July 2013 Epidemics and AIDS Update

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July 3, 2013

**After Marrow Transplants, 2 More Patients Appear H.I.V.-Free Without Drugs**

_by Donald G. McNeil Jr._

Two H.I.V.-infected patients in Boston who had bone-marrow transplants for blood cancers have apparently been virus-free for weeks since their antiretroviral drugs were stopped, researchers at an international AIDS conference announced Wednesday.

The patients’ success echoes that of Timothy Ray Brown, the famous “Berlin patient,” who has shown no signs of resurgent virus in the five years since he got a bone-marrow transplant from a donor with a rare mutation conferring resistance to H.I.V.

The Boston cases, like Mr. Brown’s, are of no practical use to the 34 million people in the world who have H.I.V. but neither blood cancer nor access to premier cancer-treatment hospitals.

But AIDS experts still find the Boston cases exciting because they are another step in the long and sofar-fruitless search for a cure. They offer encouragement to ambitious future projects to genetically re-engineer infected patients’ cells to be infection-resistant. At least two teams are already experimenting with variants on this idea, said Dr. Steven G. Deeks, an AIDS researcher at the University of California, San Francisco.

Dr. Françoise Barré-Sinoussi, a discoverer of the virus that causes AIDS and the president of the International AIDS Society, called the findings about the Boston patients “very interesting and very encouraging.” The announcement about the cases was made at the society’s annual conference in Kuala Lumpur, Malaysia.

Mr. Brown is sometimes referred to as the “first H.I.V. cure.”

But there are important differences between his case and those of the Boston patients. For example, no AIDS expert, including the doctors from Brigham and Women’s Hospital in Boston following the two patients, is using the word “cured” to describe their status.

The technique used on them involves severely weakening the immune system before a marrow transplant. It is so dangerous that it is unethical to perform it on anyone not already at risk of dying from cancer, especially because most people with H.I.V. can live relatively normal lives by taking a daily antiretroviral cocktail.

One patient stopped taking antiretroviral drugs seven weeks ago. For the other, it has been 15 weeks. No virus or antibodies to the virus have been found in their blood or other tissues since.

Normally, when a patient stops the drugs, the virus bounces back in less than a month, but each person is different.

“It could come back in a week, or in six months,” said Dr. Timothy Henrich, a doctor overseeing the two patients. “Only time will tell.”

The process the two patients underwent is risky — a third patient in the study died when his cancer returned — but somewhat less so than the procedure done on Mr. Brown.

Mr. Brown had leukemia. The three Boston patients had lymphoma.

The Boston patients’ bone marrow, where new blood cells are made, was only partially destroyed by drugs before they were given new marrow from matching donors — a process that carries a 15 to 20 percent risk of death, Dr. Henrich said.

Mr. Brown’s marrow was completely obliterated by drugs and whole-body radiation, a procedure that kills 40 percent of the patients, and he had it done twice.

Mr. Brown’s new marrow came from a donor who was a close genetic match and had a rare mutation that makes a person virtually impervious to infection with H.I.V.

The mutation, known as delta 32, creates CD4 cells — the white blood cells that the virus attacks — lacking a CCR5 surface receptor, the “door” that the virus uses to enter the cell.

The donors for the Boston patients did not have the delta 32 mutation.
Unlike Mr. Brown, the Boston patients stayed on antiretroviral therapy throughout the lengthy transplant process and for years afterward. The drugs prevent the virus from replicating itself.

“The idea was to protect the new donor cells from becoming infected,” Dr. Henrich explained. During that time, in a phenomenon known as graft-versus-host disease, the new cells were attacking their old, chemotherapy-weakened counterparts and clearing them from the body, a process that takes about nine months, Dr. Henrich said.

Because only the old cells were infected with H.I.V., the hope was that graft-versus-host disease would “mop up” all the viral reservoirs. But runaway graft-versus-host disease can be fatal, so the two patients were intermittently on and off immunosuppressive drugs and steroids to control it.

One immunosuppressive drug, sirolimus, may also have helped kill off H.I.V., Dr. Henrich said. It is known to prevent retroviruses like H.I.V. from replicating.

The two patients had transplants between two and five years ago. They had months of tests on their blood and tissues to make sure no H.I.V. or antibodies to it were found before Dr. Henrich and his research partner, Dr. Daniel Kuritzkes, proposed stopping the antiretroviral treatment.

For such tests, doctors remove immune cells and “activate” them with chemicals to make them reproduce. If any virus is hiding in the cells’ DNA, it is “spit out” and can be detected.

But doctors can never be sure that they have tested all the reservoirs where the dormant virus might hide. It is relatively easy, for example, to sample rectal but not brain tissue.

Since the patients stopped taking antiretrovirals, they “feel great and are leading completely normal lives,” Dr. Henrich said. That distinguishes them from Mr. Brown, who has survived virus-free for more than five years but still has weakness and pain from his grueling anticancer regimen.

AIDS specialists are interested in the Boston patients because they offer new insights into how the immune system can be used to attack the virus.

Dr. Anthony S. Fauci, the director of the National Institute of Allergy and Infectious Diseases, said it was “conceivable and maybe even likely” that their H.I.V. was permanently gone.

If so, he said, it would show that it is not necessary to find a matching donor who had the delta-32 mutation.

Dr. Deeks, the AIDS researcher in California, said the cases raised the question of when to say an H.I.V. patient has been “cured.”

“How should we wait six months to see if the virus rebounds?” he asked. “Or will we have to wait up to five years, as oncologists tend to do with cancer?”

Dr. Barré-Sinoussi, of the International AIDS Society, said she might eventually prefer to adopt the term oncologists use: “in remission.”

**Tory MP Mike Freer: The HPV vaccine needs to be extended to protect gay men**

by Joseph Patrick McCormick

2 July 2013, 4:28pm

Tory MP Mike Freer has announced a campaign to have the HPV vaccination extended to include teenage boys, arguing that the current scheme, which only vaccinates girls, neglects to protect gay men.

The Conservative MP for Finchley and Golders Green, is to challenge the Public Health Minister on the topic in a debate at the House of Commons tonight.

He will argue that teenage boys in state schools should be offered the HPV vaccine as well as teenage girls. The current scheme vaccinates 12 to 13 year old girls as a preventative measure against cervical cancer.

Mr Freer said he wants the scheme to be expanded in order to protect all teenagers from HPV later in life, and argues that the current scheme does not protect gay men.

He argues that the current scheme uses the concept of “herd vaccination” as a secondary motivation to protect males in relationships with vaccinated females, but that it makes no provision for the protection of men who have sex with men.

Speaking from the House of Commons in advance of the debate, Mike Freer said: “I cannot understand why the previous Government introduced a scheme that so wilfully neglected the sexual health needs of men, particularly the homosexual community. I am adamant the current Government
must review the vaccine contract and change this sorry state of affairs. I will be making this point forcefully to the Minister in tonight’s debate.”

He went on to argue that men who have sex with unvaccinated women, such as those born abroad, or are unvaccinated because they are older, are also put at risk by the current system.

Mike Freer has been working with the Terrence Higgins Trust on his campaign, and expects a number of cross-party MPs to be present to support his debate.

Daisy Ellis, Head of Parliamentary & Public Affairs at Terrence Higgins Trust, said: “We believe men have a right to the same protection from HPV as women. While the current programme of vaccinating women protects some men indirectly, gay and bisexual men will remain at risk of developing HPV-related cancers unless the programme is redefined to protect both sexes. HPV can be passed on through anal and oral sex, and – unlike most other STIs – condoms will not always stop an infection taking place. A proper, inclusive vaccination programme would be the most effective way to reduce the level of anal and oral cancers among men.”

**Ghana Cuts New HIV Infections Among Children by 76% Ahead of South Africa—UNAIDS**

*Ghana Business News*, (06.26.2013) By Ekow Quandzie

The number of HIV-infected Ghanaian children accessing HIV treatment doubled from 2009 to 2012, resulting in a 76-percent decline in HIV incidence among children since 2009. Ghana was one of 21 African countries targeted by the Joint United Nations Programme on HIV/AIDS (UNAIDS) “Global Plan towards elimination of new HIV infections among children by 2015 and keeping their mothers alive” (Global Plan). Other priority countries where HIV incidence declined more than 50 percent among children included Botswana, Ethiopia, Malawi, Namibia, South Africa, and Zambia. South Africa had 24,000 fewer new HIV infections among children in 2012 than in 2009 (a 63-percent decline). Overall, HIV infections among children in the 21 priority nations declined by 38 percent (130,000 fewer new infections) since 2009.

Michel Sidibé, UNAIDS executive director, reported progress had stalled in other African countries with high HIV incidence and that access to HIV treatment was “unacceptably low” in most of the Global Plan priority nations. HIV incidence increased in Angola, and the 2012 rate of incidence in Nigeria was “largely unchanged” from 2009. Of the 21 priority nations, Nigeria had the highest HIV incidence among children, with 60,000 new infections in 2012. Sidibé called for “urgent action” to reach the Global Plan’s 2015 goals.

UNAIDS and the President’s Emergency Plan for AIDS Relief revealed the Global Plan during the 2011 UN General Assembly High Level Meeting on AIDS. The plan’s 2015 target goals are to cut new infections among children by 90 percent and to reduce AIDS-related maternal deaths by 50 percent.

**HIV-Positive Men Show High Rates of Papillomavirus Infection at Oral, Anal, and Penile Sites**


Researchers from the Autonomous University of Barcelona, the Lluita Contra la SIDA (Fight Against AIDS) Foundation, and the IrsiCaixa Foundation in Spain report high rates of human papillomavirus (HPV) infection in the oral, anal, and penile cavities of HIV-infected men, particularly in the anal cavities of men who have sex with men (MSM).

For each year from 2005 to 2009, the researchers investigated the presence of HPV among 733 male HIV-infected patients at the Germans Trias i Pujol Hospital in Badalona, Spain, including 538 MSM. The researchers also studied the rate of new infections and clearance of the virus during the four years of study. In the sample studied, the prevalence rates were 73 percent of cases with anal HPV, 26 percent with penile, and 16 percent in oral sites; new cases during the four years were 36 percent with anal HPV, 17 percent penile, and 11 percent oral.

The results indicate high prevalence and incidence of HPV in the three sites and a low clearance rate. MSM presented higher prevalence (84 percent) and incidence in the anal canal, and lower clearance percentage than heterosexuals, but prevalence in heterosexuals was also high at 42 percent. The researchers commented that the prevalence in MSM was expected, but the prevalence in the heterosexual group was unexpected. In other sites, prevalence, incidence, and clearance were similar and coinfection in all three sites was similar at 7 percent in heterosexuals and 6 percent in MSM. The study found lower
prevalence of HPV infection in anal sites of patients treated with antiretrovirals, but this was a weak effect.

The researchers recommended routine oral, penile, and anal examinations for all HIV-positive patients during annual clinic regardless of sexual behavior and practices.

The full report, “Natural History of Human Papillomavirus Infections Involving Anal, Penile, and Oral Sites Among HIV-Positive Men,” was published online in the journal Sexually Transmitted Diseases (2013; doi: 10.1097/OLQ.0b013e31827e87bd).

**Shape-shifting disease proteins may explain variable appearance of neurodegenerative diseases**

**Targeting distinct alpha-synuclein strains a potential treatment approach**

PHILADELPHIA—Neurodegenerative diseases are not all alike. Two individuals suffering from the same disease may experience very different age of onset, symptoms, severity, and constellation of impairments, as well as different rates of disease progression. Researchers in the Perelman School of Medicine at the University of Pennsylvania have shown one disease protein can morph into different strains and promote misfolding of other disease proteins commonly found in Alzheimer’s, Parkinson’s and other related neurodegenerative diseases.

Virginia M.Y. Lee, PhD, MBA, professor of Pathology and Laboratory Medicine and director of the Center for Neurodegenerative Disease Research, with co-director, John Q. Trojanowski MD, PhD, postdoctoral fellow Jing L. Guo, PhD, and colleagues, discovered that alpha-synuclein, a protein that forms sticky clumps in the neurons of Parkinson’s disease patients, can exist in at least two different structural shapes, or "strains," when it clumps into fibrils, despite having precisely the same chemical composition.

These two strains differ in their ability to promote fibril formation of normal alpha-synuclein, as well as the protein tau, which forms neurofibrillary tangles in individuals with Alzheimer’s disease.

Importantly, these alpha-synuclein strains are not static; they somehow evolve, such that fibrils that initially cannot promote tau tangles acquire that ability after multiple rounds of "seeded" fibril formation in test tubes.

The findings appear in the July 3rd issue of *Cell*.

**Morphed Misfolding Proteins Found In Overlapping Neurodegenerative Diseases**

Tau and alpha-synuclein protein clumps are hallmarks of separate diseases – Alzheimer’s and Parkinson’s, respectively. Yet these two proteins are often found entangled in diseased brains of patients who may manifest symptoms of both disorders.

One possible explanation for this convergence of Alzheimer’s and Parkinson’s disease pathology in the same patient is a global disruption in protein folding. But, Guo and Lee showed that one strain of alpha-synuclein fibrils which cannot promote tau fibrillization actually evolved into another strain that could efficiently cause tau to fibrillize in cultured neurons, although both strains are identical at the amino acid sequence level. Guo and Lee called the starting conformation "Strain A," and the evolved conformation, "Strain B."

To figure out how A and B differ, Guo showed that the two strains folded into different shapes, as indicated by their differential reactivity to antibodies and sensitivity to protein-degrading enzymes. The two strains also differed in their ability to promote tau fibrillation and pathology in mouse brains, mimicking the results from cultured cells. When analyzing post-mortem brains of Parkinson’s patients, the team found at least two distinct forms of pathological alpha-synuclein.

Lee and her team speculate that in humans, alpha-synuclein aggregates may shift their shapes as they pass from cell to cell (much like a cube of silly putty being re-shaped to form a sphere), possibly developing the ability to entangle other proteins such as tau along the way. That process, in turn, could theoretically yield distinct types of alpha-synuclein pathologies that are observed in different brain regions of Parkinson’s disease patients.

While further research is needed to confirm and extend these findings, they have potentially significant implications for patients afflicted with Parkinson’s and other neurodegenerative diseases. For example, Lee explains, they could account for some of the heterogeneity observed in Parkinson’s disease. Different strains of pathological alpha-synuclein may promote formation of distinct types of alpha-synuclein aggregates that may or may not induce tau pathology in different brain regions and in different patients. That, in turn, could explain why some Parkinson’s patients, for example, experience only motor impairments while others ultimately develop cognitive impairments.
The findings also have potential therapeutic implications, Lee says. By recognizing that pathological alpha-synuclein can exist in different forms that are linked with different impairments, researchers can now selectively target one or the other, or both, for instance with strain-selective antibodies. "What we’ve found opens up new areas for developing therapies, and particularly immunotherapies, for Parkinson’s and other neurodegenerative diseases," Lee says.

**Single Men, Smokers at Higher Risk for Oral Human Papillomavirus Infection, Moffitt Cancer Center Study Shows**
Jul 02, 2013

**TAMPA, Fla.** – Smokers and single men are more likely to acquire cancer-causing oral human papillomavirus (HPV), according to new results from the HPV Infection in Men (HIM) Study. Researchers from Moffitt Cancer Center, the National Cancer Institute, Mexico and Brazil also report that newly acquired oral HPV infections in healthy men are rare and when present, usually resolve within one year.

The study results appeared in the July issue of *The Lancet*.

HPV infection is known to cause virtually all cervical cancers, most anal cancers and some genital cancers. It has recently been established as a cause of the majority of oropharyngeal cancers, a malignancy of the tonsils and base of tongue.

HPV-related oropharyngeal cancer is rare, but rates have been increasing rapidly, especially among men. To determine the pattern of HPV acquisition and persistence in the oral region, researchers evaluated the HPV infection status in oral mouthwash samples collected as part of the HIM Study, which was originally designed to evaluate the natural history of genital HPV infections in healthy men.

"Some types of HPV, such as HPV16, are known to cause cancer at multiple places in the body, including the oral cavity," said study lead author Christine M. Pierce Campbell, Ph.D., M.P.H., a postdoctoral fellow in Moffitt’s Center for Infection Research in Cancer. “We know that HPV infection is associated with oropharyngeal cancer, but we don’t know how the virus progresses from initial infection to cancer in the oral cavity. One aspect of the HIM Study is to gather data to help us understand the natural history of these infections.”

During the first 12 months, nearly 4.5 percent of men in the study acquired an oral HPV infection. Less than 1 percent of men in the study had an HPV16 infection, the most commonly acquired type, and less than 2 percent had a cancer-causing type of oral HPV.

Their findings are consistent with previous studies showing a low prevalence of oral HPV cancers. However, this study shows the acquisition of cancer-causing oral HPV appeared greater among smokers and unmarried men.

"Additional HPV natural history studies are needed to better inform the development of infection-related prevention efforts,” said Anna R. Giuliano, Ph.D., director of Moffitt’s Center for Infection Research in Cancer. “HPV16 is associated with the rapid increase in incidence of oropharyngeal cancer, most noticeably in the United States, Sweden and Australia, where it is responsible for more than 50 percent of cases. Unfortunately, there are no proven methods to prevent or detect these cancers at an early stage.”

The researchers note that persistent oral HPV16 infection may be a precursor to oropharyngeal cancer, similar to how persistent cervical HPV infection leads to cervical pre-cancer.

**Maintaining immune balance involves an unconventional mechanism of T cell regulation**

St. Jude Children's Research Hospital study challenges prior understanding of the process regulating specialized T cells that are essential for a balanced immune system

New findings from St. Jude Children’s Research Hospital reveal an unconventional control mechanism involved in the production of specialized T cells that play a critical role in maintaining immune system balance. The research appears in the current online edition of the scientific journal *Nature*.

The work focused on white blood cells known as regulatory T cells. These cells are crucial for a balanced immune response. Regulatory T cells suppress other immune system components in order to protect healthy tissue from misguided immune attacks or to prevent runaway inflammation.

St. Jude researchers showed that a molecular complex called mTORC1 uses an unconventional process to serve as a rheostat, controlling the supply and function of regulatory T cells. Loss of mTORC1 activity impairs the regulatory T cells that suppress the immune system’s inflammatory response. The
mTORC1 complex is part of the mTOR pathway, which was thought to inhibit rather than promote the number and function of regulatory T cells.

"These results challenge the prior view of the mTOR pathway as an inhibitor of these key immune cells and highlight the role of the mTORC1 complex in regulating the T cells that are vital for controlling inflammation," said Hongbo Chi, Ph.D., an associate member of the St. Jude Department of Immunology and the paper's corresponding author.

The findings also identified the mechanism mTORC1 uses in programming regulatory T cells to function as immune suppressors. Chi said the results should aid efforts to develop new drugs for use in organ transplantation or for treatment of autoimmune disorders.

For this study, researchers used specially bred mice to explore the mTOR pathway's role in the function of regulatory T cells. Investigators demonstrated mTORC1's importance by selectively deleting genes that carry instructions for making key elements of mTORC1 and a related complex. The deletion that targeted mTORC1 resulted in dramatically reduced immune suppression by regulatory T cells and the mice rapidly developed a fatal inflammatory disorder.

Researchers also showed that mTORC1 works by integrating signals from two immune receptors on the cell surface with cholesterol metabolism. With the right input, mTORC1 promoted production of regulatory T cells and cemented their role as suppressors of immune activity.

In another twist, investigators linked that suppressive function to cholesterol and lipid metabolism. Rather than relying on more conventional strategies of immune regulation, researchers showed how regulatory T cells depend on the metabolic pathway to control production of molecules CTLA4 and ICOS, which are responsible for immune suppression. Production of CTLA4 and ICOS by regulatory T cells decreased as lipid metabolism dropped. "We are just starting to appreciate the importance of lipids in the immune system, particularly in the function of regulatory T cells," Chi said.

Lifesaving HIV Treatment Could Reach Millions More People Following Landmark Study
July 4, 2013 — Millions more people could get access to life-saving HIV drug therapy, following a landmark study led by Australian researchers based at the Kirby Institute at the University of New South Wales (UNSW).

The researchers have found a lower daily dose of an important HIV drug therapy is safe and as effective in suppressing the virus as the standard recommended dose.

The findings have been presented at the International AIDS Society Conference in Kuala Lumpur, Malaysia.

"This has the potential to affect the treatment of millions of HIV positive people," says UNSW Professor Sean Emery, the protocol chairperson of the study, known as ENCORE1 and Head of the Therapeutic and Vaccine Research Program at the Kirby Institute.

"A reduced daily dose should translate into a lower cost of treatment and permit more effective and efficient use of health care resources. Essentially, more people could receive this life-saving treatment for the same amount of funding."

HIV-positive people from 13 countries in Africa, Asia, Australia, Europe and Latin America took part in the trial. Half these people took two-thirds of the current standard daily dose of the antiretroviral (ART) efavirenz, a commonly used treatment for HIV; the other half took the standard daily dose. The 630 participants were observed regularly for a year. The results indicate that a reduction in daily dose of one third is both safe and effective compared to the higher dose currently recommended for people with HIV infection.
Gene That Controls Aggressiveness in Breast Cancer Cells Identified

July 3, 2013 — In a discovery that sheds new light on the aggressiveness of certain breast cancers, Whitehead Institute researchers have identified a transcription factor, known as ZEB1, that is capable of converting non-aggressive basal-type cancer cells into highly malignant, tumor-forming cancer stem cells (CSCs). Intriguingly, luminal breast cancer cells, which are associated with a much better clinical prognosis, carry this gene in a state in which it seems to be permanently shut down.

The researchers, whose findings are published this week in the journal Cell, report that the ZEB1 gene is held in a poised state in basal non-CSCs, such that it can readily respond to environmental cues that consequently drive those non-CSCs into the dangerous CSC state. Basal-type breast carcinoma is a highly aggressive form of breast cancer. According to a 2011 epidemiological study, the 5-year survival rate for patients with basal breast cancer is 76%, compared with a roughly 90% 5-year survival rate among patients with other forms of breast cancer.

“We may have found a root source, maybe the root source, of what ultimately determines the destiny of breast cancer cells—their future benign or aggressive clinical behavior,” says Whitehead Founding Member Robert Weinberg, who is also a professor of biology at MIT and Director of the MIT/Ludwig Center for Molecular Oncology.

Transcription factors are genes that control the expression of other genes, and therefore have a significant impact on cell activities. In the case of ZEB1, it has an important role in the so-called epithelial-to-mesenchymal transition (EMT), during which epithelial cells acquire the traits of mesenchymal cells. Unlike the tightly-packed epithelial cells that stick to one another, mesenchymal cells are loose and free to move around a tissue. Previous work in the Weinberg lab showed that adult cancer cells passing through an EMT are able to self-renew and to seed new tumors with high efficiency, hallmark traits of CSCs.

Other earlier work led by Christine Chaffer, a postdoctoral researcher in the Weinberg lab, demonstrated that cancer cells are able to spontaneously become CSCs. Now Chaffer and Nemanja Marjanovic have pinpointed ZEB1, a key player in the EMT, as a gene critical for this conversion in breast cancer cells.

Breast cancers are categorized into at least five different subgroups based on their molecular profiles. More broadly these groups can be subdivided into the less aggressive 'luminal' subgroup or more aggressive 'basal' subgroup. The aggressive basal-type breast cancers often metastasize, seeding new tumors in distant parts of the body. Patients with basal breast cancer generally have a poorer prognosis than those with the less aggressive luminal-type breast cancer.

Chaffer and Marjanovic, a former research assistant in the Weinberg lab, studied non-CSCs from luminal- and basal-type cancers and determined that cells from basal cancers are able to switch relatively easily into CSC state, unlike luminal breast cancer cells, which tend to remain in the non-CSC state.

The scientists determined that the difference in ZEB1’s effects is due to the way the gene is marked in the two types of cancers. In luminal breast cancer cells, the ZEB1 gene is occupied with modifications that shut it down. But in basal breast cancer cells, ZEB1’s state is more tenuous, with repressing and activating markers coexisting on the gene. When these cells are exposed to certain signals, including those from TGFß, the repressive marks are removed and ZEB1 is expressed, thereby converting the basal non-CSCs into CSCs. (Credit: Image courtesy of Whitehead Institute for Biomedical Research.)
TGFβ, the repressive marks are removed and ZEB1 is expressed, thereby converting the basal non-CSCs into CSCs.

So what does this new insight mean for treating basal breast cancer?

"Well, we know that these basal breast cancer cells are very plastic and we need to incorporate that kind of thinking into treatment regimes," says Chaffer. "As well as targeting cancer stem cells, we also need to think about how we can prevent the non-cancer stem cells from continually replenishing the pool of cancer stem cells. For example, adjuvant therapies that inhibit this type of cell plasticity may be a very effective way to keep metastasis at bay."

Marjnaovic agrees but cautions that the model may not be applicable for every cancer.

"This is an example of how adaptable cancer cells can be," says Marjanovic, who is currently a research assistant at the Broad Institute. "We have yet to determine if ZEB1 plays a similar role in all cancer types, but the idea that cancer cells reside in a poised state that enables them to adapt to changing environments may be a mechanism used by many cancers to increase their aggressiveness."

Journal Reference:

Genomes of Cholera Bacteria from Haiti Confirm Epidemic Originated from Single Source

July 2, 2013 — The strain of cholera that has sickened thousands in Haiti came from a single source and was not repeatedly introduced to the island over the past three years as some have thought, according to a new study published in mBio®, the online open-access journal of the American Society for Microbiology.

The results of this latest study are consistent with earlier findings that indicate Vibrio cholerae bacteria were introduced to Haiti by United nations soldiers between July and October 2010, when Nepalese soldiers arrived to assist recovery efforts after the January 2010 earthquake in that country. The genome sequences of V. cholerae strains from Haiti reveal they have not gained any new genetic material since their introduction and that they have a limited ability to acquire genes from other organisms through a process called transformation.

This new information may help public health authorities understand future cholera outbreaks in Haiti and elsewhere, according to the authors. "The use of high resolution sequence data that is amenable to evolutionary analysis will greatly enhance our ability to discern transmission pathways of virulent clones such as the one implicated in this epidemic," write the authors.

The earthquake in January 2010 killed tens of thousands of Haitians, and it was followed several months later by an outbreak of cholera, a disease that had never before been documented in Haiti. Studies of the outbreak indicate that poor sanitation at a United Nations camp resulted in sewage contamination of local water supplies, and phylogenetic analysis of the Haiti V. cholerae strains and strains from around the globe indicate the strain was most likely accidentally brought to the camp by U.N. troops from Nepal.

Earlier "fingerprinting" of Haiti’s V. cholerae isolates using pulse-field gel electrophoresis (PFGE) has shown the bacterium has changed somewhat since the epidemic began in October 2010, but because of the nature of PFGE, the significance of those changes was not known. Were the changes meaningful? Were the bacteria gaining or losing genes that could impact the course of disease? Did they gain genes from other bacteria in the environment? Are their genomes rearranged? The answers could make a difference in the severity of future outbreaks.

The authors of the study in mBio® set out to study in greater detail how V. cholerae may have evolved since its introduction to the island nation, and whether it has acquired genes that bestow new abilities. They sequenced the genomes of 23 different V. cholerae isolates from Haiti that represent multiple PFGE "fingerprint" patterns and were taken from a variety of locations and at various time points during the epidemic.

When compared with the genome sequences of V. cholerae strains from around the world, the Haiti isolates and three Nepal isolates are tightly related, forming a monophyletic group to which no other genome sequences belong.

This result indicates that "Nepalese isolates are the closest relatives to the Haiti strain identified to date, even when placed into a phylogeny with a larger collection of isolates representing recent cholera epidemics," write the authors. This means that the outbreak originated from a single introduction of bacteria, and PFGE variants arose from gradual evolution of the organisms, not from any secondary introduction.
The Haiti strains also have a limited ability to acquire new genes through the process of transformation, by which genetic material is picked up from other bacteria or from the environment. There is some evidence that transformation is an important mechanism for bacteria to acquire the necessary abilities to adapt to a particular environment, so the fact that the Haiti strains are deficient in this respect raises the question of whether they will be able to adapt to life in Haiti or if they might go extinct once the epidemic has ended.

The Haiti isolates belong to a type of *V. cholerae* called "Atypical El Tor" strains, a group that, in locations in Asia and Africa, has managed to acquire multidrug resistance and enhanced virulence traits that result in higher infection rates and harsher symptoms. The authors argue that to avert larger and more difficult to treat outbreaks of cholera, it is necessary to track the ongoing and unpredictable evolution of the organism in Haiti and elsewhere with surveillance of *V. cholerae* via tools like whole genome sequencing.

**New Method for Mapping the Protein Signals Between Healthy and Diseased Cells**

July 2, 2013 — Researchers at Memorial Sloan-Kettering Cancer Center in New York, working in collaboration with researchers at the Proteome Center Tuebingen (PCT), have developed a new method for identifying the cell of origin of intracellular and secreted proteins within multicellular environments. This technological advancement is particularly exciting because it will provide investigators with a new tool for comprehensive mapping of cell-cell communication, which is especially important in all aspects of cancer development, maintenance, and response to therapy. For example, this method could be used to study cell signaling events between normal and malignant cells in order to better understand the molecular mechanisms by which surrounding normal cells alter tumor growth and response to treatment.

The technique, named cell type specific labeling using amino acid precursors (CTAP), exploits the inability of vertebrate cells to synthesize essential amino acids normally required for growth and homeostasis. A team headed by Dr. Nicholas Gauthier and Dr. Martin Miller at the Memorial Sloan-Kettering Cancer Center engineered cells to express amino acid biosynthesis enzymes, which enabled cells to grow on their own supply of amino acids produced from supplemented precursors.

The team went on to show that supplementing heavy stable isotope-labeled forms of these precursors led to incorporation of heavy amino acids into proteins produced in enzyme expressing cells. Dr. Boumediene Soufi and Dr. Boris Macek from the PCT designed experiments that utilized quantitative mass spectrometry to search for proteins that contained these stable isotope labels. In this way, the cell of origin of both intracellular and secreted proteins identified in multicellular culture could be determined. By providing a means to link proteins directly to specific cell types, the authors believe that this new method will be useful in studies of cell-cell communication and biomarker discovery.

Journal Reference:

**A Potentially Life-Saving Protein Takes Shape**

July 2, 2013 — A tiny protein called ubiquitin—so named because it is present in every cell of living things as dissimilar as hollyhocks and humans—may hold the key to treatment for a variety of diseases from Parkinson’s to diabetes. The protein, found in all eukaryotes (organisms with membranous cells), was considered unimportant when it was described in 1975. But scientists now know ubiquitin takes many
different forms and is important in basic cellular processes, from controlling cells’ circadian clocks to clearing away the harmful build-up of cells found in cancer and other diseases.

To maximize ubiquitin’s potential for treating diseases, researchers are working to identify the protein’s dizzying array of structures, and to understand each form’s function. Ubiquitin forms polymeric chains linked by specific amino acids. Each ubiquitin protein can connect to its neighbor through one of eight different amino acids, and each combination appears to do something different in a normal cell, says University of Maryland structural biologist David Fushman, whose lab studies these ubiquitin chains and their linkages.

Imagine the cell as a dance floor, thronged with proteins seeking partners, says Fushman, who has studied ubiquitin since 2000. When two ubiquitins join through a lysine, “it’s like two hands meeting, but just with a single finger touching that’s specific to that lysine.” The choice of lysine determines the shape of the ubiquitin chain, and probably also determines its function.

Fushman and his colleagues' newly published research focuses on one of the most common and least studied linkages, the polymeric chain formed by the amino acid Lysine-11. The ubiquitin chains linked by Lysine-11 "are directly involved in cell cycle regulation," Fushman says. To turn that knowledge into medically useful information, “we have to understand exactly how they form and with whom they interact.”

Most work of this type is done in a test tube and uses x-ray crystallography to map the structures. Fushman’s lab uses a different method that he says produces an environment somewhat closer to nature. The team used nuclear magnetic resonance spectroscopy (NMR) and other techniques to map the Lysine-11-linked chains.

The researchers found these chains take on a different shape in solution than in crystals, and are more flexible than was previously thought. Ubiquitin chains linked via Lysine-11 can form various three-dimensional shapes, and as salt concentrations change, the chains’ shape also changes, the team found.

Researcher Carlos Castañeda and others in the Fushman lab reported their results in a paper published July 2 in the biological journal *Structure*.

The most-studied ubiquitin chain, linked via Lysine-48, is known as a "protein destroyer" because it labels cellular proteins to be broken up for later recycling. The UMD team found the cellular receptors responsible for breaking down proteins interact with Lysine-11 chains, but not as efficiently as with Lysine-48 chains. Therefore protein destruction does not appear to be the main task of the Lysine-11 linked chain, Fushman says; its function is something different and perhaps equally vital to maintaining healthy cells.

**Journal Reference:**

**Terrence Higgins Trust: ‘Very few’ gay men will ever bug chase for HIV**
by Scott Roberts
9 July 2013, 11:11am

UK sexual health charity Terrence Higgins Trust has commented to PinkNews about a story in The Mirror, which claims a small number of gay men have willingly had unprotected sex in order to become HIV positive.

A 30-year-old man only identified as “Nick” from the Midlands told the Sunday Mirror that hundreds of men in the UK are meeting on online forums, Facebook and Twitter in an attempt to become infected with the virus.

He reportedly said: “I feel fit as a fiddle. I feel full of energy and healthier as a result of being on my medication.

“I get my liver function tests every three months, my cholesterol tested regularly and I get loads of general health checks so if there are any underlying conditions I know straight away. Even better, I get it all on the NHS.”

The paper then cites a highly questionable 2003 investigation by Rolling Stone magazine, which claimed a quarter of all new HIV transmissions in the US could be attributed to bug chasing.

The figures were widely disputed and the Human Rights Campaign, America’s largest LGBT lobby group, branded the investigation “irresponsible”.

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The figures were widely disputed and the Human Rights Campaign, America’s largest LGBT lobby group, branded the investigation “irresponsible”.
Cary James, head of health improvement programmes at Terrence Higgins Trust, said: “The term ‘bug chasing’ first came to public attention about ten years ago. However, research has so far found no evidence to suggest that it is practiced on any scale.

“We are aware of a tiny minority of people who meet online to discuss bug chasing as a sexual fantasy, but the reality is that very few will act on this in the real world. We recommend that gay and bisexual men protect themselves against HIV and other sexually transmitted infections by using condoms.”

Yusef Azad, director of policy at the National AIDS Trust, said: "It is hard to separate fact and fantasy when it comes to reports of ‘bug-chasing’. If it exists at all, it can only involve a tiny minority of gay men. Most gay men understand HIV remains a long-term serious condition, and transmission should be avoided.”

In February, the UK Health Protection Agency blamed “a return of risky sexual practices” for rising HIV cases in gay men.

Figures from the Agency showed a record number of gay and bisexual men were diagnosed with HIV in 2011.

**Typhoid’s lethal secret revealed**

Typhoid fever is one of the oldest documented diseases known to have afflicted mankind but what makes it so lethal has remained a mystery for centuries. In a study appearing online July 10 in the journal *Nature*, Yale researchers offer an explanation of how the devastating disease marked by delirium and stupor still kills 200,000 people every year—and also suggests the basis of a future vaccine.

The culprit appears to be a powerful toxin possessed by *Salmonella typhi*, the bacterium that causes typhoid fever. Yale scientists for the first time describe the structure of the typhoid toxin and show that it causes disease in mice. The toxin helps explain why typhoid fever has such different symptoms than an infection by its close genetic cousin Salmonella, the common cause of food poisoning.

"What makes this so exciting for us is that vaccines and therapeutics that target toxins have an excellent track record of success," said Jorge Galan, Lucille P. Markey Professor of Microbial Pathogenesis and senior author of the paper.

Typhoid fever is believed to have killed Athenian leader Pericles and a third of the population of the Greek city in 430 B.C. during the Peloponnesian War and has perplexed doctors ever since. Untreated, it kills up to 20 percent of those it infects, however many of those who survive remain carriers for life but show no symptoms. This fact explains why fever, illness and death followed from job to job the notorious carrier Mary Mallon, best known as Typhoid Mary. A cook for wealthy New England families, she is believed to have unwittingly infected several dozen people in the early 20th century.

Although the cause of typhoid fever has been known for over a century, what makes *Salmonella typhi* so deadly has remained a mystery. Galan and his team showed that the answer to this mystery may be typhoid toxin, a lethal toxin created from the merger of two separate and powerful toxins. The atomic structure of the toxin and its receptor reported in this study, may pave the way to new life-saving therapeutics.

**Toward a safer form of acetaminophen**

Efforts to develop a safer form of acetaminophen — the pain and fever-reducer that is one of the most widely used drugs — have led to discovery of substances that may have less potentially toxic effects on the liver. A report on the research appears in *ACS Medicinal Chemistry Letters*.

Roman Shchepin and colleagues explain that a link exists between acetaminophen and liver damage. The damage may be severe and can occur with intentional and accidental overdoses, as well as when susceptible individuals take the drug. Indeed, acetaminophen has been implicated in almost 50 percent of all acute liver failure cases in the United States alone. Scientists have known the biochemical basis of acetaminophen’s liver toxicity, and Shchepin and colleagues set out to develop safer versions of acetaminophen.

They describe the design and testing of two compounds that have a similar architecture to acetaminophen, but aren’t toxic to liver cells grown in the laboratory. The researchers say that, although further testing is needed, these compounds are promising candidates for acetaminophen replacements.
Pandemic Risk? Troubling Traits of H7N9 Avian Flu Virus
July 10, 2013 — The emerging H7N9 avian influenza virus responsible for at least 37 deaths in China has qualities that could potentially spark a global outbreak of flu, according to a new study published July 10, 2013 in the journal *Nature*.

An international team led by Yoshihiro Kawaoka of the University of Wisconsin-Madison and the University of Tokyo conducted a comprehensive analysis of two of the first human isolates of the virus from patients in China. Their efforts revealed the H7N9 virus's ability to infect and replicate in several species of mammals, including ferrets and monkeys, and to transmit in ferrets—data that suggests H7N9 viruses have the potential to become a worldwide threat to human health.

"H7N9 viruses have several features typically associated with human influenza viruses and therefore possess pandemic potential and need to be monitored closely," says Kawaoka, one of the world’s leading experts on avian flu.

Normally, avian influenza viruses do not infect humans, with the exception of the highly pathogenic H5N1 strains. However, the H7N9 virus has so far infected at least 132 humans, killing more than 20 percent of those infected, and several instances of human-to-human infection are suspected.

The new study suggests that the ability of the H7N9 virus to infect and replicate in human cells may be due to just a few amino acid changes in the genetic sequence of the virus. "These two features are necessary, although not sufficient, to cause a pandemic," says Kawaoka, explaining that the influenza virus depends on host cells, which it hijacks to make new virus particles and sustain the chain of infection.

In monkeys, the H7N9 virus was shown to efficiently infect cells in both the upper and lower respiratory tract. Conventional human flu viruses are typically restricted to the upper airway of infected nonhuman primates.

"If H7N9 viruses acquire the ability to transmit efficiently from person to person, a worldwide outbreak is almost certain since humans lack protective immune responses to these types of viruses," according to Kawaoka.

Transmission studies conducted by Kawaoka’s group in ferrets—animals that, like humans, infect one another through coughing and sneezing and that are a standard model for studies of influenza in mammals—showed that one of the H7N9 strains isolated from humans can transmit via respiratory droplets, though not as efficiently as human influenza viruses. The limited aerosol transmission observed in ferrets adds to concerns about the potential threat as avian flu viruses typically lack that ability, Kawaoka notes.

"H7N9 viruses combine several features of pandemic influenza viruses, that is their ability to bind to and replicate in human cells and the ability to transmit via respiratory droplets," Kawaoka says.

Complicating the H7N9 picture is the fact that the H7N9 virus does not kill poultry, which promises to make surveillance much more difficult. "We cannot simply watch out for sick or dead birds. Rather, tests have to be performed to determine whether or not a bird is infected. Considering the vast number of poultry, this is a daunting task."

The positive news conveyed in the new Nature report is that most of the H7N9 strains tested were somewhat sensitive to antiviral drugs effective against the seasonal flu virus, although one isolate, which appears to be a mix of two variants of the H7N9 virus, seemed to resist neuraminidase inhibitors like Tamiflu.

Further research is needed, Kawaoka argues, to support vaccine development, to assess the risks, and to better understand why the H7N9 viruses infect humans so efficiently.

**Journal Reference:**

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Researchers Identify New Source of Powerful Immunity Protein
July 10, 2013 — Researchers at UT Southwestern Medical Center report the identification of a new cellular source for an important disease-fighting protein used in the body's earliest response to infection.
The protein interferon-gamma (IFN-γ) keeps viruses from replicating and stimulates the immune system to produce other disease-fighting agents. Neutrophils, the newly identified cellular source of the protein, are the major component of the pus that forms around injured tissue.

The researchers also report that the neutrophils appear to produce IFN-γ through a new cellular pathway independent of Toll-like receptors (TLRs): the body's early warning system for invasion by pathogens. This finding indicates that mammals might possess a second early-alarm system—the sort of built-in redundant engineers would envy, said Dr. Felix Yarovinsky, assistant professor of immunology and senior author of the study published online in the Proceedings of the National Academy of Sciences in June.

"We believe our mouse study provides strong evidence that neutrophils, white blood cells created in the bone marrow, produce significant amounts of IFN-γ in response to disease," Dr. Yarovinsky said. "The finding of a new and essential cellular source for IFN-γ challenges a long-held belief in the field and is significant because neutrophils are the most common kind of white blood cell."

Two pathogens were used in this study: the parasite Toxoplasma gondii—which can cause brain damage in humans and other mammals that have compromised immune systems—and a type of bacterium that causes gastroenteritis, Salmonella typhimurium.

Innate immunity is the body's first line of defense against pathogens, including those that it has never before encountered. Adaptive immunity is the secondary system that battles pathogens to which the body has previously been exposed and to which it has developed antibodies.

Textbooks list natural killer (NK) cells and T cells as the body's significant sources of IFN-γ. Although large numbers of neutrophils have long been observed to congregate at the site of a new infection, they were commonly thought to be first responders or foot soldiers rather than generals in the battle against disease, as this study indicates they are, Dr. Yarovinsky explained.

About 20 years ago, there were clinical reports in humans and animals suggesting that neutrophils might produce IFN-γ, but the idea was largely ignored by the scientific community until the last decade, he said.

Since then, studies at UT Southwestern and elsewhere have found that mice lacking NK and T cells, and therefore expected to be unable to produce IFN-γ, somehow continued to withstand infections better than mice genetically unable to make any IFN-γ. These observations suggested the possibility of an unknown source of the protein, he explained.

In a series of experiments, the UT Southwestern researchers identified neutrophils as the major source of IFN-γ in mice lacking NK and T cells. "Based on what we know about neutrophils, their large numbers and rapid deployment to the site of infection should provide an important means of very early, robust, and rapid elimination of disease-causing agents," the researchers wrote. Although neutrophil-derived IFN-γ alone is insufficient to achieve complete host protection, the protein significantly extended the survival of mice in this study, Dr. Yarovinsky said.

In related news, the Burroughs Wellcome Fund in June announced that Dr. Yarovinsky had been selected for its 2013 Investigators in the Pathogenesis of Infectious Disease Award to further investigate mechanisms of host defense against various infectious diseases mediated by IFN-γ produced by neutrophils. The award will provide $500,000 over five years to pursue this line of research.

Others involved include first author Carolyn Sturge, a graduate student of immunology; former research assistant Alicia Benson; research assistant II Megan Raetz; graduate student Cara L. Wilhelm; Dr. Julie Mirpuri, assistant professor of pediatrics; and Cancer Immunobiology Center Director Dr. Ellen Vitetta, professor of immunology and of microbiology.

Journal Reference:

Researchers Identify Specific Fetal Antigens Attacked by Maternal Antibodies

July 9, 2013 — UC Davis MIND Institute researchers have identified the specific antibodies that target fetal brain proteins in the blood of a subset of women whose children are diagnosed with autism. The finding is the first to pinpoint a specific risk factor for a significant subset of autism cases, as well as a biomarker for drug development and early diagnosis. The researchers have named autism related to these antibodies "Maternal Autoantibody-Related," or MAR autism.
The study found that the mothers of children with autism were more than 21 times as likely to have the specific MAR antibodies in their systems that reacted with fetal brain proteins, or antigens, than were the mothers of children who did not have autism. In fact, specific combinations of MAR antibodies were not found in the blood of mothers whose children were typically developing.

The research, "Autism-specific maternal autoantibodies recognize critical proteins in developing brain," is published online today in Translational Psychiatry, a Nature journal.

The study was led by principal investigator and immunologist Judy Van de Water, a researcher affiliated with the MIND Institute. Earlier studies by Van de Water and her colleagues found that women with certain antibodies in their bloodstreams are at greater risk of having a child with autism and that their children exhibited more severe language delays, irritability and self-injurious behaviors than did the autistic children of mothers whose blood did not have the antibodies.

"Now we will be able to better determine the role of each protein in brain development," said Van de Water, professor of internal medicine. "We hope that, one day, we can tell a mother more precisely what her antibody profile means for her child, then target interventions more effectively."

To identify the exact antigens targeted by the mothers' antibodies, Van de Water and her colleagues conducted the research in Northern California using blood samples from 246 mothers of children with autism and of a control group of 149 mothers of children without autism to examine their reactivity with the candidate antigens.

Seven antigens were significantly more reactive to the blood of mothers of children with autism than to that of the control mothers. The study found that the mothers with antibodies that reacted with any one of these antigens, either individually or in combination with other antigens, were more than three times as likely to have a child with autism spectrum disorder.

Several combinations of antibodies in the blood from mothers of children with autism were not found in the control mothers' blood. Nearly 23 percent of mothers of children with autism had certain combinations of autoantibodies against the target antigens, compared with less than 1 percent of mothers of children without the disorder.

The specific antigens identified in the study are lactate dehydrogenase A and B, cypin (guanine deaminase), stress-induced phosphoprotein 1, collapsing response mediator proteins 1 and 2, and Y-box binding protein. All are found throughout the body, but also are expressed at significant levels in the human fetal brain and have established roles in neurodevelopment. For example, cypin is an enzyme that plays an important role in normal neurite branching, a fundamental function in the developing brain, whereas the CRMP proteins are critical later in neuron development for axon outgrowth.

Maternal antibodies are known to cross the placenta during pregnancy and can be detected in a fetus as early as 13 weeks. By 30 weeks, maternal antibody levels in the fetus are about half that of the mother, and at birth, the concentration is even greater in the newborn than in the mother herself. The maternal antibodies stay in the baby's bloodstream for about 6 months after birth, after which the baby's own immune system takes over.

Once in the fetal bloodstream, the antibodies then may enter the brain and attack cells that have corresponding proteins that act as antigens. This antigen-antibody response is an important defense against foreign invaders, such as bacteria or viruses, but is not normally directed against oneself. When directed against one's own tissue, the antibodies are known as autoantibodies.

"It is important to note that women have no control over whether or not they develop these autoantibodies, much like any other autoimmune disorder," Van de Water said. "And, like other autoimmune disorders, we do not know what the initial trigger is that leads to their production."

Understanding which proteins and which pathways are implicated in MAR autism can help elucidate the causes of autism and possibly lead to new therapies, such as administering 'antibody blockers' to the mother during pregnancy to prevent damage to the developing fetal brain, Van de Water said.

These findings are leading to the development of a MAR diagnostic test for autism, which would be available to the mothers of young children who are showing signs of developmental delay. If the test were positive, the child would be a candidate for early behavioral intervention.

"These findings are incredibly important because they establish a cause for a significant portion of autism cases, thereby opening up new lines of inquiry into possible biological treatments," said MIND Institute Director Leonard Abbeduto. "In addition, the findings demonstrate that a diagnostic test is within reach. This test would be invaluable for women who are considering becoming pregnant and could lead to earlier and more accurate diagnosis of children with developmental challenges and help get them into behavioral interventions at younger ages."
A MAR diagnostic test also would assess a mother's risk of having a child with autism prior to conception, which is particularly important for women who already have a child with the disorder. UC Davis has patented this technology and licensed the exclusive worldwide rights to develop it for commercial purposes to Pediatric Bioscience, Inc.

"We know that early behavioral interventions for autism are critical," said Isaac Pessah, professor and chair of the Department of Molecular Biosciences in the UC Davis School of Veterinary Medicine and former director of the UC Davis Center for Children's Environmental Health. "Developing a predictive test for autism before symptoms become obvious could have an enormous impact on treating children with the condition."

Study participants were from the CHARGE (Childhood Autism Risks from Genetics and the Environment) study, an ongoing study that was launched in 2001 by the MIND Institute and the UC Davis Center for Children's Environmental Health, of which Van de Water now is director. Children with autism spectrum disorder, children with developmental delay and typically developing children between the ages of 2 and 5 years are studied with the goal of better understanding the causes of autism.

A related study is the MARBLES (Markers of Autism Risk in Babies — Learning Early Signs) study, also being conducted at the MIND Institute and the Center for Children's Environmental Health. This study follows pregnant women who already have a child with autism. Multiple factors related to genetics and the environment is under study in an effort to uncover predictors for having a child with autism.

Van de Water said knowing the specific protein targets of the maternal antibodies enables researchers to develop more precise animal models of autism.

**Journal Reference:**

**“Tipping point” reached after rise in Philippines HIV/AIDS cases?**
MANILA, 11 July 2013 (IRIN)—Consistent increases in HIV infections in the Philippines cannot be reversed without appropriate interventions, say health experts, following the recent release of the country’s highest monthly infection rate recorded thus far.

In May 2013 415 new HIV cases were recorded, with 55 percent of cases being among those aged 20-29.

Since 2007, the Department of Health’s National Epidemiology Centre (DOH-NEC) has noted a steady increase in HIV cases. In 2000, there was one case registered every three days; in 2011, this number grew to one case every three hours.

“The nature of the HIV epidemic has changed. Transmission is still primarily through unprotected sex, but infections are now mostly through same sex transmission whereas previously, it was heterosexual,” said Teresita Bagasiao, the Joint UN Programme on HIV/AIDS (UNAIDS) country coordinator in the Philippines. **Concentrated epidemic**

Part of the problem is that current interventions have not kept pace with change.

“Most interventions are still focused on heterosexual transmission. There is an opportunity for focused interventions [on men having sex with men and injecting drug users] to reach the recommended 60-80 percent of key affected populations,” said Bagasiao.

But even with these interventions, any drop in HIV infections would not occur for another 3-5 years, she added.

Though Philippines is a low-prevalence country with less than 1 percent of the nearly 95 million population infected, Bagasiao said the epidemic is “concentrated” with an average 4-5 percent rate of infection among what donors call “key” populations, including sex workers, men who have sex with men and injecting drug users.

Since 1987 when HIV was first discovered in the Philippines, DOH-NEC has registered 13,594 infections.

**“Tip of the iceberg”**

But others say this official number is just the “tip of the iceberg”. Stigma continues to surround HIV and with less than 1 percent of the general population getting tested for HIV, officially recorded cases most likely do not accurately reflect the epidemic.

“We project that the number of infected will reach 39,000-50,000 by 2015,” said Jonas Bagas, executive director of The Library Foundation Sexuality, Health and Rights Educators Collective, Inc (TLF-
Share), an NGO member of the Philippine National AIDS Council (PNAC), the country’s central advisory body on HIV/AIDS.

“HIV is still considered a gay disease and equated to a death sentence. Prevention messages are mostly scare tactics making people afraid of getting tested. They’d rather not know their status, especially the young people,” said Bagas.

Barriers discouraging testing among youths include a provision in the 1998 Philippine AIDS Prevention and Control Act that requires parental consent for anyone under 18 to be tested for HIV. Advocates are fighting to amend the requirement.

Rather, added Bagas, an appropriate response to halt the spread of HIV is to give youths sex education that focuses on prevention strategies such as delaying first sexual encounters, as well as a nationwide publicity campaign on HIV information and services.

“We have yet to have a nationwide campaign on HIV on the scale that we have for other diseases like dengue,” Bagas said. As of early June there have been at least 42,000 dengue infections reported in 2013 nationwide, with nearly 200 deaths.

**Concerted effort needed**

But budgets for such an HIV prevention campaign are hard to secure.

Though government agencies such as DOH and the Department of Social Welfare and Development have increased spending in recent years for HIV prevention, funds from international agencies for HIV have shrunk dramatically.

In 2009, international donors funded almost 73 percent of the country’s budget for HIV prevention and control; in 2010, the contribution barely reached 40 percent.

The 2008 Commission on AIDS Report recommended US$1 per capita annual spending for HIV prevention and control—nearly $95 million based on the current estimated population.

According to the Philippines National AIDS Council 2012 report, $37 million was spent on HIV from 2009-2011, or an average of about $12.4 million per year.

“We need the help of the LGUs [local government units, the country’s smallest unit of government] in appropriating funds in their local budgets for HIV and STI [sexually transmitted disease] prevention and information. The DOH cannot do it alone,” said Genesis Samonte, the department’s chief epidemiologist for HIV.

“We are at a tipping point. We have a choice [to determine] how big this problem will be, but we cannot go back any more. It is our response to HIV that will now dictate its magnitude,” Samonte concluded.

**Boy, 12, dies after undergoing historic transplant at U to treat HIV and leukemia**

Article by: ROSE FRENCH, Star Tribune, Updated: July 12, 2013—11:53 PM

A boy with HIV and leukemia, who underwent an experimental cell transplant at University of Minnesota’s Amplatz Children’s Hospital, has died of complications from the procedure, his doctors said Friday.

Twelve-year-old Eric Blue, of Alexandria, La., had not been publicly identified until Friday, when university officials released a statement confirming that he died July 5, nearly three months after the risky — and potentially historic — procedure.

Blue was in line to become the second person in the world to be cured of both deadly illnesses by the extraordinary type of bone marrow transplant, doctors said.

“He was incredibly brave and courageous, and understood he was participating in something historic,” said Dr. Michael Verneris, a transplant specialist at the university, who treated Blue.

In June, Blue developed a severe complication called graft-versus-host disease, which occurs when the immune cells of the donor attack various tissues of the body.

“Sometimes the disease is a very treatable problem,” said Verneris. “Unfortunately in his circumstance, for whatever reason, it was worse than it would otherwise be ... he had an especially bad form of it.”

Until Friday, Blue’s identity had been withheld for privacy reasons. But his mother granted permission to reveal his name following his death to acknowledge her son’s pioneering medical contributions, according to U officials.

The procedure, which was performed April 23, involved injecting Blue with blood cells from a donor with a rare genetic resistance to HIV, the virus that causes AIDS. Less than 1 percent of the population is
born with this genetic resistance, according to Verneris. Doctors hoped the transplant would rid the boy’s body of both the leukemia and HIV and help fight off any recurrence.

While not yet conclusive, tissue and blood tests obtained through Blue’s treatment have shown an absence of HIV, even after his medications were discontinued, Verneris said.

“There was no sign of leukemia either for that matter,” he said. “Things were looking quite bright. Of course, that makes it even more bittersweet in some ways ... that he was almost there.”

“This patient absolutely needed to have this transplant,” Verneris added. “And if he hadn’t developed a very common side effect of bone marrow transplant and died from it, we were hopeful this was all going to work well.”

Both the boy and his doctors knew his case was going to be a challenge, and success was never a guarantee. Still, physicians say Blue’s case helped them advance the science of the novel treatment.

“The promise we made to the child ... was that we were going to learn from this, and we still are learning,” said Verneris. “This is the first step in many, [and] hopefully we’ll be able to really improve the outcomes of not only people with HIV, but people with leukemia through understanding these processes better.”

**Prior flu exposure dictates your future immunity, allowing for new, rationally developed regimens**

**Findings offer alternative approach to creating a universal influenza vaccine**

A team of scientists, led by researchers at The Wistar Institute, has determined that it might be possible to stimulate the immune system against multiple strains of influenza virus by sequentially vaccinating individuals with distinct influenza strains isolated over the last century.

Their results also suggest that world health experts might need to re-evaluate standard tests used for surveillance of novel influenza strains. Their findings are published in the *Journal of Experimental Medicine*, available online now.

According to the Wistar researchers, their analysis could lead to an alternative approach to creating a "universal" flu vaccine—a vaccine that would provide resistance to seasonal and pandemic influenza strains over many years, negating the need for an annual flu shot.

"Influenza vaccines are very safe and provide good protection. However, we need to continuously update seasonal flu vaccines because influenza viral proteins change over time," said Scott Hensley, Ph.D., an assistant professor at The Wistar Institute and corresponding author on the study. "Since influenza viruses are constantly changing, we all have unique pre-exposure histories that depend on when we were born and the specific types of viruses that circulated during our childhood."

Vaccines work by stimulating the immune system to produce antibody proteins against particles (called antigens) from an infectious agent, such as bacteria or a virus. The immune system saves the cells that produce effective antibodies, which then provide immunity against future attacks by the same or similar infectious agents. Despite the availability of a vaccine, seasonal influenza typically kills 36,000 Americans, alone, and nearly a half million individuals around the world, in total.

Most current efforts to create universal vaccines hinge on the idea of generating antibodies against a portion of the virus that is relatively unchanged year-to-year.

"Our studies demonstrate that individuals that are infected sequentially with dramatically different influenza strains mount antibody responses against a conserved region of influenza virus," Hensley said. "Since we now know that pre-exposure events can influence vaccine responsiveness in a predictable way, we can begin to design vaccine regiments that preferentially elicit antibody responses against conserved regions of influenza virus."

The researchers began their current work by studying human antibody responses against the 2009 pandemic H1N1 virus. The 2009 strain is antigenically distinct from recently circulating seasonal H1N1 strains, and a distant relative of the virus that caused the devastating "Spanish Flu" of the early 20th century. The most effective antibodies are those that bind to a particular portion (or "epitope") of hemagglutinin (HA), a protein produced by the influenza virus.

According to Hensley, however, their chief insight occurred when his team hit the "sort" button on a spreadsheet document, thereby arranging all samples by age of the donor. Different aged people, they found, mount vastly different antibody responses to pandemic H1N1, depending on whether or not they were exposed to a seasonal H1N1 years earlier. "We can now accurately predict how individuals will respond to the pandemic H1N1 strain based on the year that they were born," Hensley said.
Their investigation also suggests that ferrets with no prior influenza exposure might not be the most reliable predictor of human immune responses. Anti-sera—or blood containing antibodies—created in these "naïve" ferrets are commonly used for influenza surveillance. The researchers found that naïve ferrets mount a response to an epitope in a decidedly different portion of HA than do most humans, but subsequently infecting these ferrets with other historical influenza strains can shift the antibody response toward the epitope that human antibodies recognize. This shift might also be replicable in humans through multiple infections or vaccinations, the researchers believe.

According to Hensley, one strategy would be to sequentially vaccinate children with antigenically distinct viral strains. "Babies are born with an immunological blank slate," Hensley said. "We may be able to strategically vaccinate our children with antigenically diverse influenza strains to elicit antibodies against conserved viral epitopes."

**Black-legised ticks linked to encephalitis in New York state**

Researchers urge citizens and healthcare providers to be vigilant

The number of tick-borne illnesses reported to the U.S. Centers for Disease Control and Prevention is on the rise. Lyme disease leads the pack, with some 35,000 cases reported annually. In the Northeast, the black-legged ticks (*Ixodes scapularis*) that spread Lyme disease also infect people with other maladies, among them anaplasmosis, babesiosis, and – as a new paper in the journal *Parasites and Vectors* reports – Powassan encephalitis.

Powassan encephalitis is caused by Powassan virus and its variant, deer tick virus. The virus is spread to people by infected ticks, and can cause central nervous system disruption, encephalitis, and meningitis. There is a 10-15% fatality rate in reported cases, with many survivors suffering long-term neurological damage.

Rick Ostfeld, a disease ecologist at the Cary Institute of Ecosystem Studies and one of the paper’s authors, comments: "We’ve seen a rise in this rare but serious illness in parts of New York State that are hotspots for Lyme disease. And we suspected it was tied to an increase in black-legged ticks carrying deer tick virus, particularly on the east side of the Hudson River."

This is precisely what a 5-year assessment bore out. Researchers surveyed ticks at sites east and west of the river. Fieldwork involved collecting ticks off of small mammals and birds, as well as dragging tick cloths near animal burrows and known tick habitats. Routine surveys performed by the New York State Department of Health were also incorporated in the study.

More than 13,500 ticks of seven species were assessed. Black-legged ticks made up almost all of the collection. Nymphs and adults were sampled, as these life stages are most likely to infect people. Counties surveyed included Dutchess, Putnam, Westchester, Sullivan, Ulster, Orange, and Rockland. In addition, blood samples were taken from a variety of birds and mammals, to identify deer tick virus hosts.

Throughout the study, lab analyses performed at Wadsworth Center’s Arbovirus Laboratories found deer tick virus in black-legged ticks. Areas east of the Hudson River had the highest concentration of infected adult ticks – on the order of 4-5% in Westchester, Putnam, and Dutchess counties. Virginia opossums, striped skunks, and raccoons were among the animals found to transmit deer tick virus to feeding ticks.

Laura Kramer, one of the paper’s authors and a research scientist at the Wadsworth Center, notes: "Our findings are consistent with deer tick virus infection rates in people revealed in clinical tests by the New York State Department of Health. Of fourteen individuals testing seropositive for deer tick virus, ten were residents of Westchester, Putnam, or Dutchess counties. Another two were from Albany and Suffolk counties, areas with burgeoning black-legged tick populations."

Adding to the problem: unlike Lyme disease, anaplasmosis, and babesiosis – which take feeding black-legged ticks hours to transmit – deer tick virus transmission can occur in just 15 minutes. This leaves very little 'grace period' for removing ticks, and underscores the importance of vigilance in tick habitat.

Ostfeld concludes: "When patients present with encephalitis symptoms in areas with high levels of Lyme disease, especially during the summer, physicians need to consider Powassan encephalitis. While rare, it’s associated with significant complications. There is no vaccine or specific antiviral therapy, the best strategy remains prevention."

Our knowledge of Powassan encephalitis has grown largely as a result of West Nile virus surveillance. Both are part of the flavivirus group of arboviruses. More extensive arbovirus testing may reveal that deer tick virus is more widespread than previously thought.
Biochemists uphold law of physics

Experiments by biochemists at the University of California, Davis show for the first time that a law of physics, the ergodic theorem, can be demonstrated by a collection of individual protein molecules—specifically, a protein that unwinds DNA. The work will be published online by the journal *Nature* on July 14.

Using technology invented at UC Davis for watching single enzymes at work, Bian Liu, a graduate student in the Biophysics Graduate Group and professor Steve Kowalczykowski, Department of Microbiology and Molecular Genetics and UC Davis Cancer Center, found that when they paused and restarted a single molecule of the DNA-unwinding enzyme RecBCD, it could restart at any speed achieved by the whole population of enzymes.

"It’s pretty impressive," said Daniel Cox, a physics professor at UC Davis who was not involved in the work. "The laws of physics should apply to biological systems, and it turns out they do."

The results also have implications for understanding how proteins fold into their correct shape, for exploring interactions between drugs and their targets, and for engineering enzymes for new functions.

The ergodic theorem, proposed by mathematician George Birkhoff in 1931, holds that if you follow an individual particle over an infinite amount of time, it will go through all the states that are seen in an infinite population at an instant in time. It’s a fundamental assumption in statistical mechanics—but difficult to prove in an experiment.

Liu and Kowalczykowski weren’t attempting to test laws of physics when they began the work. They wanted to know why RecBCD, an enzyme that unwinds DNA in *E. coli* bacteria, showed so much variability in its rate of action.

RecBCD attaches to and moves along DNA, unwinding the double helix into two separate strands. It has two jobs in the cell: to allow damaged DNA to be repaired, and to break down invading "foreign" DNA from viruses.

In 2001, Kowalczykowski’s laboratory, with the late professor Ronald Baskin at UC Davis, developed a technique to trap single molecules of RecBCD and watch them at work on a strand of DNA in real time. They have since exploited the method to study how DNA is repaired—in humans, a vital process in protecting against cancer and developmental defects.

"Ever since the original experiments, we've noticed RecBCD molecules have quite a broad range of speeds," Kowalczykowski said.

Liu used the single-molecule visualization technique to measure the rates of hundreds of RecBCD molecules, finding bell-shaped curves for the whole population.

One explanation could be that a large proportion of the proteins were not folded properly and were "trapped" in an inefficient state. However, mild heat or unfolding treatments, which should have allowed the proteins to relax into their correct folded state, had no effect.

RecBCD usually runs for about a minute before stopping spontaneously. Liu found that he could stop the enzyme early by taking away ATP, the chemical fuel that makes the enzyme work.

When he brought back the fuel, he found that the enzymes started up again—but at a random speed, not related to their previous rate. Overall, the individual RecBCD proteins could restart at any speed within the bell-shaped spread shown by all the proteins.

The experiment shows that RecBCD can move through a wide range of slightly different conformations in which it works at slightly different speeds. However, when it is attached to a step on the DNA ladder, it is locked in shape. Because the time for the enzyme to move from step to step along DNA is shorter than the time it needs to change conformation (about one second), it remains in the same conformation as long as it is moving along DNA, Kowalczykowski said.

What is the point? Why not just have all the enzymes work at one, optimal rate? Having this important enzyme able to operate at a range of speeds might give the cell flexibility to respond to rapidly changing conditions, Kowalczykowski said. For example, degradation of foreign DNA is a process that needs to go quite fast: copying and repairing DNA might require the enzyme to work more slowly, in combination with other proteins.
How cranberries impact infection-causing bacteria
Research points to potential role for cranberry derivatives in implantable medical devices

Consuming cranberry products has been anecdotally associated with prevention of urinary tract infections (UTIs) for over 100 years. But is this popular belief a myth, or scientific fact?

In recent years, some studies have suggested that cranberries prevent UTIs by hindering bacteria from sticking to the walls of the urinary tract, thanks to phytochemicals known as proanthocyanidins (PACs). Yet the mechanisms by which cranberry materials may alter bacterial behaviour have not been fully understood.

Now, researchers in McGill University’s Department of Chemical Engineering are shedding light on the biological mechanisms by which cranberries may impart protective properties against urinary tract and other infections. Two new studies, spearheaded by Prof. Nathalie Tufenkji, add to evidence of cranberries’ effects on UTI-causing bacteria. The findings also point to the potential for cranberry derivatives to be used to prevent bacterial colonization in medical devices such as catheters.

In research results published online last month in the Canadian Journal of Microbiology, Prof. Tufenkji and members of her laboratory report that cranberry powder can inhibit the ability of Proteus mirabilis, a bacterium frequently implicated in complicated UTIs, to swarm on agar plates and swim within the agar. The experiments also show that increasing concentrations of cranberry powder reduce the bacteria’s production of urease, an enzyme that contributes to the virulence of infections.

These results build on previous work by the McGill lab, showing that cranberry materials hinder movement of other bacteria involved in UTIs. A genome-wide analysis of an uropathogenic E. coli revealed that expression of the gene that encodes for the bacteria’s flagellar filament was decreased in the presence of cranberry PACs.

The team’s findings are significant because bacterial movement is a key mechanism for the spread of infection, as infectious bacteria literally swim to disseminate in the urinary tract and to escape the host immune response.

"While the effects of cranberry in living organisms remain subject to further study, our findings highlight the role that cranberry consumption might play in the prevention of chronic infections,” Tufenkji says. "More than 150 million cases of UTI are reported globally each year, and antibiotic treatment remains the standard approach for managing these infections. The current rise of bacterial resistance to antibiotics underscores the importance of developing another approach.”

Another recent study led by Tufenkji in collaboration with McGill professor Showan Nazhat, a biomaterials expert at the Department of Mining and Materials Engineering, finds that cranberry-enriched silicone substrates impaired the spread of Proteus mirabilis. Those results, published online in the journal Colloids and Surfaces B: Biointerfaces, point to potential use for cranberry derivatives to hinder the spread of germs in implantable medical devices such as catheters, which are frequently implicated in UTIs.

"Based on the demonstrated bioactivity of cranberry, its use in catheters and other medical devices could someday yield considerable benefits to patient health," Tufenkji says.

In Children With Fever, Researchers Distinguish Bacterial from Viral Infections
July 15, 2013 — In children with fever but no other symptoms of illness, it is difficult to know whether a child has a viral infection that will resolve on its own or a potentially serious bacterial infection that requires antibiotics.

Now, researchers at Washington University School of Medicine in St. Louis report that they can distinguish between viral and bacterial infections in children with fever by profiling the activity of genes in a blood sample. In a small study, analyzing genes in white blood cells was more than 90 percent accurate, far better than the standard diagnostic test, which is only correct about 70 percent of the time.

The research is published July 15 in the Proceedings of the National Academy of Sciences Online Early Edition.

While more work is needed, the study’s results support the notion that analyzing the activity of the body’s genes in response to childhood infections could help to identify the cause of illness and ensure that children get the right treatment.

"It's a common problem that children develop a fever without any apparent cause," says senior author Gregory Storch, MD, the Ruth L. Siteman Professor of Pediatrics and chief of the Division of Pediatric Infectious Diseases at Washington University School of Medicine and St. Louis Children’s Hospital.
"Some of these kids have serious bacterial infections that can be life threatening, but the largest number have viral infections. The trouble is, from a practical standpoint, it's hard to know which is which."

As a precaution, many children who have a fever without an apparent cause are treated with antibiotics even though the drugs don't work against viruses and overprescribing them contributes to antibiotic resistance.

The new study involved 30 children ages two months to 3 years who had fevers above 100.4° F but no obvious signs of illness, like a cough or diarrhea. Twenty-two of the children were known to have viral infections based on previous extensive genomic testing that is not yet practical to use in a clinic setting, and eight others children had bacterial infections.

But Storch and his colleagues at the university's Genome Institute and the Genome Technology Access Center wanted to know whether a test called a gene expression microarray could identify patterns of gene activity in white blood cells that could discriminate children with viral infections from those with bacterial infections. White blood cells are the immune system's first line of defense against foreign invaders, and the scientists theorized that they would respond differently to viruses than to bacteria.

The researchers also had access to results of a standard diagnostic test performed when the children initially were evaluated with fevers at St. Louis Children's Hospital. That test involves analyzing the number of white blood cells in a blood sample. Generally, the counts are elevated for bacterial infections and either low or normal for viral infections.

"We know there are many exceptions to that rule, and we certainly saw that in this study," Storch said. "A lot of patients with viral infections had elevated white-blood cell counts so doctors thought they had bacterial infections and prescribed antibiotics, which in fact were not necessary."

Using microarray technology, the researchers could easily distinguish bacterial infections from viral infections based on distinctive patterns of gene expression. "That's really important for clinicians because if they see a pattern of gene expression that indicates a viral infection, they could feel comfortable not prescribing antibiotics," Storch added.

As a comparison, the research team performed the microarray analysis on blood samples from 35 children without fever, also ages 2 months to 3 years, who were having outpatient surgery. Earlier genomic testing showed that eight of those children had viruses, even though they didn't cause any symptoms.

"In the kids with a virus and a fever, many genes were very active, compared with kids who had viruses and no fever, whose genes were quiet," Storch explained. "The microarray basically tells us how a patient is reading the infection. The very active genes tell us that an infection is making a patient sick, while quiet genes tell us either there's no infection or maybe a bacterium or virus is there, but it's not causing fever or illness."

This distinction is important because when standard tests suggest a child has a virus, doctors don't know whether virus is producing a child's illness or whether it's an innocent bystander. According to Storch, "the danger of attributing symptoms to a virus that is actually an innocent bystander is that the child might not receive needed antibiotics."

This points to the potential benefit of using tests that measure the response of genes to get more conclusive answers to illness. This would help to ensure that antibiotics are targeted to those children who really need them, he added.

Next, Storch hopes to refine the microarray technology, which simultaneously analyzes all 25,000 genes in the body. This makes the test too time consuming and expensive to use in a clinic setting. But he and his co-workers want to identify a smaller number of critical genes that could be used to distinguish between viral and bacterial infections and evaluate a second-generation test in children as part of a new study.

Journal Reference:

**Surprise Finding Reveals How Adaptive Our Immune Systems Can Be**

July 15, 2013 — Studies of patients with immunodeficiencies involving single gene mutations can reveal a great deal about our immune systems, especially when actual symptoms do not accord with clinical expectations.

Australian scientists acknowledge such a gap between expectation and reality in a new study, which examines people with 'Autosomal Dominant Hyper IgE Syndrome'. 
Hyper IgE Syndrome arises from a mutation in the STAT3 gene. This makes patients slightly more susceptible than normal to blood cancers known as 'lymphomas', and exposes them to recurrent skin infections and pneumonia. While these symptoms are certainly a problem, laboratory experiments and animal models predict far more susceptibility to viruses and cancers than is actually the case.

PhD student Megan Ives, Dr Elissa Deenick and Associate Professor Stuart Tangye, from Sydney's Garvan Institute of Medical Research, discovered that the immune systems of people with Hyper IgE Syndrome have much more redundancy, or compensatory capacity, than expected. Specifically, the research team expected patients to be significantly less able to create effective 'killer T cells', the class of immune cells that destroy invading microbes and cancers. Their results are published in the Journal of Allergy and Clinical Immunology, now online.

"Under normal circumstances, the STAT3 molecule passes biochemical signals in T cells which instruct them to turn on their killing machinery. In Hyper IgE patients, who lack the gene, the signal just appears to take a diversion most of the time, and that seems to work," said Dr Elissa Deenick.

"There are certain molecules that Killer T cells need in order to become effective—and possibly in the case of a very few viruses and lymphomas, Hyper IgE patients are unable to generate the signals necessary to make these molecules. However they do make effective responses against most viruses and cancers." Associate Professor Stuart Tangye believes the study is important because it underlines the differences between results obtained through mouse model work and real human infectious diseases. "I believe it's just as useful to find an explanation for a prediction that didn't happen as it is to have a prediction confirmed," he said.

"Megan's work allows us to understand why Hyper IgE patients are not super unwell, as you would expect. That understanding is vital clinically, because it may allow us to address the actual symptoms more effectively."

**Journal Reference:**
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**Changing View on Viruses: Not So Small After All**
Chantal Abergel and Jean-Michel Claverie. Electron microscopy image of a Pandoravirus particle. The virus is 1,000 times bigger than the flu virus and has nearly 200 times as many genes.

**By Carl Zimmer**

**Published: July 18, 2013**

There was a time not that long ago when it was easy to tell the difference between viruses and the rest of life. Most obviously, viruses were tiny and genetically simple. The influenza virus, for example, measures about 100 nanometers across, and has just 13 genes.

Those two standards, it’s now clear, belong in the trash. Over the past decade, scientists have discovered a vast menagerie of viruses that are far bigger, and which carry enormous arsenals of genes. French researchers are now reporting the discovery of the biggest virus yet. The pandoravirus, as they’ve dubbed it, is 1,000 times bigger than the flu virus by volume and has nearly 200 times as many genes — 2,556 all told.

Making the discovery all the more startling is the fact that, of all the genes that pandoraviruses carry, only six percent match any gene known to science.

“We believe we’re opening a Pandora’s box – not so much for humanity but for dogma about viruses,” said Dr. Jean-Michel Claverie of the University of Mediterranée, co-author of the paper that was published online Thursday in the journal Science. “We believe we’re touching an alternative tree of life.”

Giant viruses would be important enough simply for the way they have blurred the line between viruses and the rest of life. But they excite scientists for another reason. Utterly unknown a decade ago, they turn out to be everywhere, including in our own bodies. What effect they have on the world’s ecosystem — or our own health — is anyone’s guess right now.

It was the very giant-ness of giant viruses that allowed them to be overlooked for so long. Scientists first discovered viruses in the late 1800s when they were puzzled by a disease that beset tobacco plants. They mashed up wilted tobacco leaves with water and passed the mixture through fine porcelain filters that trapped bacteria and fungi. The clear liquid could still make healthy tobacco leaves sick. The Dutch botanist Martinus Beijerinck dubbed it “a contagious living fluid.”

In the 1930s, the invention of powerful microscopes finally allowed scientists to see viruses. They found that viruses were unlike ordinary cells: they didn’t generate their own fuel; they didn’t grow or divide. Instead, viruses invaded cells, hijacking their biochemistry to make new copies of themselves. Being small and simple seemed like part of the viral way of life, allowing them to replicate fast.

It wasn’t until 2003 that a team of French researchers discovered the first giant virus. They had been puzzling over sphere-shaped objects that were the size of bacteria but contained no bacterial DNA. Eventually they realized that they were looking at a monstrously oversized virus, containing 979 genes.

Those first giant viruses were isolated from amoebae living in water from a cooling tower. Once scientists realized that viruses could be so large, they changed their search parameters and started finding other species in all manner of places, from swamps to rivers to contact lens fluid.

And along the way the biggest viruses got bigger. In 2011, Dr. Claverie and his colleagues set a new record with megaviruses, a type of giant virus with 1,120 genes they discovered in sea water off the coast of Chile. They then dug into the sediment below that sea water and discovered pandoraviruses, with more than twice as many genes.

Dr. Claverie speculates that pandoraviruses and other giant viruses evolved from free-living microbes that branched off from other life several billion years ago. “The type of cells they may have evolved from may have disappeared,” he said.

The idea that giant viruses represent separate branches on the tree of life is a controversial one that many other experts aren’t ready to embrace. “They provide no evidence for that notion, so it seems a distraction to me,” said T. Martin Embly, a professor of evolutionary molecular biology at Newcastle University.

Despite those reservations, Dr. Embly and other researchers hail pandoraviruses as an important discovery. “I think it’s wonderful that such crazy and divergent lifeforms continue to be discovered,” said Tom Williams, Dr. Embly’s colleague at Newcastle University.

The new study also drives home the fact that giant viruses are far from rare. Shortly after discovering pandoraviruses in sea floor sediment, Dr. Claverie and his colleagues found them in water from a lake in Australia, 10,000 miles away. “It definitely indicates that they must not be rare at all,” said Dr. Claverie.

Giant viruses may be so common, in fact, that they may be hiding inside of us, too. In a paper published online on July 2 in The Journal of Infectious Diseases, French researchers offered evidence that giant viruses dwell in healthy people. They isolated a new giant virus from blood donated by a healthy volunteer, and then found antibodies and other signs of the virus in four other donors.
Giant viruses may lurk harmlessly in our bodies, invading the amoebae we harbor. Whether they can make us sick is an open question. “I don’t believe we have the proof at the moment that these viruses could infect humans,” said Dr. Claverie.

“But again,” he added, “never say never.”

That’s wise advice when it comes to giant viruses.

July 22, 2013

**Post-Cure Hep C Reinfection Is Common Among Prisoners**

Prisoners who achieve a cure for hepatitis C virus (HCV) are at high risk for reinfection, especially if they continue to inject drugs on the inside. Reporting their findings in the journal Hepatology, researchers studied 119 prisoners who had achieved a sustained virologic response (SVR, considered a cure) following treatment for hep C.

The study population was 98 percent male, with a median age of 33, and 81 percent of them had a history of injection drug use (IDU). Following a median follow-up period of 1.4 years, nine of the participants were reinfected with hep C, with seven of them switching virus genotypes, for an overall reinfection rate of 5.27 cases per 100 person-years. The incidence of reinfection was 12.47 times higher among those actively using drugs compared with those not using post-treatment, 9.95 times higher among those coinfected with HIV compared with HIV-negative participants, and 7.47 times higher among those engaging in more than one risk behavior following treatment compared with those engaging in either one or no risk behaviors.

The investigators stressed that “preventative interventions at diagnosis and during and after HCV treatment should be strongly reinforced.”

To read the study abstract, [click here](#).

**'This Is a Public Health Failure': New York City’s Troubling Silence on Morning-After HIV Meds**

*By Tim Murphy*

Last Wednesday, in scorching midday heat, about twenty members of the longtime HIV/AIDS activist group ACT UP held a protest in front of Mt. Sinai Medical Center’s entrance at Madison Avenue and 100th St., their shirts soaked in sweat. They carried posters reading “Why Didn’t Your E.R. Know About HIV Morning-After Drugs?” and “How Many More Infected Before You Get It Right?” Walking in a circle, they chanted: “What do we want? PEP on demand. When do we want it? Now!”

Passersby, when I asked them, generally had no idea what PEP was. Nearby, Mt. Sinai staffers on their lunch break watched the protest impassively. “There was a mix-up that night,” said a female employee who asked not to be named. “We’ve never had a problem with this before.”

Well, what is PEP? Short for “post-exposure prophylaxis,” it is the practice of starting a month-long course of HIV meds within 72 hours of possible exposure to the virus to prevent permanent infection. In 2005, the U.S. Centers for Disease Control issued evidence of PEP’s effectiveness plus guidelines for PEP usage, and the New York State health department did the same in 2008 – and again as recently as this year – for ERs throughout the state, requiring them to administer PEP to medically qualified patients who request it.

The protest last week was a response to an incident involving a gay man who, according to ACT UP, went to the Mount Sinai Medical Center emergency room earlier this month after fearing he had been exposed to HIV during sex, asked for PEP, and had difficulty obtaining his starter dose. (The man wants to remain anonymous and ACT UP would not put me in touch with him.) As many as three Mt. Sinai ER staffers told the man there was no such treatment, says ACT UP, and it was only after the man called up the activist group, which then got in touch with a doctor with admitting privileges at Mt. Sinai, that the man was given PEP medication.

Mt. Sinai, for its part, has issued a statement claiming that the man “promptly received his initial dose of medication. The patient has returned to Mount Sinai for follow-up care to ensure the treatment’s success.” Mt. Sinai also included portions of an email it said the patient later sent them: “At the ER after the initial confusion over talking to the right person, at one point I may have spoken to a janitor on accident, I was checked in I was given the medicine within 20 minutes and then referred to [your] clinic so I think they will handle my costs without a problem.”

Regardless of the details of this one case, ACT UP alleges that people seeking PEP often face unnecessary hurdles in ERs. Around midnight one night this week, I spent an hour calling fifteen different
ERs throughout the asking if they had PEP. The person answering the phone in most cases didn’t know what “PEP” was until I explained it, at which point they told me that they had it, and that I should come in. Sometimes I had to talk to two or three people before someone knew what I was talking about.

ACT UP thinks that’s a sign there are gaps in the system. “The state has got to enforce their own guidelines,” said member Jim Eigo. “They’ve done a terrible job.”

But perhaps a bigger question is: Eight years into the CDC putting its stamp of approval on PEP as a measure to block HIV infection, why do so few people — especially gay men, who continue to make up the city’s highest rate of HIV infections — know what it is or where to get it? Especially in a city that ranks with L.A. and Miami as having the highest HIV rates in the country. Not to mention a city whose health department obviously cares about preventing HIV and has put considerable money and effort into widely distributing its own branded condoms.

A 2011 study done in gay bathhouses found that, while 63 percent of the men reported unprotected sex in the past 90 days, only 36 percent knew about PEP or PrEP (which is the practice of taking an HIV med all the time in order to block HIV infection, a bit like the Pill; the FDA approved a drug for PrEP last year).

The bathhouse study comes at a time when national HIV rates have flatlined overall but have risen 22 percent among young gay men, with young gay and bi- men of color most affected. The CDC recently calculated that, if current rates continue, half of young gay men will have HIV by age 50.

All the more reason to let more gay men know about PEP as a prevention option. However, "I've never seen a big PR push to make people aware of PEP," says Dr. Dan Egan, an emergency doctor at St. Luke’s-Roosevelt, whose PEP program follows up with ER visitors to make sure they get the full course of meds. "There should be. People should know about it. This has been a public-health failure."

According to several sources, there has never been a major poster campaign in New York City subways, bus shelters or gay venues, such as ones that have appeared in the U.K. or Australia.

The New York State Department of Health says that they are "in the process of developing consumer/public education materials" about PEP, but at deadline didn’t get back with further details.

The New York City Department of Health, which funds Mt. Sinai and five other health centers in the city to provide various HIV prevention tools (including, if they choose, PEP), has nothing about PEP on its HIV/AIDS page. Via email, the department said it was "in discussions to develop materials for broader awareness around [PEP] availability."

Gay Men’s Health Crisis (GMHC), the city’s best-known HIV/AIDS group, confirmed that they’ve never done one. "That could change," said the group’s Krishna Stone. Due to decreased budgets, she said, a campaign might take the form of flyers or palm cards rather than posters. She also said the group would create PEP ads for gay hookup apps like Grindr if the apps would give them free ad space.

Recently, GMHC ran information about PrEP on Grindr, the popular gay hookup app, but the app has never run something on PEP. Similarly, Next Magazine, the popular gay nightlife weekly, has never run a PEP ad, according to its editor, Alex Erikson.

Today, if you search "PEP HIV NYC" on Google, you come up with pepnow.org, a site founded by ACT UP member and Yale Med research intern James Krellenstein. There’s also pep411.com, a site started by St. Luke’s-Roosevelt HIV doctor Antonio Urbina, and GMHC’s page. All three point readers to places in the city where they can get PEP.

During the Mt. Sinai protest, ACT UP members pointed out that, from a public-health perspective, it’s far cheaper to give someone HIV meds for one month — an average cost of up to $1,000, but usually covered by insurance or an assistance program — than for a lifetime, the cost of which runs above $1 million.

"The city is sitting on a goldmine in terms of using PEP to find people at highest risk for HIV and connecting them to care," said Eigo.

But, he said, the city wasn’t doing all it could to get out the word about PEP.

Dr. Egan echoes that thought. "There are so many avenues now with easy access to large-scale populations — hookup apps, magazines, posters in bars. The city’s got their NYC condoms. They could put a message on them: 'If the condom breaks, go get PEP.'"

**Drug to Treat HIV/AIDS Side Effect Mired in Lawsuit**

*New York Amsterdam News* (07.18.2013) By Glenn Townes

Napo Pharmaceuticals has filed a lawsuit against Salix Pharmaceuticals that has delayed availability of the drug Fulyzaq, which the two firms partnered in 2008 to develop. The US Food and Drug Administration
(FDA) approved Fulyzaq in 2012 for the prevention of “excessive” diarrhea, a common side effect of HIV antiretroviral therapy (ART).

Salix announced in May that the company was working with FDA to expedite Fulyzaq distribution, and stated in June that the drug was available from Walgreens Specialty Pharmacies. However, many doctors treating HIV-infected people reported that their patients were unable to obtain Fulyzaq at Walgreens or any other pharmacy, according to Napo’s legal representative.

Until the legal issues are settled, patients with excessive gastrointestinal adverse reactions to ART will continue to suffer. One such individual is a Washington, DC, resident who reported that he has lived with HIV since the 1980s. He noted that ART successfully reduced his viral load to virtually undetectable levels, but he still had “severe intestinal and bowel issues” because of his drug regimen.

A new study from researchers at the University of Alabama at Birmingham and colleagues has examined the impact of aflatoxins on the AIDS epidemic in Africa. Aflatoxins are poisons produced by aspergillus fungi that can be found on damp grains, nuts, and beans, usually in hot humid climates. Federal law limits the allowable amount of these highly dangerous toxins in food to 20 parts per billion. High doses of aflatoxins can be deadly; exposure even to low doses could cause liver cancer. Aflatoxins also have been found to be immunosuppressive, possibly causing increased immunosuppression in HIV-positive individuals.

Because African countries rely heavily on several crops that develop aspergillus, researchers investigated the association between aflatoxins and HIV. They measured the blood levels of aflatoxins and the disease in 314 HIV-positive Ghanaians who had never been on antiretroviral therapy. Results showed that higher aflotoxin levels in participants’ blood often coincided with higher HIV blood levels, even for individuals with high levels of CD4 blood cells. These participants with high CD4 blood cells had not been infected long and were not eligible to begin antiretroviral therapy, under World Health Organization guidelines. Researchers believed that aflatoxins either produced proteins that contributed to HIV reproduction or reduced the number of white blood cells in some way, making the virus’s attack on the immune system more powerful.


**OraSure Founders Reunite for New Project** *Morning Call (Allentown, PA)* (07.18.2013) By Sam Kennedy
Two founders of Bethlehem, Pa.-based OraSure Technologies, maker of the OraQuick HIV test, will work together to create a TB test. In 1988, Sam Niedbala and Mike Gausling partnered with two other friends to form what would become OraSure. Niedbala’s new company, TB Biosciences, recently announced it received $1.5 million from a group of investors led by Originate Ventures, the venture capital fund co-founded by Gausling. TB Biosciences is working on a means of diagnosing the disease using antibodies found in the blood of patients with active TB. Niedbala believes that developing an improved TB test is part of the larger fight against HIV because so many HIV-positive individuals actually died of TB.

According to TB Biosciences, preliminary trials indicated a 90-percent accuracy rate for the company’s technology, which the New York University (NYU) School of Medicine has developed throughout the past two decades. “This would be a major breakthrough in tuberculosis testing and could go a long way to saving many lives each year,” said Frank Rimalovski, executive director of the NYU Innovation Venture Fund.

**Natural pest control protein effective against hookworm: A billion could benefit**
A benign crystal protein, produced naturally by bacteria and used as an organic pesticide, could be a safe, inexpensive treatment for parasitic worms in humans and provide effective relief to over a billion people around the world. Researchers from the University of California, San Diego, La Jolla, CA, report on this potentially promising solution in a study published ahead of print in the journal *Applied and Environmental Microbiology*.

Hookworms, and other intestinal parasites known as helminths infect more than 1 billion people in poverty-stricken, tropical nations, sucking the vitality from the body, and leaving hundreds of millions of
children physically and mentally stunted. Current drugs are insufficiently effective, and resistance is rising, but little effort has been made to develop better drugs because the relevant populations do not represent a profitable market for drug companies.

"The challenge is that any cure must be very cheap, it must have the ability to be mass produced in tremendous quantities, safe, and able to withstand rough conditions, including lack of refrigeration, extreme heat, and remote locations," says Raffi Aroian, a researcher on the study.

In earlier research, Aroian and his collaborators described a protein, Cry5B, that can kill intestinal nematode parasites—such as human hookworms—in infected test animals (hamsters). Cry5B belongs to a family of proteins that are generally accepted as safe for humans. These proteins are produced naturally in Bacillus thuringiensis (Bt), a bacterium which is applied to crops as a natural insecticide on some organic farms, and CryB proteins have been engineered into food crops such as corn and rice, to render them pest resistant.

As shown for the first time in this paper, Cry5B can also be expressed in a species of bacterium, Bacillus subtilis, which is closely related to Bacillus thuringiensis, and which is also related to bacteria which are present in some probiotics, says Aroian. In the current research researchers showed that a small dose of Cry5B, expressed in this bacterium can achieve a 93 percent elimination of hookworm parasites from infected hamsters. That, says Aroian, is substantially better than current drugs.

The scientific significance of the research, he says, is that "bacteria similar to those that are food grade—which are cheap and can readily be mass produced—can be engineered to produce molecules that can cure parasitic diseases."

Study Lays Groundwork for Norovirus Anti-Viral Treatments
July 22, 2013 — An animal model of the human norovirus created at the University of Michigan Health System lays the groundwork for understanding the biology of the pesky virus and developing antiviral drug treatment.

Well-known as the virus that impacts cruise ship vacations, norovirus leads to misery on land too. The virus spreads quickly from person to person in any closed-in space, such as schools, nursing homes, or day-care centers.

"The first virus in this group was discovered in 1972 following a disease outbreak at a school in Norwalk, Ohio in 1968. Since then research has been underway to culture noroviruses in the laboratory and develop animal models," says lead researcher Christiane Wobus, Ph.D., assistant professor in the Department of Microbiology and Immunology at the University of Michigan Medical School.

An international group of scientists from the U.S. and Germany authored the study published in mBIO, a journal of the American Society of Microbiology.

"Norovirus research has been hampered by the absence of a norovirus cell culture and a genetically manipulable small animal model," Wobus says. "This new model gives us the tool to test potential antiviral compounds and may lay the foundation to culture these viruses in the lab."

The new model was developed by determining whether human noroviruses can infect "humanized" mice, that is mice containing human immune cells. These mice are widely used for study of the human immunodeficiency virus (HIV), a virus which can only infect human cells.

The study identified macrophages, a vital immune cell in the body, as the cell type infected by the virus.

Very few particles of the virus can lead to infection. Estimates are as few as 18 particles can cause gastroenteritis (inflammation of the stomach and intestines) and lead to diarrhea, vomiting and stomach pain. In the U.S. norovirus causes approximately 21 million cases of acute gastroenteritis a year, and 800 deaths.

"Most people can cope with the symptoms, but deaths are more likely among the elderly mainly because of dehydration," Wobus says.

Only the common cold is more widespread than the norovirus, which can remain on surfaces for weeks, ready to cause more infections. Because it lacks a lipid envelope, norovirus is not susceptible to common disinfectants and alcohol-based sanitizers.

The economic impact of these infections is staggering with an economic cost for norovirus associated food-borne outbreaks alone of $5.8 billion in the U.S.

There is no vaccine for preventing norovirus infection and no drug to treat it. But the Centers for Disease Control and Prevention offers some tips for prevention, including handwashing with soap and
water, washing fruits and vegetables properly and cleaning and disinfecting surfaces, and if you are sick not preparing food or caring for others.

**Journal Reference:**

**A Bad Alliance: Rare Immune Cells Promote Food-Induced Allergic Inflammation in the Esophagus**

July 21, 2013 — Food is an integral part of life; but, for some, it can be harmful. Allergic inflammation caused by inappropriate immune responses to some types of food has become a major public health issue. Over the past ten years, the prevalence of food allergies has increased by nearly 20 percent, affecting an estimated six million people in the U.S.

Eosinophilic esophagitis (EoE) is a food allergy-associated disease that affects children and adults and is caused by inflammation in response to such trigger foods as eggs, nuts, milk, wheat, and soy. Inflammation of the esophagus, as seen in EoE patients, can eventually lead to debilitating esophageal dysfunction, causing difficulty in swallowing, esophageal fibrosis, and food impaction. However, current treatment options for EoE, including adherence to strict diets, are non-specific and disruptive to patients' lifestyle.

Until recently, the mechanisms underlying the development of EoE were unclear, but a new study from the Perelman School of Medicine at the University of Pennsylvania and The Children’s Hospital of Philadelphia (CHOP) shows that a type of rare immune cell and specific reactions to allergenic foods team up—in a bad way—to cause EoE. However, this association does point to new ways to possibly treat inflammation associated with EoE.

The presence of large populations of immune cells in the esophagus of human patients with EoE suggests that the immune system might contribute to the pathogenesis of this disease. In earlier work, researchers from CHOP, along with collaborators at Cincinnati Children’s Hospital, found that genetic mutations in the gene that encodes for thymic stromal lymphopoietin (TSLP), a protein that is produced by epithelial cells that line the esophagus and directs the activities of various types of immune cells, are highly associated with EoE in children. These results suggested that TSLP played an important role in the development of this disease, but how this factor contributed to esophageal inflammation in response to food was unknown.

Now, David Artis, PhD, associate professor of Microbiology at Penn, two postdoctoral researchers in the Artis lab, Mario Noti, PhD and Elia Tait Wojno, PhD, and colleagues, have identified one mechanism by which TSLP might contribute to the development of EoE. They describe their work this week online ahead of print in *Nature Medicine*.

Using a mouse model of EoE, Artis’s group found that sensitization to egg and peanut protein, in association with increased levels of TSLP, led to the mobilization of a rare type of immune cell called basophils. In healthy people, these cells comprise less than 1 percent of the total immune cells in the body.
In EoE, however, these rare cells pack a punch—when mice with EoE were treated with therapeutic reagents that limited TSLP and basophil responses to food allergens, esophageal inflammation in these animals improved dramatically. "The use of this new mouse model has revealed that TSLP production, and the resulting basophil responses, may be critical in promoting EoE in response to exposure to allergy-triggering foods," says Noti.

Supporting experiments in mouse models, the research team also found exaggerated TSLP and basophil responses in the esophageal biopsy tissues of pediatric and adult patients with EoE. What's more, pediatric EoE patients with a genetic mutation in the TSLP gene were more likely to have increased basophil responses in their blood compared to EoE patients that lacked this mutation. "The identification of TSLP and basophil responses in the esophagus and peripheral blood of human patients with EoE supports our mouse model studies and indicates that these factors may play a key role in EoE in patients," says Tait Wojno.

The findings from both mouse and human studies suggest that TSLP and basophils may promote the development of inflammation in the esophagus in response to foods that trigger an allergic response in some individuals, or that TSLP and basophils could contribute to the persistence of inflammation. These factors could potentially be targeted using novel therapeutics to treat EoE in patients, say the researchers. "Although more research is required, these studies suggest that we may be able to target TSLP and basophils to treat esophageal inflammation associated with EoE," adds Artis.

**Journal Reference:**
Mario Noti, Elia D Tait Wojno, Brian S Kim, Mark C Siracusa, Paul R Giacomini, Meera G Nair, Alain J Benitez, Kathryn R Ruymann, Amanda B Muir, David A Hill, Kuulakwase R Chikwawa, Amin E Moghaddam, Quentin J Sattentau, Meera G Nair, Alain J Benitez, Kathryn R Ruymann, Mario Noti. Thymic stromal lymphopoietin—elicited basophil responses promote eosinophilic esophagitis. Nature Medicine, 2013; DOI: 10.1038/nm.3281

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**Evolutionary Forensics Used On Hepatitis C Virus Outbreak**
July 19, 2013 — The rapid molecular evolution of hepatitis C virus (HCV) has been used to help incriminate the source of an outbreak in two Spanish hospitals in the late nineties. The evolutionary techniques used, described in BioMed Central's open access journal BMC Biology, also helped separate those who were infected by the person in question from those infected elsewhere during the same time period.

In the days before deep sequencing became a cheap option scientists used partial sequencing of HCV to help convict an anesthetist of infecting 275 patients with this virus. Back in 1998 an anesthetist was alleged to have injected himself with opioid painkillers, using some of the dose meant for his patients, before giving them the rest using the same needle and syringe. It is only now, after the experts' testimony and appeals, that the science used to track the outbreak and the spread of the virus is being made public. The main difficulty in establishing a link between the source and the infected patients is that the virus continues to evolve in its host. Also unlike HIV, HCV can remain silent in an infected patient for years even though it is still capable of being transmitted.

Prof Fernando González Candelas from the Universidad de Valencia, who led this multicentre study explained, "We sequenced 322 patients who were suspected to have been infected by the donor and 44 local, unrelated controls. Our analysis of over 4000 sequences from the E1-E2 region of the viral genome allowed us to exclude 47 patients as having been infected elsewhere. Because we knew the dates of infection for some patients we were able to use their data to validate a molecular clock and construct an estimated date of infection for each patient and of the source."

The patients were all infected between 1988 and 1998 shortly after the estimated date of infection of the source.

Improvements in sequencing techniques and computing now make it easier to obtain whole viral genomes and the phylogenetic analysis of RNA viruses, especially the molecular clock technique, is increasingly used today to analyze disease outbreaks in order to help plan control measures.

Prof González Candelas continued, "Naturally, there are very limited possibilities for a single infected patient of infecting so many recipients, but the recent case of a medical technician in New Hampshire (USA), and eight other states, shows that more such events might be revealed."

**Journal Reference:**
Antibiotic-Resistant Bacteria Widespread in Hudson River, Study Finds

July 19, 2013 — The risk of catching some nasty germ in the Hudson River just started looking nastier. Disease-causing microbes have long been found swimming there, but now researchers have documented antibiotic-resistant strains in specific spots, from the Tappan Zee Bridge to lower Manhattan. The microbes identified are resistant to ampicillin and tetracycline, drugs commonly used to treat ear infections, pneumonia, salmonella and other ailments.

The study is published in the current issue of the Journal of Water and Health.

"If you find antibiotic-resistant bacteria in an ecosystem, it’s hard to know where they’re coming from," said study co-author Andrew Juhl, a microbiologist at Columbia University’s Lamont-Doherty Earth Observatory. "In the Hudson, we have a strong case to make that it’s coming from untreated sewage."

On repeated visits to 10 locations on the Hudson, the researchers found microbes resistant to ampicillin 84 percent of the time, and resistant to tetracycline 38 percent of the time. The stretches harboring the most sewage-indicator bacteria also generally contained the most antibiotic-resistant ones. These were led by Flushing Bay, near LaGuardia Airport, followed by Newtown Creek, on the border of Brooklyn and Queens; and sewage outfall pipes near Piermont Pier in Rockland County, N.Y.; West 125th Street in Manhattan; and Yonkers, in Westchester County, N.Y.. The antibiotic-resistant bacteria found include potentially pathogenic strains of the genera Pseudomonas, Acinetobacter, Proteus and Escherichia.

"They could be difficult to treat in people with compromised immune systems," said Dr. Stephen Morse, an infectious disease epidemiologist at Columbia's Mailman School of Public Health, who was not involved in the study. "If I were inclined to swim in the Hudson, quite truthfully I’d look to this paper for the places to stay away from."

Though people routinely catch infections while swimming, only severe illnesses are typically treated with antibiotics. And an antibiotic-resistant infection would be noted only if the illness failed to respond to treatment — a scenario that probably happens, but is not well documented or reported, said Morse. One exception was an outbreak on the Indonesian island of Borneo in 2000 when 32 athletes competing in a swimming event in the Segama River came down with leptospirosis. Transmitted by animal urine, the infection is marked by fever, chills and pink eye.

Previous studies in the Hudson have shown that microbe counts go up after heavy rains, when raw sewage is commonly diverted into the river. Some 27 billion gallons of raw sewage and rainwater are released into the Hudson each year by wastewater treatment plants. Lacking the capacity during heavy rains to simultaneously pump runoff from city streets and sewage from buildings, many sewage-treatment plants are forced to divert both streams into the river, in what is known as a combined-sewer overflow, or CSO. In an ongoing partnership with the environmental group Riverkeeper, scientists at Lamont-Doherty and Queens College at the City University of New York have been tracking water quality in the Hudson and making their results public on Riverkeeper’s website. Their work has confirmed that CSOs remain a serious problem, even though the Hudson is generally cleaner than it has been in the past.

The Hudson has gotten so much better," said the study’s lead author, Suzanne Young, a former student at Lamont and Queens College, now at the University of South Florida. "If we came up with a sustainable solution, water quality could continue to improve."

This is not the first time that antibiotic-resistant bacteria have been found in a river. A 2002 study in the journal Emerging Infectious Diseases found ampicillin-resistant bacteria in the Hudson, as well as 15 other U.S. rivers, including the Mississippi, Ohio and Colorado. However, this is the first study to firmly link specific microbes to sewage in the Hudson, and to compare results at different locations.

It is not just a matter of swimming safely. Rivers can incubate bacteria, allowing them to transfer their drug-resistant genes to normal bacteria. "If these resistant genes are transferred, they can develop into disease-causing bacteria," said Ronald J. Ash, a microbiologist and professor emeritus at Washburn University, lead author of the 2002 paper.

Bacteria can also play an important role in the environment. As more antibiotic-resistant microbes replace native bacteria, those changes could eventually have an impact on plants and animals. "Microbial communities can affect the health of the entire ecosystem," said Young, who is now studying how Mississippi water snakes respond to infection with antibiotic-resistant pathogens.

Antibiotic resistance has become a public health crisis. About 100,000 people die each year from hospital-acquired infections, most of which are due to antibiotic-resistant pathogens, according to the...
Infectious Diseases Society of America. Superbugs resistant to methicillin kill about 19,000 people each year, more than HIV/AIDS. The development of resistance has been linked to overuse of antibiotics to treat minor infections in humans, and to industrial feedlots, where low levels of antibiotics are fed to chicken, cattle and pigs to promote growth and prevent infection. The Natural Resources Defense Council estimates that 80 percent of antibiotics in the U.S. are fed to livestock.

There are signs that the tide is turning, at least in the Hudson. In a landmark deal with the state, New York City agreed last year to spend $187 million to replace some parking lots and city streets with porous pavement, and to plant more vegetation on rooftops and other impervious surfaces to reduce runoff. An additional $2.4 billion will be spent on infrastructure to eliminate 1.5 billion gallons of CSOs by 2030. "There's now a timeline for answering the question, 'How much sewage overflow reduction is needed and when?' " said Larry Levine, a senior attorney at the Natural Resources Defense Council, which pushed for the settlement.

Public awareness may also help. In 2012, New York Gov. Andrew Cuomo signed the Sewage Pollution Right to Know Law requiring public notification of sewage spills in New York waters. Not long after the law passed, Westchester County announced a "controlled discharge" at Sleepy Hollow, sparking a debate about whether the National Ironman competition should cancel its swimming leg 15 miles to the south. (The swim went ahead as planned).

"The results from this study are significant because they help us to understand the processes involved in the spread of antibiotic resistant bacteria through the environment, but also because they provide added incentive to reduce sewage pollution into our waterways" said coauthor Gregory O'Mullan, a microbiologist with joint appointments at Lamont and Queens College who oversees the laboratory where the study was done.

**Journal Reference:**

**HIV/AIDS Vaccines: Defining What Works**
July 18, 2013 — Designing an effective HIV/AIDS vaccine is something of a paradox: a good vaccine would be safe and look enough like HIV to kick-start the immune system into neutralizing the virus—but the problem is that this is exactly what the human immune system has trouble doing even when it's exposed to the real thing.

Now a team of researchers led by scientists at The Scripps Research Institute in La Jolla, CA has developed a strategy for inducing a key part of an effective immune response to HIV. By tracing the evolution of HIV-recognizing molecules called antibodies taken from the blood of rare individuals whose immune systems are naturally able to target and neutralize the virus, they may have found a way to replicate this for everybody.

At a talk next week at the American Crystallographic Association meeting in Hawaii, the team will present multiple crystal structures, which like detailed architectural blueprints show how the virus interacts with components of the immune system. Examining these structures has allowed them to reverse engineer molecules that specifically activate the precursors of effective, neutralizing antibodies against the virus—molecules that may be components of a future vaccine against HIV.

"What we tried to do was to learn how those [effective] antibodies developed over the course of natural infection and attempt to guide the immune response in the direction of what we know works in certain HIV-infected individuals," said structural biologist Jean-Philippe Julien, who is presenting the work in Hawaii.

He conducted the research under the direction of Professors Ian Wilson and William Schief of The Scripps Research Institute. The work was funded by the International AIDS Vaccine Initiative Neutralizing Antibody Center, the Scripps Center for HIV/AIDS Vaccine Immunology and Immunogen Discovery and the National Institute of Allergy and Infectious Diseases (one of the National Institutes of Health). Additional support was provided through a Canadian Institutes of Health Research fellowship.

Julien cautioned that the work might not, by itself, be the final answer that shows how to make an effective HIV/AIDS vaccine—but it is a step in the right direction. Most likely, Julien said, any future HIV/AIDS vaccine would combine multiple biological components in order to give the broadest possible protection against the virus.

He added that their candidate molecule was able to achieve the desired immune reactions in the test tube, and they are currently testing it in animals to see if it is able to kick-start the desired immune response. If those experiments go well, he said, further studies will examine whether it can protect...
animals against infection, and human trials for safety and vaccine efficacy would be next—though it may be years before those results are known.

While designing a vaccine against any pathogen is a long, hard process, HIV has been particularly difficult, and despite decades of efforts and hundreds of millions of dollars spent in the process, we still do not yet have an effective vaccine that can prevent infection.

**Every Three Hours Someone in the Philippines Gets HIV**
By Anjani Trivedi, July 24, 2013

Chris Lagman vividly remembers the night that the human immunodeficiency virus (HIV) came into his life. It was two years ago, and the LGBT activist was at a pre-Christmas dinner party in Quezon City with dozen gay Filipino professionals. During the course of the evening, a fellow guest and close friend asked if he could speak privately to Lagman. He told Lagman that he had recently tested positive for HIV. Over the next few months the young man seemed to vanish from their social circle. The next time Lagman saw him was at his funeral, where relatives said the friend had died from a “mysterious” ailment. That was the first of many funerals Lagman would attend in 2011 as people he knew began to succumb to complications arising from HIV. “I would hear from other friends, [they would ask] why is it that suddenly a lot of my friends are passing away,” Lagman says.

Although there is still a low prevalence of HIV in the Philippines, with just under 24,000 cases reported in 2012, transmission is now growing rapidly. A decade ago, a new case of HIV was being reported every three days. These days, it’s every three hours—a terrifying statistic that makes the Southeast Asian nation one of only nine in the world where transmission is on the rise. The latest data from the Department of Health shows 415 new HIV cases in May, a 52% spike over the same period last year and the highest ever since 1984. According to the department, 9 out of 10 new cases are men, mostly under 30. The reason, many health workers say, is poor “prevention coverage”—things like the use of condoms and the availability of HIV testing—coupled with an increase in risky behavior. (Even Lagman admits to an unprotected sexual encounter in 2008 with a man he knew to be HIV positive. Fortunately Lagman later tested negative.)

It doesn’t help that overbearing cultural and social stigma is attached to HIV and homosexuality in the Philippines. “It’s almost like a refusal to acknowledge that this is a sexually related disease and that we have to acknowledge that people have sex,” Senator Pia Cayetano tells TIME. “Seriously, it’s as basic as that.” Lagman, who also used to have a popular blog with thousands of gay readers, believes that this stigma helps the epidemic to spread by driving gay sex underground, fueling risky behavior and forcing gay men to act in a “very stealthy and in a very shadowy way.”

The government’s response, in the form of the Reproductive Health Bill that covers the “prevention, treatment and management” of HIV and AIDS, among other sexually transmitted diseases, was stymied earlier this year when the apex court of this deeply Catholic nation held up the bill’s implementation after conservatives and religious groups filed objections. “The Reproductive Health Bill, if passed into law in its present form, will put the moral fiber of our nation at risk,” the national Catholic Bishops’ Conference of the Philippines said in a statement.

The Philippines already has an AIDS prevention and control law that mandates education programs and monitoring systems. However, activists say the 15-year-old law is antiquated, given the changing nature of the disease. “When the HIV law was enacted in 1998, it was designed to address an epidemic for overseas Filipinos and among sex workers,” Jonas Bagas, head of a sexual- and health-rights NGO in the Philippines, tells TIME. “That’s not the situation right now.” Bagas says intervention programs for stigmatized communities—MSMs [men who have sex with men], transgender, freelance sex workers, injection drug users and drug users in general—need to be intensified. If the Reproductive Health Bill is upheld by the Supreme Court, the situation should improve. Reproductive and sexual health care will be offered at village-level and free condoms provided.

Other health issues compete for scarce funds and government attention, however. Only about $15 million a year is spent on AIDS-related programs (far less than the dollar per head of population recommended by the Report of the Commission on AIDS in Asia in 2008). Peter Mosende of UNAIDS points out that containing HIV and AIDS will not become a high policy priority while diseases like tuberculosis, which kills 75 people a day in the Philippines, rampage through the country. But things could change fast. Estimates suggest that there will be 50,000 people with HIV in the Philippines in the next three years, with more than 50% of them in need of treatment, unless remedial measures are taken...
now. HIV testing needs to be drastically expanded, for example. It currently reaches less than 5% of intravenous drug users and MSMs — the two groups most prone to infection.

Chris Lagman saw his own HIV test as a rite of passage. “It was such a horrible experience. It was kind of an outing experience for me,” he remembers. Not long afterward, Lagman, with a group of friends, started an NGO called Love Yourself. “When people take care of themselves and they love themselves, they will stay away from risky behavior that can expose them to the virus,” he says, explaining the group’s name. There’s no doubt that a little love can go a long way, but in the fight against HIV the Philippines needs more. It needs money, political will and a readiness to dismantle decades of stigma.

A quick test for the Black Death
Sugar-based detection method enables easy and accurate identification of the Yersinia pestis bacterium
July 24, 2013
Diagnosing the presence of Yersinia pestis, the cause of plague, may soon be easier than ever before. Scientists working with Peter Seeberger, Director at the Max Planck Institute of Colloids and Interfaces (MPIKG) in Potsdam and Professor at the Freie Universität Berlin, have come up with a simple, inexpensive and reliable method of detecting the bacterium. The research team, specialising in glycochemistry glycobiology, first identified and synthesised an oligosaccharide structure on bacterial surface before combining it with a protein to heighten the immunological effect. The presence of antibodies against this surface glycan in the blood of infected patients can be a biomarker of diagnostic value in Yersinia pestis infections. The Potsdam-based scientists also used the antigen to create antibodies which can directly detect the plague pathogen in infected samples.

The Black Death is best known as a devastating medieval disease which affected Europe, Central Asia and China. The plague killed more than 200 million people through the ages. Yet it is by no means completely eradicated. In 2002 there was an outbreak of plague in the Indian state of Himachal Pradesh, and 2008 saw 18 cases reported in Madagascar. Ziketan, a city in north-west China, was quarantined after an outbreak in 2009, and in the same year there were 16 cases in Tobruk, Libya. Cases are also repeatedly reported in New Mexico, USA. As it is extremely infectious and indeed deadly, plague is one of the most dangerous bioweapons.

Although plague can be treated with antibiotics, survival rates decrease with every hour the disease remains undetected. Left untreated, plague can often lead to death within a very short time, depending on the disease type. “Early identification of an infection is of paramount importance for survival,” says Chakkmukal Anish, Leader of the Glycobiology Research Group at the Max Planck Institute in Potsdam, “So our work may have direct and positive consequences on patient survival rates.”

Antibodies are created with a glycoprotein as antigen
In order to specifically detect the plague pathogen, the scientists first had to identify an oligosaccharide in a lipopolysaccharide on the surface of Yersinia pestis. This oligosaccharide would serve as a specific antigen. They then synthesised the complex compound in a multi-step process. Subsequently, the chemists bound the sugar molecule to a protein which is used in many vaccines to heighten the immune reaction. The resulting glycoprotein produced by the sugar-protein compound was used to trigger an immune reaction in mice. The scientists used this circumstance to create antibodies to the plague pathogen using murine immune cells.

The antibodies can identify plague bacteria with high selectivity (accuracy) without the result being distorted by other bacteria biochemically related to plague. Thus the scientists have effectively produced a quick test for the Black Death. There are, in fact, many ways of using this particular research finding in medical practice. On the one hand, the glycan or its glycoconjugates can be applied to test strips where it acts as an antigen and catches antibodies from the blood of infected patients. The antigen-antibody complexes are very easy to detect with fluorescing proteins. On the other hand, the antibodies could provide a way of directly detecting the plague pathogen in infected tissue. Here, too, fluorescing proteins are used to identify whether the antibodies have docked onto the bacterial surface.

Advances in glycomics have a practical value in medicine
“These reliable tests are simple and economical to administer,” says Peter Seeberger. This gives the new approach major advantages over the testing methods used to date. In the past, plague pathogens were detected by phenotyping or gene testing. The problem with these methods is that they are complex, expensive and slow—and, what’s more, they have a high failure rate.
The new method is a direct result of research successes in glycomics. This field is dedicated to the study of carbohydrates, which includes all sugars, and their role in biology. Scientists are now able to identify and synthesise ever more complex carbohydrate molecules. “We have the ability to synthesise complex molecules from simple chemical building blocks, much like children using Lego bricks to build a spaceship,” explains Chakkumkal Anish. “This is just the start – we have only just begun exploiting the opportunities this brings.” The chemical methods signify much more than just scientific advances. They also help scientists to come up with new methods of diagnosis and treatment, and to develop vaccinations for various diseases. “Basic research has an intrinsic value,” says Peter Seeberger. “But in the field of glycomics, we are increasingly able to translate our research directly into applications with a practical value, very much like the value our latest development has for the medical world.”

Pressurized virus blasts its infectious DNA into human cells
The virus that causes those painful lip blisters known as cold sores has an internal pressure eight times higher than a car tire, and uses it to literally blast its infectious DNA into human cells, scientists are reporting in a new study. Discovery of the pressure-driven infection mechanism — the first in a human virus — opens the door to new treatments for viral infections, they add in a study in the Journal of the American Chemical Society.

Alex Evilevitch and colleagues point out that the viruses responsible for influenza, AIDS and other infections that affect millions of people annually are quick to develop resistance to drugs that target viral proteins. Through genetic mutations, these proteins can quickly disguise themselves and evade anti-viral drugs. That has led to a search for vulnerabilities that don’t involve viral proteins. Evilevitch’s team looked at the pressure inside the herpes simplex virus 1 (HSV-1), the virus that causes cold sores.

They describe how HSV-1 enters cells, docks with portals on the nucleus and injects DNA with high pressure caused by tight packing of the capsid, the tough shell that houses the viral genome. Researchers already knew that several viruses that infect bacteria, called bacteriophages, use the same high-pressure mechanism to shoot their DNA into bacteria nuclei. Evilevitch and colleagues conclude that evolution has preserved this effective technique as a key step in viral infection — making it a desirable target for future treatments to defeat HSV-1 and other viruses that work the same way. The same mechanism exists in eight related viruses, including those responsible for mononucleosis and chickenpox in children, and shingles in adults. Drugs that interfere with it thus could limit “the potential for development of drug resistance that can occur due to rapid adaptive mutations of viral genomes,” the scientists state.

Changes in Gut Bacteria May Promote Inflammation and HIV Disease Progression
Published on Wednesday, 24 July 2013 00:00
Written by Liz Highleyman
Changes in intestinal bacteria may contribute to disease progression and development of non-AIDS conditions in people with HIV, even those on effective antiretroviral therapy (ART), according to a report in the July 10, 2013, issue of Science Translational Medicine.

A growing body of evidence indicates that chronic HIV infection is associated with greater risk and earlier development of non-AIDS conditions such as cardiovascular disease, which are linked to persistent inflammation and ongoing immune activation.

Ivan Vujkovic-Cvijin, Susan Lynch, Joseph McCune, and colleagues from University of California at San Francisco compared populations of gut bacteria in treated and untreated people with HIV infection and healthy HIV negative individuals.

HIV infection is characterized by dysregulation of the intestinal immune barrier and translocation—or spilling out of the gut—of bacteria and microbial products that stimulate ongoing immune activation and a state of chronic systemic inflammation, the researchers noted as background.

"We thought the gut microbiome might be different in HIV-infected individuals, and that the high degree of immune activation in the patients might be associated with and possibly due to the presence of specific members of the bacterial community," Lynch explained in a UCSF news release.

The authors collected rectal tissue biopsy samples from 6 untreated HIV positive people with active infection, 1 HIV positive long-term non-progressor, 18 people with HIV on ART with undetectable plasma viral load and varying degrees of immunological recovery, and 9 HIV negative individuals matched for other health risks. All were men.
Results

- Total levels of intestinal bacteria were similar regardless of HIV status.
- High-resolution bacterial genetic profiling identified "a dysbiotic mucosal-adherent community," or abnormal population of bacteria on the intestinal mucosa, in untreated HIV positive individuals, which contained more Proteobacteria but fewer Bacteroidiaan Clostridia species.
- In particular, people with HIV had more pro-inflammatory and opportunistic bacteria including Salmonella, Escherichia (i.e., E. coli), Serratia, Shigella, Klebsiella, Staphylococcus, Pseudomonas, and Campylobacter species.
- Bacterial "dysbiosis" was evident even among HIV positive people on suppressive ART.
- Changes in bacterial populations in people with HIV were not strongly associated with CD4 T-cell counts or levels of HIV RNA or DNA in the gut.
- However, these changes were associated with elevated T-cell activation, mucosal disruption (including levels of indoleamine 2,3-dioxygenase, an enzyme that can impair the gut's ability to act as a barrier), and plasma biomarkers of inflammation.
- Among HIV positive people on ART, the extent of dysbiosis correlated with tryptophan catabolism through the kynurenine pathway and plasma concentrations of the inflammatory cytokine interleukin 6, which are 2 established markers of disease progression.

"These observations demonstrate a link between mucosal-adherent colonic bacteria and immunopathogenesis during progressive HIV infection that is apparent even in the setting of viral suppression during [ART]," the authors concluded. "This link suggests that gut-resident microbial populations may influence intestinal homeostasis during HIV disease."

These findings point to a vicious cycle in which gut inflammation leads to bacterial abnormalities, which in turn promote chronic systemic inflammation, as previously seen in mice.

The researchers do not believe there is a single bacterial species responsible for disrupting intestinal integrity, nor do they propose specific probiotic therapies to restore a healthy gut, according to the UCSF release, though Lynch said manipulating bacterial populations is a promising idea.

"It appears that changes in the microbiome perpetuate a vicious cycle that drives inflammation in HIV-infected patients," she said. "We are considering a restoration ecology approach to restore appropriate microbial colonization patterns and healthy functioning of the gut microbiome."

"Our dream is to be able to make the virus go away, allowing HIV-infected people to lead longer lives without the need for life-long therapy," McCune added. "Perhaps restoring the microbiome to normal will be one strategy to make that happen." 7/24/13

Reference

Other Sources

Bacterial Blockade: How Gut Microbes Can Inactivate Cardiac Drugs

July 25, 2013 — For decades, doctors have understood that microbes in the human gut can influence how certain drugs work in the body—by either activating or inactivating specific compounds—but questions have remained about exactly how the process works.

Harvard scientists are now beginning to provide those answers.

In a paper published July 19 in Science, Peter Turnbaugh, a Bauer Fellow at the Center for Systems Biology in the Faculty of Arts and Sciences (FAS), and Henry Haiser, a postdoctoral fellow, identify a pair of genes that appear to be responsible for allowing a specific strain of bacteria to break down a widely prescribed cardiac drug into an inactive compound, as well as a possible way to turn the process off.

"The traditional view of microbes in the gut relates to how they influence the digestion of our diet," Turnbaugh said. "But we also know that there are over 40 different drugs that can be influenced by gut microbes. What's really interesting is that although this has been known for decades, we still don’t really understand which microbes are involved or how they might be processing these compounds."

To answer those questions, Turnbaugh and his colleagues chose to focus on digoxin, one of the oldest known cardiac glycosides. The medicine is typically prescribed to treat heart failure and cardiac arrhythmia.
"It's one of the few drugs that, if you look in a pharmacology textbook, it will say that it's inactivated by gut microbes," Turnbaugh said. "John Lindenbaum's group at Columbia showed that in the 1980s. They found that a single bacterial species, *Eggerthella lenta*, was responsible."

Researchers in the earlier study also tried—but failed—to show that testing bacterial samples from a person's gut could be used to predict whether the drug might be inactivated.

"To some degree the research was stalled there for a number of years, and the findings in our paper help to explain why," Turnbaugh said. "Originally, it was hoped that we would simply be able to measure the amount of *E. lenta* in a person's gut and predict whether the drug would be inactivated, but it's more complicated than that."

Beginning with lab-grown samples of *E. lenta*—some cultured in the presence of digoxin, some in its absence—Turnbaugh and Haiser tested to see if certain genes were activated by the presence of the drug.

"We identified two genes that were expressed at very low levels in the absence of the drug, but when you add the drug to the cultures ... they come on really strong," Turnbaugh said. "What's encouraging about these two genes is that they both express what are called cytochromes—enzymes that are likely capable of converting digoxin to its inactive form."

Though he warned that more genetic testing is needed before the results are definitive, Turnbaugh said other experiments support these initial findings.

The researchers found only a single strain of *E. lenta*—the only one that contained the two genes they had earlier identified—was capable of inactivating digoxin. In tests using human samples, bacterial communities that were able to inactivate the drug also showed high levels of these genes.

"We were able to confirm that simply looking for the presence of *E. lenta* is not enough to predict which microbial communities inactivate digoxin," Turnbaugh said. "We found detectable *E. lenta* colonization in all the human fecal samples we analyzed. But by testing the abundance of the identified genes we were able to reliably predict whether or not a given microbial community could metabolize the drug."

In addition to being able to predict whether a given microbial community would inactivate the drug, Turnbaugh and colleagues identified a possible way to halt the process.

"It was previously shown that in the lab *E. lenta* grows on the amino acid arginine and that as you supply more and more arginine, you inhibit digoxin inactivation," he said.

Tests conducted with mice showed that animals fed a diet high in protein, and thereby arginine, had higher levels of the drug in their blood than mice fed a zero-protein diet.

"We think that this could potentially be a way to tune microbial drug metabolism in the gut," Turnbaugh said. "Our findings really emphasize the need to see if we can predict or prevent microbial drug inactivation in cardiac patients. If successful, it may be possible someday to recommend a certain diet, or to co-administer the drug with an inhibitor like arginine, ensuring a more reliable dosage."

**Journal Reference:**
Researcher Digs Into the Contested Peanut-Allergy Epidemic

July 25, 2013 — The path of the peanut from a snack staple to the object of bans at schools, day care centers and beyond offers important insights into how and why a rare, life-threatening food allergy can prompt far-reaching societal change, according to a Princeton University researcher.

Before 1980, peanut allergies were rarely mentioned in medical literature or the media, said Miranda Waggoner, a postdoctoral researcher at the Office of Population Research in the Woodrow Wilson School of Public and International Affairs. Her article on the subject, "Parsing the peanut panic: The social life of a contested food allergy epidemic," was published recently in the journal Social Science & Medicine.

Starting around 1990, articles in medical journals began discussing the seriousness of peanut allergies, Waggoner said. At the same time, advocacy groups were emerging to raise awareness of the issue. By the mid-1990s, newspapers were printing articles with headlines such as "Nut Allergy Girl's Terror; Girl Almost Dies from Peanut Allergy."

And the 21st century brought descriptions of peanut allergies—in medical journals and the media—as an epidemic.

For those with a peanut allergy, ingesting the legume can lead to anaphylactic shock and, if untreated, death. But the allergy is quite rare and it isn't clear whether it is becoming more common, Waggoner said.

The increased focus on peanut allergies in the medical community, the media and society in general combined to push changes like peanut bans in schools, Waggoner said.

"All of this was happening at about the same time to produce this big societal problem that is based on what is a small problem in terms of the population affected," Waggoner said. "One physician has written that the same number of people die each year from peanut allergies as from lightning strikes, yet the perception of peanut allergy risk has invaded the common social spaces we all inhabit—airlines, day cares and schools."

In 2002, Massachusetts became the first state to enact guidelines for the management of food allergies in schools, calling for "peanut-free" tables in the lunchroom under some circumstances. Many schools and day care centers have banned peanuts, and some baseball parks now offer peanut-free zones.

"This was part of a broader concern about food risks, changing perceptions of food production, as well as changes in the way we think about child risk," Waggoner said. "If you ask adults about peanut allergies..."
when they were in school, most of them will say it wasn’t an issue. Peanut butter and jelly sandwiches were a staple, healthy snack. It’s the classic American kid snack.

"The fact that this sort of mundane food is under attack is really a potent moment for us as a society," Waggoner said. Several factors make it difficult to assess the prevalence of peanut allergies or whether the problem is becoming more common, Waggoner said. Before the 1990s, little data were collected on peanut allergies. And peanut allergy numbers are generally based on self-reporting, which leaves them open to interpretation and influence by increased media attention.

"There’s definitely increased awareness about it," Waggoner said. "There’s more medical research being done. There’s more medical awareness, but what is contested is the prevalence, because it is based on self-reporting. We don’t have a good sense of long-term change over time."

Experts now say about 1 percent of the American population has a reported peanut allergy, Waggoner said.

Another unknown is the cause of peanut allergies, Waggoner said, adding that researchers are using genetic and molecular testing in the search for a cause.

Peter Conrad, a medical sociologist at Brandeis University who is an expert on the medicalization of society, said Waggoner's research offers important insights into the evolution of peanut allergies as a public problem.

"This paper helps us understand how a relatively rare disorder, peanut allergies, has become seen as a public risk and even as a childhood epidemic," Conrad said. "While the individual risk is high, the risk on a population level is small.

"Sometimes the public’s response to a disorder may significantly outpace the actual public health risk potential. Papers like this help us understand how the sociological nature of the disorder may well shape the public response more than its medical and epidemiological nature."

Along with continuing medical research into the causes and prevalence of peanut allergies, Waggoner said another important area for future research is why it is the peanut allergy that has sparked this level of public interest and resulting changes in society.

"While eight foods account for over 90 percent of food allergy reactions, including milk, eggs, peanuts, tree nuts, fish, shellfish, soy and wheat, the peanut allergy has arguably received the largest share of medical and social attention," Waggoner writes in the paper.

Among the possible explanations: the severity of allergic reactions to peanuts and the harmful potential of such a mundane food, Waggoner said.

Journal Reference:

Platelet Activation Inhibits the Host Cell Entry of HIV
July 23, 2013 — Infection biologists of the German Primate Center (DPZ) under the direction of Stefan Pöhlmann have found evidence that platelets (thrombocytes) might constitute an innate defense against infection with the human immunodeficiency virus (HIV). HI-viruses are the cause of the immune deficiency disease AIDS.

In cooperation with colleagues from the Hannover Medical School and the Institute of Molecular Virology, Ulm University Medical Center, the scientists of the DPZ have shown in a recent study that platelet activation suppresses HIV type 1 (HIV-1) infection of cell cultures and might thus reduce viral spread in patients. The paper was published in the scientific journal Retrovirology.

Platelets, the smallest particles of the blood, are activated through contact with the vascular connective tissue, which leads to a change of their shape and the release of biological active substances from inside the platelets. One of these substances is the messenger protein CXCL4, which blocks the host cell entry of HIV-1. This finding was reported in 2012 by Auerbach and colleagues, National Institute of Allergy and Infectious Diseases, Bethesda, USA, for purified CXCL4. The efficiency of HIV-1 inhibition by platelets in patients is currently unclear and might depend on platelet numbers and activation status.

There are two types of the HI-virus: HIV-1 and HIV-2. Additionally, there is a closely related virus, which occurs in monkeys and apes—the simian immunodeficiency virus (SIV). The current study has demonstrated that HIV-1 is inhibited upon platelet activation while HIV-2 and SIV are not. HIV-1 is the most frequent and also the most aggressive type of HI-virus.

"Our research indicates that platelets might constitute an innate barrier against HIV-1 infection, a function that was largely unknown," says Stefan Pöhlmann, head of the Infection Biology Unit at the German Primate Center and senior author of the new study. "Further research needs to uncover how
efficiently CXCL4 released by platelets inhibits HIV-1 spread in patients. Another goal should be to identify substances with a CXCL4-like antiviral activity and to develop them as novel therapies against HIV-1 infection."

Journal Reference:

**Mosquito Transmission Regulates Malaria Virulence**

Malaria parasites transmitted via mosquitoes elicit a more effective immune response and cause less severe infection than those directly injected into red blood cells.

By Dan Cossins | May 29, 2013

Malaria-causing parasites transmitted to mice through mosquitoes are less virulent and induce a more protective immune response than parasites injected directly into the blood stream, according to a new study published today (May 29) in *Nature*. The researchers also identified a set of antigen-encoding genes whose expression is modified by mosquito transmission, suggesting that the parasite may accrue changes in the mosquito vector that in turn provoke a more effective immune response in its mammalian host.

“This is an extraordinarily stimulating paper, and should be the start of quite a lot of new work,” said Andrew Read of Penn State University, who wrote an accompanying commentary for *Nature* but was not involved in the study.

“We’ve shown that something happens [to the parasite] in that cycle from the mosquito through to the blood-stage infection that is attenuating virulence,” said Jean Langhorne of the MRC National Institute for Medical Research in London, U.K., who led the study. “That opens up a new set of experiments to investigate the interplay between the changes that occur in the parasite and the type of immune response in the host. If we can dissect that mechanism, then we will have good handle on what might be a good target for a human vaccine.”

When malaria parasites are transmitted to their mammalian hosts via mosquitoes, the parasites head for the liver, where they replicate before re-entering the bloodstream and infecting red blood cells. Until now, largely for convenience, most studies of malaria in humans and animal models have focused on the blood stage of infection—by directly injecting parasites into red blood cells—and therefore largely ignored the liver and mosquito phases of the parasite lifecycle. So Langhorne, along with postdoc Philip Spence and colleagues, set up a mouse model to compare infections caused by injecting the rodent-infecting malaria parasite *Plasmodium chabaudi* directly into the bloodstream with infections initiated by mosquito bite.

The team found that parasites transmitted via mosquitoes did not replicate as well and generated lower-grade infection that lasted longer but did not cause severe disease. The team also demonstrated that vector-transmitted parasites induced a different immune response, characterized by better inhibition of replication and the release of fewer inflammatory molecules.

To understand what’s behind these variations in virulence and immune response, the researchers looked at the gene expression in the parasites. They discovered that mosquito transmission strongly regulates a family of genes encoding antigens that trigger an immune response. This suggests that by altering antigen expression, mosquito transmission may change the interplay between the parasite and the immune system.

However, although it seems that the environment experienced by the parasite during mosquito transmission is responsible, it remains to be seen where and when the changes take place—inside the mosquito itself, in the skin soon after injection, or during migration to the liver. And Langhorne points out that it is not yet clear whether this attenuated phenotype is actually caused by these modified expression profiles, or merely correlated with them.

Nevertheless, “these results suggest that genes that are being affected by the mosquito transmission route are key players in determining how nasty the parasites are and how sick people get,” said Read. “We now need to understand what is causing these changes in expression profiles and how they vary [during the parasite life cycle] to understand variation in virulence. From the vaccine point of view, it really matters what’s going on here.”

“It’s thrown up some important unknowns,” said Langhorne. “[But] it puts the mosquito, and these antigen expression variants, right at the center of what we should be looking at [with regard to vaccine development].”
Read is equally excited by what the findings mean for how natural selection might work on the expression of antigen-encoding genes to produce parasites that are more or less virulent depending on the epidemiological situation.

“This suite of genes is raw material by which evolution can shape the parasite to be nice or nasty,” he said. “For example, it can be made nasty in epidemic situations during a long rainy season, when main thing is to move to new hosts. And then in places with a long dry season, where the parasites have to survive for a long time so causing acute sickness in host is not a good idea, selection could favor the antigen profile that causes chronic infections of low virulence.”


**The Elixir Tragedy, 1937**

A mass poisoning of 105 patients treated with an untested medication spurred Congress to empower the US Food and Drug Administration to monitor drug safety.

By Jef Akst | June 1, 2013

In 1937, the S.E. Massengill Company of Bristol, Tennessee, began selling bottles of Elixir Sulfanilamide, a liquid version of a popular antibiotic of the day. But more than 100 people died after taking the drug, and investigators from the US Food and Drug Administration (FDA) identified the drug’s solvent, diethylene glycol, as the killer. The US Food and Drug Administration’s role in the regulation of novel medicines was born out of tragedy. Seventy-one adults and 34 children died in the fall of 1937 after taking a drug called Elixir Sulfanilamide to treat a variety of ailments, from gonorrhea to sore throat. At that time, the FDA, which had been launched in 1906 as the Bureau of Chemistry, served simply to police claims made about food and drug ingredients. No formal government approval was required to market new drugs.
“The initial 1906 legislation was relatively weak,” says Paul Wax, a medical toxicologist at the University of Texas Southwestern Medical Center. “There had to be some truth to what [drug companies] were selling . . . but in terms of safety, let alone efficacy, that wasn’t part of the equation.”

That all changed in 1938, after the deaths linked to Elixir Sulfanilamide had become a national scandal. Six years earlier, German pathologist and bacteriologist Gerhard Domagk discovered that a chemical called prontosil protected against certain bacterial infections in mice. Further research demonstrated that the compound’s active ingredient, sulfanilamide, could fight streptococcal infections in humans, prompting several pharmaceutical companies—including Merck, Squibb, and Eli Lilly—to begin making sulfanilamide drugs. These medicines were mostly formulated as capsules and tablets, but the S.E. Massengill Company of Bristol, Tennessee, decided that a liquid form of sulfanilamide could also be a big seller.

Massengill’s chief chemist concocted a solution of 10 percent sulfanilamide, 72 percent diethylene glycol, and 16 percent water. The company’s internal control lab approved the solution’s appearance, taste, and fragrance—it was flavored with raspberry extract, saccharin, and caramel, among other ingredients—and by September 1937, Massengill had distributed 240 gallons of the liquid, called Elixir Sulfanilamide, across the country.

Although the FDA didn’t regulate drug safety at the time, and therefore had no authority to reprimand the company, the agency was able to track down and seize bottles of Elixir Sulfanilamide on a technicality—“elixir” was a designation reserved for drugs containing ethanol—an operation that saved as many as 4,000 lives. But commercial success soon soured, as the first deaths were reported in October: six patients in Tulsa, Oklahoma, died of renal failure following treatment with the drug. FDA Commissioner Walter Campbell immediately ordered the vast majority of the agency’s 239 inspectors and chemists to investigate, and researchers quickly fingered the medicine’s solvent, diethylene glycol, as the cause of the deaths. But under the regulations of the time, Massengill hadn’t really done anything wrong: analyses of the concoction taken by the Tulsa patients revealed the ingredients to be exactly what the company had said they were. (The company had only broken the law by calling the medicine an “elixir,” a designation that was reserved for drugs containing ethanol.)

The disaster provoked a public outcry that led to the passage of the 1938 Food, Drug, and Cosmetics Act, which gave the FDA power to monitor the safety of new drugs. “Unfortunately, it took a disaster like this to get the senators to vote and empower the FDA like it should have been empowered to begin with,” says Wax, who has studied the Elixir Sulfanilamide tragedy (Ann Intern Med, 122:456-61, 1995). It wasn’t until October 1962, however, that Congress passed the Kefauver-Harris Drug Amendments, requiring companies to provide evidence of efficacy, in addition to safety, for drug approval.

Bird Flu Mutation Risk
Some H5N1 and H7N9 bird flu viruses could be one mutation away from spreading efficiently between humans.
By Ed Yong | June 6, 2013

Two types of bird flu—H5N1 and H7N9—have sparked concerns about a potential pandemic. Both viruses can cause severe illness and death in people, but mercifully, neither can spread easily from person to person.

That might soon change, however. According to a study today (June 6) in Cell, some strains of both viruses are just one mutation away from getting a better grip on the cells in our upper airways. If wild
viruses accrue those mutations, they may find it far easier to spread from infected to uninfected people, increasing the risk of a pandemic.

Ram Sasisekharan of the Massachusetts Institute of Technology, who led the study, hopes that the findings will help public health officials monitor wild viruses, as well as aid in vaccine development. “These viruses are rapidly evolving and our stockpiles of vaccine are largely based on outdated strains,” he said. “We hope that our discoveries will help us to stay ahead of the curve by ensuring that vaccines are stockpiled against strains that are closest to adapting to humans.”

Flu infections begin when the hemagglutinin (HA) protein on the virus’s surface recognizes glycan molecules on host cells. Birds and mammals have different glycan receptors, and wild H5N1 and H7N9 viruses bind more strongly to the bird versions. This creates a natural barrier that prevents the viruses from easily spreading between humans.

Last year, Ron Fouchier from the Erasmus Medical Centre in Rotterdam, The Netherlands, and Yoshihiro Kawaoka at the University of Wisconsin–Madison identified two sets of amino-acid-changing mutations in HA that allow H5N1 to spread through the air between caged ferrets. Both sets included mutations that help HA stick to human receptors.

But these studies started with old H5N1 strains from 2004 and 2005, however. When Kannan Tharakaraman, a postdoc in Sasisekharan’s team, analyzed the HAs of more recent strains, he found that the receptor-binding mutations that Fouchier and Kawaoka identified were not enough to cause the viruses to preferentially bind to human receptors. “If those mutations were incorporated into current circulating strains, we didn’t see that switch,” said Sasisekharan.

Thus, rather than focusing on these previously identified mutations, the team took a new approach. They modeled the way HA interacts with different glycans, and identified four structural features that bestow the protein with a preference for human receptors over bird ones.

Next, they rated each of HA’s amino acids according to how strongly it interacts with the rest of the protein and the host receptors. “You pay a huge penalty for mutating a heavily-networked amino acid,” explained Sasisekharan, “but less networked ones have more flexibility to change.” By studying these networks, the team determined a list of mutations that were most likely to produce a receptor switch.

Finally, they analyzed the diversity of existing H5N1 strains and found that many wild viruses are already tantalizingly close to becoming potentially contagious among humans. Almost all H5N1 lineages already have at least one of the four key structural features that could allow the virus to more easily bind human glycans, and some strains have two or even three of these features. One strain, which was isolated in Egypt in 2010, will prefer human receptors over birds ones if a single amino acid were to change from glycine to leucine.

The team applied the same approach to H7N9—a recently emerged strain of flu that has infected more than 130 people in China this year. The virus’ proteins show many signs of adaptation to humans, and previous studies predicted that its HA should bind strongly to human receptors.

But that’s now what Sasisekharan found. “The wild [H7N9] virus bound very poorly to the human upper airway tissues,” he said. This is consistent with a recent report suggesting that the virus can spread through the air between ferrets, but inefficiently so. However, the team also found that a single change—swapping the 228th amino acid from glycine to serine—was enough to give the viral HA an affinity for human receptors. “Should this mutation happen, we would expect that the virus would transfer between humans more efficiently,” said Sasisekharan.

“Structural analyses of HA have made a tremendous impact on our understanding of influenza adaptation,” said Fouchier. However, he cautioned that “we know very little about the actual receptors in human airways that are relevant for flu viruses.” Even the so-called “human” receptors can vary significantly, and it’s unclear which ones are found in different parts of the airways, or how common they are.

Sasisekharan suggested that viruses carrying the mutations he identified should be tested in ferret experiments to see if they genuinely are more efficient at spreading between mammals. However, given the controversy surrounding the development of potentially contagious flu strains, Fouchier wonders “whether we would be allowed to empirically test this newly acquired knowledge in animal models.”


Platelets Help Tackle Bacteria
The cell fragments play a role in the body’s first line of defense against bacterial infection, helping white blood cells grab blood-borne bacteria in the liver.
By Sabrina Richards | June 16, 2013
Platelets may contribute to protection against bacterial infection, according to new research published today (June 16) in Nature Immunology. Scientists found that in the livers of mice, platelets collaborated with specialized white blood cells to capture and engulf blood-borne bacteria, and this interaction helped protect the animals from bacterial infection.

“It’s an extremely exciting paper,” said Steve Watson, a platelet cell biologist at the University of Birmingham, who did not participate in the research. Though previous research had demonstrated that bacteria can activate platelets, “this work emphasizes that platelets play a day-to-day role in innate immune defense by helping remove bacteria in the liver.”

Growing evidence suggests that platelets, in addition to slowing bleeding, contribute to protection against infection. In addition to expressing many receptors important to combating pathogens, platelets have been shown to aggregate with and kill bacteria in vitro. In invertebrates, immune responses and bleeding prevention are taken care of by a single cell type, and it appears that platelets have retained both functions even as vertebrate animals evolved more specialized immune cells.

The notion that platelets may cooperate with other cells to clear blood-borne bacteria came from observations in the liver, said study lead Paul Kubes, an immunologist at the University of Calgary. While performing microscopy of specialized liver phagocytes called Kupffer cells, Kubes’ team noticed an unusual interaction between the phagocytes and platelets.

Kupffer cells sit in liver blood vessels, helping to capture and kill bacteria streaming past in the blood. Using intravital microscopy, the scientists saw that in uninfected mice, platelets performed what they termed a “touch and go” maneuver—interacting briefly with the Kupffer cells, but quickly disengaging and flowing away in the blood. But when mice were infected with certain types of bacteria—either Bacillus cereus or methicillin-resistant Staphylococcus aureus (MRSA), though not methicillin-susceptible S. aureus—the platelets formed long-term interactions with the Kupffer cells, engulfing the bacteria snagged from the blood.

Two different platelet receptors—already known to be important in platelets’ ability to staunch bleeding—mediated the two types of interactions the researchers saw. Kupffer cells collect von Willebrand Factor (vWF), a free-floating blood glycoprotein, on their surface. The “touch and go” behavior Kubes’s team saw involved the binding of vWF to platelets’ glycoprotein Ib (GpIb), which facilitates platelet aggregation at wound sites. In mutant mice missing GpIb, platelet “touch and go” no longer occurred. But the long-term congregating the scientists saw in B. cereus-infected mice required vWF-binding by the integrin GpIIb-GpIIIa, which also aids platelet aggregation.

The platelets, it turns out, were performing a life-enhancing function. When the researchers depleted platelets or used GpIb-deficient mice and infected them with B. cereus, these mice succumbed to infection at much higher rates. Platelet-depleted mice all died within 4 hours of infection, while over 80 percent of GpIb-deficient mice died within 4 hours—though less than 10 percent of wild type mice died at 4 hours. Unlike wild type mice, mice lacking GpIb also couldn’t clear bacteria from their blood within 4 hours, suggesting that the platelets’ ability to interact with Kupffer cells was integral to fighting bacterial infection.

GpIb and GpIIb are “carrying members of the hemostatic repertoire of platelets—but here they fulfill an innate immune function”—highlighting platelets’ dual role, said Guy Zimmerman, an immunologist at the University of Utah, who did not participate in the study. However, he cautioned that it’s too soon to know whether platelets and Kupffer cells collaborate similarly in humans. Additionally, Zimmerman noted that the work will need to be extended to more types of bacteria, as the research focused on two gram-positive species, but many types seen by the liver will be gut-associated gram-negative species such as Escherichia coli.

If human platelets perform a similar function, patients with already-suppressed immune systems might suffer further from drugs that inhibit platelet function, such as aspirin, Kubes said. It’s an important question, acknowledged Watson. But he noted that there are many ways of activating GpIb binding, making it unlikely that aspirin drastically affects platelets’ immune functions in healthy people. Instead, he said, the work suggests that in cases of trauma, it may be that platelets must be replaced to prevent both bleeding and infection.
Understanding the mechanism behind the platelets’ decision to aggregate on the bacteria-covered Kupffer cells could allow researchers to design a therapy to “favorably affect immune defenses without out affecting thrombotic potential,” speculated Zimmerman.


Decoding Bacterial Methyllomes

A new technique could soon spur unprecedented insight into the role of bacterial epigenetics in the evolution of pathogen virulence.

By Kate Yandell | May 15, 2013

Scientists have sequenced thousands of bacterial genomes, and even demonstrated that it is possible to sequence whole genomes of emerging pathogens within days. But they are now beginning to uncover another layer of information that appears to be critical for understanding—and maybe controlling—bacterial pathogenicity: epigenetic modifications.

The ability to detect epigenetic additions to bacterial genomes is relatively new, supported by a sequencing machine from Pacific Biosciences (PacBio) that has been available commercially for just 2 years and supportive software released less than 7 months ago. But already, the technique is making waves in microbiology.

In the midst of the 2011 *Escherichia coli* outbreak in Germany that killed more than 50 people, Eric Schadt, director of the Icahn Institute for Genomics and Multiscale Biology at the School of Medicine at Mount Sinai and former chief scientific officer of PacBio, rapidly sequenced the dangerous bacteria using the PacBio sequencer. His team—along with other groups that were also sequencing the bacterium—discovered that it had acquired a Shiga toxin from a phage that could mostly explain the microbe’s increased virulence. But at first, they did not look at the methylation information. “We didn’t appreciate at the time that the methylation may be associated with the virulence!” Schadt wrote in an email to *The Scientist*.

Upon revisiting the data—and applying the new software—Schadt and colleagues discovered that along with the Shiga toxin, this particular *E. coli* had also adopted a methylase from the same phage. This methylation-laying enzyme resulted in a complete epigenetic makeover, the team learned. The group is still working on characterizing the effects of these epigenetic modifications, but Schadt said that the various pathways that were upregulated and downregulated in the bacterium, including changes in swarming and growth patterns, could have contributed to making it more virulent.

The technology is transforming the study of bacterial genome modification, said Richard Roberts, chief scientific officer at New England Biolabs, who started collaborating with PacBio to investigate bacterial epigenetics in 2010. In addition to simply mapping the epigenomes of hundreds of bacteria species, including emerging pathogens, Roberts is adding to his library of knowledge on how bacteria use methylation to protect their genomes from the restriction enzymes they release to cut up invading viral DNA. Other researchers are working on understanding the role of methylation in the cell cycle.

“It’s like you’ve been in a closed room for a long time, and you open the window and look out,” said Roberts. “And there’s a whole lot of stuff out there, and you don’t know where to look.”

Fortuitous discovery

PacBio was founded in 2004, and it commercially released its first sequencer, the PacBio RS, in April 2011. The PacBio RS II was released last month (April 2013). But since 2009, scientists at the company had suspected that its technology, in addition to sequencing nucleotides, could also detect DNA modifications.

The most commonly studied type of eukaryotic modification, methylation of the carbon-5 position of cytosine, is relatively easy to identify through bisulfite sequencing. Treating DNA with bisulfite replaces all nonmethylated cytosines with uracil, making it possible to detect methylation through sequencing the treated DNA. But bacteria are often methylated at the nitrogen-6 position of adenine and the nitrogen-4 position of cytosine as well, and there have been no good techniques for locating these modifications in the genome.

PacBio’s technology, SMRT sequencing, functions by detecting fluorescently labeled nucleotides in real time as DNA polymerase adds them to the DNA template strand. The original purpose of this alternative type of sequencing was to produce longer reads of DNA than traditional next-generation sequencing methods can. For instance, Illumina’s popular sequencer, which detects fluorescently labeled nucleotides that reversibly terminate DNA synthesis, can read 250 base pairs in a row, while SMRT sequencing produces reads of 3,000 to 5,000 base pairs on average, and can even generate reads as many...
as 20,000 base pairs in length. But PacBio scientists realized that SMRT sequencing had another advantage: DNA polymerase would synthesize DNA at slightly different speeds depending on whether the template strand was epigenetically modified or not.

Sure enough, the nucleotides emit pulses of fluorescent light as they are added to the DNA, and by calculating the lengths of the pulses and distances between them, it is possible to identify not only carbon-5-methylated cytosines, but also nitrogen-6-methylated adenines and nitrogen-4-methylated cytosines. PacBio got funding from the Human Genome Research Institute to develop software to translate these pulses into information about DNA modification, and in May 2010 they published a paper in *Nature Methods* introducing the method.

“There was no technique until PacBio came along,” said Bart Weimer, who studies foodborne pathogens at the University of California, Davis. Now, researchers can begin to use methylation patterns to differentiate closely related bacterial strains, he said, or shed light on variation among pathogens that gene sequencing has failed to explain.

“We now systematically include this type of information in our characterizations of pathogenic strains of bacteria,” Schadt said.

**The epigenetics of pathogens**

The 2010 paper immediately caught the attention of Roberts, a 1993 winner of the Nobel Prize in Physiology or Medicine for the discovery that genes can be discontinuous, divided by stretches of noncoding DNA called introns. He contacted PacBio asking how he could try out the technology, then began testing it on a few sets of methylation patterns he already knew well. He quickly found the technique to be highly accurate. In fall 2012 he and collaborators at PacBio published a summary of the methylomes of six bacterial species, from the pathogenic *Campylobacter jejuni* to the metal-reducing *Geobacter metallireducens*, discovering many new methylation motifs. He has collaborated with 10 different groups and is beginning to work with four more. So far, they have characterized methylation patterns for 150 species of bacteria, with 50 additional species currently being analyzed.

Roberts and others are also tantalized by the idea that the PacBio sequencer can detect DNA modifications besides methylation. According to current PacBio chief scientific officer Jonas Korlach, the kinetics of DNA polymerase change during sequencing as it passes over a diverse set of modifications, including one to DNA’s phosphate backbone called phosphorothioation in bacteria, formyl and carboxyl modifications in eukaryotes, and various signatures of DNA damage. However, software is not yet available to automatically identify and read off these modification types. Researchers have also noticed delays in DNA synthesis that may represent yet-undiscovered types of DNA modifications, Roberts said. “Maybe it will lead us into new ways of modifying DNA.”

But the most commonly used application of the new technology is in understanding and tracking disease. The US Department of Agriculture, for example, is investigating the epigenomes of bacteria involved in bovine respiratory disease, while researchers at the Allegheny-Singer Institute in Pennsylvania are trying to understand the epigenetics of an antibiotic-resistant *Streptococcus pneumoniae* strain called PMEN1 that was first identified in the 1980s and has sickened people across the globe. Marc Allard of the US Food and Drug Administration (FDA) has published methylation details for 12 serovars of *Salmonella enterica* and found that their methylation patterns vary considerably, even between closely related strains. And Weimer and his colleagues are now incorporating methylation data into the 100K Foodborne Pathogens project, which he leads in partnership with the FDA and Agilent Technologies. They will look at methylomes of about 1 percent of the 100,000 genomes they plan to sequence, he said, and have already selected *Listeria, Salmonella, Campylobacter*, and a species of *Vibrio* involved food poisoning from shellfish to focus on first.

Weimer says he and others are in the very earliest stages of their research, but he is convinced the methylation data will yield copious new information on why closely related pathogens vary in virulence and pathogenicity. “This has never been done at this scale before,” said Weimer, who will participate this weekend (May 18) in a *Microbial Epigenetics Workshop* at the American Society for Microbiology annual meeting in Denver. “[This new technique] will enable huge amounts of work by lots of folks around the world to be done.”

Roberts agreed that the possibilities for future research are bountiful. “I feel like a kid in a candy store looking for interesting stuff to follow through on at the moment.”

*Correction (May 15): This story has been updated from its original version to reflect that DNA polymerase synthesizes DNA at different speeds depending on epigenetic modification of the template strand, not the primer strand.*
Protective Phages
Viruses that attack bacteria may be an important component of our gut microbiota.
By Edyta Zielinska | May 20, 2013
As research surfaces supporting the role of beneficial bacteria in human health, immunity, and normal childhood development, some scientists are beginning to look at even smaller biological entities in our gut. In a study published today (May 2) in Proceedings of the National Academy of Sciences, researchers have shown that bacteria-attacking viruses, called bacteriophages, reside in the protective mucus layer of many animal species and can help keep bacterial populations in check.

“The study is ground breaking and quite novel,” Rick Bushman, a microbiome researcher at the University of Pennsylvania Perelman School of Medicine, told The Scientist in an email. “The finding that phage can bind mucous and thereby protect cells from bacterial infection is convincing and exciting.”

Working with corals, fish, and human samples, researchers from Forest Rohwer’s lab at San Diego State University began to notice there was a much higher percentage of bacteriophages in mucosal linings than in surrounding surfaces. Many animals use mucus as a protective layer at the interface between the environment and their own cells, such as those in our mouths and gut. Mucus is also home to many strains of beneficial bacterial and provides the ideal wet and warm environment, as well as nutrients, for microbial growth.

The team found that bacteriophages expressing an Ig receptor on their surface are more likely to get stuck in the mucus layer. Although mucus is normally quite sticky, phages are small enough to “diffuse throughout without getting stuck. With the receptor, they get stuck longer,” said first author Jeremy Barr, a postdoc in the Rohwer lab. An estimated 25 percent of sequenced bacteriophages contain a gene for the Ig receptor, although Barr thinks that the total number of phages in nature with the receptor may be much higher.

To test whether mucus-bound bacteriophages could protect against bacterial infection, researchers added E. coli to mucus-producing human cell lines that had or hadn’t been pretreated with bacteriophage. Cells with a pretreated mucus layer were protected from the bacterial infection, whereas those that lacked the phage were not. Although it’s unclear whether mucus-bound phages can specifically kill pathogenic bacteria or if they simply expand an animal’s normal innate immune mechanisms of keeping beneficial bacteria growth in check, Barr said it’s a question the lab is interested in pursuing.

“The possibility that humans aggregate phage on our mucosal surfaces to help our immune system defend against bacteria, and that phage may benefit by gaining easier access to bacteria within the mucus, suggests a new type of mutualism is occurring between humans and phage,” microbiome researcher Justin Sonnenburg from Stanford School of Medicine, who was not involved in the research, said in an email. “The implications for human health and the resident microbiota are intriguing and warrant further investigation.”


Silver Boosts Antibiotic Efficacy
Silver makes bacteria more susceptible to antibiotics by weakening their cell membranes and inducing overproduction of DNA-damaging oxidative radicals.
By Dan Cossins | June 20, 2013
The antimicrobial properties of silver have been known for thousands of years, but it was not clear how the metal wreaked havoc on pathogenic invaders. Now, researchers have explained the cellular processes by which the precious metal weakens bacteria and makes them more susceptible to antibiotics, according to a study published yesterday (June 19) in Science Translational Medicine. The findings suggest that silver could be used to enhance the effectiveness of antibiotics against drug-resistant bacteria.

A team lead by Jim Collins, a biomedical engineer at Boston University, showed that dissolved silver ions interfere with several cellular processes in bacteria, including disulfide-bond formation, iron homeostasis, and metabolism. These changes not only make the cell membrane more permeable, but also lead to increased production of reactive oxygen species, which can induce cell death via DNA damage. (Last month, a report from a different group found that vitamin C has a similar effect on the bacteria that cause tuberculosis.)

When Collins and his colleagues supplemented antibiotics with a small amount of silver, both in vitro and in a mouse model of a urinary tract infection, the combination killed up to 1,000 times more bacteria than the antibiotics did on their own. In addition, the researchers showed that silver sensitizes Gram-
negative bacteria to vancomycin, a large-molecule antibiotic that usually can’t breach the outer coating on the bacterial cell membranes.

“This work shows that silver can be used to enhance the action of existing antibiotics against Gram-negative bacteria, thus strengthening the antibiotic arsenal for fighting bacterial infections,” the authors wrote.

Vance Fowler, an infectious disease physiologist at Duke University in North Carolina, told Nature that the study is “really cool” but warned that silver can be toxic. But Collins said that even with non-toxic doses of silver, his team observed enhanced antibiotic efficacy in mouse models of infection. Collins added that other researchers might now attempt to find non-toxic compounds that mimic silver's bacteria-weakening mechanisms.

Bacterial DNA in Human Genomes

A new study finds strong evidence that bacteria can transfer genes into human genomes, especially in cancer cells.

By Ed Yong | June 20, 2013

A team of scientists from the University of Maryland School of Medicine has found the strongest evidence yet that bacteria occasionally transfer their genes into human genomes, finding bacterial DNA sequences in about a third of healthy human genomes and in a far greater percentage of cancer cells. The results, published today (20 June) in PLOS Computational Biology, suggest that gene transfer from bacteria to humans is not only possible, but also somehow linked to over-proliferation: either cancer cells are prone to these intrusions or the incoming bacterial genes help to kick-start the transformation from healthy cells into cancerous ones.

“It really does seem that human genome sequence data from somatic cells show signs of LGT events from bacteria, and so do cancer cells,” said Jonathan Eisen from University of California, Davis, who coordinated the peer review of the new study but was not involved in the work. “Wild stuff does happen.”

The trillions of bacteria in our bodies regularly exchange DNA with each other, but the idea that their genes could end up in human DNA has been very controversial. In 2001, the team that sequenced the first human genome claimed to have found 113 cases of such lateral gene transfers (LGT), but their conclusion was later refuted.

This high-profile error “had a chilling effect on the field,” according to Julie Dunning Hotopp who led the new study. Although her team has since found several cases of LGT between bacteria and invertebrates, “it’s still difficult to convince people that it may be happening in the human genome,” she said.

Rather than looking for bacterial genes that had become permanent parts of the human genome, Dunning Hotopp’s team searched for traces of microbial DNA in somatic cells—the cells of the body that do not form gametes.

Lab members David Riley and Karsten Sieber scanned publicly available data from the 1000 Genomes Project and found more than 7,000 instances of LGT from bacteria, affecting around a third of the people they studied. When they analyzed sequences from the Cancer Genome Atlas, they discovered 691,000 more instances of LGT 99.9 percent of these came from tumor samples rather than normal tissues.

Acute myeloid leukaemia cells were particularly rife with bacterial sequences. A third of the microbial genes came from a genus called Acinetobacter, and had been inserted into the mitochondrial genome.

Stomach cancer cells also contained lots of bacterial DNA, especially from Pseudomonas. Most of this DNA had been inserted into five genes, four of which were already known to be proto-oncogenes that can give rise to cancer, emphasizing a possible link between LGT and cancerous growth. “Finding these integrations in multiple individuals, as well as in the proto-oncogenes, really spoke to how significant this might be,” said Dunning Hotopp.

“We know already that a significant proportion of cancers are due to insertion of genetic material from viruses,” said Etienne Danchin from the French National Institute for Agricultural Research, who reviewed the paper. “But this is the first time, as far as I know, that HGT from bacteria could be suspected as a cause of cancer.”

However, Dunning Hotopp is very clear that her results tell us nothing about whether the inserted bacterial DNA contributed to causing the cancers, or were just along for the ride. To get at the question of causation, researchers could deliberately add bacterial DNA into the same sites within human cell lines to see if they turn cancerous, she said. But even if the bacterial LGT can initiate over-proliferation, it would be hard to prevent such transfers with antibiotics. “You don’t know when these transfers occur, and you
can’t give people antibiotics their entire life,” said Dunning Hotopp. “A vaccine would be nice, but that is assuming these are causative.”

“LGT is incredibly important in evolution but many claims of specific cases of LGT have been seriously flawed,” said Eisen. “I came into this as a serious skeptic. It just seemed so improbable.”

But the team won him over. They ran an extensive set of checks to make sure that these bacterial sequences were not laboratory artifacts and had not come from contaminating microbes.

For example, they showed that LGT was more common in cancer cells than healthy tissue, and two out of ten cancer types were particularly hard hit. If the bacterial integrations were artifacts of the methodology, it should be equally common in any tissue sample. The team also focused on sequences with high coverage—that is, those which had been read many times over. When the team found evidence of LGT, it was consistent across all of these reads. “In the end, the authors addressed every single question that I and the reviewers raised,” said Eisen.

Hank Seifert from Northwestern University, who was not involved in the study, remains cautious. “This paper is very interesting and potentially important,” he said. “However, until the direct analysis of specific tumor cells can be performed to validate that these are real events, this work [is] still speculative.”

But Dunning Hotopp’s team cannot do these validation studies herself. For privacy reasons, they cannot access the original tumor samples that their data came from. “People with access to the samples need to validate that the integrations are correct,” she said.

Danchin agrees that the results need to be validated but said, “I am personally convinced what they have found by screening the different databases is true. I think LGT happens much more frequently than we imagine but, most of the time, is just not detectable.”


New Viruses Attack Asia and Africa

A poorly studied family of viruses with circular genomes may be behind cases of brain inflammation in Vietnam and paraplegia in Malawi.

By Chris Palmer | June 24, 2013

Two members of a mysterious family of viruses known for their circular genomes have recently popped up in Asia and Africa. According to two independent studies published last week, the disease agents, called cycloviruses, may be involved in outbreaks of brain inflammation in Vietnam and paraplegia in Malawi. However, the causative link between the viruses and the diseases has yet to be determined.

Scientists searching for the cause of central nervous system (CNS) infections in Vietnam turned to next-generation sequencing methods to hunt for potential pathogens. One sample from more than 100 patients with undiagnosed CNS infections turned up a possible suspect: a virus in the *Circoviridae* family, dubbed CyCV-VN for cyclovirus-Vietnam. After broadening their search for the virus, the researchers found that 4 percent of the samples from an additional 642 individuals with CNS infections tested positive for the virus. The results were published in *mBio* on Tuesday (June 18).

Because other *Circoviridae* viruses are found in some animals, the scientists speculated that humans picked up CyCV-VN from nearby livestock. Indeed, tests of dozens of chickens, ducks, and pigs found that nearly 60 percent were positive for the virus. Since these animals are “commonly held in Vietnam in backyards and on small farms,” Rogier van Doorn, a clinical virologist with the Oxford University Clinical Research Unit in the Hospital for Tropical Diseases in Ho Chi Minh City, told *ScienceNOW*, “there is a lot of possible contact between these viruses and humans.”

In the southeast African nation of Malawi, a cyclovirus has also been identified as a possible cause of paraplegia, which is known to sometimes stem from infection. Tests from a group of 58 paraplegia patients revealed that a cyclovirus was present in 15 percent of blood serum samples and in 10 percent of cerebrospinal fluid samples, according to a study published online last week in *Emerging Infectious Diseases*.

Le Van Tan, a medical researcher at the Oxford University Clinical Research Unit and the first author on the Vietnam study, told *ScienceNOW* that initial analyses of the genomes from the two cycloviruses suggest they are two different species. Eric Delwart, a researcher at the Blood Systems Research Institute in San Francisco, told *ScienceNOW* that neither study can confirm whether cycloviruses are actually the cause of the illnesses. There was no control group in the Malawi study and other factors may explain the lack of positives in the control group tested in the Vietnam study, he said.
Darwin Cleared of Plagiarism

A new book by an evolution historian asserts that Darwin and Wallace developed their theories of evolution independently.

By Chris Palmer | June 26, 2013

Historian John van Wyhe of the National University of Singapore has released a new book that claims to resolve the debate over whether Charles Darwin cribbed his theory of evolution through natural selection from Alfred Wallace. Most believe that the two scientists discovered the theory of evolution independently: Darwin during his trip to the Galapagos Islands and Wallace during his 8-year trip to the Malay Archipelago. However, uncertainty about exactly when Wallace mailed a draft of his essay outlining his discovery to Darwin led to speculation that Darwin plagiarized his work from Wallace.

Van Wyhe used historical documents to trace the route of Wallace’s letter and demonstrated that Darwin came up with his theory before receiving Wallace’s letter, confirming that the men developed their theories independently. Van Wyhe documents his research in his new book, *Dispelling the Darkness: Voyage in the Malay Archipelago and the discovery of evolution by Wallace and Darwin*, to be released July 28.

Van Wyhe’s book, also chronicles other aspects of Wallace’s journey to Malaysia, including the fact that his eureka moment in his thinking of evolution came during a malarial fever on the island of Ternate near New Guinea.

“Although the theory of evolution story has been told thousands of times in books and documentaries, several long-standing mysteries and many myths and legends have distorted our picture of the most important revolution in the history of science,” said van Wyhe in a statement. “This book aims to shed light on Wallace’s less well-known voyage and reveal the true story of how evolution was unveiled to the world.”

Cure for HIV-Infected Newborns?

A clinical trial will test the strategy a Mississippi doctor used to cure an infant.

By Kate Yandell | June 27, 2013

A treatment that previously eliminated HIV infection from a Mississippi infant will be tested in a clinical trial, *Nature* reported. Newborns with possible HIV infections will be given a course of three antiretroviral drugs in hopes of knocking down the virus before it has time to take hold.

The treatment was first successfully attempted in 2010, when an HIV-infected mother gave birth in Mississippi. Most HIV-infected women in the United States are given anti-retroviral drugs during pregnancy that greatly lessen the risk of passing on the infection to their children, but the Mississippi mother had not received prenatal care.

Under ordinary circumstances, infants born to HIV-positive women would only be given one or two anti-retroviral drugs until HIV tests came back. But because University of Mississippi Medical Center doctor Hannah Gay judged the infant to be at extraordinarily high risk, she administered a cocktail of three drugs immediately. The initial test given soon after birth indicated the child had been infected, but later tests showed that the treatment had eliminated the virus.

Now International Pediatric Adolescent Aids Clinical Trials (IMPAACT) Group is planning a formal clinical trial testing the strategy and hopes to start administering treatment to potentially infected
newborns before the end of the year. The researchers will give the three-drug cocktail to at-risk infants and will add a fourth drug if the children test positive for HIV. When the children are around 3 years old, the researchers will test for viral particles and antibodies to HIV in their blood. If the children appear not to recognize the virus, the researchers will take them off the drugs and see if the virus returns.

The theory behind the treatment is that immature immune systems do not include many central memory T cells, where HIV infections take hold. Also, immature immune systems have a weaker inflammatory response than adult immune systems. Attacking the HIV virus quickly, before it has time to hole up in immune cells, may be key to preventing a permanent infection.

Researchers will present on the planned clinical trial at the International AIDS Society biennial meeting in Malaysia on June 29.

The Downside of Antibiotics?
Bacteria-killing antibiotics might also damage a person’s tissues.
By Ruth Williams | July 3, 2013

In addition to the growing threat of antibiotic-resistant bugs, there may be another reason doctors should refrain from freely prescribing antibiotics. According to a paper published online today (July 3) in Science Translational Medicine, certain antibiotics cause mammalian mitochondria to fail, which in turn leads to tissue damage.

“What the authors are suggesting is that in addition to the bactericidal properties of antibiotics, they also affect . . . the mitochondria,” said Navdeep Chandel, a professor of medicine and cellular biology at Northwestern University in Chicago, who was not involved in the work. “And what’s fascinating about that is that mitochondria are thought to be [ancient] bacteria themselves.”

Indeed, mitochondria, the organelles responsible for energy production in the cell, have bacteria-like DNA and other molecules, suggesting that mitochondria are the product of an ancient endosymbiotic event, in which a bacterium was engulfed by another cell. The important implication of this, said Ronald DePinho, president of the MD Anderson Cancer Centre in Houston, Texas, who also did not participate in the research, is that “drugs targeted to [bacterial] physiology might also impinge on mitochondrial biology.”

This concern led Jim Collins, a professor of biomedical engineering at Boston University, to study the effect of antibiotics of mitochondria. His team had previously reported that antibiotics cause a surge in the production of reactive oxygen species (ROS)—highly reactive and potentially damaging molecules—inside bacteria, which may be part of the drugs’ bacteria-killing mechanism. Collins and his team therefore asked whether antibiotics also lead to an increase in ROS production in mammalian mitochondria.

The team treated human cell lines from a variety of tissues with three different types of bactericidal antibiotic: ciprofloxacin, ampicillin, and kanamycin. “We found that at clinical levels each of the antibiotics generated ROS,” said Collins, “and we showed that they do this in part by disrupting mitochondrial function.” On the other hand, an antibiotic called tetracycline, which does not kill bacteria but merely prevents their growth, did not cause an increase in ROS.

The cells given the bactericidal antibiotics also exhibited oxidative stress—the damage caused by ROS binding and oxidizing various cellular components. Indeed, there were signs of DNA, protein, and lipid damage, said Collins. And when the same three antibiotics were given to mice, increased ROS levels and oxidative damage to tissues was also apparent.

For the average person who might be prescribed a short course of antibiotics, DePinho reckoned there would probably be nothing to worry about. “We have very robust DNA damage repair mechanisms that in the short term may attenuate any clinical impact,” he said. However, he added, “it could be different in the context of chronic administration of antibiotics.”

To see if they could prevent such damage, Collins and his colleagues treated the human cells and the mice with an antioxidant called N-acetyl-L-cysteine (NAC) in addition to the antibiotics. The NAC mitigated the antibiotic-induced ROS increase and oxidative stress, but importantly it didn’t affect the antibiotics’ bactericidal activities. Mice given a urinary tract infection, then treated with an antibiotic, cleared the bacteria just as effectively whether or not they were given NAC along with an antibiotic treatment.

The lack of NAC’s effect on the bactericidal ability of the antibiotics “is likely specific to this antioxidant,” said Collins, because other antioxidants that his group has since tested did reduce antibiotic
activity. Collins speculated that NAC might not penetrate the bacteria themselves, and reduce only the mitochondrial-derived ROS, thereby protecting the cells but not the bacteria. But, Collins said, “more work is needed to find out what would be the effective and appropriate antioxidant to take.”

For now the paper’s immediate message, said Collins, is that “coupled with the concerns about drug-resistance... one should only use antibiotics when you really need antibiotics.”


Two Patients Rid of HIV?
Bone marrow transplants appear to have eliminated HIV from Boston cancer patients.
By Kate Yandell | July 3, 2013

Timothy Ray Brown, the “Berlin patient,” was the first person reported to be cured of HIV. He has shown no signs of infection for five years since receiving a bone marrow transplant to treat leukemia. Now doctors are reporting that two more patients show no signs of HIV after receiving bone marrow transplants as treatment for lymphoma.

The two men received the transplants several years ago at the Dana-Farber/Brigham and Women’s Cancer Center in Boston, researchers said at the AIDS Society Conference in Kuala Lumpur today (July 3). Throughout the transplants and the recovery, the patients took anti-retroviral medication. But following extensive testing, they both recently went off their AIDS medicines. One of the patients has remained free of signs of HIV for the last seven weeks, while the other has remained HIV-free in the 15 weeks since he went off his medication.

“While these results are exciting, they do not yet indicate that the men have been cured,” said Timothy Henrich, one of the Brigham and Women’s Hospital doctors who treated the patients, according to The Guardian. “Long-term follow up of at least one year will be required to understand the full impact of a bone marrow transplant on HIV persistence.”

“These findings clearly provide important new information that might well alter the current thinking about HIV and gene therapy,” Foundation for AIDS Research head Kevin Frost told BBC News. “While stem-cell transplantation is not a viable option for people with HIV on a broad scale because of its costs and complexity, these new cases could lead us to new approaches to treating, and ultimately even eradicating, HIV.”

The transplants given to the Boston patients were a bit different from the transplant that Brown, the Berlin patient, received. Brown was given bone marrow from a donor who had a rare mutation that confers resistance to HIV, but Brown did not receive anti-retroviral therapy throughout the transplant process, The New York Times explained. The Boston patients received transplants from ordinary donors without the anti-HIV mutation and remained on AIDS medications throughout the transplant process and afterwards. It is encouraging that the Boston transplants appear to have been effective, since it indicates that finding rare bone marrow donors with the anti-AIDS mutation might not be necessary for eradicating the disease.

Still, the treatment is too dangerous to be attempted on ordinary HIV patients who do not also have life-threatening cancer. The type of bone marrow transplant that the Boston men received carries a 15 percent risk of death.

Doctors believe that the treatment was effective because the patients took anti-retroviral therapy in conjunction with the bone marrow transplant. HIV often hides in bone marrow cells. Researchers
speculate that once the transplanted bone marrow cells replaced the patients’ original cells, the anti-retroviral drugs kept the new cells from becoming infected. Meanwhile, the transplanted cells were killing off the patients’ original HIV-infected cells in a phenomenon called graft-versus-host disease, eliminating the disease’s reservoir from the body.

It remains to be seen whether the HIV could still be hiding somewhere in the two Boston patients’ body, such as in the brain or the gut, Henrich said.

**Gut Microbes for Life**

*Most strains of gut microbes stay with us for decades, which may prove useful for tracking our health.*

By Ed Yong | July 4, 2013

We all have trillions of microbes inside our guts, which outnumber our own cells by a factor of 10. Now, a team from Washington University School of Medicine in St. Louis (WUSTL) has shown that this microscopic community is extraordinarily stable. In healthy people, once these microbes are established in the gut early in life, presumably due to contact from close family members, most strains are unwavering in their presence, staying in the gut for decades or longer.

“We have this part of ourselves that’s assembled from outside but stays inside for decades and decades, and it contributes to our uniqueness as individuals and our health,” said WUSTL’s Jeffrey Gordon who led the study.

Although the team only studied healthy adults, their results have big implications for our understanding of disease, Gordon added. Many studies have shown that conditions such as obesity or autoimmune disorders are associated with dramatic changes in the gut microbiota. But, Gordon said, “if we don’t know what the normal variations are in healthy people, we can’t tell how an individual with disease deviates.”

If these communities are usually steady, it may be possible to monitor a person’s health by analyzing stool samples each year, said Jacques Ravel, a microbiologist from the University of Maryland School of Medicine who was not involved in the study. “This is the future of medicine: a genome once and a microbiome at each annual exam.”

While other studies have tracked changes in the gut microbiota over time, Gordon’s team wanted to get a more detailed picture. “In the past, people have gone down to the species level,” said former postdoc Jeremiah Faith, now at the Icahn School of Medicine at Mount Sinai. “But everyone has *E. coli* in their guts for sure. The difference between those *E. coli* strains can be pretty big.”

Current techniques have such a high error rate that it can be unclear if a variation in sequence is due to the presence of a new strain or a mistake in the sequencing. Faith surmounted this problem by developing a new technique called LEA-Seq (low-error amplicon sequencing). It tags a small fraction of the DNA fragments within a sample and makes exponentially more copies of them than traditional sequencing methods, so that each can be sequenced many times over greatly improving the accuracy of the resulting sequences.

Two years ago, Faith first tested his new technique on stool samples collected from a single volunteer on multiple occasions. “I was completely floored,” he said. “Over the course of 3 months, they had virtually identical sets of microbes.” This pattern held when he looked at 175 stool samples, taken from 37 healthy US adults over 5 years.

On average, each volunteer harboured around 200 strains representing 100 different bacterial species. More than 70 percent of these stayed the same after a year, and 60 percent remained across the entire 5-year span. “If you extrapolate from the rate of change, it looks like the strains we harbor in our guts last decades or maybe a lifespan,” said Gordon.

The team also found that people share gut microbe strains with relatives, but not unrelated people. This suggests that family members, through touching each other or sharing the same environments, are colonized by the same microbes during their early years. “There’s an early period of assembly for the gut microbe community, and your physiology as an adult is likely a legacy of this event,” said Gordon.

But this stability can be disrupted. During the study, four of the volunteers lost around 10 percent of their weight through a special liquid diet, and their microbe communities very quickly became less stable. When the team analyzed all the volunteers, they saw that those whose weight fluctuated the most over time had the least stable gut microbes. “This shows that strains in our gut, and how they change over time, could act as a biomarker that helps us track our health status,” said Faith. “Now, we need to know which strains are good or bad.”

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“We have this part of ourselves that’s assembled from outside but stays inside for decades and decades, and it contributes to our uniqueness as individuals and our health,” said WUSTL’s Jeffrey Gordon who led the study.

Although the team only studied healthy adults, their results have big implications for our understanding of disease, Gordon added. Many studies have shown that conditions such as obesity or autoimmune disorders are associated with dramatic changes in the gut microbiota. But, Gordon said, “if we don’t know what the normal variations are in healthy people, we can’t tell how an individual with disease deviates.”

If these communities are usually steady, it may be possible to monitor a person’s health by analyzing stool samples each year, said Jacques Ravel, a microbiologist from the University of Maryland School of Medicine who was not involved in the study. “This is the future of medicine: a genome once and a microbiome at each annual exam.”

While other studies have tracked changes in the gut microbiota over time, Gordon’s team wanted to get a more detailed picture. “In the past, people have gone down to the species level,” said former postdoc Jeremiah Faith, now at the Icahn School of Medicine at Mount Sinai. “But everyone has *E. coli* in their guts for sure. The difference between those *E. coli* strains can be pretty big.”

Current techniques have such a high error rate that it can be unclear if a variation in sequence is due to the presence of a new strain or a mistake in the sequencing. Faith surmounted this problem by developing a new technique called LEA-Seq (low-error amplicon sequencing). It tags a small fraction of the DNA fragments within a sample and makes exponentially more copies of them than traditional sequencing methods, so that each can be sequenced many times over greatly improving the accuracy of the resulting sequences.

Two years ago, Faith first tested his new technique on stool samples collected from a single volunteer on multiple occasions. “I was completely floored,” he said. “Over the course of 3 months, they had virtually identical sets of microbes.” This pattern held when he looked at 175 stool samples, taken from 37 healthy US adults over 5 years.

On average, each volunteer harboured around 200 strains representing 100 different bacterial species. More than 70 percent of these stayed the same after a year, and 60 percent remained across the entire 5-year span. “If you extrapolate from the rate of change, it looks like the strains we harbor in our guts last decades or maybe a lifespan,” said Gordon.

The team also found that people share gut microbe strains with relatives, but not unrelated people. This suggests that family members, through touching each other or sharing the same environments, are colonized by the same microbes during their early years. “There’s an early period of assembly for the gut microbe community, and your physiology as an adult is likely a legacy of this event,” said Gordon.

But this stability can be disrupted. During the study, four of the volunteers lost around 10 percent of their weight through a special liquid diet, and their microbe communities very quickly became less stable. When the team analyzed all the volunteers, they saw that those whose weight fluctuated the most over time had the least stable gut microbes. “This shows that strains in our gut, and how they change over time, could act as a biomarker that helps us track our health status,” said Faith. “Now, we need to know which strains are good or bad.”
Gut Microbes Exacerbate HIV?  
Particular microbes in the colons of HIV patients may worsen disease progression.  
By Ruth Williams | July 10, 2013

Gut microbes in patients with HIV differ from those in uninfected individuals—and may promote disease progression, according to a paper published today (July 10) in *Science Translational Medicine*. The report suggests that HIV infection in the gut actually selects for bacteria that promote immune dysfunction, even if a patient receives antiretroviral treatment.

“What’s nice about this paper is that it shows there is a stereotyped dysregulation of the microbiota in HIV patients, meaning there is a signature to the change,” said Dan Littman, a professor of molecular immunology at New York University’s Langone Medical Center, who was not involved in the study.

Furthermore, “they have gone on to show that the degree of dysbiosis and the type of bacteria present correlate with some of the important markers of immune activation and disease progression,” added Danny Douek, an immunologist at the National Institute of Allergy and Infectious Diseases in Bethesda, Maryland, who also did not participate in the research.

HIV is a retrovirus that primarily infects and replicates inside immune cells and can ultimately lead to immune dysfunction (AIDS). Because HIV makes a home inside immune cells, “it likes to replicate in the context of inflammation,” when such cells are rapidly proliferating, said Mike McCune, Chief of the Division of Experimental Medicine at the University of California, San Francisco, who led the study.

Indeed, the major indicator that an HIV infection is progressing towards AIDS is chronic systemic inflammation.

Microbes of the gut, while normally peaceful inhabitants, can also cause inflammation if they leak into the body through the intestinal walls. And, such leakage, or translocation, has been observed in HIV patients. “We’ve known for a long time that the virus replicates most avidly in the mucosa of the intestine . . . and that leads to a disruption of the immune system in that location,” said Littman. This disruption weakens the mucosal barrier, making it more porous, he explained, and one hypothesis has been that “this increased permeability of the intestinal barrier and the leakage of microbial products . . . would lead to an overall activation of the immune system.”

But while this microbial translocation explains the systemic immune activation, what isn’t clear is why systemic inflammation can sometimes continue in patients receiving antiretroviral treatment and in whom the virus itself may be almost undetectable in the blood.

To find an answer McCune’s team took colon biopsies from untreated HIV patients, patients on antiretrovirals, and healthy people and found that the bacterial communities differed between the three groups. Relative to the healthy controls, the samples from untreated HIV-infected individuals tended to be enriched for members of the Proteobacteria family and depleted for members of Clostridia and Bacteroidia families. Patients infected with HIV that were being treated with antiretrovirals, on the other hand, exhibited a spectrum of microbial communities in the ir colonic mucosa, some resembling healthy controls, others resembling untreated patients—a result that correlated with the occurrence of systemic inflammation: the more similar a person’s gut microbiome was to that of the untreated patients, the greater immune cell activity and the higher the levels of inflammatory molecules in their blood.

The team also found that the disease-associated microbial communities tended to be particularly abundant in bacterial species that could digest the amino acid tryptophan. Products of this digestion can inhibit the development of certain immune cells, particularly those that produce the cytokine interleukin-17 (IL-17). “IL-17 is critically important for the maintenance and integrity of the mucosal boundary in the gut,” explained McCune. Indeed, HIV is thought to promote breakdown of the mucosal barrier by activating tryptophan catabolism and suppression of IL-17–producing cells as they replicate in the gut.

“Very few bacteria can catabolize tryptophan,” explained McCune, so finding these tryptophan-digesting bacteria at the site of HIV replication was “a surprise,” he said. It suggests that while HIV initiates the immune dysfunction at the mucosal barrier, the bacteria recruited there during infection...
Gut Microbes Treat Illness

Oral administration of a cocktail of bacteria derived from the human gut reduces colitis and allergy-invoked diarrhea in mice.

By Chris Palmer | July 10, 2013

An astounding array of microorganisms colonizes the human gut; our large intestines alone are home to $10^{14}$ bacteria from more than 1,000 species. Though scientists have long attempted to manipulate these microbial populations to affect health, probiotics have failed to reliably treat disease. However, a new study published today in *Nature* reports that a blend of specially selected strains of *Clostridium* bacteria derived from humans can significantly reduce symptoms of certain immune disorders in mice.

“This work shows that microbes can influence the balance and architecture of the immune system of their host,” said Sarkis Mazmanian, an immunologist at the California Institute of Technology who did not participate in the research. “I think it has tremendous potential for ameliorating human disease.”

Mammalian gut microbiota—the community of microorganisms that inhabit the gastrointestinal tract—have a long, intimate, and mostly symbiotic history with their hosts. The ubiquitous bugs are integral to some of the most basic of physiological functions, including metabolism and immune system development and function. However, specific gut microbes have also been linked to autoimmune disorders, obesity, inflammatory bowel disease, and possibly even neurological disorders. “It’s clear that gut microbes can affect many, many aspects of our physiology,” said Mazmanian.

Senior author Kenya Honda and his team previously reported that colonization of germ-free mice—mice that lack a microbiota—with a cocktail of a few dozen strains of *Clostridium* bacteria derived from wild-type mice promoted the activity of regulatory T cells (T$_{reg}$) in the colon. T$_{reg}$ cells produce important anti-inflammatory immune molecules, including interleukin-10 and inducible T-cell co-stimulator, to prevent an overreaction of the immune system, and disruption of T$_{reg}$ cells is known to play a role in autoimmune disorders such as colitis, Crohn’s disease, food allergies, and type II diabetes. Indeed, mice treated with the *Clostridium* cocktail appeared more resistant to allergies and intestinal inflammation.

Clostridia bacteria include the well-known tetanus and botulism toxins. “Clostridia are very diverse bacteria, and include some pathogens,” said Alexander Rudensky, an immunologist at the Memorial Sloan–Kettering Cancer Center in New York and a cofounder, of Vedanta Biosciences, which he launched with the paper authors in 2010. “So, their role [in disease] may be surprising to immunologists and public, but not to microbiologists.”

To extend the clinical relevance of the previous results, Honda’s group repeated their experiment using *Clostridium* derived from a sample of human feces. As in the previous study, germ-free mice treated with specially selected strains of human-derived Clostridia displayed a significant increase in T$_{reg}$ cells. The treated mice also displayed reduced symptoms of colitis and allergy-induced diarrhea.

“This is a terrific advance to their previous studies where they showed that mouse microbiota can induce regulatory T cells,” said Mazmanian. “In this paper they’ve extended that to bacteria that come from humans, which they have tested in mice.”

The researchers used RNA sequencing of gut tissue samples of mice treated with human microbes to identify 17 specific non-virulent strains of *Clostridium* responsible for the increased production of T$_{reg}$ cells. They then sequenced the metagenomes of human ulcerative colitis patient guts, and found that they tended to carry lower levels of the 17 strains, with 5 out of the 17 showing a statistically significant reduction. “This work lays out the first instance of a rationally designed drug candidate isolated from human microbiota, which can be given to animals to treat autoimmune disease,” said study coauthor Bernat Olle, the chief operating officer of Vedanta Biosciences, which is developing therapies based on the new research.

Investigations into the mechanisms underlying T$_{reg}$-cell induction pointed to small chain fatty acids and bacterial antigens that are cooperatively produced by the 17 strains of *Clostridium*. The small chain fatty acids and antigens in turn activate a transforming growth factor (TGF-beta) response that drives T$_{reg}$ cell differentiation and expansion.

“It’s very valuable to see studies like this one, where detailed analysis of microbial compositions is linked to biology,” said Rudensky.

An Ocean of Viruses
Viruses abound in the world’s oceans, yet researchers are only beginning to understand how they affect life and chemistry from the water’s surface to the sea floor.

By Joshua S. Weitz and Steven W. Wilhelm | July 1, 2013

There are an estimated \(10^{31}\) viruses on Earth. That is to say: there may be a hundred million times more viruses on Earth than there are stars in the universe. The majority of these viruses infect microbes, including bacteria, archaea, and microeukaryotes, all of which are vital players in the global fixation and cycling of key elements such as carbon, nitrogen, and phosphorus. These two facts combined—the sheer number of viruses and their intimate relationship with microbial life—suggest that viruses, too, play a critical role in the planet’s biosphere.

Of all the Earth’s biomes, the ocean has emerged as the source for major discoveries on the interaction of viruses with their microbial hosts. Ocean viruses were the inspiration for early hypotheses of the so-called “viral shunt,” by which viral killing of microbial hosts redirects carbon and nutrients away from larger organisms and back toward other microorganisms. Furthermore, researchers analyzing oceanic life have discovered many novel viruses that defy much of the conventional wisdom about what a virus is and what a virus does.

Among these discoveries are “giant” marine viruses, with capsid cross-sections that can exceed 500 nm, an order of magnitude larger than prototypical viruses. Giant viruses infect eukaryotic hosts, including the protist Cafeteria and unicellular green algae. These viruses also carry genomes larger than nearly all previously identified viral types, in some cases upwards of 1 million base pairs. In both marine and nonmarine contexts, researchers have even identified viruses that can infect giant viruses, the so-called virophages, a modern biological example of Jonathan Swift’s 17th-century aphorism: “a flea/ Hath smaller fleas that on him prey;/ And these have smaller fleas to bite ’em;/ And so proceed ad infinitum.”

It is apparent that we still have much to learn about the rich and dynamic world of ocean microbes and viruses. For example, a liter of seawater collected in marine surface waters typically contains at least 10 billion microbes and 100 billion viruses—the vast majority of which remain unidentified and uncharacterized. Thankfully, there are an increasing number of high-throughput tools that facilitate the study of bacteriophages and other microbe-infecting viruses that cannot yet be cultured in the laboratory.
Indeed, studying viruses in natural environments has recently gone mainstream with the advent of viral metagenomics, pioneered by Forest Rohwer and colleagues at San Diego State University in California. More recently, culture-free methods have enabled insights into questions beyond that of characterizing viral diversity. For example, Matthew Sullivan’s group at the University of Arizona and colleagues recently developed an adapted “viral tagging” method, by which researchers can now characterize the genotypes of environmental viruses that infect a host of interest, even if those viruses cannot be isolated in culture. These and other techniques—and the increasingly interdisciplinary study of environmental viruses—bring the scientific community ever closer to a clearer understanding of how viruses shape ocean ecology.

**Not so picky**

Researchers have long believed viruses to be extremely host-specific, meaning they should infect a taxonomically narrow subset of the microbial community at a given time in any given environment. But recent evidence suggests that marine viruses may not be so picky after all, and may be capable of infecting multiple microbial species or even more distantly related organisms. For example, a 2003 study demonstrated that certain cyanophage genotypes can infect not only different strains within the same cyanobacteria species, but different cyanobacterial genera as well. And a 2011 analysis of more than 20 years of viral-host infection assays revealed that naturally occurring viruses from a diversity of taxa range from specialists to generalists. Hence, viruses are certainly not limited to a single host genotype, nor to a particular species, and perhaps not even to a genus!

Ostensibly, viruses should decrease the oceanic abundance of the targeted microbial lineage. Quantitative estimates of virus-mediated killing demonstrate that viruses are, in some cases, as important as grazers, such as protists and zooplankton, in selectively killing microbes. Such a relationship might, as a consequence, lead to dynamic fluctuations in viral and microbial populations, as viruses deplete susceptible bacteria. Indeed, new viral subtypes arise frequently and rapidly, and previously rare subtypes can quickly increase in abundance.

The sheer number of viruses and their intimate relationship with microbial life suggest that viruses play a critical role in the planet’s biosphere. Nonetheless, direct evidence for coupled oscillations in virus-microbe systems in the oceans is limited. It’s even possible that viruses do not play a strong role in controlling a microbe’s population. Or, in some instances, marine viruses that actively infect and lyse microbes may simply not have been accounted for in prior surveys. For example, until recently, the most abundant marine bacterial lineage, SAR11—estimated to make up a third of all prokaryotic cells in surface waters—had no documented viruses that were known to infect it, leading to speculations that SAR11’s observed high abundance was due, in part, to its lack of a phage predator. However, scientists recently discovered a group of non-tailed podoviruses that can and do kill SAR11. These viruses, previously unknown to science, are now estimated to be the most abundant viral type in the oceans and could be an important factor in driving changes in SAR11 populations.
Where do all the nutrients go?
Viruses in the ocean can affect the marine ecosystem in a number of ways. First and foremost, viral killing of microbes could be as important in reducing the abundance of targeted lineages as are grazers, like protists and zooplankton. Furthermore, during the infection process, a virus can alter the host cell’s metabolism by increasing the rate of photosynthesis, for example, thereby changing the rate of carbon fixation. And when a virus causes host lysis, not only are new viral particles released, but so are the carbon and other organic nutrients that were trapped inside the cell. These materials then become available for utilization by nearby microbes, a potentially beneficial process known as viral priming. Finally, under certain conditions, a virus may become a long-term resident in its host cell, integrating its genomic material into that of its host to form a “lysogen.”

The death of a host cell and the release of viral progeny are but one part of the story of how viruses affect the ocean ecosystem. Lysis of microbes also releases carbon and other organic nutrients, previously tied up as cellular materials, back into the environment. Marine microbes can assimilate these organic materials, leading to a paradoxical consequence of viral infection: the death of one host may indirectly benefit other microbes.

This hypothesis, which we term “viral priming,” has been documented in experimental model systems using microbes that predominantly occur near the ocean surface. In one illustrative example, viral lysis of a bacterium infected in the lab released organic-iron complexes that were rapidly taken up by other marine bacteria, as well as by diatoms (unicellular eukaryotic algae). This assimilation increased growth rates of the nontargeted organisms. In a second example, the removal from an experimental system of viruses that infect and lyse heterotrophs slowed *Synechococcus* cell growth and proliferation, presumably due to a decrease in virus-mediated nutrient release. Thus, what is bad for one microbial cell may indeed be good for others. In the deep ocean, however, we still do not yet know what happens to virus-released organic matter. Is it assimilated, buried, or otherwise exported? What happens to organic matter miles below the surface is important because it closes the loop of the global carbon cycle. Free carbon in the deep ocean is “ancient” (4,000–6,000 years old) and largely recalcitrant to assimilation by microbes, suggesting there may be another supply of this material. Viral lysing of deep-ocean microbes may be a potential source.

Furthermore, even before lysis, the infection of microbes alters host metabolism. Virus-induced changes in host metabolism can be so significant that the resulting infected particle is, biochemically and metabolically, a very different cell. For example, phage-infected cyanobacteria exhibit a higher rate of photosynthesis than their noninfected counterparts, presumably changing their rate of fixation of carbon from the environment until they are eventually killed by the infection. Bacterial cells undergoing active phage infections can also have altered distributions of other major elements, such as nitrogen and phosphorus, making them biochemically unique.

Moreover, viruses can establish persistent infections within their microbial host cells—similar to infections established by viruses within large eukaryotic hosts, as occurs in the case of retroviral infections—by integrating their genomic material into that of their host, forming what is called a “lysogen.” (See diagram above.) The fate of infected cells may itself be coupled with the availability of carbon and nutrients in the environment. A recent study found that marine phages were more likely to initiate lysogeny, instead of lysis, when their hosts were nutrient-depleted. Hence, viruses that may “want” to lyse their hosts may not be able to—or, perhaps, they have evolved to respond to host physiology so as to kill their hosts only when it is more likely that other healthy hosts will be available to infect, which may be indicated by the physiological status of their current host. However, lysogeny is often harder to detect than lysis because the viruses are largely “hidden” within the host. In future, our understanding of viral-host interactions will need to take into account not just who infects whom, but what happens after that.

Viruses, in theory
A liter of seawater collected near the Galapagos Islands contains at least 10 billion microbes and 100 billion viruses—the vast majority of which remain unidentified and uncharacterized. Given the difficulties in quantifying the role viruses play in complex environments, researchers have turned to mathematical models to help shed light on what viruses might be doing to their hosts and the consequences of such interactions for the ocean system. Like many mathematical models in biology, these models can be very
It's a microbial and viral world

The potential role of viruses in marine biogeochemical cycles has been discussed for nearly 2 decades now, yet the quantitative influence that viruses have at regional and global scales remains largely unresolved. Fortunately, there is a growing interest in the ecological role of ocean viruses. Indeed, as marine microbiologist Mya Breitbart of the University of South Florida posed it, the science of environmental viruses is entering into an exciting period of “truth or dare.” That is to say, there are many established tenets of viral-host interactions in the oceans that are often-repeated, but that are just now being put to the test. There are also many tenets that researchers should be “dared” to prove, or at least further substantiate. Indeed, a working group that we organized to study ocean viral dynamics at the University of Tennessee’s National Institute for Mathematical and Biological Synthesis is but one example of collaborations amongst experimentalists and modelers to characterize viral-host interactions and their consequences on a global scale. If the working group is any guide, future work on ocean viruses will include efforts to combine virus-driven biogeochemical processes, molecular biological data, and mathematical models in a unified context.

Ocean viruses may turn over as much as 150 gigatons of carbon per year—more than 30 times the standing abundance of carbon in marine plankton.

A better quantitative assessment of the role of viruses in the ocean will have important implications for understanding past trends in, and future changes to, the Earth system. Curtis Suttle of the University of British Columbia has estimated that ocean viruses may turn over as much as 150 gigatons of carbon per year—one more than 30 times the standing abundance of carbon in marine plankton. This recycling of carbon and other nutrients suggests that viruses need to be considered in quantitative, dynamic models of global change.
Global-change models integrate geophysical processes with the biology of microbes and metazoans to predict the dynamics of carbon nutrients and biodiversity. However, the smallest yet most abundant biotic agents on the planet—viruses—are rarely, if ever, included in such models. As the Intergovernmental Panel on Climate Change noted in a 2007 report (our emphasis): “The overall reaction of marine biological carbon cycling (including processes such as nutrient cycling as well as ecosystem changes including the role of bacteria and viruses) to a warm and high-CO₂ world is not yet well understood. Several small feedback mechanisms may add up to a significant one.”

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1. References

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Medical Procedures May Be Useless, or Worse
By Nicholas Bakalar

We usually assume that new medical procedures and drugs are adopted because they are better. But a new analysis has found that many new techniques and medicines are either no more effective than the old ones, or worse. Moreover, many doctors persist in using practices that have been shown to be useless or harmful.

Scientists reviewed each issue of The New England Journal of Medicine from 2001 through 2010 and found 363 studies examining an established clinical practice. In 146 of them, the currently used drug or procedure was found to be either no better, or even worse, than the one previously used. The report appears in the August issue of Mayo Clinic Proceedings.

More than 40 percent of established practices studied were found to be ineffective or harmful, 38 percent beneficial, and the remaining 22 percent unknown. Among the practices found to be ineffective or harmful were the routine use of hormone therapy in postmenopausal women; high-dose chemotherapy and stem cell transplant, a complex and expensive treatment for breast cancer that was found to be no better than conventional chemotherapy; and intensive glucose lowering in Type 2 diabetes patients in intensive care, which not only failed to reduce cardiovascular events but actually increased mortality.

In some instances, doctors routinely refused to give beneficial therapies despite a lack of evidence that they were harmful. Vaccines were unnecessarily withheld from multiple sclerosis patients in the belief that they increased flare-ups; women with lupus were denied oral contraceptives for fear they increased
the severity of the disease; and epidural anesthesia was delayed during childbirth on the theory it increased the rate of Caesarean sections. Yet good studies showed that none of these fears was justified.

“Contradicted practices don’t disappear immediately,” said the lead author, Dr. Vinay Prasad. “There’s an inertia, a 10-year period of time when the contradicted procedure continues to be practiced.”

Dr. William E. Boden, chief of medicine at the Stratton VA Medical Center in Albany, who was not involved in the work, found the study useful and provocative. “It’s challenging us to look at things we’ve done and attempt to find whether there’s evidence to support their use,” he said. “There’s going to be increasing pressure to come forward with making sure that the health care dollars we’re allocating are being well utilized.”

Dr. Prasad, chief fellow in medical oncology at the National Cancer Institute, said that new medical appliances present a special problem. “Devices are particularly bad because they can be approved if they’re similar to ones already on the market,” he said. He cited as an example the Swan-Ganz catheter, a device threaded into the heart to monitor heart function and blood flow. It gives accurate information, Dr. Prasad said, “but that information doesn’t help. We continue to introduce new catheters all the time, lacking good evidence that they work. This is a tremendous waste of resources.”

Often doctors persist with procedures that lack evidence because they seem to make sense, Dr. Prasad said. “They all sound good if you talk about the mechanisms,” he said. “You have cholesterol-clogged arteries, it makes sense that if you open them up it will help. But when that was studied, it didn’t improve survival.”

Patients, too, like to talk about mechanisms, Dr. Prasad added. “They tend to gravitate toward the nuts and bolts — what does it do, how does it work?” he said. “But the real question is: Does it work? What evidence is there that it does what you say it does? What trials show that it actually works? You shouldn’t ask how does it work, but whether it works at all.”