed that blocking glycolysis by treating cells with inhibitors lowered the percentage of infection in cells at high glucose concentrations by 80 to 90 percent. The drugs’ effects were reversed when ATP—the product of glycolysis used for energy—was added to the cells.

The scientists then used human cells—both HeLa and A549 lung carcinoma cells—to show that high glucose concentrations or directly added ATP both increased the assembly and activity of a proton pump called the vacuolar-type H+ ATPase (V-ATPase). That pump is known to play a role in viral entry into cells by regulating the pH in endosomes that must be acidic so that influenza genome can be released. They found that cells at high glucose concentrations had more acidic intracellular compartments. Plus, inhibiting glycolysis caused the disassembly of the V-ATPase, and using a drug to disassemble the pump resulted in a decrease in the percentage of infected cells, even at high glucose concentrations. The authors proposed that targeting glucose metabolism could help fight influenza infections in patients.

At high doses, the inhibitors that the researchers used adversely affected the cells, but Adamson is hopeful that they will be able to find the right amount to use. “We’re looking at trying to do very minimal doses to have the least bad effect on the cell [and] that could cause the greatest decrease in infection,” she says.
Charles Russell, an associate member in the department of infectious disease at St. Jude Children’s Research Hospital in Memphis, Tennessee, who did not participate in this research, said he found the work interesting “because they’re looking at host-based mechanisms that control flu replication.”

Experts also pointed out that there is previously published work in humans and mice suggesting that diabetes might increase susceptibility to influenza infection, and that this work could help explain that relationship. Russell said he would like to see follow-up studies that use a mouse model with diabetes “to tease out how it affects the replication of the virus.”

Perwitasari also remarked that the emphasis on cell culture over animal work is a limit to these findings that should be addressed in the future. “They can probably see if this is a really viable therapeutic strategy,” she said, but “what happens if you put it in vivo?”

Prove Antibacterials are Safe: FDA
The Food and Drug Administration is asking companies to produce evidence that their antimicrobial washes do no harm.
By Kerry Grens | December 17, 2013
Hand washes and other cleansers labeled “antibacterial” invoke a sense of cleanliness and healthiness. But don’t jump to any conclusions, says the US Food and Drug Administration (FDA). After a review of the available safety information, the agency is floating a rule to require manufacturers of consumer antimicrobial products to demonstrate that they are indeed safe.

“New data suggest that the risks associated with long-term, daily use of antibacterial soaps may outweigh the benefits,” Colleen Rogers, a lead microbiologist at FDA, said in a statement. There isn’t any evidence that antibacterial soaps are any better at preventing disease than regular soap, and, according to the FDA, “there are indications that certain ingredients in these soaps may contribute to bacterial resistance to antibiotics, and may have unanticipated hormonal effects that are of concern to FDA.”

Of particular concern is a chemical called triclosan that is added to consumer products to ward off bacteria. The FDA’s proposed rule would require manufacturers of consumer products labeled as antibacterial to demonstrate the safety and effectiveness of their soaps. The rule would apply only to products used with water—not hand sanitizers or hand wipes that don’t require washing.

Public health experts seem pleased with the FDA’s move. “These antimicrobials have taken on a life all of their own,” Rolf Halden, the director of the Center for Environmental Security at Arizona State University, told The New York Times. “Their use has really proliferated.”

The American Cleaning Institute and Personal Care Products Council issued a statement saying that they “are perplexed that the Agency would suggest there is no evidence that antibacterial soaps are beneficial as industry has long provided data and information about the safety and efficacy of these products.” However, the groups applaud the FDA’s move, and they intend to reaffirm the safety of these products. The FDA is gathering feedback on the proposed rule for 180 days.

Epigenetics enigma resolved
First structure of enzyme that removes methylation
Scientists have obtained the first detailed molecular structure of a member of the Tet family of enzymes.

The finding is important for the field of epigenetics because Tet enzymes chemically modify DNA, changing signposts that tell the cell’s machinery “this gene is shut off” into other signs that say “ready for a change.”

Tet enzymes’ roles have come to light only in the last five years; they are needed for stem cells to maintain their multipotent state, and are involved in early embryonic and brain development and in cancer.

The results, which could help scientists understand how Tet enzymes are regulated and look for drugs that manipulate them, are scheduled for publication in Nature.

Researchers led by Xiaodong Cheng, PhD, determined the structure of a Tet family member from Naegleria gruberi by X-ray crystallography. The structure shows how the enzyme interacts with its target DNA, bending the double helix and flipping out the base that is to be modified.

"This base flipping mechanism is also used by other enzymes that modify and repair DNA, but we can see from the structure that the Tet family enzymes interact with the DNA in a distinct way," Cheng says.

Cheng is professor of biochemistry at Emory University School of Medicine and a Georgia Research Alliance Eminent Scholar. The first author of the paper is research associate Hideharu Hashimoto, PhD. A team led by Yu Zheng, PhD, a senior research scientist at New England Biolabs, contributed to the paper by analyzing the enzymatic activity of Tet using liquid chromatography–mass spectrometry.

Using oxygen, Tet enzymes change 5-methylcytosine into 5-hydroxymethylcytosine and other oxidized forms of methylcytosine. 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC) are both epigenetic modifications of DNA, which change how DNA is regulated without altering the letters of the genetic code itself.

5-mC is generally found on genes that are turned off or on repetitive regions of the genome. 5-mC helps shut off genes that aren’t supposed to be turned on (depending on the cell type) and changes in 5-mC’s distribution underpin a healthy cell's transformation into a cancer cell.

In contrast to 5-mC, 5-hmC appears to be enriched on active genes, especially in brain cells. Having a Tet enzyme form 5-hmC seems to be a way for cells to erase or at least modify the "off" signal provided by 5-mC, although the functions of 5-hmC are an active topic of investigation, Cheng says.

Alterations of the Tet enzymes have been found in forms of leukemia, so having information on the enzymes’ molecular structure could help scientists design drugs that interfere with them.

N. gruberi is a single-celled organism found in soil or fresh water that can take the form of an amoeba or a flagellate; its close relative N. fowleri can cause deadly brain infections. Cheng says his team chose to study the enzyme from Naegleria because it was smaller and simpler and thus easier to crystalize than mammalian forms of the enzyme, yet still resembles mammalian forms in protein sequence.

Mammalian Tet enzymes appear to have an additional regulatory domain that the Naegleria forms do not; understanding how that domain works will be a new puzzle opened up by having the Naegleria structure, Cheng says.

**New drug candidates show promise for cure for Chagas disease**

A team of researchers from Canada has developed a class of compounds which may help eradicate a neglected tropical disease that is currently hard to kill in its chronic form. The research was published ahead of print in Antimicrobial Agents and Chemotherapy.

Chagas disease or American trypanosomiasis, caused by Trypanosoma cruzi, affects about 18 million people living mostly in Latin America. The parasite is transmitted to humans by blood-sucking reduviid bugs, also known as kissing bugs due to their predilection for feeding on the faces of their victims. In the United States, Chagas disease is considered one of the neglected parasitic infections, a group of five parasitic diseases that have been targeted by CDC for public health action.

"While historically infection was largely confined to poor and rural populations in Central and South America, it has been emerging in the U.S., Canada, Europe, Japan, and Australia, due to immigration, and nonvectorial transmission is becoming a public health threat," says Deborah Nicoll-Griffith of the Merck Frosst Centre for Therapeutic Research in Kirkland, Quebec, a researcher on the study. One 2005 estimate put the number of people infected within the U.S. at 300,000 (1/1000).
There are two phases of Chagas disease: the acute phase and the chronic phase. Both phases can be symptom free or life threatening. Left untreated the disease can lead to cardiac and digestive disorders, as the parasite burrows into the heart, esophagus and colon tissue where it causes damage over time.

The current standard of care, the drug benznidazole, has significant activity against the parasite during the acute phase, but is less effective once the disease becomes chronic.

Efforts to find new drugs focus on disrupting an enzyme, cruzipain, which the parasite uses for digestion, to produce other cellular machinery, to evade the host's immune system, and to invade heart and gastrointestinal tissues.

Nicol-Griffith and her colleagues identified two compounds known as reversible cysteine protease inhibitors that fit cruzipain like jigsaw puzzle pieces, jamming the enzyme. In the study, they tested the efficacy of the compounds in mice against that of benznidazole. While all treatment groups showed a marked reduction in parasite burden, in all tissues, the two experimental compounds had greater cure rates of acute infections (90% and 78%) compared to benznidazole (71%).

"The efficacy shown in these T. cruzi murine studies suggests that nitrile-containing cruzipain inhibitors show promise as a viable approach for a safe and effective treatment of Chagas disease," write the researchers.

**Toys, books, cribs harbor bacteria for long periods, study finds**

*Streptococcus biofilms persisted on objects and surfaces in a daycare center, in some cases after a cleaning*

BUFFALO, N. Y. – Numerous scientific studies have concluded that two common bacteria that cause colds, ear infections, strep throat and more serious infections cannot live for long outside the human body. So conventional wisdom has long held that these bacteria won’t linger on inanimate objects like furniture, dishes or toys.

But University at Buffalo research published today in *Infection and Immunity* shows that *Streptococcus pneumoniae* and *Streptococcus pyogenes* do persist on surfaces for far longer than has been appreciated. The findings suggest that additional precautions may be necessary to prevent infections, especially in settings such as schools, daycare centers and hospitals.

"These findings should make us more cautious about bacteria in the environment since they change our ideas about how these particular bacteria are spread," says senior author Anders Hakansson, PhD, assistant professor of microbiology and immunology in the UB School of Medicine and Biomedical Sciences. "This is the first paper to directly investigate that these bacteria can survive well on various surfaces, including hands, and potentially spread between individuals."

*S. pneumoniae*, a leading cause of ear infections in children and morbidity and mortality from respiratory tract infections in children and the elderly, is widespread in daycare centers and a common cause of hospital infections, says Hakansson. And in developing countries, where fresh water, good nutrition and common antibiotics may be scarce, *S. pneumoniae* often leads to pneumonia and sepsis, killing one million children every year.

*S. pyogenes* commonly causes strep throat and skin infections in school children but also can cause serious infection in adults.

The UB researchers found that in the day care center, four out of five stuffed toys tested positive for *S. pneumoniae* and several surfaces, such as cribs, tested positive for *S. pyogenes*, even after being cleaned. The testing was done just prior to the center opening in the morning so it had been many hours since the last human contact.

Hakansson and his co-authors became interested in the possibility that some bacteria might persist on surfaces when they published work last year showing that bacteria form biofilms when colonizing human tissues. They found that these sophisticated, highly structured biofilm communities are harder than other forms of bacteria.

"Bacterial colonization doesn’t, by itself, cause infection but it’s a necessary first step if an infection is going to become established in a human host," he explains. "Children, the elderly and others with compromised immune systems are especially vulnerable to these infections."

**IMAGE:** This SEM image shows a mature pneumococcal biofilm: the nearly round structures of *S. pneumoniae* bacteria are organizing together a matrix of smaller, oddly shaped material surrounding them that makes...
He explains that studies of how long bacteria survive on inanimate objects have used cultures grown in laboratory media, called broth-grown planktonic bacteria, and invariably show that bacteria die rapidly.

"But we knew that this form of bacteria may not represent how they actually grow in the host," says Hakansson. "Since discovering that biofilms are key to the pathogenesis of S. pneumoniae, we wanted to find out how well biofilm bacteria survive outside the body."

The UB experiments found that month-old biofilm of S. pneumoniae and S. pyogenes from contaminated surfaces readily colonized mice, and that biofilms survived for hours on human hands and persisted on books and soft and hard toys and surfaces in a daycare center, in some cases, even after being well-cleaned.

"In all of these cases, we found that these pathogens can survive for long periods outside a human host," says Hakansson. But, he says, the scientific literature maintains that you can only become infected by breathing in infected droplets expelled through coughing or sneezing by infected individuals.

"Commonly handled objects that are contaminated with these biofilm bacteria could act as reservoirs of bacteria for hours, weeks or months, spreading potential infections to individuals who come in contact with them," concludes Hakansson. He cautions that more research should be done to understand under what circumstances this type of contact leads to spread between individuals.

"If it turns out that this type of spread is substantial, then the same protocols that are now used for preventing the spread of other bacteria, such as intestinal bacteria and viruses, which do persist on surfaces, will need to be implemented especially for people working with children and in health-care settings," he adds.

Genetic Clue to Fighting New Strains of Flu
Dec. 24, 2013 — Researchers at the University of Melbourne have discovered a genetic marker that can accurately predict which patients will experience more severe disease in a new strain of influenza (H7N9) currently found in China.

Published in the journal Proceedings of the National Academy of Sciences, senior author, Associate Professor Katherine Kedzierska from the Department of Microbiology and Immunology said that being able to predict which patients will be more susceptible to the emerging influenza strain, will allow clinicians to better manage an early intervention strategy.

"By using genetic markers to blood and lung samples, we have discovered that there are certain indicators that signal increased susceptibility to this influenza. Higher than normal levels of cytokines, driven by a genetic variant of a protein called IFITM3, tells us that the severe disease is likely," she said.

"We call this a Cytokine Storm and people with the defective genetic variant of the protein IFITM3 are more likely to succumb to severe influenza infection.

Professor Peter Doherty, AC, Laureate Professor and a lead author of the study from the University of Melbourne said predicting how influenza works in individuals has implications for the management of disease and the resources on our health system.

"We are exploring how genetic sequencing and early identification can allow us to intervene in treating patients before they become too unwell. As new cases of influenza emerge in the Northern Hemisphere, we try to keep a season ahead and prepare to protect the most vulnerable in our community," he said.

Though the H7N9 strain has not been found in Australia, researchers from the University of Melbourne are collaborating closely with Prof Jianqing Xu and his group from the Shanghai Public Health Clinical Center in China. In addition to this, Dr Zhongfang Wang, an NHMRC Australian-China Exchange Fellow is also working closely with Melbourne experts.

Journal Reference:
Essential Factor for Lyme Disease Transmission Identified

Dec. 19, 2013 — *Borrelia burgdorferi*, the bacterium that causes Lyme disease, hitchhikes in ticks for dissemination to mammalian hosts—including humans. An article in the 19 December issue of *PLOS Pathogens* identifies HrpA, an RNA helicase, as a crucial player in the transmission from ticks to mammals.

George Chaconas, from the University of Calgary, Canada, and a member of the university's Snyder Institute for Chronic Diseases, and colleagues had previously identified HrpA as a modulator of *B. burgdorferi* protein expression. For this study, Chaconas' group joined forces with Justin Radolf and Melissa Caimano from the University of Connecticut Health Center, USA, to analyze the molecular function of the HrpA protein and further explore its role in the bacterium's complicated life cycle, in particular for transmission of the pathogen.

Its DNA sequence suggests that HrpA is an RNA helicase, a protein that can harvest energy from the cell's stores, use it to unwind RNA, and so regulate translation of RNA into protein. Most bacteria have several putative helicases, including one from the HrpA family, but nothing was known about the actual HrpA function from other species. HrpA is the only putative RNA helicase in *B. burgdorferi*, and the scientists found that it indeed possesses the multiple activities characteristic of a helicase: it can bind to RNA and use its ATPase activity to harvest energy, which in turn is used to unwind the RNA strand. They also showed that these activities are involved in the regulation of target RNAs.

When the scientists tested whether mutant *B. burgdorferi* that lacked the hrpA gene could infect mice, they found that the mutant bacteria could not. For this experiment, the scientists injected normal or mutant bacteria directly into mice, and subsequently tested mouse blood, skin, bladder, or joint tissue for the presence of bacteria. Normal bacteria could be recovered from all tissues after a week and up to 4 weeks post injection, but mutant bacteria were undetectable even after one week, suggesting that they were unable to survive or multiply in the mammalian host.

HrpA-deficient bacteria were also unable to infect mice using the natural route, i.e. via a bite from an infected tick. This was not because the mutant bacteria were unable to grow or survive in the ticks. Rather the mutants could not exit the tick midgut or enter the salivary glands, where Borrelia needs to be for successful transmission during feeding; even right after the engorged ticks fell off, mutant bacteria were not detectable in the mouse skin around the attachment site.

The authors say, "We now know that HrpA is involved in both parts of the *B. burgdorferi* lifecycle: animal infection and tick transmission, making it a very important protein in *B. burgdorferi* gene regulation and establishing gene regulation through an RNA helicase as an important regulatory pathway in the Lyme spirochete."


Elucidating Biological Cells' Transport Mechanisms

Dec. 20, 2013 — A new study focuses on the motion of motor proteins in living cells, applying a physicist's tool called non-equilibrium statistical mechanics.

Motion fascinates physicists. It becomes even more intriguing when observed in vivo in biological cells. Using an ingenious setup, Japanese scientists have now calculated the force of molecular motors acting on inner components of biological cells, known as organelles. In a new study, published in EPJ E,
the focus is on mitochondria—akin to micrometric range cellular power plants—travelling along microtubules in a cell. These findings by Kumiko Hayashi, from Tohoku University, Sendai, Japan, and team could contribute to elucidating the transport mechanism in biological cells by multiple motors.

Hayashi and colleagues have investigated, for the first time, the so-called Einstein relation for the motion of mitochondria transported by motor proteins—called kinesin and dynein—in living cells. The Einstein relation describes how micro-sized beads follow a random motion under the influence of thermal noise, when diffusing in aqueous solutions. This relation stems from the fluctuation dissipation theorem studied in the field of non-equilibrium statistical mechanics.

The researchers observed the motion of a mitochondrion transported by motor proteins using fluorescence microscopy. They applied a single-particle tracking algorithm to the images of fluorescently tagged mitochondria. They then compared this motion with the random motion of a bead artificially incorporated into a cell, observed by using fluorescence correlation spectroscopy.

By comparing both evaluations of the diffusion coefficient, from both mitochondria and bead estimates, they found that the value of the medium's viscosity obtained using the beads was slightly lower than that obtained using the organelle motion. This discrepancy is linked to the fact that physical laws such as the Einstein relation are not sufficient to fully describe the organelles' motion, which is subjected to many simultaneous complex biological processes, such as the chemical reaction of motor proteins and the interaction with the cell's vesicles.

Journal Reference: