May 2013 Epidemics and AIDS Update

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HIV-Infected Children: Less-Used Regimen Is More Effective for Children in Low-Resource Settings

Apr. 30, 2013 — Researchers from The Children’s Hospital of Philadelphia and the Perelman School of Medicine at The University of Pennsylvania, along with colleagues at the Botswana-Baylor Children’s Clinical Centre of Excellence, conducted the first large-scale comparison of first-line treatments for HIV-
positive children, finding that initial treatment with efavirenz was more effective than nevirapine in suppressing the virus in children ages 3 to 16. However, the less effective nevirapine is currently used much more often in countries with a high prevalence of HIV. The results of the study of more than 800 children are published today in the Journal of the American Medical Association (JAMA).

There are more than 3 million HIV-positive children in the world, and more than 90 percent of them live in sub-Saharan Africa. Currently, the World Health Organization (WHO) recommends both efavirenz and nevirapine for first-line pediatric use in resource-limited settings such as sub-Saharan Africa. Lead author Elizabeth Lowenthal, MD, MSCE of The Children’s Hospital of Philadelphia, says this study has the potential to change the standard of care in the parts of the world where most HIV-infected children live.

"Because nevirapine costs less than efavirenz and is more widely available in pediatric formulations, it is currently the more frequent choice for initial treatment in these children. However, our study suggests that efavirenz produces better outcomes," said Dr. Lowenthal.

Senior author Robert Gross, MD, MSCE, an associate professor of Infectious Diseases and Epidemiology at Penn Medicine, adds, "Given this evidence, it is very reasonable to adjust pediatric HIV treatment guidelines. However, as we move towards such changes, more work should be done to make efavirenz a more financially viable option for children on anti-retroviral therapy in these resource-limited settings."

Previous studies favoring efavirenz over nevirapine in adults have resulted in treatment guidelines for adults in many countries, including a few in resource-limited settings, to recommend the use of efavirenz over nevirapine. "In these low-resource settings, Non-Government Organizations typically work with countries’ medical programs to forecast their HIV-related drug needs and lobby companies to lower prices for bulk purchases," explained Lowenthal. "Through such programs, drugs that were once more expensive can become cost-effective."

Doctors Lowenthal and Gross applaud the work that the government of Botswana has done to both bring high-quality HIV treatment to its citizens and to facilitate the generation of knowledge to help improve treatment options. "Botswana has been extremely supportive of clinical trials and epidemiological studies, and is very forward thinking in its willingness to inform the world. For such a small country, the amount of research that comes out of Botswana on HIV and tuberculosis is tremendous, which has not only benefitted their public health, but public health for all."

**Journal Reference:**
Elizabeth D. Lowenthal et al. Association Between Efavirenz-Based Compared With Nevirapine-Based Antiretroviral Regimens and Virological Failure in HIV-Infected Children. JAMA, 2013 DOI: 10.1001/jama.2013.3710

**Immunogenicity of 2 Doses of HPV Vaccine in Younger Adolescents vs 3 Doses in Young Women**

**A Randomized Clinical Trial**

**Importance** Global use of human papillomavirus (HPV) vaccines to prevent cervical cancer is impeded by cost. A 2-dose schedule for girls may be possible.

**Objective** To determine whether mean antibody levels to HPV-16 and HPV-18 among girls receiving 2 doses was noninferior to women receiving 3 doses.

**Design, Setting, and Patients** Randomized, phase 3, postlicensure, multicenter, age-stratified, noninferiority immunogenicity study of 830 Canadian females from August 2007 through February 2011. Follow-up blood samples were provided by 675 participants (81%).

**Intervention** Girls (9-13 years) were randomized 1:1 to receive 3 doses of quadrivalent HPV vaccine at 0, 2, and 6 months (n = 261) or 2 doses at 0 and 6 months (n = 259). Young women (16-26 years) received 3 doses at 0, 2, and 6 months (n = 310). Antibody levels were measured at 0, 7, 18, 24, and 36 months.

**Main Outcomes and Measures** Primary outcome was noninferiority (95% CI, lower bound >0.5) of geometric mean titer (GMT) ratios for HPV-16 and HPV-18 for girls (2 doses) compared with young women (3 doses) 1 month after last dose. Secondary outcomes were noninferiority of GMT ratios of girls receiving 2 vs 3 doses of vaccine; and durability of noninferiority to 36 months.

**Results** The GMT ratios were noninferior for girls (2 doses) to women (3 doses): 2.07 (95% CI, 1.62-2.65) for HPV-16 and 1.76 (95% CI, 1.41-2.19) for HPV-18. Girls (3 doses) had GMT responses 1 month after last vaccination for HPV-16 of 7736 milli-Merck units per mL (mMU/mL) (95% CI, 6651-8999) and HPV-18 of 1730 mMU/mL (95% CI, 1512-1980). The GMT ratios were noninferior for girls (2 doses) to girls (3 doses): 0.95 (95% CI, 0.73-1.23) for HPV-16 and 0.68 (95% CI, 0.54-0.85) for HPV-18. The GMT ratios for girls (2 doses) to women (3 doses) remained noninferior for all genotypes to 36 months.
Antibody responses in girls were noninferior after 2 doses vs 3 doses for all 4 vaccine genotypes at month 7, but not for HPV-18 by month 24 or HPV-6 by month 36.

Conclusions and Relevance Among girls who received 2 doses of HPV vaccine 6 months apart, responses to HPV-16 and HPV-18 one month after the last dose were noninferior to those among young women who received 3 doses of the vaccine within 6 months. Because of the loss of noninferiority to some genotypes at 24 to 36 months in girls given 2 doses vs 3 doses, more data on the duration of protection are needed before reduced-dose schedules can be recommended.

Scientists' Hope for HIV Cure

The Telegraph (London), (04.27.2013) By Jake Wallis Simons

Researchers at Aarhus University Hospital in Denmark are conducting clinical trials with humans to test a “novel strategy” as a cure for HIV. The technique includes unmasking reservoirs of virus hiding in resting immune cells and bringing the virus to the surface of the cells to be destroyed by the body's natural immune system. In vitro studies of the technique were so successful that the Danish Research Council provided the funding for the clinical trials. According to Dr. Ole Søgaard, senior researcher at the Aarhus University Hospital and a member of the research team, early signs are promising.

The technique uses drugs called HDAC inhibitors that are commonly used to treat cancer, to drive the HIV from the patient’s DNA to the surface of infected cells. Søgaard is confident that they will be successful in activating HIV from the reservoirs; he sees the challenge as getting the patients’ immune system to recognize the virus and destroy it. He noted that this depends on the strength and sensitivity of individual immune systems and how large a proportion of the virus is exposed. Fifteen patients are participating in the trial.

Five universities in Britain—Oxford; Cambridge; Imperial College, London; University College, London; and King's College, London—have formed the Collaborative HIV Eradication of Reservoirs UK Biomedical Research Centre group (CHERUB) with the goal of finding an HIV cure. The group is researching the same technique as the researchers at Aarhus Hospital, but the research has not advanced to the clinical trial stage. CHERUB will combine the technique of releasing virus reservoirs with “immunotherapy” to give patients’ bodies a greater chance of destroying the virus. Also, their research is focusing on recently infected patients, as the researchers believe this will improve chances of a cure.

Ebola’s Secret Weapon Revealed

May 2, 2013 — Researchers have discovered the mechanism behind one of the Ebola virus' most dangerous attributes: its ability to disarm the adaptive immune system.

University of Texas Medical Branch at Galveston scientists determined that Ebola short-circuits the immune system using proteins that work together to shut down cellular signaling related to interferon. Disruption of this activity, the researchers found, allows Ebola to prevent the full development of dendritic cells that would otherwise trigger an immune response to the virus.

"Dendritic cells typically undergo a process called 'maturation' when they're infected by a virus—they change shape and present antigens on their surface that tell T-cells to attack that particular virus, thus generating an adaptive immune response," said UTMB professor Alexander Bukreyev, senior author of a paper on the discovery now online in the Journal of Virology. "But Ebola prevents dendritic-cell maturation and produces a severe infection without an effective adaptive immune response. We found that its ability to do this depends on several specific regions of two different proteins."

Bukreyev’s research group made the discovery after a series of procedures that started with a clone of the Ebola Zaire virus strain. Working under maximum-containment conditions in a biosafety level 4 facility in UTMB's Galveston National Laboratory, the team introduced mutations into the virus’ genetic code at four locations thought to generate proteins that affected immune response.

They then infected human dendritic cells with each of the resulting new strains and compared the results with those produced by unmutated Ebola Zaire. Each of the four new viruses, they found, was unable to suppress dendritic-cell maturation.

"We saw two very interesting things," Bukreyev said. "First, that these mutations restore maturation of dendritic cells very effectively, and second, that a mutation in even one of these genetic domains makes the virus unable to suppress maturation. That means that the virus needs multiple combined effects in order to undermine the immune system in this way."

Ebola's ability to evade the human immune response is one of the factors that accounts for its high mortality rate—up to 90 percent in humans—and the notoriety that it gained after its first appearance in
Zaire in 1976, in an outbreak that killed 280 people. Zaire—now the Democratic Republic of the Congo—is the home country of Ndongala Lubaki, lead author on the paper and a postdoctoral fellow at UTMB.

Journal Reference:

**Nyaope: dangerous drug, dangerous misconceptions**

Melody Hu
7 May 2013

Despite evidence that disproves the popular belief that antiretroviral (ARVs) drugs are an ingredient in nayope or “whoonga”, media coverage on the street drug continues to perpetuate this misconception.

This week a story on community action against whoonga in the *Sowetan* (6 May 2013) listed ARVs as one of the primary ingredients after heroin.

Even internationally-renowned sources such as NPR make the mistake of describing whoonga as a “concoction of an AIDS medication and a street drug, like marijuana or heroin” as recently as December 2012.

While the spread of whoonga use is certainly a newsworthy and distressing societal ill, many news outlets have been negligent in stopping the spread of another problem: the inaccurate perception that antiretroviral (ARV) drugs – in particular, the drug efavirenz – are an active ingredient in the street drug.

Despite the majority of nayope-related coverage falling into this trap, an edition of last week’s *The Star* (30 April 2013) bucked the trend and dismissed ARVs as an active component of the street drug. This example of considered and critical coverage proves that it is possible for the media to cut the ties between nayope and ARVs.

Stories about the street drug whoonga, also known as nayope, have been a fixture in local media since the drug first surfaced in 2006 in local South African townships. In recent years whoonga-related coverage grew as the side effects of the drug – severe addiction and a rise in crime to finance the habit – are increasingly felt by users, their families and communities.

The unfortunate link between whoonga and ARVs was first established in early reports on the street drug, which listed ARVs amongst a laundry list of ingredients, including heroin, rat poison, and marijuana. The fact that efavirenz, when taken orally, can sometimes produce side effects such as double vision and vivid dreams may contribute to its being believed to be an ingredient, according to the [Harvard School of Public Health](https://www.hsph.harvard.edu/)’s Dr. David Grelotti.

However, a number of experts have recently provided compelling evidence to the contrary.

Laboratory analyses of numerous local batches of whoonga in 2011 found no traces of ARVs in any of the samples, instead pinpointing heroin and rat poison as the major and active components of the street drug.

Furthermore, the heavy molecular weight of efavirenz makes it difficult to vaporise and thus almost impossible to smoke, according to UKZN’s Dr. Thavender Govender. President Jacob Zuma has also spoken out against this misconception.

It is no wonder then that reports of whoonga-related ARV theft from HIV clinics and even individual HIV-positive patients have made news in the past.

These incidents pointlessly damage treatment outcomes for the millions of HIV-positive South Africans who depend on ARVs to stay healthy – and coverage of these incidents that does not actively dispel the underlying misconceptions will only continue to perpetuate the misinformation and its negative consequences.

**Chinese Women With HIV Suffer Harassment and Stigma**

Human rights and press freedom are crucial if China is to achieve its stated AIDS prevention goals, particularly when it comes to safeguarding women and children, a leading AIDS specialist has said.

According to U.S.-based dissident doctor Wan Yanhai, AIDS prevention is intimately bound up with human rights and the empowerment of women, and China is no exception.

"During the late 1990s, a large number of women became infected with HIV through blood transfusions, and then passed the virus on to their unborn children, particularly in central China’s Henan province," Wan said in a commentary broadcast on RFA’s Cantonese Service on Monday.
Wan accused Beijing of deliberately obscuring the truth about the extent to which AIDS is transmitted through tainted blood transfusion and donation clinics in poorer areas of the country.

"They don't take the initiative to tell people of the dangers of HIV infection through blood donation or blood transfusions, which leads to a situation in which a lot of women are infected but do not know it, and pass [HIV] onto their children," he said.

"At the same time, a lot of men are infected without knowing it, and pass the virus on to their wives, and she to the children ... resulting in countless tragedies," Wan said.

Rampant discrimination
He said the government's stated aim to achieve zero mother-to-child infections is hampered by rampant discrimination against those who are found to be HIV positive in rural areas, where there is a widespread lack of education about transmission through blood transfusions.

"If a pregnant woman is found in the hospital to be infected with HIV, they could be subjected to serious victimization and social discrimination once the news gets out into the community," Wan said.

"The infected person can be evicted from their home or expelled from their village, while the pressure will rise on the woman to have an abortion," he said.

Such mothers, even if they do elect to give birth, will then be transferred to infectious diseases hospitals to have their baby, who will then carry this stigma on their record through school and into the labor market, regardless of their HIV status.

Goal of zero mother-to-child transmissions
Wan said that while the United Nations-backed targets of zero new infections, zero discrimination, and zero AIDS-related deaths are still a long way off for many governments, the target of zero mother-to-child transmissions set by the State Council in 2010 as part of the 12th Five Year Plan should be achievable for China.

But any prevention measures should take human rights protection as their cornerstone, he warned.

"The Chinese government’s efforts to prevent mother-to-child transmission of HIV need to be established on the basis of the protection of the rights of women and children," Wan said.

"Humans, unlike animals, have complicated social and economic relationships," Wan said.

Control and surveillance
The government view of people living with HIV as legitimate targets for the nationwide "stability maintenance" surveillance system is a serious barrier to prevention work, he added.

"Policies towards AIDS patients and those living with HIV often take the attitude of control and surveillance, while widespread stigma and poverty hamper the effectiveness of prevention efforts in the area of mother-to-child transmission," Wan said.

Wan also cited widespread discrimination against the population of migrant workers in the provision of medical treatment and assistance for women and children, and a lack of equal access to medications, medical treatment, and social welfare payments, compared to men.

"The rights of children born to couples who are not married must also be taken into account through the provision of household registration documents, free medical treatment, and child welfare payments," he said.

Blood-selling schemes
Activists estimate that at least 100,000 people in Henan alone have been infected with HIV through the blood-selling schemes run by local governments, which bought blood donations from impoverished rural residents, but also took a cut of the proceeds.

Collectors paid villagers to give their blood, pooled it without testing for HIV or any other infections, extracted the valuable plasma, and then re-injected the blood back into those who sold it.

Around 40,000 of them have now died of AIDS, leaving around 60,000 still living with HIV.

Under government measures announced in 2009, orphans whose parents have both died of AIDS should receive 600 yuan (U.S. $96) in subsidies every month, while single parent families with one parent lost to AIDS would get 200 yuan (U.S. $32) a month.

Many of those infected by tainted blood transfusions linked to blood-selling schemes or by mother-to-baby transmission have tried unsuccessfully to sue local authorities for failing to deliver promised treatment packages and adequate compensation, however.

Last August, hundreds of AIDS patients tore down the gates of the Henan provincial government buildings in a bid to get officials to take heed of their demands.

03-05-2013
Correction to HIV story
During the past week a story originating in the Telegraph entitled "Scientists on brink of HIV cure" has been published in other medias.

The article's title and subtitle suggests that a cure for HIV is expected in months. But this is not the case, according to the Danish researchers from Aarhus University Hospital whose work was cited in the Telegraph.

- We are not on the brink of an HIV cure, says Dr. Ole Søgaard from Aarhus University Hospital.
- We have an exciting study in which a potential anti-HIV latency reversing agent is tested in persons with HIV. We are making good progress, but there is still a long way to go."

The authors state that they regret if anyone got the impression from reading the article that there may be a cure for HIV in the immediate future. Like many others, the researchers believe that a cure for HIV is an achievable goal, but most likely it will take many years, numerous basic science discoveries, and several phase 1/2 trials before a HIV cure may actually be reached.

Immune cells that suppress genital herpes infections identified
Discovery has implications for development of a vaccine to prevent and treat HSV-2 and similar infections
SEATTLE – Fred Hutchinson Cancer Research Center and University of Washington scientists have identified a class of immune cells that reside long-term in the genital skin and mucosa and are believed to be responsible for suppressing recurring outbreaks of genital herpes. These immune cells also play a role in suppressing symptoms of genital herpes, which is why most sufferers of the disease are asymptomatic when viral reactivations occur.

The discovery of this subtype of immune cells, called CD8αα+ T cells, opens a new avenue of research to develop a vaccine to prevent and treat herpes simplex virus type 2, or HSV-2. Identifying these T cells' specific molecular targets, called epitopes, is the next step in developing a vaccine.

The findings are described in the May 8 advance online edition of Nature.

Better understanding of these newly identified CD8αα+ T cells may also play a critical role in developing effective vaccines against other types of skin and mucosal infections, according to senior author Larry Corey, M.D., Ph.D., an internationally renowned virologist and president and director of Fred Hutch.

"The discovery of this special class of cells that sit right at the nerve endings where HSV-2 is released into skin is changing how we think about HSV-2 and possible vaccines," said Corey. "For the first time, we know the type of immune cells that the body uses to prevent outbreaks. We also know these cells are quite effective in containing most reactivations of HSV-2. If we can boost the effectiveness of these immune cells we are likely to be able to contain this infection at the point of attack and stop the virus from spreading in the first place. We’re excited about our discoveries because these cells might also prevent other types of viral infections, including HIV infection."

There is currently no effective vaccine for genital herpes. "While antiviral treatment is available, the virus often breaks through this barrier and patients still can transmit the infection to others," Corey said. "In addition, newborn herpes is one of the leading infections transmitted from mothers to children at the time of delivery. An effective genital herpes vaccine is needed to eliminate this complication of HSV-2 infection."

The long-term persistence of CD8αα+ T cells where initial infection occurs may explain why patients have asymptomatic recurrences of genital herpes because these cells constantly recognize and eliminate the virus, according to Jia Zhu, Ph.D., corresponding author, research assistant professor in Laboratory Medicine at the University of Washington and an affiliate investigator in the Fred Hutch Vaccine and Infectious Disease Division.

"The cells we found perform immune surveillance and contain the virus in the key battlefield where infection occurs, which is the dermal-epidermal junction," said Zhu. "These cells are persistent in the skin and represent a newly discovered phenotype distinguished from those of CD8+ T cells circulating in the blood."

The dermal-epidermal junction (DEJ) is where the dermis (outer skin layer) connects to the epidermis (the tissue layers just beneath the skin). This junction is important because of the roles it plays in cellular communication, nutrient exchange and absorption, and other skin functions.

Scientists examined the DEJ for T cell activity because this is where the genital herpes virus multiplies after reactivating and traveling from its hiding place in the body's sensory neurons. Previous research by
the same research group showed that the nerve endings reach the dermal-epidermal junction and release the virus that infects the skin and can cause lesions.

Prior to this research, CD8αα+ T cells were known to exist in the gut mucosa. Much of the research on CD8+ T cells has focused on studying them in the circulating blood, which has a dominant phenotype of CD8αβ+. Fred Hutch and UW scientists compared the two types of CD8+ T cells and found that only the CD8αα+ T cells persist in the skin while CD8αβ+ T cells diminished from the tissue after healing of a herpes lesion.

"We did not expect to find CD8αα+ T cells in the skin," Zhu said. "This was a surprise."

The research involved using novel technologies to examine the T cells in human tissues. In all, the work provides a roadmap that can be applied to other human diseases, according to Zhu.

Zhu said the studies the research group performed in humans are unique. "To our knowledge, we are the only research group to use sequential human biopsies to study CD8+ T cell function in situ, in their natural spatial distribution and at their original physiological state," she said.

According to the federal Centers for Disease Control and Prevention, 776,000 people in the United States are newly infected with herpes annually. Nationwide, 16.2 percent, or about one out of six people aged 14 to 49 years have genital HSV-2 infection. Generally, a person can only get HSV-2 infection during sexual contact with someone who has a genital HSV-2 infection. Transmission can occur from an infected partner who does not have a visible sore and may not know that he or she is infected.

Most individuals infected with HSV-2 or the related HSV-1, which causes genital herpes and cold sores, experience either no symptoms or have very mild symptoms that go unnoticed or are mistaken for another skin condition. Because of this, most people infected with HSV-2 are not aware of their infection.

Antibiotics Could Cure 40 Percent of Chronic Back Pain Patients
Posted on May 9, 2013

Danish scientists have discovered that many cases of chronic back pain are caused by bacterial infections, which means they could be cured cheaply with antibiotics rather than surgery.

Peter Hamlyn, a surgeon at University College London hospital whom The Guardian describes as “one of the UK’s most eminent spinal surgeons,” said the discovery is the most significant he has witnessed in his career and deserving of a Nobel Prize. “This is vast. We are talking about probably half of all spinal surgery for back pain being replaced by taking antibiotics,” he said.

Specialists have long known that infections are sometimes to blame for chronic back pain, but such cases were thought to be exceptional.

Experts caution that the breakthrough will not help sufferers of normal, acute or sub-acute back pain. But for people with chronic pain, the discovery could radically improve their lives. “These are people who live a life on the edge because they are so handicapped with pain,” said Dr. Hanne Albert of the Danish research team. “We are returning them to a form of normality they would never have expected.”

—Posted by Alexander Reed Kelly.

Ian Sample at The Guardian:

The Danish team describe their work in two papers published in the European Spine Journal. In the first report, they explain how bacterial infections inside slipped discs can cause painful inflammation and tiny fractures in the surrounding vertebrae.

Working with doctors in Birmingham, the Danish team examined tissue removed from patients for signs of infection. Nearly half tested positive, and of these, more than 80% carried bugs called Propionibacterium acnes.

The microbes are better known for causing acne. They lurk around hair roots and in the crevices in our teeth, but can get into the bloodstream during tooth brushing. Normally they cause no harm, but the situation may change when a person suffers a slipped disc. To heal the damage, the body grows small
blood vessels into the disc. Rather than helping, though, they ferry bacteria inside, where they grow and cause serious inflammation and damage to neighbouring vertebrae that shows up on an MRI scan.

Up to 40% of patients with chronic back pain could be cured with a course of antibiotics rather than surgery, in a medical breakthrough that one spinal surgeon says is worthy of a Nobel prize.

Surgeons in the UK and elsewhere are reviewing how they treat patients with chronic back pain after scientists discovered that many of the worst cases were due to bacterial infections.

The shock finding means that scores of patients with unrelenting lower back pain will no longer face major operations but can instead be cured with courses of antibiotics costing around £114.

One of the UK's most eminent spinal surgeons said the discovery was the greatest he had witnessed in his professional life, and that its impact on medicine was worthy of a Nobel prize.

"This is vast. We are talking about probably half of all spinal surgery for back pain being replaced by taking antibiotics," said Peter Hamlyn, a consultant neurological and spinal surgeon at University College London hospital.

Hamlyn recently operated on rugby player Tom Croft, who was called up for the British and Irish Lions summer tour last month after missing most of the season with a broken neck.

Specialists who deal with back pain have long known that infections are sometimes to blame, but these cases were thought to be exceptional. That thinking has been overturned by scientists at the University of Southern Denmark who found that 20% to 40% of chronic lower back pain was caused by bacterial infections.

In Britain today, around 4 million people can expect to suffer from chronic lower back pain at some point in their lives. The latest work suggests that more than half a million of them would benefit from antibiotics.

"This will not help people with normal back pain, those with acute, or sub-acute pain – only those with chronic lower back pain," Dr Hanne Albert, of the Danish research team, told the Guardian. "These are people who live a life on the edge because they are so handicapped with pain. We are returning them to a form of normality they would never have expected."

Claus Manniche, a senior researcher in the group, said the discovery was the culmination of 10 years of hard work. "It's been tough. There have been ups and downs. This is one those questions that a lot of our colleagues did not understand at the beginning. To find bacteria really confronts all we have thought up to this date as back pain researchers," he said.

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The microbes are better known for causing acne. They lurk around hair roots and in the crevices in our teeth, but can get into the bloodstream during tooth brushing. Normally they cause no harm, but the situation may change when a person suffers a slipped disc. To heal the damage, the body grows small blood vessels into the disc. Rather than helping, though, they ferry bacteria inside, where they grow and cause serious inflammation and damage to neighbouring vertebrae that shows up on an MRI scan.

In the second paper, the scientists proved they could cure chronic back pain with a 100-day course of antibiotics. In a randomised trial, the drugs reduced pain in 80% of patients who had suffered for more than six months and had signs of damaged vertebrae under MRI scans.

Albert stressed that antibiotics would not work for all back pain. Over-use of the drugs could lead to more antibiotic-resistant bacteria, which are already a major problem in hospitals. But she also warned that many patients will be having ineffective surgery instead of antibiotics that could alleviate their pain.

"We have to spread the word to the public, and to educate the clinicians, so the right people get the right treatment, and in five years' time are not having unnecessary surgery," she said.

Hamlyn said future research should aim to increase the number of patients that respond to antibiotics, and speed up the time it takes them to feel an improvement, perhaps by using more targeted drugs.

The NHS spends £480m on spinal surgery each year, the majority of which is for back pain. A minor operation can fix a slipped disc, which happens when one of the soft cushions of tissue between the bones in the spine pops out and presses on nearby nerves. The surgeons simply cut off the protruding part of the disc. But patients who suffer pain all day and night can be offered major operations to fuse damaged vertebrae or have artificial discs implanted.
"It may be that we can save £250m from the NHS budget by doing away with unnecessary operations. The price of the antibiotic treatment is only £114. It is spectacularly different to surgery. I genuinely believe they deserve a Nobel prize," said Hamlyn. Other spinal surgeons have met Albert and are reviewing the procedures they offer for patients.

**Scientists confirm that the Justinianic Plague was caused by the bacterium Yersinia pestis**

**Ancient DNA analyses of skeletal remains of plague victims from the 6th century AD provide information about the phylogeny and the place of origin of this pandemic**

10.05.2013

From the several pandemics generally called 'pestilences' three are historically recognized as due to plague, but only for the third pandemic of the 19th-21st centuries AD there were microbiological evidences that the causing agent was the bacterium *Yersinia pestis*. "For a long time scholars from different disciplines have intensively discussed about the actual etiological agents of the past pandemics. Only ancient DNA analyses carried out on skeletal remains of plague victims could finally conclude the debate", said Dr. Barbara Bramanti of the Palaeogenetics Group at the Institute of Anthropology at Johannes Gutenberg University Mainz (JGU). About two years ago, she headed the international team which demonstrated beyond any doubt that *Y. pestis* also caused the second pandemic of the 14th-17th centuries including the Black Death, the infamous epidemic that ravaged Europe from 1346-1351.

Bramanti and her Mainz colleague Stephanie Hänsch now cooperated with the University of Munich, the German Bundeswehr, and international scholars to solve the debate as to whether *Y. pestis* caused the so-called Justinianic Plague of the 6th-8th centuries AD. The results of ancient DNA analyses carried out on the early medieval cemetery of Aschheim in Bavaria were published last week in *PLoS Pathogens*. They confirmed unambiguously that *Y. pestis* was indeed the causing agent of the first pandemic, in contrast to what has been postulated by other scientists recently. This revolutionary result is supported by the analysis of the genotype of the ancient strain which provide information about the phylogeny and the place of origin of this plague. As for the second and third pandemic, the original sources of the plague bacillus were in Asia.

"It remains questionable whether at the time of the Byzantine Emperor Justinian only one strain or more were disseminated in Europe, as it was at the time of the Black Death," suggested Bramanti and Hänsch. To further investigate this and other open questions about the modalities and route of transmission of the medieval plagues, Bramanti has recently obtained an ERC Advanced Grant for the project "The medieval plagues: ecology, transmission modalities and routes of the infection" (MedPlag) and will move to the Center for Ecological and Evolutionary Synthesis (CEES) at the University of Oslo in Norway. The CEES, chaired by Nils Chr. Stenseth, has an outstanding and rewarded record of excellence in the research on infectious diseases and in particular on *Y. pestis*.

**New Discovery May Lead the Way to Improved Whooping Cough Vaccine**

May 10, 2013 — Scientists at Trinity College Dublin have made novel discoveries concerning the current vaccine against whooping cough that may lead to the development of an improved future vaccine. The findings could help reduce the incidence of the disease which is increasing in developed countries. The research led by Professor of Experimental Immunology, Kingston Mills has just been published in the journal *PLoS Pathogens*.

A new vaccine against whooping cough, caused by the bacteria *Bordetella pertussis* was first introduced to the routine vaccination schedule for infants and children in most developed countries, including Ireland over a decade ago. Prior to the introduction of this vaccine, children were immunised with a vaccine made from whole bacteria. Although this 'whole cell pertussis vaccine’ was effective at preventing the infection, it had been associated with side effects. Dissatisfaction with that vaccine led to the development of an 'acellular pertussis vaccine’ made from components of the bacteria combined with an adjuvant to boost immune responses.

Following its introduction in the late 1990s, the new vaccine has proved to be very safe and has been effective in controlling the potentially fatal disease of whooping cough in children. However, protective immunity conferred with the vaccine falls quite quickly, necessitating frequent booster vaccinations. This fall off in the immunity may be contributing to the number of whooping cough cases which are increasing with quite dramatic increases reported in certain countries, including the US, Australia and the Netherlands.
Professor Kingston Mills's research team at the School of Biochemistry and Immunology in the Trinity Biomedical Sciences Institute has discovered important mechanistic differences in the type of immune responses induced with the new 'acellular' and old 'whole cell' vaccine. The whole cell vaccine, although much more likely to cause adverse reactions in recipients, was capable of inducing strong cellular immune responses mediated by white blood cells called T cells, in particular a type of T cell called Th1 cells. In contrast, the new acellular vaccine, although safer, was less effective in inducing cellular immunity, but instead induced immunity mediated by antibodies and another type of T cell called a Th17 cell.

Most vaccines include a component called an adjuvant to boost immune responses to the bacterial or viral antigens in the vaccine and the acellular pertussis vaccine uses an aluminium salt, called alum. However, Dr Padraig Ross, Dr Sarah Higgins and Ms Aideen Allen in Professor Mills' laboratory, working in collaboration with Dr Rachel McLoughlin and Dr Ed Lavelle, have shown that the vaccine could be improved further through the use of a different adjuvant.

The current vaccine does not enhance the induction of Th1 cells, required for conferring optimum protective immunity against the bacteria. They showed that by switching the adjuvant from alum to an adjuvant based on bacterial DNA, they could induce the crucial Th1 cells and thereby enhance the efficacy of the vaccine against Bordetella pertussis infection in a murine model. The new vaccine has the potential to protect a higher proportion of immunised children using a lower number of doses.

Commenting on the significance of the findings, Professor Mills said: "Although it will not be an easy task to implement, our findings should pave the way for an improved vaccine against whooping cough in children."

**Journal Reference:**

**Up to a third of HIV infections in European gay men may have come from another country**

Recent infections also more likely to have crossed borders
Gus Cairns
Published: 10 May 2013

A study (Frentz) that looked at genetic similarities in the HIV from recently diagnosed people in 24 European countries found that among ‘clusters’ of closely related viruses (which indicate networks of transmission), a quarter of people who were in a cluster were connected to people diagnosed in other countries.

This was the particularly the case in gay men, where nearly a third (31%) of people in viral ‘clusters’ were connected to at least one diagnosis in another country. In contrast, only 14% of heterosexual people belonged to international clusters, indicating less mobility.

People who had been recently infected, and people with HIV subtype B, were also more likely to belong to a cluster indicating cross-border infections: but as these are both more associated with gay men than with other risk groups, only being gay remained significantly associated with belonging to an international cluster.

Since two-thirds of ‘clusters’ actually only comprised two people, this indicates that a significant proportion of Europeans diagnosed with HIV, and especially gay men, acquire their HIV either while abroad, or from someone from abroad. This phylogenetic study shows that risky sexual encounters among gay men while travelling abroad, as seen in another large European study, are indeed resulting in some cross-border HIV transmission.

On the other hand – as the researchers emphasised – the fact that a majority of connected infections, especially among heterosexuals and people with non-B HIV subtype, were in sole-country clusters, also indicates that we may need to revise assumptions that immigration from high-prevalence countries is still the biggest contributor to infections among heterosexual people.

**The SPREAD study: patient characteristics**

This study is part of an EU-funded research collaboration called the SPREAD Programme, which has been analysing the genetic makeup of HIV from recently diagnosed people in 25 European countries (samples taken from 2002 onwards).
In this study, the SPREAD researchers genetically analysed samples from 4260 recently diagnosed people, with the largest numbers from Germany, Spain, Sweden, Belgium, the Czech Republic and Denmark. Fifty-five per cent of participants gave their origin as western Europe, 21% eastern Europe, and 11% sub-Saharan Africa. Nearly half (48%) said they most likely acquired HIV from gay sex, 35% from heterosexual sex and 8% from injecting drugs. Seventy per cent of people were living in their country of origin; in Israel, Sweden, and Norway, 50% or more of the people diagnosed were immigrants from outside Europe, with Ethiopia, Zimbabwe and Thailand the most common countries of origin.

The mean viral load in these people was 63,000 copies/ml and their mean CD4 count 363 cells/mm³. Twenty-nine per cent of infections were acquired less than a year ago, though in two-thirds of cases the duration of infection was unknown. Nine per cent of the samples analysed were resistant to at least one HIV drug.

Cautions and caveats
Before commenting on clusters, it is important to mention that this study is not representative of all infections throughout western and central Europe. The UK and France are not part of the SPREAD programme, and neither are Switzerland, Hungary and several Balkan countries. It is important also to emphasise that SPREAD only looks at a sample of HIV infections in each country and that, in some countries, risk groups were over- or under-represented. Some countries, such as Poland and Cyprus, do not routinely collect data on which risk group diagnosed people belong to.

Finally, only 31% of HIV viruses were closely related to any other infection, so the majority of infections were not in ‘clusters’ and one cannot say anything about where they might come from. The definition of being in a ‘cluster’ is that a virus was more than 98% genetically similar to at least one other virus analysed by SPREAD. As the researchers say, they might have detected many more clusters given whole-country databases to analyse.

Connected infections: findings
Nonetheless, some interesting data emerged from the 457 clusters analysed, and the 1330 people who belonged to those clusters. Seventeen per cent of clusters featured people diagnosed in more than one country and these international clusters tended to be larger, containing 26% of diagnosed people.

Four in ten of these international clusters featured people only from directly neighbouring countries, but the other 60% included people diagnosed in countries not sharing a common border. The largest cluster comprised 28 people with closely similar virus who were diagnosed in Slovakia, the Czech Republic and Italy. There was also a large German/Spanish cluster.

Patients found to be in clusters were more likely to be gay men. Sixty-three per cent of people in clusters were gay versus 41% not in clusters They were also more likely to have subtype B virus, the predominant type in native Europeans (82% in clusters, 59% not in clusters), and to have been infected in the last year – 39% of people in clusters had been recently infected versus 24% not in clusters.

Thirty-one per cent of gay men in clusters were in an international one and 29% of people recently infected.

In contrast only 15% of injecting drug users, 14% of heterosexual people and 13% of people with a non-B subtype of HIV were in an international cluster.

Conclusions
This study confirms findings from a number of other national and international studies. On the one hand, studies from Belgium and Switzerland show that HIV rarely crosses between risk groups, and a study from the Netherlands found that HIV from African and Caribbean immigrants was not crossing into the general population. As the researchers comment about this study, “The small number of migrants, their relatively moderate sexual risk behaviour and low mixing with Dutch heterosexuals” means that spread of non-B-type HIV into the heterosexual population is hardly happening.

Even in gay men, 70% of clustered infections were solely in one country. However, it is also notable that gay men were twice as likely as heterosexual people to be members of internationally connected clusters of infection, and also more likely to have been recently infected. (In this connection, it is also interesting that the proportion of people [in clusters or not] who were recently infected increased from 33% of diagnoses in 2002 to 48% in 2007, though this could just as well indicate increased testing rates as an increased rate of infection.)

An analysis of the large European EMIS Study of men who have sex with men, presented at the International AIDS Conference in Washington last year (Fernández-Dávila), found that a substantial number of gay men in Europe had their last high-risk sex while not in their own country, with Spain and Berlin in Germany the most frequently cited locations (and Paris and London, not included in SPREAD, coming in as the third and fourth most popular destinations).
This study suggests both that settled immigrant communities may need better information about HIV risk in their own community but also that gay men may need better information about infection risk whilst traveling abroad.

References

Second-generation protease inhibitor faldaprevir cures up to 80% of hepatitis C

Response gap between genotype 1a and genotype 1b

Keith Alcorn
Published: 30 April 2013

A second-generation hepatitis C protease inhibitor, faldaprevir, cured up to 80% of previously untreated people with genotype 1 hepatitis C virus (HCV) infection when combined with pegylated interferon and ribavirin, Professor Peter Ferenci of the Medical University of Vienna reported on Saturday at the International Liver Congress (EASL 2013) in Amsterdam.

The findings came from the phase III STARTVerso study which randomised 656 people with chronic hepatitis C infection to receive at least 12 weeks of faldaprevir or placebo, in combination with pegylated interferon and ribavirin.

The results of STARTVerso, together with two other phase III study results, are likely to be submitted later this year to support the licensing of faldaprevir in the United States and European Union.

Faldaprevir is being developed by Boehringer-Ingelheim for use with pegylated interferon and ribavirin, and also for use in interferon-free regimens.

Speaking at a press conference on the opening day of the International Liver Congress, EASL Secretary-General Prof. Mark Thursz said that he expected faldaprevir, another second-generation protease inhibitor simeprevir, and sofosbuvir, a NS5B nucleotide polymerase inhibitor, to “totally cannibalise” the market niche for hepatitis C therapy currently occupied by the first-generation protease inhibitors boceprevir (Victrelis) and telaprevir (Incivo).

STARTVerso randomised participants to receive one of two doses of faldaprevir (120mg once daily or 240mg once daily) or placebo for at least 12 weeks, in combination with pegylated interferon and ribavirin.

At 12 weeks, participants in the 120mg arm were randomised to continue faldaprevir treatment to week 24, or to a placebo, and all participants continued pegylated interferon and ribavirin to week 24. At week 24, people who had achieved HCV RNA <25 IU/ml at week 4 and HCV RNA <25 IU/ml and undetectable at week 8 (Early Treatment Success) were eligible to stop all treatment. The remainder received pegylated interferon and ribavirin to week 48.

In the 240mg arm, all participants stopped faldaprevir at week 12 but continued pegylated interferon and ribavirin to week 24. All participants in this arm who achieved Early Treatment Success (ETS) were able to stop treatment at week 24. The remainder received pegylated interferon and ribavirin to week 48. In the control arm, all participants received pegylated interferon and ribavirin plus placebo for 24 weeks, followed by a further 24 weeks of pegylated interferon and ribavirin.

Depending on their treatment assignment, up to 80% of people who received faldaprevir achieved a sustained virologic response 12 weeks after stopping treatment (SVR12). There was no difference in efficacy between the two doses of faldaprevir but the lower dose (120mg) was better tolerated.

Around two-thirds of study participants had genotype 1b hepatitis C infection. Between 58 and 65% had a CT or TT IL28B genotype, indicating the likelihood of a poorer response to interferon-based treatment. Participants were predominantly Caucasian (78%) and in the earlier stages of liver disease (META VIR score F1-F2) (81-84%) according to study arm. Six per cent of participants had cirrhosis.

Final results showed that by intent-to-treat analysis 80% of patients in the faldaprevir 240mg arm and 75% in the 120mg arm achieved SVR12, compared with 52% in the pegylated interferon/ribavirin arm (p< 0.001).

A total of 88% achieved early treatment success and were eligible to stop all treatment at week 24. Of these patients 89% in the 240mg group and 86% in the 120mg group achieved SVR12. There was a pronounced difference in SVR rates in the faldaprevir-treated patients between those with detectable or undetectable HCV RNA at week 4. Among those with HCV RNA below the limit of quantification but still detectable at week 4, 77% in the 120mg and 72% in the 240mg achieved SVR12. Among those with undetectable HCV RNA at week 12, 91 and 94% respectively achieved SVR12.
There was a modest difference in response rates between HCV genotype 1a and 1b in the faldapevир 240mg group (76 vs 83%) favouring genotype 1b. The difference was more pronounced in the faldapevир 120mg group (69 vs 84%). This difference in SVR12 rates was not explained by higher rates of null or partial response, but by higher rates of viral breakthrough during treatment and post-treatment viral relapse.

Responses also differed according to IL28B genotype. Those with the favourable CC genotype had the best responses (90 and 95% in the 120mg and 240mg groups respectively), compared to 70 and 69% for those with the CT genotype and 76 and 79% for the TT genotype.

Adverse events leading to treatment discontinuation occurred in 4% of placebo recipients, 4% of faldapevир 120mg recipients and 5% of faldapevир 240mg recipients. Serious adverse events occurred in 7% of faldapevир recipients. The only adverse events that occurred more frequently in faldapevир-treated patients were gastrointestinal, and these occurred with highest frequency in the 240mg group.

12% of these patients experienced gastrointestinal symptoms compared with 7% in the faldapevир 120mg group and 3% in the control group who were taking pegylated interferon and ribavirin with placebo.

Mild to moderate rash was also observed in more patients taking faldapevир compared with the placebo group. 32-33% of patients taking 120mg or 240 mg faldapevир respectively experienced rash, compared with 22% in the placebo group. Severe rash was rarely observed, occurring in two patients in each study arm (6 cases overall). None of the rash events observed in this trial were life-threatening, and all events resolved.

A higher frequency of jaundice in faldapevир-treated patients (3% in the 240mg group) was accompanied by a higher frequency of bilirubin elevations (53% of patients in the 240mg group experienced bilirubin elevations, compared to 12% in the 120mg group). These increases were transient and bilirubin levels returned to normal after week 16.

There was no difference in the incidence of anaemia between the study arms up to week 24. People in the faldapevир arms were less likely to require ribavirin dose reductions.

The 240mg dose offered no clear advantages over the 120mg dose, Professor Ferenci concluded, although the finding of lower response rates in genotype 1a in the 120mg dose group may leave that conclusion open to question.

Reference

New Test for H7N9 Bird Flu in China May Help Slow Outbreak, Prevent Pandemic
May 10, 2013 — Breaking research appearing online today in Clinical Chemistry, the journal of AACC, demonstrates that a recently developed diagnostic test can detect the new strain of influenza (H7N9) currently causing an outbreak in China.

Since the end of March, 31 people have died from H7N9 infection, and the number of confirmed cases has climbed to 129. Evidence suggests that most H7N9 infections have arisen from poultry-to-human transmission, and that passage of the virus between humans is limited. However, researchers have also found mutations in the virus that are known to help avian viruses adapt to mammalian hosts. If these mutations lead to sustained human-to-human transmission, a serious pandemic could occur.

In this study, Wong et al. designed a diagnostic test with high specificity for the H7N9 virus that does not cross react with distantly related viruses, including all previously known avian and mammalian H7 viruses. They also show that this one-step quantitative real-time PCR assay enables specimen processing in about 3 hours.

According to the authors, this new test should also detect viruses closely related to the H7N9 virus. If confirmed, this capability could prove vital; it’s likely that the H7N9 virus is evolving rapidly, and there could be multiple introductions of avian H7N9 viruses from animals to humans. The test also demonstrates a detection limit of ~0.04 median tissue culture infective dose (TCID50) per reaction. This means that it should be sensitive enough to identify patients with active virus replications.

"These results suggest that the established assay should be suitable for screening H7N9 viruses in human samples," said lead investigator Leo Poon, PhD, of the University of Hong Kong, though additional evaluation using clinical specimens from H7N9 patients is needed.
If validated, this diagnostic test could help health officials avert a potential pandemic by allowing them to monitor the spread of the virus. The test could also identify H7N9 patients in the early stages of infection, improving their chances of responding to clinical treatments.

**Journal Reference:**

**Scientists Find Key to Gene-Silencing Activity**
May 8, 2013 — A team led by scientists at The Scripps Research Institute (TSRI) has found how to boost or inhibit a gene-silencing mechanism that normally serves as a major controller of cells’ activities. The discovery could lead to a powerful new class of drugs against viral infections, cancers and other diseases.

"Learning to control natural gene silencing processes will allow an entirely new approach to treating human disease," said Ian J. MacRae, assistant professor in TSRI’s Department of Integrative Structural and Computational Biology and principal investigator for the study, which appears as the cover story in the May 9, 2013 issue of the journal *Molecular Cell.*

**A Scientific Mystery and Technical Conundrum**
The gene-silencer in question is Argonaute 2, a molecular machine in cells that can grab and destroy the RNA transcripts of specific genes, preventing them from being translated into proteins. Argonaute 2 and other Argonaute proteins regulate the influence of about a third of the genes found in humans and other mammals—and thus are among the most important modulators of our cells’ day-to-day activities.

Argonautes' gene-silencing functions also help cells cope with rogue genetic activity from invading viruses or cancer-promoting DNA mutations.

Yet Argonautes' workings are complex and not yet entirely understood. For example, before it starts a search-and-destroy mission against a specific type of target RNA, an Argonaute 2 protein takes on board a target-recognition device: a short length of "guide RNA," also known as a microRNA (miRNA). The miRNA’s sequence is mostly complementary to the target RNA’s—a sort of chemical mirror-image—so that it can stick tightly to it.

But how do an Argonaute protein and its miRNA guide, having formed their partnership, manage to part company? It has been a scientific mystery and technical conundrum for researchers, who have found it hard to separate Argonaute proteins from miRNAs in the lab dish.

"That problem led us to look for a way to get Argonautes to unload these miRNAs," said Nabanita De, a postdoctoral fellow in MacRae’s laboratory who was first author of the new study.

**Matches and Mismatches**
In an initial set of experiments, the team demonstrated that when an miRNA hooks up with an Argonaute 2, the pair do remain locked together and functioning for an exceptionally long time: days to weeks, whereas solo miRNA normally is degraded within minutes.

Yet prior studies by other laboratories have hinted at the existence of mechanisms that can hasten the separation of miRNAs from Argonautes. Some viruses, for example, produce decoy target RNAs that virtually nullify the activity of the corresponding miRNAs, seemingly by destabilizing the miRNA-Argonaute pairing. A key feature of these decoy target RNAs is that they make an almost perfect complementary match to the miRNAs—especially at one end of the miRNAs, known as the three-prime or 3' end. In this respect, they match the miRNAs much better than the natural gene transcripts that the miRNAs evolved to target.

De confirmed that decoy RNAs designed to match miRNAs this way can greatly hasten the miRNAs' "unloading" from Argonautes, thus effectively dialing down these miRNAs' normal gene-silencing activities. By contrast, mismatches at the 3' end delayed unloading, enhancing the gene-silencing activity. Why do these matches and mismatches have such effects on the miRNA-Argonaute pairing? The mechanisms aren’t obvious. But De noted that mismatches at the opposite end of miRNAs—the 5' end—have the opposite effect. "Targets with 5'-end mismatches are actually better at unloading miRNAs from Argonaute," she said.

"The next thing we’re trying to figure out is how all that works," said MacRae. "We have some guesses but no clear answer."

In a study reported last year, MacRae's laboratory used X-ray crystallography to determine the first high-resolution atomic structure of an Argonaute 2-miRNA complex. Now the team is working on a structural study of the complex as it grabs a target RNA. "When we can see the structural details of that
interaction, then I think we'll have a much better handle on this loading and unloading process," said MacRae.

**Many Potential Applications**

Scientists already have begun developing gene-silencing drugs that work like miRNAs; they are taken up by Argonaute proteins as guide RNAs and lead to the silencing of targeted gene transcripts. Pharmaceutical companies also are developing drugs that bind directly to miRNAs to inhibit their activity. The findings here suggest a new and, in principle, more powerful class of miRNA inhibitors/enhancers, aimed at destabilizing or stabilizing the miRNA-Argonaute complex.

"I can think of many applications for these," said MacRae. "One of the most obvious would be against hepatitis C virus, which requires a certain miRNA in liver cells for efficient replication; an RNA-based drug that speeds up the unloading of this virus-enhancing miRNA would be a powerful approach for shutting down the virus." A better understanding of the miRNA loading and unloading process also should lead to better miRNA-type drugs, he added.

**Journal Reference:**


**Newly described type of immune cell and T cells share similar path to maturity**

Better understanding of cells' development has implications in study of inflammatory diseases

PHILADELPHIA — Labs around the world, and a core group at Penn, have been studying recently described populations of immune cells called innate lymphoid cells (ILCs). Some researchers liken them to foot soldiers that protect boundary tissues such as the skin, the lining of the lung, and the lining of the gut from microbial onslaught. They also have shown they play a role in inflammatory disease, when the body's immune system is too active.

In animal studies, group-2 innate lymphoid cells (ILC2s) confer immunity during a parasitic infection in mice and are also involved in allergic airway inflammation. A team of Perelman School of Medicine, researchers from the Departments of Medicine, Microbiology, Pathology and Laboratory Medicine, and Cancer Biology, found that maturation of ILC2s requires T-cell factor 1 (TCF-1, the product of the Tcf7 gene) to move forward. TCF-1 is protein that binds to specific parts of DNA to control transcription of genetic information from DNA to messenger RNA.

Avinash Bhandoola, PhD, professor of Pathology and Laboratory Medicine, and Qi Yang, PhD, a postdoc in the Bhandoola lab, describe in *Immunity* that one mechanism used to build ILCs is the same as that in T cells. Both cell types use a protein pathway centered on Notch that the lab of coauthor Warren Pear, MD, PhD, also in the Pathology and Laboratory Medicine, has studied for the last two decades. Other contributing authors are from the laboratory of David Artis, PhD in Microbiology, that are experts in ILC function, and Angela Haczku, MD, PhD, in the Department of Medicine, who focuses on asthma.

But what makes ILCs and T cells different in their final development? T cells are made in the thymus. ILCs don’t need the thymus, but researchers don’t know exactly where they are produced, just that the thymus isn’t essential for their normal development, unlike T cells.

In the *Immunity* study, mice without the Tcf7 gene also lack ILC2, and were unable to mount an ILC2 immune response. Forced expression of TCF-1 in bone marrow progenitor cells in the mice partially bypassed the requirement for Notch signaling in the generation of ILC2 in the mice. The researchers suggest that transcription factors such as TCF-1 that underlie early steps of T cell development are also implicated in the development of innate lymphoid cells.

The collaborators’ next steps are to better understand the basic steps of ILC development and build mouse models to test ILC function. "We want to know where ILCs develop in the body and what progenitor cells give rise to ILCs." says Bhandoola. "If we succeed in constructing mouse models missing different types of ILC, our collaborators can use them to better figure out what these cells do, and perhaps eventually how to control them."

**Cutting-Edge Bacteria Research Leads to More Effective Treatment of Complex Infections**

May 14, 2013 — Bacteria are life forms, which, like all other life forms, struggle for the best living conditions for themselves. Therefore they will try to avoid getting attacked by the human immune system,
and therefore they have developed various ways to protect themselves from the human immune system. When safe from the immune system, they can focus on breeding and multiplying, and if they become numerous enough, the human body will experience their presence as an infection. Some bacteria are relatively harmless, while others are fatal.

The bacteria avoid being attacked by the human immune system by forming a biofilm—a surface to protect them against the immune system. "The biofilm contributes to bacterial resistance, and that can cause severe, persistent infections around heart valve implants and in lungs and the urinary tract," explains postdoc. Mikkel Girke Jørgensen from the Department of Biochemistry and Molecular Biology at the University of Southern Denmark. Together with professor Poul Valentin-Hansen from the same institution and scientists from American Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, and Georgetown University Medical Center, Washington DC, he stands behind the new discovery.

The researchers now understand the underlying regulatory mechanisms behind the formation of biofilms. The mechanism involves small RNA molecules, which can affect bacterial gene expression and thus the decision of whether to form biofilm or not. Bacteria can move by using their so-called flagella to swim with. When they need to form biofilms, they "turn off" the flagella, stop moving and start to form a biofilm. "We have now established what decides whether they swim or not—and that determines whether they form biofilms or not," explains Mikkel Girke Jørgensen and continues:

"Prospects for the pharmaceutical industry are huge. This increased understanding of biofilm formation may be the first step in creating new ways to treat complicated infections in the future."

**Journal Reference:**

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**Level of Dengue Virus Needed for Transmission Defined**
May 14, 2013 — Researchers have identified the dose of dengue virus in human blood that is required to infect mosquitoes when they bite. Mosquitoes are essential for transmitting the virus between people so the findings have important implications for understanding how to slow the spread of the disease.

By defining the threshold of the amount of virus needed for transmission, the research also provides a target that experimental dengue vaccines and drugs must prevent the virus from reaching in order to be successful at preventing the spread of disease during natural infection.

Dengue, also known as 'breakbone fever', is a viral infection that is transmitted between humans by mosquitoes. In most people it causes flu-like symptoms but in a small proportion of cases the disease can become life-threatening. Recent estimates indicate that there are 390 million infections of dengue across the globe each year and with no vaccine or specific treatment available, current measures to prevent the spread of disease are focused on controlling the mosquito vector.

In research funded by the Wellcome Trust, scientists and doctors at the Oxford University Clinical Research Unit at the Hospital for Tropical Diseases in Vietnam studied the factors that influence the transmission of dengue viruses from dengue patients to the mosquitoes that feed on them. Their findings reveal that mosquitoes that feed on dengue patients with very high levels of virus in their blood are more likely to be infectious to other humans two weeks later.

"Our findings suggest that focused public health intervention strategies to prevent transmission from these 'high risk' spreaders of the virus could have a major impact in slowing the spread of disease," explains Professor Cameron Simmons, a Wellcome Trust Senior Fellow at the Oxford University Clinical Research Unit in Vietnam.

Although the levels of virus in patients who had been hospitalized by the disease were much higher, the majority of patients with mild symptoms who were treated at outpatient centres also had enough virus in their blood to support transmission.

"At the moment, dengue surveillance systems typically only count hospitalized patients but our findings confirm that less serious cases represent an equally important source of virus infection. Since these cases often remain in the community for the duration of their illness, it's important that we explore ways to prevent such patients from providing a source of further virus transmission," added Professor Simmons.

The researchers hope that understanding the level of virus needed for transmission of infection will provide a useful reference point for the development of experimental drugs and vaccines and could be used to inform the endpoints for clinical trials evaluating such interventions.
Circumcision plans go awry in Swaziland
MBABANE, 13 May 2013 (IRIN)—It was an ambitious plan to circumcise the majority of men in Swaziland, an effort to reduce the risk of HIV transmission in a country with the world’s highest HIV prevalence. How could it have gone wrong?

“First they told me that circumcision will not really protect me against HIV. Then they tell me that I cannot have sex for some weeks or months after circumcision. I told them ‘fusaki’ [get out]!” Eric Dlamini, a 22-year-old law student, told IRIN.

These views are at the heart of the failure of the Accelerated Saturation Initiative (ASI) to achieve more than a fraction of its targeted goal, the circumcision of 80 percent of Swazi males between ages 15 and 49 within a year.

The programme, a partnership between the Ministry of Health and Social Welfare and the US-based Futures Group, was launched in 2010, and extended to 30 March 2012 when initial efforts showed a failure to achieve targeted results. But only about 20 percent—or 32,000—of the targeted demographic were circumcised through the programme.

US$15.5 million was spent on the programme, or $484 per circumcised male.

“We do not believe [ASI] was a failure but an additional prevention measure that is contributing to the overall combination efforts to end the HIV/AIDS pandemic in the country,” US Embassy in Swaziland spokesperson Molly Sanchez Crowe told the local press.

Imposed from outside?
Male circumcision has been scientifically proven to reduce a man’s risk of contracting HIV through vaginal intercourse by as much as 60 percent. Follow-up studies have found that the effectiveness of male circumcision in HIV prevention is maintained for several years.

Government health officials, like Minister of Health Benedict Xaba and Khanya Mabuza, the acting director of the National Emergency Council on HIV and AIDS (NERCHA), have noted that ASI taught the country important lessons and left behind several clinics and other health infrastructure.

But a year after the programme ended, Swazi health officials are still trying to figure out what went wrong. Health workers, who spoke to IRIN on the condition of anonymity, pointed out that the programme was hastily implemented. They wondered why the short implementation time was not extended. Ending the programme, they fear, may suggest to international donors that the country is a hopeless cause.

“We have been struggling with HIV for 20 years, and we see programmes come and go. Some are fads... and some are not well thought out. The Swaziland programme came from the outside. The health ministry was willing to go along because there was money there. But it was imposed,” said Thandi Mduli, an HIV testing officer in Manzini.

Officials with health-oriented NGOs admitted to IRIN they are “terrified” of criticizing an initiative funded by the “mighty” US President’s Emergency Plan for AIDS Relief (PEPFAR) and involving the global population control NGO Population Services International (PSI).

The ASI programme was an attempt to duplicate in Swaziland the circumcision successes seen in Kenya and other countries, without apparently doing the pre-campaign ground work. Kenya has carried out an estimated 477,000 circumcisions since its programme started in 2008, according to the government.

In 2011, UNAIDS and PEPFAR launched a five-year plan to have more than 20 million men in 14 eastern and southern African countries undergo medical male circumcision by 2015.

Reasons for failure
“There were a lot of issues involving male circumcision that were not properly explained to Swazi men, so they rejected it and they talked to their friends, and word of mouth was negative instead of positive. This is the opposite of what a campaign like this needs to work,” said NERCHA’s Mabuza.

Other issues included unfamiliarity of the procedure. “When I heard I would still have to wear a condom, I said, ‘What is the point?’” said Samkelo Mduli, a university student.

A survey commissioned by the Futures Group in 2011 found that although there was a 91 percent awareness of circumcision, nationally, the largest barrier to circumcision was fear of pain. Other barriers included fear of something going wrong, and a general lack of understanding of the procedure.

Another reason for the rejection of circumcision was not anticipated by ASI promoters: belief in witchcraft, which is widespread in Swaziland. Criminals are known to seek “strengthening” potions made with human body parts. Killings associated with “ritual murder” routinely correspond with national
elections. Victims, usually children or older people, are found with body parts missing. One attack made headlines in the Swazi press recently.

“That’s also what I wanted to know, and they wouldn’t tell me—what happens to my foreskin once it is cut off?” said Mduli.

Health Minister Xaba alluded to this when he told the Times of Swaziland, “Some men feared that the foreskin could end up in wrong hands, being used by some unscrupulous people for their ulterior motives.”

“This is embarrassing and nobody wants to talk about it,” said the programme director of a faith-based HIV/AIDS initiative in Manzini. “The circumcision initiative failed because of this arrogance on the part of its promoters. It would have been easy to be honest and explain to the Swazi men that their foreskins would be incinerated like all surgical refuse. But the promoters said, ‘Oh, no, we can’t talk about witchcraft. What will the donors say?’”

**Book Review: The Plague That Refuses to Go Away**
By [PLOS Guest Blogger](https://www.plos.org)
Posted: May 15, 2013

*Jasmine Grenier and Madhukar Pai from McGill University* review “Spitting Blood: The History of Tuberculosis” by Helen Bynum

Tuberculosis is one of the oldest human diseases and remains to this day one of the world’s top killers. The [WHO](https://www.who.int) reported nearly nine million new cases of tuberculosis globally in 2011, with 1.4 million deaths worldwide. Even today, in India alone, nearly 1000 patients die of tuberculosis every day. Clearly, this is one ancient plague that continues to take a toll on humanity.

The [WHO millennium development goal](http://www.who.int) for tuberculosis is to achieve a 50% decrease in tuberculosis mortality by 2015. Recently, a *[Global Thematic Consultation on Health](http://www.who.int)* concluded with more ambitious post-2015 targets: zero new tuberculosis infections, zero tuberculosis deaths, zero tuberculosis suffering and zero tuberculosis stigma and discrimination.

Although much progress has been made in the past 20 years, there remains an enormous burden of disease and many countries are not on track to meet even the modest 2015 goal. In the past year, tuberculosis has received significant media attention, highlighting positive developments in our fight toward elimination, such as the *roll-out of a rapid molecular test for TB*, approval of the first *new drug for tuberculosis* in over 40 years.

But the media has also highlighted setbacks such as the emergence of ‘totally drug-resistant’ strains of *Mycobacterium tuberculosis* in countries like India and South Africa, and failure of the first novel *tuberculosis vaccine since BCG*. Several roadblocks still stand in the way of reducing the burden of disease, including challenges in early diagnosis of the disease, before transmission occurs in the community, as well as in providing effective shorter and more effective treatment regimens.

*Spitting Blood* by Helen Bynum is released at a pivotal time in the history of tuberculosis, where renewed efforts are being put into the control of this rampant illness. In her book, Bynum presents an in depth exposé on the history of tuberculosis, taking the reader through the medical, cultural and societal implications of the disease through time, highlighting just how much it has shaped history, and been shaped by socioeconomic development.

Starting the book by recounting George Orwell’s experience with tuberculosis and how it influenced not only his work but most of his life, Bynum draws in the reader and arouses curiosity while providing, through the narrative of Orwell’s life, basic scientific facts regarding tuberculosis. This is one of the book’s strengths, where the microbiological and medical concepts are not glossed over but rather fully explored with the help of clear, simplified yet accurate explanations. This will allow the book to reach a much wider audience.

In some ways similar to Dubos’ *The White Plague*, Bynum uses known cultural figures, such as John Keats and Charlotte Brontë, to better show the reader the complete hold that tuberculosis can have on a person’s life. Not limiting itself to an exposé of the European experience of tuberculosis, the book also details the scientific and cultural evolution of tuberculosis in other continents, adding a greater level of depth and providing a much broader account of historical facts. The level of detail in the anecdotes provided is truly impressive and shows the depth of research that went into creating this work.

Through the lives of artists, scientists and political figures, Bynum takes readers through the complete history of tuberculosis from the medieval period up until the 21st century. Several critical moments of scientific discoveries are highlighted such as when [Rene Laennec](https://en.wikipedia.org/wiki/R%C3%A9my_de_Caritat,_Comte_de_Laennec) correlated the various granulomatous
pathologies with a common etiology and when Robert Koch identified Mycobacterium tuberculosis, the causative agent.

The book also describes the evolution of the treatment for tuberculosis, until the seminal discovery of streptomycin by Selman Waksman. We learn how TB was once treated with mixtures of frankincense and myrrh, and that patients were encouraged to embark on sea voyages to warmer shores in order to aspire to a cure. We also learn that in the 19th century, tuberculosis was commonly attributed to sedentary lifestyles and excess liquor and the author describes the emergence of the stigma associated with tuberculosis, which still exists today.

Toward the end of the book, we learn of the discoveries of the first anti-tuberculosis medications and how the drug regimens evolved to counter the threat of drug-resistant strains of bacteria. We also learn of the formulation of the DOTS strategy by the WHO – direct observed therapy, short-course – to enhance compliance and successful treatment outcomes.

Unfortunately, the portion of the book allotted to the modern challenges surrounding TB is much less extensive and detailed than the older historical accounts. There is little discussion surrounding the challenges with lack of significant decline in TB incidence despite the DOTS strategy, continued reliance on antiquated vaccines, drugs and diagnostics, the rampant use of suboptimal tests in high TB burden countries or the challenges in making newer WHO-endorsed tests more affordable. The critical issue of declining budgets for TB control and research and development is barely discussed. A more lengthy discussion of current controversies and challenges surrounding modern tuberculosis control would have greatly enhanced the relevance of this fine book in 2013.


Jasmine Grenier is completing her medical training at McGill University in Montreal. She has worked on TB research projects and published on topics relating to TB diagnostics.

Madhukar Pai, MD, PhD, is an Associate Professor of Epidemiology at McGill University in Montreal and an Associate Director of the McGill International TB Centre. He also serves as a consultant for the Bill & Melinda Gates Foundation. Dr. Pai has previously served as co-chair of the Stop TB Partnership’s Working Group on New Diagnostics. He is also a member of the PLOS Medicine Editorial Board and the PLOS ONE Editorial Board.

HIV organisations silent on safe-sex discord
By GayNZ.com Daily News staff
16th May 2013 — 11:04 am

Both the NZ AIDS Foundation and Body Positive are remaining tight-lipped regarding the cover story of the most recent issue of HIV NZ which questions the NZAF’s safe-sex promotion strategy.

Under its former title, Collective Thinking, the publication was in recent years published by the NZ AIDS Foundation. Following pressure to relinquish it, and it’s Ministry of Health-derived funding, to HIV peer-support organisations, and concerns that under the NZAF it had become less a voice for HIV-positive people and more a mouthpiece for the Foundation, it was formally handed over to Body Positive last May.

Part of the agreement underpinning the handover was that Body Positive and its associated HIV+ peer support organisations would not cut across the Foundation’s core safe sex HIV prevention strategy based on the message to gay and bi men: "Use a condom and lube for anal sex every time.”

However, GayNZ.com Daily News understands there are concerns within the NZAF regarding the cover story in the current issue: "Is New Zealand's Condom-Only Policy Enough?", penned by Body Positive General Manager Bruce Kilmister.

The article, written for a readership of HIV-positive people who are primarily gay and bi men, questions the NZAF’s core message and prominently highlights an entirely different approach, adopted by Australia and enshrined in the ‘Melbourne Declaration.’ As a result Australia promotes guidelines assisting people to make their own decisions on condom use based on how they perceive their own risk factors. In particular it embraces a more liberal use of pre- and post-exposure dosing with HIV medications.

Responding to questions to the NZAF from GayNZ.com Daily News, the two organisations have released a joint statement: "The editorial panel of HIV NZ, established in the agreement between Body Positive and NZAF, will be meeting in late May to review the last issue and plan the next. It is not appropriate for any public comment about HIV NZ to be made outside of this collaborative process at this time."

At the time of the hand-over to Body Positive Kilmister said the publication would in future have total editorial independence.
In recent years the HIV epidemic amongst gay and bi men in Australia has continued the upward trend seen in similar communities world-wide whereas in New Zealand it appears to be trending down. Historically the New Zealand epidemic has never reached the high levels experienced overseas, a situation generally attributed to the NZAF’s consistent promotion of its core message, amongst other factors.

Scientists Weaken HIV Infection in Immune Cells Using Synthetic Agents

*Science Daily*, (05.01.2013)

HIV hides within certain types of cells and reproduces slowly, eventually causing chronic inflammation regardless of drug treatment. According to Servio H. Ramirez PhD, assistant professor of pathology and laboratory medicine at Temple University School of Medicine (TUSM), although antiretroviral drugs allowed persons with HIV infection to live longer, the patients continue to have extended exposure to low levels of HIV replication and associated inflammation. It is believed that this inflammatory process in the central nervous system (CNS) is the underlying cause of HIV-associated neurocognitive disorder.

Researchers at TUSM’s Department of Pathology and Laboratory Medicine and Center for Substance Abuse Research investigated the connection between inflammation and neurocognitive conditions linked to long-term HIV exposure. Ramirez and colleagues focused on the CB2 receptor, a protein located on the surface of macrophages. Macrophages are a type of white blood cell that engulfs and destroys foreign agents, and the researchers believed they are likely the primary reservoir for HIV. They are the first cells infected after sexual transmission of the virus, are found in every organ of the human body, and circulate in the blood. They believe that macrophages may be carrying HIV into the brain, and initiating HIV-associated cognitive decline.

CB2 is a binding site for cannabinoids, active compounds of cannabis (marijuana), but does not transmit the psychoactive effects of cannabis. The researchers hypothesized that CB2 may play a role in blocking inflammation in the CNS. They discovered that synthetic anti-inflammatory substances distantly related to the active ingredient in marijuana may take the strength out of HIV while the virus is hiding in macrophages. The researchers conducted experiments using a non-clinical HIV macrophage cell model. They treated the HIV-infected cells with one of three different synthetic CB2-activating compounds and sampled the cells periodically to measure the activity of the enzyme called reverse transcriptase, which is essential for HIV replication. After seven days, all three compounds had successfully attenuated HIV replication.

Results suggest that selective CB2 agonists could potentially be used along with antiretroviral drugs to create new HIV/AIDS drug treatments and that the human immune system could be enhanced to fight HIV. Yuri Persidsky, MD, PhD, chair of TUSM’s Department of Pathology and Laboratory Medicine and one of the researchers, commented that the study suggests that stimulating CB2 receptors in white blood cells could produce benefits against other viral infections.


Intestinal Bacterium Akkermansia Curbs Obesity

May 15, 2013 — A dominant and useful bacterium called *Akkermansia muciniphila* is present in the intestinal system of all humans, from babies to the elderly. This microorganism is found in the intestinal mucus layer that protects against intruders. Even more remarkable is that this bacterium has a favourable effect on the disrupted metabolism associated with obesity.

Prod. Patrice Cani from Brussels and Prof. Willem de Vos from Wageningen University, together with their colleagues, published these findings in the scientific journal *Proceedings of the National Academy of Sciences (PNAS)*. They see potential in deploying *Akkermansia* bacteria to further understand and treat obesity and medical consequences.

Obesity and type 2 diabetes are both characterised by symptoms including inflammation, changes in the composition of the intestinal bacteria and the disruption of the natural barrier in the intestines. Ten years ago, researchers at the Laboratory of Microbiology at Wageningen University, part of Wageningen UR, discovered the bacterium *Akkermansia muciniphila* (named after the Wageningen microbial ecologist Dr Antoon Akkermans, 1941-2006), which was able to grow in the mucus layer of the intestines. The bacteria were apparently present in large numbers in humans (and rodents) that were not overweight. Fewer were present in humans and rodents with inflammations or obesity. The
microbiologists at Université Louvain in Brussels and their Wageningen colleagues wondered what the role of this bacterium could be.

In the article that appeared on 13 May in the journal *Proceedings of the National Academy of Sciences*, the research team concluded that the bacteria are less frequent in mice with induced obesity and with type 2 (adult-onset) diabetes. Furthermore, administering rather indigestible fibres such as oligofructose, known for its advantageous effect on intestinal biota, resulted in a recovery of the *Akkermansia* population in mice. The presence of the bacteria strengthens the intestinal barrier and is also inversely correlated with weight increase (fat storage), inflammation reactions in fatty tissues and insulin resistance. However, is there also a causal relationship between the favourable developments and the occurrence of *Akkermansia* bacteria?

To check that, the researchers administered *Akkermansia* bacteria to ordinary mice on various diets. With a normal diet, no effect was noticed but in mice that became overweight as a result of a high-fat diet, the *Akkermansia* bacteria caused a reduction in fat development and associated metabolic defects, without affecting food intake. After the administration of *Akkermansia* bacteria, there was an increase in endocannabinoid levels, a substance that ensures blood glucose remains at the correct level. In addition, the intestinal barrier function was strengthened. Only intact, living bacteria produced these results; the researchers noticed that bacteria that had been heated beforehand had no effect. Although human studies have not yet been carried out, the results seem to show potential; a treatment with *Akkermansia* bacteria could reduce inflammation and may prevent obesity.

Journal Reference:

Study IDs Key Protein for Cell Death
To determine the location of ALKBH7 in cells, MIT researchers engineered these cells to express ALKBH7 bound to green fluorescent protein (GFP). The cells' mitochondria express a red fluorescent protein. In cells where ALKBH7 is present in the mitochondria, the green and red signals mix and appear yellow. (Credit: Jennifer Jordan and Dragony Fu)

May 14, 2013 — Findings may offer a new way to kill cancer cells by forcing them into an alternative programmed-death pathway.

When cells suffer too much DNA damage, they are usually forced to undergo programmed cell death, or apoptosis. However, cancer cells often ignore these signals, flourishing even after chemotherapy drugs have ravaged their DNA.

A new finding from MIT researchers may offer a way to overcome that resistance: The team has identified a key protein involved in an alternative death pathway known as programmed necrosis. Drugs that mimic the effects of this protein could push cancer cells that are resistant to apoptosis into necrosis instead.

While apoptosis is a tightly controlled procedure that breaks down and disposes of the dying cell in a very orderly way, necrosis is a messier process in which the cell's membrane ruptures and its contents spill out.

"People really used to think of necrosis as cells just falling apart, that it wasn't programmed and didn't require gene products to make it happen," says Leona Samson, a member of MIT's Center for
Environmental Health Sciences and Koch Institute for Integrative Cancer Research. "In the last few years it has become more clear that this is an active process that requires proteins to take place."

In the May 10 online edition of the journal *Genes and Development*, Samson and colleagues report that a protein known as ALKBH7 plays a key role in controlling the programmed necrosis pathway. Dragony Fu, a former postdoc in Samson's lab, is the paper's lead author, and postdoc Jennifer Jordan is also an author.

**Unexpected findings**

ALKBH7 belongs to a family of proteins first discovered in E. coli about a dozen years ago as part of a DNA-repair mechanism. In humans, there are nine different ALKBH proteins, which Samson's lab has been studying for several years.

Most of the mammalian ALKBH proteins appear to be involved in DNA repair, similar to the original E. coli version. In particular, they respond to DNA damage caused by alkylating agents. These agents can be found in pollutants such as fuel exhaust and tobacco smoke, and are also used to treat cancer.

In the new paper, Samson, a professor of biology and biological engineering, and her colleagues found that ALKBH7 has an unexpected effect. When the researchers lowered ALKBH7 levels in human cells grown in the lab, those cells were much more likely to survive DNA damage than cells with normal ALKBH7 levels. This suggests that ALKBH7 actually promotes cell death.

"That was a surprising finding, because previously all of these ALKBH proteins were shown to be helping the cell survive when exposed to damage," says Fu, who is now a visiting research fellow at the University of Zurich.

Upon further investigation, the researchers found that when healthy cells suffer massive DNA damage from alkylating agents, they enter the programmed necrosis pathway. Necrosis, which can also be initiated by bacterial or viral infection, is believed to help the body's immune system detect threats.

"When dying cells release their contents during necrosis, it serves as a warning signal for your body that there is a virus there and recruits macrophages and other immune cells to the area," Fu says.

**Potential drug targets**

The findings suggest that when DNA is so badly harmed that cells can't repair it, the programmed necrosis pathway kicks in to prevent cells with major genetic damage from potentially become cancerous. Other researchers have shown that some types of cancer cells have much lower ALKBH7 levels than normal cells. This suggests that the cancer cells have gained the ability to evade programmed necrosis, helping them to survive, Fu says.

The necrosis pathway appears to be initiated by an enzyme called PARP, which becomes hyperactive following DNA damage and shuts down the cell's production of two molecules that carry energy, ATP and NAD. The MIT team found that ALKBH7 prevents ATP and NAD levels from returning to normal by disrupting the function of mitochondria—the cell structures that generate energy for a cell.

Without an adequate supply of those critical energy-carrying molecules, the cell cannot survive and undergoes necrosis. In cells that lack ALKBH7, ATP and NAD levels rebound, and the cells survive, carrying a heavy burden of DNA damage.

The researchers are now investigating the molecular details of the programmed necrosis pathway in hopes of identifying ways to activate it in cancer cells.

**Journal Reference:**


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**Roadmap to an HIV Vaccine**

Researchers track the evolution of HIV in a single patient to understand what drives the production of broadly neutralizing antibodies.

By Sabrina Richards | April 3, 2013

By investigating an African patient's HIV infection, researchers have traced the development of an antibody that is effective at neutralizing many strains of HIV, according to a study published today (April 3) in *Nature*. The researchers—who identified the original HIV variant as well as the broadly neutralizing antibody, and pieced together their evolution over the course of infection—hope that a vaccine mimicking this process could encourage the development of such effective HIV-fighting antibodies.

The new research provides "really in-depth information on how a particular type of broadly neutralizing antibody emerges over the course of a natural HIV infection," said Leonidas Stamatatos, an immunologist at Seattle Biomedical Research Institute who did not participate in the study.
Broadly neutralizing antibodies—able to block many strains of HIV from binding target cells—are notoriously rare: only about 20 percent of HIV-positive people ever generate such antibodies. One of the most attractive neutralizing targets is the HIV envelope protein (Env) that binds T cells, which is present on every variant of HIV. But Env is covered in sugar molecules that often mimic host structures, making it hard for the immune system to distinguish virus from self. In order to avoid an adverse autoimmune reaction, the body produces few B cells whose antibodies can recognize these common structures. One approach to developing an effective HIV vaccine is to stimulate these rare B cells, but because Env’s sequence can vary widely between HIV strains, researchers didn’t know much about the right Env variant for the job.

In order to find an Env that could stimulate an antibody with broadly neutralizing potential, Barton Haynes at Duke University and researchers at the Center for HIV-AIDS Vaccine Immunology (CHAVI) set up eight acute infection clinics in Malawi, South Africa, Tanzania, Uganda, and one in North Carolina, where they could watch antibody and virus develop within weeks of infection.

Haynes and his team found one patient who developed a broadly neutralizing antibody within 3 years of infection. The antibody, dubbed CH103, could block infection of target cells by 55 percent of the HIV virus particles they tested, which expressed a total of 196 different types of Env. Because the team had blood samples from the patient starting 4 weeks after infection, they could isolate the original antibody, CH103’s predecessor, as well as determine the sequence of the original Env protein that first spurred the antibody’s production.

As HIV proteins accrue mutations during an infection, antibodies evolve to increase specificity and adapt to changing targets. Finding the antibody that bound the original Env allowed researchers to identify which mutations conferred broadly neutralizing activity to CH103 and identify mutations in Env that could have contributed to CH103’s development. Haynes and his colleagues hope to recreate the evolution of CH103-like antibodies using the right combination of Env variants in a vaccine.

“The study provides important information on how one might design a rational vaccination strategy,” Dennis Burton, an immunologist at The Scripps Research Institute who did not participate in the study, wrote in an email to The Scientist. “[It’s] a significant leap.” An effective vaccine will need to elicit more than one type of antibody to block HIV infection, so Haynes and his team are also examining the evolution of broadly neutralizing antibodies and their corresponding HIV proteins in other patients, as well.

Indeed, the bulk of the work is just beginning, said Stamatatos, who noted that the possible combinations of Env “are nearly infinite.” It’s also not clear yet whether the Env mutants should be given together, or provided sequentially in a fashion more akin to a natural infection. Haynes and his colleagues are currently beginning to test both strategies with different Env combinations in macaques and mice engineered to express human antibodies.


**Microbes Affect Weight Loss**

Microbial changes in the gut contribute to a patient’s ability to slim down after gastric bypass surgery.

*By Ruth Williams | March 27, 2013*

Surgically bypassing the stomach is not the only reason that patients undergoing such a procedure quickly begin to drop pounds. Changes to the microbial make-up of their intestines also play a big role, according to a paper published in *Science Translational Medicine* today (March 27). The results suggest that tweaking a person’s gut microbes to mimic the effects of bypass treatment might, one day, be an effective means of losing weight without the need for surgery.

“What they’ve shown in this paper is that gastric bypass has an effect on the bacteria of the intestine, and that if you take those bacteria and transplant them into another mouse [that hasn’t had surgery] . . . that mouse [also] loses weight, which is amazing,” said Louis Aronne, a professor of medicine at Weill Cornell Medical College in New York, who was not involved in the study.

Obesity can be accompanied by an array of life-threatening complications such as diabetes and heart disease. But, as many people will attest to, losing weight can be a real struggle.

The problem with regular dieting is that “when you are forced to eat less . . . your body reacts by reducing basal energy expenditure,” said Lee Kaplan, director of the Massachusetts General Hospital Weight Center in Boston and a senior author of the study. “So it does everything it can to make you more hungry and to burn fewer calories to try to make you regain the weight.”
One effective albeit drastic solution is gastric bypass surgery. This procedure was originally thought to work by forcibly reducing a person’s capacity for food, but the effects are more complicated than they first seemed, Kaplan explained. “We began to see, in patients that got bypass surgery, that they were not hungry and that they were not craving what they used to crave.” Studies in animals also revealed that the procedure had some unexpected metabolic effects. “Energy expenditure goes up rather than down,” said Kaplan. “So everything about bypass surgery seems to be the opposite of what would happen with a diet.”

Hints as to the cause of the unexpected physiological changes came from studies in both rats and humans that showed gastric bypass surgery altered gut microbial profiles. Kaplan and his team have now observed similar bypass-induced microbial changes in obese mice. Within 1 week of surgery, three phylogenetic groups of microbes—bacteriodetes, verrucomicrobia, and proteobacteria—were significantly enriched in the feces of the mice. And by 5 weeks, this restructuring of the microbial community had stabilized. As expected, the mice also lost 30 percent of their body weight, and exhibited metabolic changes—their blood glucose levels lowered and insulin sensitivity improved.

However, as with previous studies, it remained unclear whether these microbial and metabolic changes were directly linked. To determine if these changes shared a causal relationship, Kaplan’s team transplanted the microbial community from the guts of mice that had undergone surgery into microbe-free, normal-weight mice.

“What we saw in the recipients that were given a gastric bypass microbiota was that their food intake was not altered, but they had less adiposity [body fat] and they lost about 5 percent of their body weight,” said lead author Alice Liou, a research scientist in the laboratory of Peter Turnbaugh at Massachusetts General Hospital.

“The microbiota is only one piece of the story,” noted Kaplan, “but based on the results we report in this paper, it’s clear that it is a significant piece.” In fact, he said, “we calculate that the microbiota transfer could account for upwards of 20 percent of the effects of the surgery.”

Although it is not yet clear how the microbes are altering metabolism and energy expenditure, or whether such microbial transfers would be effective in humans, the results “imply that a probiotic that replicates the effect of gastric bypass surgery is possible, and may contribute to weight loss,” said Aronne. Indeed, added Elaine Holmes, head of computational and systems medicine at Imperial College London, “by understanding the mechanism of weight loss and adipose reduction . . . the ultimate hope is that we can identify new drug targets to achieve the same effects as a bypass, but without the risk of surgery.”


**Virus Versus Bacteria**

A newly developed drug, modeled after a bacteria-infecting virus, is less likely to become antibiotic resistant.

By Edyta Zielinska | April 17, 2013

To create a drug with a lower risk of becoming antibiotic resistant, researchers turned to viruses that naturally infect bacteria, and created a drug that mimics a cell-wall busting viral enzymes called lysins, according to a new report published last week (April 10) in *PLOS ONE*. Viral lysins appear to resist bacterial evolution that would render them ineffective over time.

“We’ve taken advantage of the evolution of viruses. It’s using nature versus nature,” author Vincent Fischetti head of Rockefeller University’s bacterial pathogenesis and immunology lab told *Healthline*. “We’ve found it’s best when you don’t fight nature. Viruses have been around a lot longer than we have.”

Antibiotic resistance has become an increasing problem in hospitals, rendering a slew of formerly effective antibiotics useless against certain deadly strains of bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA). To combat bacteria’s tendency to evolve new methods of bypassing a drug’s mechanism of action, Rockefeller University researchers turned to the viral enzyme lysin that viruses use to break through the bacterial cell wall of a cell they’ve infected as they exit in large numbers. Lysin is particularly resistant to bacterial evolution because it interacts with bacterial proteins that are essential for maintaining cell-wall integrity.

When researchers tested the new drug, dubbed Epimerox, against wild-type *Bacillus anthracis*, as well as bacteria that had been chemically altered to have a higher rate of antibiotic resistance, the cells did not appear to develop resistance. In addition, mice infected with lethal doses of *B. anthracis* were rescued by treatment with the drug.

The researchers said they hope to begin human clinical trials within 2 years.
Did Inbreeding Royals Evolve?
A new study suggests that in the Spanish Habsburg royal family, natural selection may have diminished the most harmful effects of inbreeding.
By Dan Cossins | April 22, 2013

The Habsburg royal family of Spain, which favored inbred marriages to keep titles in the family and forge alliances, may have evolved from 1450–1800 to blunt the worst effects of inbreeding, according to a study published this month in *Hereditity*. But some geneticists are not convinced, reported *Nature*.

Evolutionary theory predicts that over time, there will be a purging of the harmful mutations that result from inbreeding. But while the effect has been observed in animals and plants, there is little evidence for this phenomenon in human populations. Researchers from the University of Santiago de Compostela in Spain decided to look for that evidence by analyzing 350 years of genealogical records of the Spanish Hapsburgs, a family with a long history of inbreeding.

Inbreeding increases the probability that offspring will inherit two copies of a recessive disease-causing mutation, but infertility—which may be a consequence of inbreeding—can block such mutations from being passed down. Assuming that early deaths—in infancy, excluding miscarriages and stillbirths, and early childhood, up to the age of 10—were a result of inbreeding, the researchers hypothesized that if natural selection had purged the harmful mutations, the number of early deaths would go down over time.

Sure enough, their analysis of deaths in the written records of the Habsburg royal family revealed significantly fewer early deaths in children between 1600 and 1800 than between 1450 and 1600.

The number of infant deaths went up over the same period, however, which the authors said might be because deaths in infancy and early childhood were caused by different mutations—so the infants might have died as a result of mutations that only cause disease some of the time, and were thus purged more slowly than those that almost always cause disease and might have caused the deaths in early childhood.

But Princeton University geneticist Leonid Kruglyak told *Nature* that the observed changes in mortality may not be due to natural selection, and are more likely to be a statistical fluke caused by the small size of the sample.

Celebrating 60 Years of the Double Helix
*Genome Biology* speaks to a scientist involved in the discovery of the structure of DNA, and asks modern geneticists to highlight the key advances that have followed.
By Dan Cossins | April 25, 2013

On this day (25 April) in 1953 *Nature* published three papers describing the structure of DNA: one from James Watson and Francis Crick of Cambridge University that proposed the now famous double helix, and two accompanying papers from Rosalind Franklin and Maurice Wilkins of King’s College, London, who used X-ray diffraction images to support the helix hypothesis.

Today, 60 years later, science celebrates the ground-breaking discovery. For our part, we have published a [poster](#) outlining the history of genetics and genomics, from the initial 1953 structural findings to the completion of the draft human genome sequence 10 years ago, and especially the deluge of knowledge that has been unveiled in the last decade. We also have a [series of webinar](#) in which George Church and other leading scientists explore what the future holds for DNA research. And *Genome Biology* commemorates the landmark with a fascinating [in-depth interview with Raymond Gosling](#)—then a biophysics graduate student working under Wilkins and one of two named authors, along with James Watson, still alive to tell the tale.
In a story packed with fascinating, often humorous, details about lab research in the 1940s, Gosling describes the “serendipitous” events that led to the discovery, from acquiring the finest quality DNA samples from a Swiss biochemist to finding that paperclips were perfect for the painstaking task of stretching out the fibers in preparation for X-ray imaging in the basement of the chemistry building at King’s College. Gosling also discusses the use of a condom to seal the collimator of the camera into which they were pumping hydrogen to produce clear pictures.

As for the resulting images of DNA, Gosling recalls how “this beautiful spotted photograph . . . was the most wonderful thing. And I knew at the time that what I’d just done was to produce a crystalline state in these fibers, and if then the DNA was the gene material, I must be the first person ever to make genes crystallize.” It was at that moment Gosling realized it would only be a matter of time before the structure of DNA was revealed. “I can still remember vividly the excitement of showing this thing to Wilkins and drinking his sherry by the glass . . . by the gulpful,” he recalled.

To bring the story up to date, Genome Biology also invited 13 of the scientists currently serving on its editorial board to discuss the most important advances in the field since 1953. The list includes the discovery of introns and gene fragmentation, the rise of restriction mapping, microarrays, DNA sequencing, the Human Genome Project, ancient DNA analysis, and horizontal gene transfer. Happy birthday, DNA!

Lamarck and the Missing Lnc
Epigenetic changes accrued over an organism’s lifetime may leave a permanent heritable mark on the genome, through the help of long noncoding RNAs.

By Kevin V. Morris | October 1, 2012

Rudyard Kipling’s Just So Stories tell tales not so much of evolution, but of the magic and wonder of the animal world. He describes the wizard who gave the camel a hump for its laziness, and the alligator who snapped and stretched the nose of a naïve young elephant to its current lengthy proportion. Those delightful fables, published some 70 years after Jean-Baptiste Lamarck’s death, provide entertaining explanations for such evolved traits, and were clearly inspired by Lamarck’s description of adaptive change, not Charles Darwin’s. In his 1809 publication Philosophie Zoologique, Lamarck wrote of the giraffe, from whose habit of reaching for the green leaves of tall trees “it has resulted . . . that the animal’s forelegs have become longer than its hind legs, and that its neck is lengthened to such a degree that the giraffe, without rearing up on its hind legs . . . attains a height of six meters.”

Although biologists have generally considered Lamarck’s ideas to contain as much truth as Kipling’s fables, the burgeoning field of epigenetics has made some of us reconsider our ridicule. While no biologist believes that organisms can willfully change their physiology in response to their environment and pass those changes on to their offspring, some evidence suggests that the environment can make lasting changes to the genome via epigenetic mechanisms—changes that may be passed on to future generations.

Epigenetics: genome gatekeeper
Epigenetic changes can range from chemical modifications of histone proteins—such as acetylation and methylation—to modifications made to the DNA itself. Such changes usually cause chromatin compaction, which limits the ability of the RNA polymerase II transcription complex to access DNA, ultimately resulting in reduced messenger RNA (mRNA) and protein output. Many view epigenetics as an annotation or editing of the genome that defines which genes will be silenced in order to streamline protein production or squelch unnecessary redundancy. That annotation, they say, does not and cannot permanently change the original manuscript (i.e., DNA), but merely access to the manuscript.

A fascinating 2008 study that looked at people born during the Dutch Hunger Winter in 1944–1945 hints at the possibility that transgenerational epigenetic inheritance also occurs in humans.1 Adults who were conceived during the famine had distinct epigenetic marks that their siblings born before or after the famine did not. These marks reduced the production of insulin-like growth factor 2 (IGF2) and affected the growth of the famine-gestated children. Notably, these marks were retained for several decades in the afflicted individuals. While these observations suggest the possibility of transgenerational epigenetic inheritance, the modifications could also have occurred in utero as a result of famine conditions rather than being inherited in the germline. Therefore, whether such a distinct phenomenon occurs in humans remains to be definitively determined.

However, in model experimental systems, there is strong evidence for transgenerational epigenetic inheritance. In one study carried out in mice, an environmental stress that resulted in aggressive behavior in males caused the same behavior in their offspring.[5. T.B. Franklin, I.M. Mansuy, “Epigenetic inheritance in mammals: evidence for the impact of adverse environmental effects,” *Neurobiol Dis*, 39:61–65, 2010.] Notably, the offspring had changes in the DNA methylation patterns of particular genes. Collectively, these and other transgenerational studies all point to the notion that selective pressure can be applied from the environment and passed on to daughter cells and offspring.

**Controlling epigenetics**

While epigenetic modifications to the genome are well studied, far less is known about how particular epigenetic marks are directed to their target loci. Clearly, something is guiding the modifications, which appear to be differentially distributed based on particular stresses induced on the cell or organism. Recent studies suggest that epigenetic changes, and possibly transgenerational epigenetic inheritance, could be explained by a somewhat unexpected molecular player: long noncoding RNA.

Long noncoding RNAs (lncRNAs) are transcripts generally expressed from regions of “junk” DNA that are not thought to code for proteins. Estimates of lncRNA abundance range from 70 to 98 percent of transcripts present in the cell, and some are several thousand bases long.[6. T.R. Mercer et al., “Long non-coding RNAs: insights into functions,” *Nat Rev Genet*, 10: 155–59, 2009.]. Unlike short noncoding RNAs, such as short interfering RNA, which silence genes by cutting mRNAs in the cytoplasm, lncRNAs appear to bind to transcripts in the nucleus as they emerge from the replication fork of the DNA, and recruit enzyme complexes to induce epigenetic changes at these loci.[7. K.V. Morris, “Long antisense non-coding RNAs function to direct epigenetic complexes that regulate transcription in human cells,” *Epigenetics*, 4:296–301, 2009.]

DNA, which presumably occurs when the lncRNAs direct enzymes such as the DNA methyltransferase DNMT3a to targeted spots on the genome. Alternatively, lncRNAs can direct modifications of nearby histones, usually in the form of methylation of the histone tail.

DNA methylation itself can be passed down from a cell to its daughter cells.[11. M.S. Weinberg et al., “The antisense strand of small interfering RNAs directs histone methylation and transcriptional gene silencing in human cells,” *RNA*, 12:256–62, 2006.] In addition, it has been known for some time that such modifications can also lead to permanent changes in the genetic code. Methylation of a cytosine (C), for example, can cause that nucleic acid to change to a thymine (T) through deamination, or the removal of an amine group. Nearly 80 percent of methylation sites in the human genome occur on a cytosine that is followed by a guanine, in a CpG sequence. Deamination occurs when the methylated C undergoes a hydrolysis reaction resulting in the production of ammonia, followed by the conversion of the cytosine to a thymine at that spot in the DNA sequence. While this C-to-T conversion is considered random, the spontaneous deamination of methylated CpGs has been found to be about 2-fold faster than C-to-T conversions in nonmethylated CpG sequences,[12. J.C. Shen et al., “The rate of hydrolytic deamination of 5-methylcytosine in double-stranded DNA,” *Nucleic Acids Res.*, 22:972–76, 1994.] suggesting a bias toward CpG regions in the deamination process.

Although these ideas have yet to be substantiated by complete experimental evidence, one can envision this as a model for how the system might work—a mechanism by which epigenetic changes, guided by lncRNAs, could make permanent and heritable changes to the genome. Indeed, such a lncRNA-based DNA editing system could be driving some aspects of genetic variation and could explain the common appearance of single nucleotide polymorphisms within a species. If this is true, one has to wonder what role lncRNA-directed DNA methylation has been playing in the evolution of the genome.

**Driving diversity**

DIRECTING EVOLUTION: Epigenetic modification most often occurs on cytosines (C) that are followed by a guanine (G) (top). These methylated cytosines are more likely to undergo a chemical reaction that converts the C into a thymine (T), permanently changing the genetic sequence. When that altered sequence is replicated during cell division, the newly generated matching strand will copy this altered sequence (bottom), giving the next generation a slightly altered genomic manuscript. This new manuscript could alter the structure of the encoded protein, or change the mRNA homology sequence for lncRNA binding, rendering the lncRNA unable to bind and suppress that gene, thus allowing the altered sequence to be transcribed again.

Intriguingly, a greater frequency of targeted C-to-T changes could also result in an overall loss of complementarity between the sequence and the lncRNA that targets it. As a result, rather than initiating suppression of the target gene, the change could result in renewed transcription in subsequent generations. At the same time, this process could permit the target transcript to fold into a different conformation, thereby allowing other subsets of lncRNA interactions to occur at slightly different loci.

Alternatively, changes to the lncRNAs themselves might lead to a loss of lncRNA-protein associations, resulting in different cellular machinery being localized to the particular target loci. Thus, the over-activity of one lncRNA could doom that lncRNA to a loss of function, but simultaneously result in the evolution of a new regulatory lncRNA network with potentially different downstream effects.

Furthermore, a site frequently targeted by lncRNAs would likely contain a larger proportion of T:A bonding between the DNA strands, due to deamination events. Such permanent and heritable changes in the genetic code could change the shape of the encoded protein, its function, or its ability to be transcribed altogether.
One can begin to envision how environmental variation, by instigating epigenetic changes, could increase organismal complexity, thus giving populations a greater chance at surviving new and perhaps permanent environmental threats. In other words, epigenetics, rather than random genetic point mutations, could provide the missing link between environmental pressure and the resulting genetic variability that generates robustness of a species.

Most certainly, if such a pathway were to exist in human cells, one would expect it to be elusive purely due to the sheer complexity of the process—involving lncRNAs, epigenetic changes, DNA methylation, and deamination. Thus, it is not out of the realm of possibility that such a mechanism exists, but has yet to be elucidated by science.

The inner molecular workings of the cell are vastly complex, and the emerging realization that lncRNAs are active modulators of gene transcription and epigenetic states only complicates the picture. Clearly, as more data emerges in this exciting area of research, additional layers of regulation will need to be added to the central dogma of molecular biology. Although an organism cannot pass down specific information about its own experiences—the giraffe will not be able to help its offspring reach taller trees just by stretching its own neck—it may give succeeding generations a fighting chance in a difficult environment by offering them a slightly altered arsenal of genetic tools. *Kevin V. Morris is an associate professor at The Scripps Research Institute in La Jolla, California, and the University of New South Wales in Sydney, Australia.*

**Mapping Disease**

*Online tools could help to improve our patchy knowledge of the whereabouts of infectious diseases.*

By Ed Yong | April 29, 2013

In the past week, researchers and journalists have scrambled to map the spread of H7N9 bird flu through China to identify its source and highlight at risk areas. Mapping is a common response to outbreaks, especially of new diseases, but some scientists believe it must become a more proactive part of disease control.

Such mapping techniques have been used since the turn of the 19th century to track outbreaks in real time and understand their causes. In 1854, for example, John Snow penned a famous diagram of a cholera outbreak in London, pinpointing a water pump as the source.

Despite this long history, however, efforts to plot the locations of infectious diseases still tend to be reactive rather than proactive. And while local outbreaks are regularly and thoroughly mapped, the broader landscape is far murkier. According to a team of scientists led by Simon Hay from the University of Oxford in the United Kingdom, only 4 percent of important infectious diseases have been comprehensively mapped at a global scale. The rest are plagued by patchy data.

“We have very little information on where in the world diseases are,” said David Pigott, who is part of Hay’s team at Oxford. Such information is crucial when it comes to planning surveillance, risk assessments, vaccine programs, and outbreak responses. “[For example], if you get cases outside of a known distribution, you can rapidly see if there’s a genuine range expansion or a misdiagnosis,” said Pigott. “It’s such an integral part of disease control.”

The team audited existing maps for 174 infectious diseases of clinical importance. Following a huge systematic review, they scored the maps for each disease according to how much of the known global range is covered and the quality of the data—whether they were up-to-date and whether they relied on accurate measures like molecular diagnostics or GPS coordinates, rather than unverified expert opinion.

“It’s a very impressive study,” said Tom Koch, an expert on medical maps from the University of British Columbia, who was not involved in the study. “It brings a whole mass of data together and presents a portrait from which we can do interesting work.”

With a score of 75 out of 100 considered a passing grade, only 7 diseases met that criterion, including dengue fever, monkeypox, and two types of malaria. Most infections, including some intensely studied diseases like HIV, failed to meet the benchmark because of a trade-off between quality and scale. “There was really detailed clinical data where someone had gone to a village and done a map at a small scale,” said Pigott. “But maps that did cover the world were of lower quality and relied on an expert saying, ‘I know it’s in this country.’”

And even the highest-scoring diseases have room for improvement. After an exhaustive review of the whereabouts of dengue fever, recently published in *Nature*, Hay’s team concluded that there are 390 million infections every year, more than three times the number estimated by the World Health Organization.
Despite shortfalls like this, Hay and his colleagues optimistic. They argue that technology can help to plug the gaps in our maps in the future, and they point to several untapped sources of data. For example, both PubMed and GenBank, which collect biomedical literature and gene sequences respectively, contain geospatial information for the majority of diseases that the team reviewed. And social networks like Twitter can provide invaluable real-time clues about spreading symptoms and illnesses, often tagged with geographical information. During the 2009 outbreak of H1N1 swine flu, for example, Twitter predicted outbreaks **1 or 2 weeks ahead** of traditional surveillance measures.

However, Koch cautions that disease data are not as freely available as the team suggests. In some cases, “privacy concerns and the proprietary attitudes of governments have made it harder for us to get some types of data, such as mortality data from an outbreak,” he said. “[John] Snow would never have got his data today.”

John Brownstein from Boston Children’s Hospital, one of Hay’s team, faced these problems during his PhD work on West Nile Virus and Lyme disease. “I struggled because governments or researchers wouldn’t share their information,” he said. “But there was all this incredible knowledge on the web being discussed through professional networks or news media.”

To collate those rich but disparate data sources, Brownstein created HealthMap—a website that automatically monitors, organizes and maps information on infectious diseases from unconnected sources. These include Google news, mailing lists like ProMED Mail, and bulletins from organizations such as the World Health Organization. Another site called BioCaster, developed by Nigel Collier at the National Institute of Informatics and international collaborators, works along similar lines.

Hay’s team believes that the problem now is not a lack of data but a deluge of it. Sites like HealthMap and BioCaster are already using learning algorithms to filter online sources for information relevant to infections. They are also using crowdsourcing tools that ask online volunteers to check if flagged social media chatter actually relates to the disease of interest.

These solutions are not panaceas, however. Brownstein emphasizes the need to build regional contacts to get the right data in the first place. “The local aspect is critical,” he said. “Our team is mining Weibo [a prominent Chinese social network] for information on H7N9. The things we’ve been able to get from that information are unbelievable,”—such as a few reports of H7N9 cases that emerged well before they were officially reported. “This wasn’t available during SARS.”

**Suited to a T**
Sorting out T-cell functional and phenotypic heterogeneity depends on studying single cells.
By Kelly Rae Chi | May 1, 2013
Activated T cells are a diverse and ever-changing crew. What gives this type of white blood cell the ability to stamp out infection is also what makes it complicated to study. T cells express receptors that respond to specific antigens. Not only different subsets of T cells, but individual T cells, can react to the same antigen in different ways—for example, by rapidly expanding and differentiating, by releasing distinct sets of cytokines at certain times, or by killing other cells. And, influenced by their past and present experiences, they can change their behavior over the course of months or years.

Because T cells are so flexible in form and function, and are in a constant state of transition, researchers are realizing that extracting them from blood, mashing them together, and analyzing their overall gene expression and other characteristics doesn’t capture their nuances. A more detailed analysis might well reveal clues for improving immune monitoring and developing new therapies.

Technologies that track T cells on the single-cell level are beginning to resolve these cells’ heterogeneity and to show how T-cell populations shift through their continuum of states. “There’s a growing sense that we need to understand how individual T cells behave,” says Ton Schumacher, group leader in immunology at The Netherlands Cancer Institute in Amsterdam. Different single-cell-level techniques for doing so each bring unique angles to learning about the many personalities of T cells.

*The Scientist* sought out experts who are using techniques new and old to study single T cells. Here’s what they said.

**Microwells/Serial Microengraving**

**User:** J. Christopher Love, associate professor of chemical engineering, Massachusetts Institute of Technology’s Koch Institute for Integrative Cancer Research

**Technology:** Love’s group created an array of 85,000 or more wells—each of which holds less than 1 nanoliter—that allows them to separate individual T cells and quantify their secretions over time. They deposit a suspension of T cells over the array and allow the cells to settle by gravity, then seal the chip with a glass slide coated with one or more antibodies specific for secreted proteins, repeating this step at different time points. The antibodies on the glass slide capture the secreted proteins, which are subsequently labeled with fluorescent antibodies. The T cells themselves are fluorescently labeled either before or after the glass slide captures their secreted proteins.

Standard automated microscopes read out labeled proteins and cells. Although Love’s arrays are customized, anyone can use soft lithography methods to make their own, he says.

**Application:** By combining microwell arrays with high-resolution automated imaging, Love’s group was able to examine the sequence and time scale in which polyfunctional human T cells release a set of particular cytokines. They learned that release occurs in a well-defined order, which may help further define these T-cell populations according to function (*PNAS*, 109:1607-12, 2012).

**Pros:**

• *Sensitive.* By isolating large numbers of cells (around 100,000), the technology allows “fine resolution of rare events,” Love says.
• **Nondestructive.** The individual cells can be recovered for additional analysis. Love has successfully expanded them in culture.

• **Affordable.** A typical experiment costs about $100–$200 in materials and reagents, including antibodies. Materials and cost of the master mold run about $5–$10 per array, plus 1 hour’s worth of labor. You will also need access to a wide-field epifluorescence microscope (preferably automated). The master molds can be reused about 100 times before they become damaged, and the arrays themselves 3–4 times.

**Cons:**

• **Low throughput.** One chip can process only a few clinical samples.

• **Low temporal resolution.** It takes 1–2 hours to take measurements of an entire array—sufficient for the half-lives of the secreted molecules Love’s group is studying, but not for studies of intracellular T-cell signaling, which unfolds over minutes.

• **Limited multiplexing.** The number of molecules you can measure at once is limited to 4 or 5.

**Considerations:** The challenge comes in data analysis. With a grant from the National Institutes of Health to develop better software that links all of the measurements of a cell back to its unique spatial location on the chip, Love hopes to help researchers interested in serial microengraving integrate their data more quickly.

**Mass Cytometry**

**User:** [Mark Davis](https://www.dvssciences.com/), director, Stanford Institute for Immunology, Transplantation and Infection (ITI); professor of microbiology and immunology, Stanford University; Howard Hughes Medical Institute investigator.

**Technology:** Mass cytometry is similar to flow cytometry in principle, except that in mass cytometry, antibodies are tagged with rare-earth heavy metal isotopes instead of fluorophores. (The instrument, called CyTOF—for “cytometry by time of flight”—is manufactured exclusively by DVS Sciences in Sunnyvale, California; [www.dvssciences.com/](https://www.dvssciences.com/).) Just as in flow cytometry, cells with molecular features of interest labeled by tagged antibodies move single file through a narrow tube, but at the end they are sprayed into a mass spectrometer, which vaporizes them to their atoms, flings them against a detector—the lightest arriving first—and counts the heavier ions present in or on the cell.

CyTOF’s key advantage over flow cytometry is its ability to analyze 30–40 markers simultaneously. In contrast, flow cytometry can tackle about 15 markers tops, says Davis, in part because of the corrections needed for the fluorophores’ spectral overlap.

DVS Sciences is continuing to expand its catalog of metal-tagged antibodies, making the process of developing an antibody panel to profile T cells much less daunting than it was a few years ago.

**Applications:** Using CyTOF to phenotype CD8+ T cells from human blood, Davis showed that these cells exhibited an unexpected gradation of phenotypes and secreted a more complex range of cytokines than previously thought. Even cells specific for a given antigen show a variety of phenotypes and functions ([Immunity, 36:142–52, 2012](https://www.dvssciences.com/)). Better understanding such differences might help researchers predict T-cell responses.

**Pros:**

• **Unmatched multiplexing.** 30 markers produce more than a billion possible combinations, providing a huge boost in potential resolution, Davis says, and T cells are complex enough to make that worthwhile. “This is going to be a quantum leap in understanding phenotypic and functional variation in lymphocytes and white blood cells in general.”

• **Efficient.** CyTOF can make more measurements in a smaller volume of blood, compared with flow cytometry. This allows the group to phenotype pediatric samples, which usually come in small volumes—something they couldn’t do with flow cytometry, Davis says.

**Cons:**

• **Low throughput.** It takes 15–30 minutes to analyze one sample.

• **Destructive.** Cells are burned by the analysis—in fact, about 60–70% are vaporized without getting measured at all—so you can’t analyze them over time or go back and phenotype them further. However, you could sort cells into populations ahead of time using fluorescence-activated cell sorting (although then you’re back to fluorophores, and consequently a cap of 10–12 parameters).

• **Expensive.** Reagents for a single sample cost more than $300. A CyTOF machine costs roughly $650,000. However, the data obtained is well worth these extra costs, Davis says.

**Considerations:** Traditional flow cytometry data-analysis software can’t handle 40 parameters’ worth of data. Scientists have come up with two ways of dealing with the complex data from CyTOF: 3-dimensional principal component analysis (3D-PCA), which reduces the dimensionality of the data by...
homing in on its most variable features, and SPADE, a type of clustering that distributes data and reduces its variability. Each analysis tool has its own disadvantages and advantages, but together they are complementary, Davis says.

**Cellular Barcoding**

Researchers label each progenitor T cell with a unique genetic tag (shown in red or blue) that is passed on to all daughter cells. Scientists can then tell whether two descendant cell populations have a common or distinct origin. If the populations have a common origin, they will have both red and blue tags (top panel). If they have separate origins, only one tag will be found in each population (bottom).

**Technology:** To uniquely tag individual cells with molecular barcodes, Schumacher’s group generates lentiviral or retroviral plasmid libraries containing thousands of different plasmids, each of which contains a short stretch of noncoding DNA (the barcode) as well as a gene for green fluorescent protein.

The group introduces these libraries into T cells, and then injects those cells back into mice, allowing them to differentiate and expand. After the experiment, the group harvests various tissues from mice, and uses a second-generation sequencing-based detection system to parse them according to their lineages.

**Application:** If you want to follow how particular subsets of T cells or other immune cells are formed, or how heterogeneity arises, cellular barcoding is particularly powerful, says Schumacher.

However, the cells you follow should have the ability to expand substantially for the tracing to work. Using barcoding, Schumacher’s group recently reported that the fate of single naive T cells from mice is surprisingly unpredictable, given how uniformly the populations of these cells appear to differentiate and expand during an infection (*Science*, Epub ahead of print, 14 March 2013).

**Pros:**
- **Goes the distance.** Great strategy for longitudinally tracking the progeny of cells and their long-range migration in a living animal
- **Casts a wide net.** Unlike lineage tracing, the technique gives each unique parent T cell a label which allows researchers to track the fate of many cells and their progeny.

**Cons:**
- **Lengthy set-up time.** Getting the technique up and running can take several months, even for a lab that’s already familiar with mice and lentiviral transduction.
- **Destructive.** Readout requires lysing the cells, so any additional analysis must occur before barcoding.

However, you can preselect a population of cells to barcode and introduce into the animal, Schumacher says.
Considerations: The setup, which involves creating a large series of primers, costs a few thousand dollars. The cost of sequencing is the major limiting factor and depends how much sequencing and coverage you need—a deep sequencing answer can cost as much as $10,000. Schumacher is open to sharing his plasmid libraries with newcomers. Keep in mind, however, that accurate results require a tag sampling control—to assess whether you can recover the full repertoire of tags—as well as a tag distribution control, which tests the degree to which individual naive T cells share similar tags by chance (Nature Rev Immunol, 10:621-31, 2010).

Intravital Microscopy

User: Thorsten Mempel, assistant professor of medicine, Massachusetts General Hospital, Charlestown

Technology: Intravital microscopy (IVM) enables in vivo, live-cell imaging of a small part of an animal that has been immobilized on a stage. (See “Eyes on Cancer,” The Scientist, April 2012.) It’s not new, but because of the great improvements in microscopy and imaging software in recent years, scientists are able to peer into tissue to greater depths without sacrificing resolution.

Depending on the area imaged, experiments can last 1 to 12 hours. Mempel’s group can look at four different cell populations, or parameters, using fluorescence labeling.

Application: The group was able to see how T cells migrated within lymph nodes and tumor tissues of living mice, and how their interaction with other cells affected their gene expression and behavior (Immunity, 38:237-49, 2013).

Pros:
• In vivo. For T cells, this technique is especially powerful because cells can be studied in their natural surroundings—which hugely affect their function.
• Real-time. You can continuously observe cells (every second or minute), unlike cellular barcoding, where you get a single time point. (On the other hand, most IVM experiments can’t be done on a time scale of months.)

Cons:
• Difficult to master. The microsurgical techniques and the maintenance of tissue for long-lasting experiments can be enormously challenging. Even in a lab with IVM experience, expect 6 months to consistently generate reproducible results and a year to become an expert, Mempel says.
• Narrow focus. The technique scopes out an area of 100–600 μm² and 40–100 μm deep, which captures only tens or hundreds of cells. T cells can migrate over longer distances.
• Expensive. Mempel’s setup, a multiphoton laser-based microscope, costs about $700,000, but expect to pay a minimum of $350,000 for the essentials: an infrared laser and microscope.

Considerations: Mempel finds that some people want to try IVM but have no clear idea of what they would like to learn. “It only makes sense if you have a very defined scientific question” that can’t be answered using other methods, he says.

Re-sensitizing Resistant Bacteria

Researchers use a protein-lipid complex found in human breast milk to increase the activity of otherwise-ineffective antibiotics against drug-resistant pathogens.

By Dan Cossins | May 2, 2013

A protein-lipid complex that naturally occurs in human breast milk can increase the sensitivity of methicillin-resistant Staphylococcus aureus (MRSA) and other drug-resistant strains to multiple classes of antibiotics in animal models, according to a study published yesterday (May 1) in the PLOS ONE.

The findings suggest that the molecule—known as HAMLET (human alpha-lactalbumin made lethal to tumor cells)—could be useful as an antimicrobial adjuvant to boost otherwise ineffective antibiotics against MRSA and other “superbugs” that can cause lethal infectious outbreaks in hospitals.

HAMLET is one of the first antimicrobial adjuvant therapies to show efficacy in vivo, said Anders Hakansson, a microbiologist at the University of Buffalo and lead author of the study. “This molecule can help sensitize [resistant] bacteria to antibiotics we already have,” said Hakansson, “so we can again start using the treatment arsenal that was once available but that has been rendered ineffective.”

Additionally, if the compound does indeed prove effective in humans, HAMLET could be used to lower the doses of antibiotics needed to fight ordinary bacteria, which could slow the build up of resistance, he said.

But Karen Bush of Indiana University in Bloomington, who was not involved in the study, warned that “there are some problems with making this a viable therapeutic option. The main concern is that they
are going to have to use high concentrations of HAMLET in order to affect all the MRSA strains out there,” which may not be cost effective and could bring problems with solubility and, potentially, toxicity.

But such problems may be avoided if HAMLET is used as a topical treatment for wound infections, she said. “I think this could be used topically, in treating wound infections, something where you spread it on the skin or on a dressing. Something like that could be effective.”

Hakansson began working with HAMLET—a lab-made combination of alpha-lactalbumin and one of two lipids, all of which are found in human breast milk—as a graduate student in Sweden. In 1995, he demonstrated that the protein complex killed tumor cells, and later showed that it does so by infiltrating the tumor cell’s mitochondria, depolarizing the inner membrane and inducing cell death.

In 2011, Hakansson’s group showed that HAMLET could also kill Streptococcus pneumoniae via a similar mechanism—a sodium-dependent influx of calcium ions that led to membrane depolarization. And last year they demonstrated that even at non-lethal concentrations, HAMLET sensitized the same bacterial pathogen to antibiotics.

To see if HAMLET would have the same potentiating effect on S. aureus, in which it did induce calcium influx but not apoptosis, Hakansson and his University of Buffalo colleagues Laura Marks and Emily Clementi used in vitro assays to show that the presence of HAMLET could make MRSA sensitive to methicillin, and render other resistant strains sensitive to vancomycin, erythromycin, and gentamycin. With HAMLET, these antibiotics not only inhibited the growth of the bacteria, but also killed them. The molecule had the same affect on antibiotic activity against S. aureus biofilms.

The team then tested the HAMLET/antibiotic combination on MRSA in the nasal passage of living mice, and found that while methicillin alone failed to reduce colonization, the combination produced a significant decrease.

Hakansson said that by blocking the cell’s hydrogen ion pump, HAMLET dissipates the proton gradient across the membrane. “This means the cell has a harder time making energy, so the cell’s efflux pumps—the usual mechanism for expelling antibiotics—would not work as effectively,” he said.

Methicillin works on the outside of the cell, however, so it is not clear how its efficacy is improved by HAMLET. Hakansson speculated that changes to the ion concentrations across the membrane could result in structural alterations to the cell wall that allow methicillin to bind more effectively. “It appears there are some changes in the cell wall that make it an easier target,” he said.

Finally, the researchers demonstrated that HAMLET inhibits the evolution of methicillin resistance even as the bacteria are repeatedly exposed to increased concentrations of the antibiotic.

“They certainly show there is a slower development of resistance, but they’re still seeing resistance,” Bush noted. “There is no way you’re going to shut down resistance completely.”

Nevertheless, Hakansson has confidence in the approach, and has cofounded a company to test HAMLET on different bacteria and infection models. “I’m hoping this [work] will trigger more thought in this direction—rather than trying to find new antibiotics, looking at adjuvants would not be a bad idea.”

L. Marks et al., “Sensitization of Staphylococcus aureus to methicillin and other antibiotics in vitro and in vivo in the presence of HAMLET,” PLOS ONE, 8:e63158, 2013.

Telomeres Affect Gene Expression

As telomeres shorten with age, genes as far as 1,000 kilobases away could be affected, including one responsible for an inherited muscle disease.

By Ed Yong | May 5, 2013

dux4, a gene responsible for the genetic disease facioscapulohumeral muscular dystrophy (fshd), is normally silenced because it sits next to a telomere—a protective dna sequence that caps the ends of chromosomes, according to a study published today (may 5) in nature structural and molecular biology. But as telomeres shorten, as they do with age, dux4 expression climbs, which may explain the late onset of fshd. Another gene, called frg2, which sits 100 kilobases away from the telomere, is also affected by telomere length.

“This was completely unexpected,” said coauthor Guido Stadler at the University of Texas Southwestern Medical Center in Dallas, since earlier
studies showed that telomeres only silence genes a few kilobases away. Stradler and his colleagues even found preliminary evidence that telomeres can also influence a gene even more distant on the chromosome, 1,000 kilobases away—an effect that disappears as the telomere shortens.

“We think that DUX4 and FRG2 are the tip of an iceberg,” he said: due to shrinking telomeres, many genes might gradually become more active as we get older, which may be important for several diseases of old age. “This represents a very significant general advance in our understanding of how telomere shortening may affect human biology.”

FSHD is an inherited disease that causes the upper body muscles to gradually waste away. Most such genetic disorders manifest during early childhood, but FSHD is unusual in symptoms usually appear when people are in their teens or early 20s.

To investigate this delay, Stadler created lines of muscle-making cells using muscle tissues from FSHD patients and unaffected family members, and manipulated the lengths of their telomeres. As the telomeres got shorter, DUX4 gradually became over 10 times more active, in a greater proportion of cells, explaining late onset of FSHD symptoms in people who inherit the disease-associated version of DUX4.

It is possible that doctors may eventually consider telomere length when offering a prognosis or advice about FSHD, or try to control the disease with treatments that lengthen telomeres. Of course, Stadler said, “before patients may benefit from our findings, we need to do a lot of work, especially to confirm that our in vitro findings hold true in vivo.”

Alexandra Belayew from the University of Mons in Belgium, who was not involved in the study, said the results aren’t a “major conceptual change” for FSHD researchers, who largely agree that DUX4 activation is the major cause of the disease. “What is new is the proposed mechanism by which aging contributes to disease progression,” she said.

And the experiments with FRG2, which showed a similar pattern of increased activity with shortening telomeres, emphasized that even genes more distant from the chromosome’s ends could be affected in this way as we age.

Stadler’s results also solve another mystery about FSHD—why so few people carrying the known FSHD mutations actually develop the disease. DUX4 lies within a repetitive stretch of DNA at one end of chromosome 4. People with FSHD only have 1 to 10 copies of DUX4 (most have up to 100), as well as mutations in the gene that allow it to be stably expressed. But this combination of features is found in around 1 percent of the population, and FSHD only affects 1 in 20,000 people.

Stadler suggests that people who carry FSHD mutations but never show symptoms might be born with exceptionally long telomeres, or have telomeres that shorten slowly thanks to genetic or environmental factors.

“The paper highlights the complexity of FSHD, which cannot be explained with the classical model in which a mutated gene causes a disease,” said Rosella Tupler from the University of Massachusetts Medical School, who was not involved in the study.

The team will now study people with FSHD to see if the length of their telomeres correlates with the onset and progression of their disease. They also want to work out how the length of telomeres affects the activity of other nearby genes. Belayew added, “Does telomere shortening play a role in infantile FSHD in which clinical manifestations are seen much earlier?”


The Science of Stretch
The study of connective tissue is shedding light on pain and providing new explanations for alternative medicine.

By Helene M. Langevin | May 1, 2013

It joins your thigh to your calf; your hand to your arm; your breastbone to your clavicle. As you move, it allows your muscles to glide past one another. It acts like a net suspending your organs and a high-tech adhesive holding your cells in place while relaying messages between them. Connective tissue is one of the most integral components of the human machine. Indeed, one could draw a line between any two points of the body via a path of connective tissue. This network is so extensive and ubiquitous that if we were to lose every organ, muscle, bone, nerve, and blood vessel in our bodies, we would still maintain the same shape: our “connective-tissue body.”

Despite increasing evidence of its role in chronic pain and other diseases, connective tissue is not very well studied. I arrived at researching connective tissue by a circuitous route. Working as a clinical endocrinologist, I would see patients suffering from chronic pain, and quickly became frustrated with the
treatment options I could offer—usually some combination of physical therapy and analgesics, which often were not very effective. Some of my patients would ask about trying acupuncture. But, having done research in neuroscience and being firmly rooted in the practice of Western medicine, I was skeptical. Eventually, I decided to learn more, if only to be able to respond to patient questions more intelligently.

In 1986, I took evening classes at the Tri-State Institute of Traditional Chinese Acupuncture in Stamford, Connecticut (now the Tri-State College of Acupuncture in New York City), which offers hands-on experience in acupuncture. The teacher described how to twirl the inserted acupuncture needles just enough to elicit a particular sensation in the patient, usually described as an ache in the area surrounding the needle, which can radiate some distance away from it. I was told that the acupuncturist is supposed to feel tightness or tugging on the needle, akin to when a fish gets caught on a hook. When I felt that tug myself, I became curious about the physical mechanism that was causing it. The teachers explained it as muscle contracting around the needle, but I could feel it in locations, such as the wrist, where there was no muscle at all. The needles had to be interacting with connective tissue.

A decade later, after I had moved to the then Department of Neurology at the University of Vermont (UVM) College of Medicine in Burlington, I had the opportunity to begin research on the acupuncture “needle grasp.” Here was a physiological phenomenon that one could feel with one’s fingers, but which had no obvious biological explanation. I started collaborating with Martin Krag, an orthopedic surgeon at UVM who had some experience testing alternative-medicine approaches using scientific methods. The logical first step was to quantify the tugging response to acupuncture needling. With the help of David Churchill, a biomedical engineer in the Orthopedic Department at UVM who designed a robotic acupuncture-needling instrument, we began measuring the force needed to pull out the needles in a reproducible manner from 16 different points on the body. We measured the “pullout force” in 60 human subjects and found that it did indeed increase after needle rotation, at times so dramatically that it exceeded the capacity of our 500 g load-measurement device.1

We then tested the possible mechanisms that could cause this phenomenon, starting with simple experiments in which we inserted and rotated a needle in a piece of rat abdominal wall. What we saw under the microscope was quite striking: when acupuncture needles were rotated, the loose connective tissue under the skin became mechanically attached to the needle. Even a small amount of rotation caused the connective tissue to wrap around the needle, like spaghetti winding around a fork.2 This winding caused the surrounding connective tissue to become stretched as it was pulled by the needle’s motion. Using ultrasound, we confirmed that the same phenomenon occurs in live tissue.3

In the years that followed, I became part of a small but growing community of scientists who were joining the ranks of molecular and physiological researchers dedicated to studying this neglected tissue. Connective tissue has been relegated to the role of passive viscoelastic material in traditional biomechanical models, but researchers are now beginning to demonstrate just how many systems of the body may be affected by mechanical changes in connective tissue, and some of these findings are beginning to inform clinical practice.

A growing field

Connective tissue is something of an orphan child in medicine: although it is an integral part of the musculoskeletal system, connective tissue is basically absent from orthopedic textbooks, which deal principally with bones, cartilage, and muscles. Orthopedic interest is almost exclusively restricted to the “specialized” connective tissues such as tendons and ligaments, which connect bone to muscles and to other bones, respectively. Nonspecialized connective tissues, which form what’s known as the fasciae and envelop all muscles, nerves, bones, and blood vessels, are typically allotted a short paragraph in current textbooks, if mentioned at all.

However, interest in the field has been growing. One area that has attracted many researchers at the cellular level is the study of mechanotransduction: how the integrin family of adhesion molecules forms a physical and informational link between the extracellular matrix and the interior of cells. Through these cell-matrix connections, cells sense forces and transform these mechanical signals into cellular responses such as the activation or deactivation of signaling molecules, translocation of transcription factors into the nucleus, and ultimately, changes in gene expression.4 In addition, substantial evidence supports the notion that mechanical signals can be transmitted directly through the cytoskeleton into the interior of the nucleus. (See “Full Speed Ahead,” The Scientist, December 2009.)

Some of the most well-established work in this field has involved the study of fibroblasts—the cells that are responsible for synthesizing all the proteins that make up the extracellular matrix. These cells reside within the matrix they create, responding to mechanical stimulation by regulating the amount of collagen and other matrix proteins produced, and by secreting matrix-degrading enzymes in response to...
chronic changes in tissue forces. Such changes can be induced by repetitive motion and are thought to be an important factor in work-related musculoskeletal injuries such as tendinitis of the shoulder or wrist.5

Here was a physiological phenomenon that one could feel with one's fingers, but which had no obvious biological explanation.

Fibroblasts also play a major role in the response to acute injury, particularly when they transform into myofibroblasts. Before the availability of surgery and surgical sutures, gaping wounds needed a powerful mechanism in order to pull shut and heal. Myofibroblasts serve this function by secreting large amounts of collagen and expressing α-smooth muscle actin protein, which make the cells contractile.6 Then, by exerting tension on the collagen matrix, these cells pull the edges of the wound together. Myofibroblasts normally die once this job is done and a stable scar has formed. However, during chronic inflammation, myofibroblasts can drive an excessive deposition of collagen, and the increased tissue tension can result in the development of tissue contractures that restrict full range of motion. This response is also thought to play a role in the development of some types of tissue fibroses and cancer. Indeed, fibrotic, or scarred tissues, become stiffer, and cancer cells have been shown to spread more easily on fibrotic matrices.7

Although much of the work in this area to date has been performed in cell culture, rather than in whole tissue, some of this basic research is beginning to inform clinical research and practice, especially in the area of chronic musculoskeletal pain, including low-back pain. One of the reasons that low-back pain is so difficult to manage is that large numbers of patients have no detectable abnormalities of the spine and associated tissues, and the source of their pain is unknown. Some groups have begun to investigate the possibility that the pain is arising from the nonspecialized connective tissues on either side of the spine.

Indeed, researchers at the University of Heidelberg found in 2008 that connective tissues contain sensory nerve endings that can transmit pain when these tissues are stretched in the presence of inflammation.8 Until then, it had not been clear whether connective tissue had its own sensory nerve supply capable of generating sensations. Subsequently, ultrasound studies in my laboratory demonstrated that the connective tissues that surround the muscles of the back are, on average, thicker in people with chronic low back pain.9 Normally, these connective tissues are composed of alternating layers of tightly woven dense fibers that can bear substantial loads, and loose areolar tissue, which contains large quantities of water and allows the adjacent dense layers to glide past one another. In addition to having thicker connective tissue overall, people with low-back pain show a decreased gliding motion of dense layers, suggesting that a fibrotic process could cause the decreased mobility.

**Connecting the dots**
Despite these recent advances, the overwhelming majority of research on connective tissue still involves cells grown in culture dishes. And recent studies suggest that, especially for fibroblasts, the mechanical behavior of cells may be quite different when cells are grown on 2-D surfaces compared to cell behavior in a 3-D environment that is more similar to that of whole tissue, such as a thick collagen gel. For example, it is becoming apparent that the ubiquitous intracellular “stress fibers” characteristic of fibroblasts grown on 2-D surfaces are not present in fibroblasts grown in 3-D-culture environments or in whole tissue, and that these fibers may in fact be an artifact of cell culture, rather than a phenomenon that has physiological meaning. The fact that the study of fibroblasts in whole tissue is lagging far behind that of fibroblasts in vitro, combined with the general lack of attention to nonspecialized connective tissue at the whole-body level, has limited the understanding of natural connective-tissue function.

I began my research into connective tissue on the whole-animal level, but quickly began to investigate the cellular components involved in the winding response to acupuncture needles. After dissecting some of the tissue we had manipulated, we saw that the fibroblasts residing in the connective tissue as far as several centimeters away from the needle began to reorganize their internal cytoskeleton and change shape, becoming large and flat. We also found that the same reorganization response could be elicited by simply stretching a piece of connective tissue between two grips and holding the tissue in the stretched position for about 30 minutes, or even stretching an anesthetized mouse by bending its body to one side. Interestingly, 30 minutes is typically the amount of time that needles are left in place during an acupuncture treatment. Furthermore, if one lets go of the needle after rotating it, the needle does not unwind right away. Thus, the “whorl” of connective tissue remains intact as long as the needle remains under the skin, causing the tissue to be stretched for a prolonged period.

Ongoing studies in my lab are addressing why the fibroblasts change shape in response to sustained stretching. So far we have found that the changes are associated with a large-scale relaxation of the connective tissue. We also saw that the fibroblasts initiated a specific Rho-dependent cytoskeletal reorganization that was required for the tissue to fully relax. Rho is an intracellular signaling molecule known to play a role in cell motility and the remodeling of cell-surface proteins that connect the fibroblast to its surrounding matrix. The molecule’s involvement in fibroblast shape change suggested that the cells are able to reduce the tissue tension by adjusting how strongly and where they are gripping the surrounding connective tissue or muscle. (See illustration above.) In addition, we found that the shape change is also associated with a sustained release of ATP from the fibroblast. Within the cell, ATP acts as fuel, but outside of the membrane, ATP can function as a signaling molecule. Extracellular ATP can be converted to other purines such as adenosine, which can act as a local analgesic, thus providing a possible cellular and physiological mechanism to explain the pain relief experienced by some acupuncture patients. (See “Puncturing the Myth,” The Scientist, September 2011.)

Acupuncture-needle manipulation results in sustained stretching, and therefore constitutes a useful tool that can be used to study this biomechanical function. The possibility that connective tissue dynamically regulates its level of tension is intriguing, as it could dampen fluctuations in tissue tension. Connective tissue surrounds nerves, blood vessels, and lymphatics,
and reducing changes in tissue tension could affect how these structures function. Importantly, fibroblast cytoskeletal reorganization is a rather slow process, taking several minutes, and therefore would occur in response to sustained changes in tissue length such as changes in posture and body positions. Remarkably little is known about the effects of static tissue stretching, though repetitive, cyclical stretching has been extensively studied because of its relevance to breathing, walking, and cardiovascular pulsations. Acupuncture-needle manipulation results in sustained stretching, and therefore constitutes a useful tool that can be used to study this biomechanical function.

In contrast to the general neglect of connective tissue in the conventional medical and scientific fields, “alternative-medicine” researchers, and especially clinical practitioners, have for many years recognized the potential importance of connective tissue in health and disease. In conventional physical therapy, stretching of surgical scars and joint tissue that has contracted and stiffened after prolonged immobilization is widely believed to cause remodeling of connective tissue. Alternative therapies such as myofascial release and Rolfing focus on stretching as a treatment modality for musculoskeletal pain, even in the absence of an obvious past injury or scarring. Indeed, a variety of alternative manual and movement-based therapies work under the collective assumption that connective-tissue pathology lies at the source of musculoskeletal pain, and that this can be ameliorated with manual treatments.

**Connection to acupuncture meridians**

The mysterious “acupuncture meridians,” defined as lines or tracks connecting acupuncture points, also may be related to connective tissue, as they seem to be preferentially located along connective-tissue planes between muscles, or between muscle and bone. We have found that more than 80 percent of acupuncture points in the arm are located along connective-tissue planes. This makes sense, since loose connective tissue houses blood vessels and nerves, suggesting that mechanical stimulation of connective tissue generated by needle manipulation could transmit a mechanical signal to sensory nerves, as well as intrinsic sensory afferents directly innervating connective tissue.

Clearly, connective tissue needs more attention. A simple PubMed search illustrates this problem, as specific subject headings for “nonspecialized connective tissue” do not exist. By default, alternative medicine has become a motivating force in connective-tissue research and clinical practice. This is an example of an area in which the combination of conventional and alternative medicine, typically referred to as “integrative medicine,” should be understood in a broader sense as integration within medicine itself, inspired by alternative-medicine concepts. The growth and maturation of the field of connective-tissue research will no doubt benefit from exciting new developments resulting from this integration.

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**References**


**New Guardians Against Diabetes?**

A new class of immune cell could protect against type 1 diabetes by suppressing other immune cells.

By Ed Yong | May 20, 2013
Our bodies are protected by white blood cells called T-cells that detect and destroy infections, tumors, and other threats. But these watchmen are themselves watched by regulatory T-cells, which suppress the proliferation of other T-cells and prevent our immune systems from turning against our own bodies.

Now, a team of Australian scientists has discovered a new type of these suppressors that are distinguished by a protein called CD52, which they produce in large quantities and release. These “CD52hi cells” appear to play a key in type 1 diabetes. In humans with the disease, these cells are rare and ineffective at suppressing other T-cells. Furthermore, depleting these cells in diabetes-prone mice quickly triggers the disease.

“We can’t claim yet that these cells are important for preventing diabetes in humans, but the evidence suggests that they are,” said Len Harrison from the Walter & Eliza Hall Institute of Medical Research in Australia, who led the research, published today (May 19) in Nature Immunology.

“This finding will assist in the development of targeted therapies to manipulate the immune response and improve human health,” added Jane Buckner from the Benaroya Research Institute in Seattle, who was not involved in the study. However, she added that we don’t know how important these suppressor cells are compared to more familiar types of regulatory T-cells.

The new discovery of CD52hi cells could also explain some puzzling side effects of alemtuzumab (or Campath)—an antibody that binds to CD52. Discovered in the 1980s, the drug has been used to successfully treat leukaemia and lymphoma—possibly because it stops cancer cells from using CD52 to suppress other T-cells and sneak past the immune system.

More recently, alemtuzumab has been used to treat multiple sclerosis, but some clinical trials have shown that 30 percent of patients who take the antibody develop a different autoimmune disease. This may be because the antibody lifts the suppressive effects of CD52hi cells, allowing pathogen-attacking immune cells to go off the rails. This may also explain why people on alemtuzumab tend to be protected against serious infections.

People with type 1 diabetes often produce antibodies against GAD65, one of their own proteins. Harrison’s team knew that injections of GAD65 can activate regulatory T-cells that prevent diabetes in mice. But when the researchers examined these activated cells, they discovered one unusual finding after another.

The T-cells activated by GAD65 differed from all known varieties. Their distinguishing feature was a high level of CD52, a protein with no clear physiological role that is thought to sit in the membranes of white blood cells. But the suppressive CD52hi cells can release CD52 to control other T-cells at a distance. Once it reaches other cells, it binds to the T-cell surface protein Siglec-10, one of several proteins that “have only been put on the map in the last decade,” said Harrison. “CD52 is one of the few known [substances] that works through them.”

So, these new cells act through mysterious protein that binds to an equally mysterious receptor through an unexpected route. “It’s surprising,” said Harrison, “and a new mechanism of suppression.”

This chain of events could be important for preventing type 1 diabetes. When Harrison’s team depleted CD52 from lymphocytes and injected the cells into young diabetes-prone mice, all of them developed diabetes within a month, far sooner than they normally would.

Conversely, boosting CD52hi cells, or perhaps targeting the Siglec-10 receptor directly, could be interesting new avenues for treating type 1 diabetes. But Harrison also thinks that the opposite strategy—blocking CD52—might be useful for boosting immune responses, and is now doing experiments to see if this phenomenon can improve vaccine effectiveness.

E. Bandala-Sanchez et al., “T cell regulation mediated by interaction of soluble CD52 with the inhibitory receptor Siglec-10,” Nature Immunology, doi:10.1038/ni.2610, 2013.

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 mucus 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.”


**Vitamin C Slays TB Bacteria**

**The essential nutrient can kill drug-resistant *Mycobacterium tuberculosis* by producing oxidative radicals that damage DNA.**

By Dan Cossins | May 21, 2013

High doses of vitamin C can rapidly wipe out entire populations of drug-resistant strains of the bacteria that cause tuberculosis (TB) by inducing a chemical reaction that produces high levels of DNA-damaging oxidative radicals, according to a study published today (May 21) in *Nature Communications*.

The study was done on *Mycobacterium tuberculosis* in lab cultures, but if the results hold up in vivo, it raises the possibility that vitamin C could be added to existing TB drugs to speed up treatment. Moreover, by elucidating the mechanism by which vitamin C kills *M. tuberculosis*, the work suggests a promising route for the development of new TB drugs to tackle these hard-to-kill bacteria.

“[The research] shows that perhaps *M. tuberculosis* doesn’t have quite the iron-clad armor that we often associate with it; that is does perhaps have some Achilles heels and weak underbellies,” said William Bishai, director of the KwaZulu-Natal Research Institute for Tuberculosis and HIV in Durban, South Africa, who was not involved in the study.

But Bishai warned that it will be difficult to achieve the high concentrations of vitamin C required in humans, and added that “there is still a little bit more proof of principle needed” before large-scale drug discovery efforts centered around this mechanism can begin. “I think the question would be, can we identify a more potent reductant that would be delivered at lower concentrations but still stimulate the oxidative radicals?”

“We’re not saying vitamin C is the cure for TB,” said Catherine Vilcheze of the Albert Einstein Medical College at Yeshiva University in New York, who was first author on the study. “But we should look at trying to find ways to mimic the way it kills TB bacteria [in vitro], because it is very, very effective.”

The initial discovery of vitamin C’s TB-killing ability came about by accident. To test their hypothesis that the amino acid cysteine helps the TB drug isoniazid (INH) kill off *M. tuberculosis* by acting as a reductant to produce oxidative radicals, Vilcheze and colleagues decided to see if vitamin C, another reducing agent, would have the same effect. It did. But the researchers were shocked to see that vitamin C alone, used as a control, also wiped out the bacteria. “I was not expecting any effect at all, so that was very surprising,” Vilcheze said.
The researchers then demonstrated that at a certain concentration, vitamin C could sterilize cultures within just a few weeks. It had the same effect on drug resistant strains and extensively-drug-resistant strains. "If you start with about 10 million cells, after 3 weeks [of vitamin C treatment] there is nothing left in your culture," said Vilcheze. "It kills everything." Concentrations had to be much higher to achieve that same effect in other pathogens, however, so *M. tuberculosis* is uniquely susceptible.

The team hypothesized that vitamin C killed the bacteria by driving an iron-dependent reaction that produces reactive oxygen species (ROS), which can induce cell death via DNA damage. Sure enough, when added to the bacteria in an environment that lacked oxygen or was depleted for iron, the vitamin C lost its sterilization capacity. What’s more, levels of ROS and oxidative damage to DNA and lipids in cell walls and membranes were far higher in cultures treated with vitamin C than controls that were either untreated or treated with vitamin E.

The study also demonstrated that vitamin C's effect was increased 60-fold in *M. tuberculosis* strains deficient for mycothiol, which protects the cells from oxidative damage. This suggests that a combination therapy pairing a mycothiol inhibitor with vitamin C, or similar compounds that produce an oxidative burst, could potentially kill the pathogen more quickly than available treatments, said Vilcheze. And although Bishai cautioned that the high concentrations of vitamin C used in the experiment would be hard to replicate in the human body, he added that it might be possible to develop a more potent but equally well-tolerated reductant to do the same job.

In the meantime, study leader Bill Jacobs, also at Albert Einstein Medical College, wants to carry out clinical trials to see if adding vitamin C supplements to current drug regimens could benefit TB patients. “We don’t know whether vitamin C will work in humans, but we now have a rational basis for doing a clinical trial,” said Jacobs in a press release. “At the very least,” added Jacobs, “this work shows us a new mechanism that we can exploit to attack TB.”

C. Vilcheze et al., “*Mycobacterium tuberculosis* is extraordinarily sensitive to killing by a vitamin C-induced Fenton reaction,” *Nature Communications*, doi:10.1038/ncomms2898, 2013.

**TB programs affected by isoniazid shortage**


A shortage of isoniazid interfered with patient care and may contribute to the spread of tuberculosis in the United States, according to a report by the CDC.

“Interruptions in the supply of second-line anti-TB medications have been ongoing in the United States for several years,” the researchers wrote in *Morbidity and Mortality Weekly Report*. “But since November 2012, TB control programs have experienced the first sustained generalized supply interruption of a first-line anti-TB medication.”

According to the report, a shortage of 300 mg isoniazid tablets was first reported in November, which was attributed to difficulty procuring the active ingredient. In December, isoniazid became available in 100 mg tablets. Suppliers anticipated that the 300-mg tablets would be available by January, but they still are not available, and the 100-mg tablets also are limited.

The National Tuberculosis Controllers Association conducted a survey of 68 jurisdictions to identify issues related to medication procurement, medication supply and TB treatment related to the isoniazid shortage. There were 42 respondents, and 33 reported difficulty in obtaining isoniazid within the previous mount. The shortage led to 18 of 25 TB programs prioritizing high-risk patients for treatment of latent TB infection. Twenty-two TB programs changed to alternate treatment regimens.

“How the increased use of alternative regimens and the rising cost of isoniazid driven by increased demand might affect the future supply of isoniazid and other first-line anti-TB medications is uncertain,” the researchers wrote. “CDC is continuing to work on developing a sustainable solution that will maintain an uninterrupted supply of anti-TB drugs in the United States.”

**The 'Undetectable' Paradox**

To combat the spread of HIV, we need a way to talk honestly about what it means to be 'undetectable.'

BY Tyler Curry

May 24 2013 6:00 AM ET

It is impossible to have a modern conversation about HIV and HIV stigma without having the term “undetectable” used, misused, and abused. Those involved in HIV activism certainly have strong opinions on how the term that refers to an HIV-positive person’s undetectable viral load should be used (and who is using it incorrectly). Some herald the term as a badge of honor worn by those who are compliant in
treatment and open with their HIV status, while others would scold the same group of people for using the term as an excuse to engage in unsafe sexual behavior.

Either way, oversimplified accolades and mudslinging moral judgments have no place in a conversation about HIV stigma, prevention, and the term that is a result of compliance with medication. With many gay men still unclear about what being “undetectable” truly constitutes, how do we get to a place where we can discuss what it does and doesn’t mean without all of us looking dirty in the end?

For those who are still unsure: An HIV-positive person can achieve undetectable viral levels after undergoing antiretroviral therapy. The viral load affects the chance that they will transmit HIV. According to an article in Journal Watch HIV/AIDS Clinical Care, one study indicated that early antiretroviral therapy reduced the likelihood of transmission by 96%. Once antiretroviral meds help a person achieve an undetectable viral load, it is possible to remain at this level provided the person continues to take the medication as directed.

An education on the specifics of HIV as it is today, including the meaning of being undetectable, should be mandatory reading for gay men, regardless of status. It is critical to the entire community to understand where we are in terms of HIV research. No matter how far removed you are from the HIV pandemic, you are still susceptible to the virus (especially if you think you aren’t).

Now, unless we find a way to infuse subliminal HIV messaging into the speakers of every H&M in the country, casual conversation among our peers is the most effective method of education. But, as with many discussions concerning HIV, the discussion quickly turns into the blame game. So who loses?

Everyone.

For the sake of conversation, let’s liken a person with an undetectable viral load to a person who is HIV-negative. With both classifications, you get tested regularly to make sure that you are still safely in your category. But unlike being HIV-negative, discussing the meaning of an undetectable status almost immediately gets bogged down by shame-mongering and moral accusations. Use of the term is often ridiculed, immediately placing judgment on the HIV-positive person who speaks about his undetectable status.

The following quote was taken from a post that asked people how they think we can make progress in eliminating HIV stigma:

“I’d like to hear more responsible discussion in our community about how dangerous and reckless it is to use the term ‘undetectable’ given the implications of treating ‘undetectable’ status as if it were really something different from being positive.”

This claim wasn’t made with malicious intent, but does give a lucid demonstration of the difficulty of discussing HIV-related topics without subconsciously casting judgment.

In fact, people who are undetectable should never stop talking about their status. At the gym, on the subway, and even at Sunday services (if that’s your sort of thing).

“Did you catch the last inning of the Rangers game last night?”

“Hell no, I don’t watch sports. But my viral load is 57!”

A person discussing their undetectable status is a beautiful thing because it means they have been tested, are on treatment, and are open and honest about their HIV status. The idea that the term “undetectable” is used only to lure unsuspecting prey into performing high-risk sexual acts with someone who is positive is both stigmatizing and criminalizing. This notion removes all responsibility from the other party when they have just been given the information they need to protect their own health. And it is, in fact, their responsibility to protect their own health (and no one else’s).

Far too often, our community mistakes silence as an admission of innocence. If no one asks a person’s HIV status, no one tells. Worse, some will assert their HIV-negative status even if it’s been months or even years since their last test.

Yet these proverbial question marks walk around every day, unscathed by denunciations associated with their bedtime behavior. They aren’t reduced to sweeping stereotypes of being sexual pariahs even though their pseudo-negative HIV status could possibly place a person at much greater risk than that of someone who is undetectable.

In the realm of sex and dating, the responsibility lies with you to make the appropriate choices to protect your health. Unfortunately, people are slutty, nobody likes using condoms, and everybody is a liar. But that doesn’t mean we have to muddle the value of an undetectable viral load and debase a group of people who are at least willing to be up front with their status.

The sexual acts of gay men do not exist in two separate vacuums. If they did, it would certainly be much easier to end the transmission of the virus. Therefore the conversation about what it will take to
decrease stigma and increase testing must also exist without uninformed generalizations that could silence many before they even speak.

In order for a conversation about HIV and HIV stigma to have substantive meaning, assumptions, accusations, and generalizations need to become “undetectable.”

**Antibiotics: A new understanding of sulfonamide nervous system side effects**

Scientists at EPFL have uncovered the molecular basis behind some of the neurological side effects of sulfonamide antibiotics, providing doctors with possible means to minimize them in patients.

Since the discovery of Prontosil in 1932, sulfonamide antibiotics have been used to combat a wide spectrum of bacterial infections, from acne to chlamydia and pneumonia. However, their side effects can include serious neurological problems like nausea, headache, dizziness, hallucinations and even psychosis. In a recent Science publication, EPFL researchers have shown for the first time how sulfonamides can interfere with a patient's nervous system.

The problem is that, even though we know how sulfonamides work, we do not understand the actual molecular mechanics behind their side effects. Consequently, it is difficult to modify drug structure or customize therapeutic regimes in order to better serve the needs of patients. That is the critical issue addressed by a team of EPFL scientists lead by Kai Johnsson at EPFL's Laboratory of Protein Engineering.

The team drew from previous research showing that blocking the activity of a certain enzyme (sepiapterin reductase) affects the levels of an important molecule called tetrahydrobiopterin (BH4) in cells. BH4 is critical for the production of neurotransmitters like serotonin and dopamine, and BH4 deficiency causes similar neurological problems to those associated with sulfonamide side effects.

The EPFL scientists showed for the first time that sulfonamides actually bind to the part of the enzyme that makes BH4. Using a high-throughput drug screening system, the researchers identified ten sulfonamides that strongly inhibit the enzyme. Taking advantage of the expertise of Florence Pojer at EPFL's Global Health Institute, the scientists were able to solve the enzyme's molecular structure and determine how sulfonamides bind to it.

Sulfonamides also seem to act on the actual biochemical pathway that synthesizes BH4, as increasing doses of the drugs decreased BH4 concentrations in cultured human cells. The critical finding, however, was that, along with BH4, sulfonamides also reduced the actual production of dopamine. By giving cultured human nerve cells different sulfonamides, the researchers found that their natural production of dopamine decreased in proportion to the sulfonamide doses. In addition, it was clear that the impact on dopamine production was different between sulfonamides.

The group's work shows for the first time that sulfonamides interfere with the biosynthesis of neurotransmitters, which can account for their reported neurological side effects. It also helps us understand how the activity of these drugs relates to their molecular structure, and suggests ways of improving their clinical use.

"Once you know what's happening you can begin to think about strategies to address the problem – and that is the impact of this work", says Kai Johnsson. "Historically, I don't think that there is a more important class of drugs than sulfonamides, and now we can understand them better. It also reminds us that surprising discoveries can be made even for drugs this old."

**Vaccine blackjack: IL-21 critical to fight against viral infections**

Woodruff Health Sciences Center | May 22, 2013

Scientists at Emory Vaccine Center have shown that an immune regulatory molecule called IL-21 is needed for long-lasting antibody responses in mice against viral infections.

The results are published in the *Journal of Virology*.

"Our findings highlight how IL-21 could be important in the development of antiviral vaccines," says research associate Ata Ur Rasheed Mohammed, PhD, the first author of the paper. The senior author is Rafi Ahmed, PhD, director of the Emory Vaccine Center and a Georgia Research Alliance Eminent Scholar.

The findings could lead scientists designing future vaccines to incorporate IL-21 directly or to use the ability to stimulate IL-21 as a gauge of vaccine activity. IL-21 was discovered in 2000. Its effects have also been studied in the area of immune responses against HIV, and it has been in clinical trials for skin cancer and kidney cancer and auto-immune disorders.
A main objective of vaccination is to make the recipient’s immune system develop antibodies that can neutralize infecting viruses. Signals from IL-21 appear to be necessary for generating long-lived plasma cells, which reside in the bone marrow and secrete antibodies.

Rasheed and his colleagues probed mice that were unable to respond to IL-21, because the mice were engineered to lack the gene for the IL-21 receptor. They examined the altered mice in the context of three different types of viral infections: LCMV (lymphocytic choriomeningitis virus), VSV (vesicular stomatitis virus), and influenza.

When infected with each of the three viruses separately, the altered mice did start to produce antibodies, but antibody levels faded out over the course of around two months. The mice "exhibited a profound defect in generating long-lived plasma cells and in sustaining antibody levels over time," the authors write.

Rasheed’s team demonstrated that IL-21 is playing a role in germinal centers, structures in the lymph nodes and spleen where cells that produce high-affinity antibodies are selected. In the IL-21 receptor deficient mice, germinal centers form but are not sustained. IL-21 signals are important both for the antibody-producing cells and for T helper cells that support them, the researchers showed.

The research was supported by the National Institutes of Allergy and Infectious Diseases (P01 AI097092-01A1 and RO1 A1030048) and the Center for HIV/AIDS Vaccine Immunology and Immunogen Discovery (UM1AI100663).


First drug to improve heart failure mortality in over a decade
Coenzyme Q10 decreases all cause mortality by half in randomized double blind trial
Lisbon, 25 May 2013: Coenzyme Q10 decreases all cause mortality by half, according to the results of a multicentre randomised double blind trial presented today at Heart Failure 2013 congress. It is the first drug to improve heart failure mortality in over a decade and should be added to standard treatment, according to lead author Professor Svend Aage Mortensen (Copenhagen, Denmark).

Heart Failure 2013 is being held from 25-28 May in Lisbon, Portugal. It is the main annual meeting of the Heart Failure Association of the European Society of Cardiology (1).

Coenzyme Q10 (CoQ10) occurs naturally in the body and is essential to survival. CoQ10 works as an electron carrier in the mitochondria, the powerhouse of the cells, to produce energy and is also a powerful antioxidant. It is the only antioxidant that humans synthesise in the body.

CoQ10 levels are decreased in the heart muscle of patients with heart failure, with the deficiency becoming more pronounced as heart failure severity worsens. Statins are used to treat many patients with heart failure because they block the synthesis of cholesterol, but these drugs also block the synthesis of CoQ10, which further decreases levels in the body.

Double blind controlled trials have shown that CoQ10 improves symptoms, functional capacity and quality of life in patients with heart failure with no side effects. But until now, no trials have been statistically powered to address effects on survival.

The Q-SYMBIO study (2) randomised 420 patients with severe heart failure (New York Heart Association (NYHA) Class III or IV) to CoQ10 or placebo and followed them for 2 years. The primary endpoint was time to first major adverse cardiovascular event (MACE) which included unplanned hospitalisation due to worsening of heart failure, cardiovascular death, urgent cardiac transplantation and mechanical circulatory support. Participating centres were in Denmark, Sweden, Austria, Slovakia, Poland, Hungary, India, Malaysia and Australia.

CoQ10 halved the risk of MACE, with 29 (14%) patients in the CoQ10 group reaching the primary endpoint compared to 55 (25%) patients in the placebo group (hazard ratio=2; p=0.003). CoQ10 also halved the risk of dying from all causes, which occurred in 18 (9%) patients in the CoQ10 group compared to 36 (17%) patients in the placebo group (hazard ratio=2.1; p=0.01).

CoQ10 treated patients had significantly lower cardiovascular mortality (p=0.02) and lower occurrence of hospitalisations for heart failure (p=0.05). There were fewer adverse events in the CoQ10 group compared to the placebo group (p=0.073).

Professor Mortensen said: "CoQ10 is the first medication to improve survival in chronic heart failure since ACE inhibitors and beta blockers more than a decade ago and should be added to standard heart failure therapy."

He added: "Other heart failure medications block rather than enhance cellular processes and may have side effects. Supplementation with CoQ10, which is a natural and safe substance, corrects a
deficiency in the body and blocks the vicious metabolic cycle in chronic heart failure called the energy starved heart."

CoQ10 is present in food, including red meat, plants and fish, but levels are insufficient to impact on heart failure. CoQ10 is also sold over the counter as a food supplement but Professor Mortensen said: "Food supplements can influence the effect of other medications including anticoagulants and patients should seek advice from their doctor before taking them."

Patients with ischaemic heart disease who use statins could also benefit from CoQ10 supplementation. Professor Mortensen said: "We have no controlled trials demonstrating that statin therapy plus CoQ10 improves mortality more than statins alone. But statins reduce CoQ10, and circulating CoQ10 prevents the oxidation of LDL effectively, so I think ischaemic patients should supplement statin therapy with CoQ10."

**New research shows that potatoes provide one of the best nutritional values per penny**

May 24, 2013 — A frequently expressed concern in the ongoing public health debate is the lack of affordability of fresh vegetables, especially those that are nutrient dense. A new study, "Vegetable Cost Metrics Show That Potatoes and Beans Provide Most Nutrients Per Penny," published in the journal *PLOS ONE*, shows that potatoes are one of the best nutritional values in the produce aisle, providing one of the better nutritional values per penny than most other raw vegetables and delivering one of the most affordable source of potassium of the more frequently consumed vegetables, second only to beans.

Dr. Adam Drewnowski and colleagues from the University of Washington used a combination of nutrient profiling methods and national food prices data to create an "affordability index," which was then used to examine the nutrients per unit cost of 98 individual vegetables as well as five vegetable subgroups including dark green, orange/red, starchy, legumes (beans and peas) and "other" vegetables.

The results indicated while dark green vegetables had the highest nutrient density scores, after accounting for cost, starchy vegetables (including potatoes) and beans provided better nutritional value for the money. Potatoes, in particular, provide one of the lowest cost options for four key nutrients including potassium, fiber, vitamin C and magnesium. Among the most frequently consumed vegetables, potatoes and beans were the lowest-cost sources of potassium and fiber—nutrients of concern, as identified by the 2010 USDA Dietary Guidelines.

"The ability to identify affordable, nutrient dense vegetables is important to families focused on stretching their food dollar as well as government policy makers looking to balance nutrition and economics for food programs such as the school lunch program and WIC," said lead researcher Adam Drewnowski, PhD. "And, when it comes to affordable nutrition, it’s hard to beat potatoes."

The study was funded by the United States Potato Board and adds to the growing database of nutrition science that supports potatoes in a healthful diet. In addition, one medium-size (5.3 ounce) skin-on potato contains just 110 calories per serving, boasts more potassium (620g) than a banana (450g), provides almost half the daily value of vitamin C (45 percent), and contains no fat, sodium or cholesterol.

What the Smallest Infectious Agents Reveal About Evolution

May 23, 2013 — Radically different viruses share genes and are likely to share ancestry, according to research published in BioMed Central’s open access journal *Virology Journal* this week. The comprehensive phylogenomic analysis compares giant viruses that infect amoeba with tiny viruses known as virophages and to several groups of transposable elements. The complex network of evolutionary relationships the authors describe suggests that viruses evolved from non-viral mobile genetic elements and vice versa, on more than one occasion.

The recent discovery of virophages inside the giant viruses, which in turn infect amoeba, has led to speculation about their origin and their relationship to other viruses and small transposable genetic elements. To try to answer this question a research team including Eugene Koonin from the NIH and Didier Raoult from URMITE compared the genetic material from virophages, such as the Mavirus, Sputnik, or OLV (which was isolated from an Antarctic organic lake), to eukaryotic self replicating transposable elements known as Polintons or Mavericks.

Eugene Koonin explains: "Between the known virophages there are six conserved genes, arranged in a similar way. Five of these have counterparts in the Polintons, but their sequence and arrangement are sufficiently different to discount suggestions that Polintons evolved directly from a Mavirus-like
ancestor. Rather our data suggests that Maviruses have evolved from a fusion between a Politon/Maverick-like transposable element and an unknown virus."

Including information about other viruses and virus-like elements: adenoviruses that infect animals and are one of the causes of the common cold; certain bacteriophages that infect bacteria; transpovirons which infect giant viruses; and a Tetrahymena transposable element (Tlr1), the virus "evolutionary tree" appears as a network of swapped genes.

Didier Raoult, whose team discovered the transpovirons, says: "It appears that viruses have evolved from non-viral genetic elements and vice versa on more than one occasion. Viral evolution is more complex than we thought."

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**New Coating Method Accelerates Bonding With Bone Three Times Faster**

May 24, 2013 — Researchers at the International Center for Materials Nanoarchitectonics (MANA) and Tokyo Medical and Dental University have developed a coating method which accelerates bonding with bone by three times.

Dr. Masanori Kikuchi, Group Leader of the Bioceramics Group, International Center for Materials Nanoarchitectonics (MANA) and a research group at Tokyo Medical and Dental University succeeded in developing a coating method which accelerates bonding with bone by three times.

Dr. Masanori Kikuchi, Group Leader of the Bioceramics Group, International Center for Materials Nanoarchitectonics (MANA; Director-General: Masakazu Aono), National Institute for Materials Science (President: Sukekatsu Ushioda), and a research group including Masayoshi Uezona (graduate student), Prof. Kazuo Takakuda (Institute of Biomaterials and Bioengineering), Prof. Keiji Moriyama (School of Dentistry, Maxillofacial Orthognathics), and others at Tokyo Medical and Dental University (President; Takashi Ohyama) succeeded in developing a coating which accelerates bonding with bone by three times.

Orthodontic subperiosteal devices are superior in terms of low invasiveness, but because bonding with bone on the surface of the bone is necessary, a minimum waiting time of approximately three months had been required until medical use was possible, even when coating treatment was performed with hydroxyapatite (HAp). In order to shorten this time, the device shape was optimized and a new coating method was developed in joint work by NIMS and Tokyo Medical and Dental University. As a result, a coating method which realizes in only one month the same bone coverage as after 3 months with the conventional device was established.

**Journal Reference:**

*Photographs of tissue preparation during 4 weeks after surgery. In the upper 2 photos, soft tissue (dyed pink) exists between titanium material (black) and bone tissue (dyed brown); however, with the HAp/Col in the lower photos, direct bonding has occurred between the material and the bone. (Credit: Image courtesy of National Institute for Materials Science)*

**Accidental Find Shows Vitamin C Kills Tuberculosis** *Economic Times,* (05.21.2013)

Researchers from Albert Einstein College of Medicine in New York have discovered that Vitamin C kills the TB bacteria. They report that they made the discovery accidentally while investigating how the bacteria develop resistance to the anti-TB drug isoniazid.

The researchers added isoniazid and the reducing agent cysteine to the TB bacteria in a test tube with the expectation that the bacteria would develop resistance. Instead, the researchers killed the TB culture. Next, the researchers replaced the cysteine with another reducing agent, Vitamin C, and it killed the bacteria also. When the researchers omitted the TB drug isoniazid and used Vitamin C alone, the outcome was the same—it killed the bacteria. They tested Vitamin C with drug-resistant TB strains and had the same result. Also, the TB bacteria never developed resistance to Vitamin C in the laboratory tests.

William Jacobs, the study's senior author, emphasized that so far, researchers have demonstrated these results only in a test tube. The researchers did not know if it would work with humans and, if so, at what dosage. The authors urged additional research into potential uses of Vitamin C in TB treatment, noting that it was “inexpensive, widely available, and very safe to use.”

The full report, “Mycobacterium tuberculosis is Extraordinarily Sensitive to Killing by a Vitamin C-Induced Fenton Reaction,” was published in the journal *Nature Communications* (2013; doi:10.1038/ncomms2898).

**Elimination of Self-Reactive T Cells in the Thymus: A Timeline for Negative Selection**

Ivan Lilyanov Dzhagalov, Katherine Grace Chen, Paul Herzmark, Ellen A. Robey

As an important safeguard against autoimmunity, T cells bearing autoreactive T cell antigen receptors are eliminated during their development in the thymus, a process known as negative selection. Although much is known about the molecular events involved in negative selection, surprisingly little is known about the dynamic aspects of the process. Here we examine a synchronized population of developing T cells (thymocytes) undergoing negative selection within three-dimensional living thymic tissue. We show that the initial encounter with negative selecting ligands results in migratory arrest, but in spite of this synchronous early response, individual thymocytes then undergo delayed and asynchronous entry into the death program between 2 and 12 hours thereafter. Using time-lapse two-photon imaging, we reveal that thymocyte death and the clearance of the dead cells invariably occur together, with many thymocytes already engulfed by a macrophage before the cell death-related changes in chromatin and membrane permeability are evident. These data provide a timeline of the major events during negative selection, and suggest close coupling between thymocyte death and clearance by macrophages.

May 27, 2013

**New Tools to Hunt New Viruses**

By DONALD G. MCNEIL JR.

A new flu, H7N9, has killed 36 people since it was first found in China two months ago. A new virus from the SARS family has killed 22 people since it was found on the Arabian Peninsula last summer.

In past years, this might have been occasion for panic. Yet chicken and pork sales have not plummeted, as they did during flus linked to swine and birds. Travel to Shanghai or Mecca has not been curtailed, nor have there been alarmist calls to close national borders.

Is this relatively calm response in order? Or does the simultaneous emergence of two new diseases suggest something more dire?

Actually, experts say, the answer to both questions may well be yes.

“We’ve done a great job globally in the last 10 years,” said Dr. William B. Karesh, a wildlife veterinarian and chief of health policy for the EcoHealth Alliance, which tracks animal-human outbreaks. “Compared to H5N1 and SARS, we’re getting on top of these diseases much, much faster.”

But he added that “people have become desensitized over time — it’s ‘Oh, O.K., another one.’ ”

And scientists say the world cannot afford to relax. The threat is real. New diseases are emerging faster than ever.

Peter Daszak, a parasitologist and president of the EcoHealth Alliance, has even put a number on it: 5.3 new ones each year, based on a study using data from 1940 to 2004. He and his co-authors blamed population growth, deforestation, antibiotic overuse, factory farming, live animal markets, bush meat hunting, jet travel and other factors.
Some aspects of the new viruses are scary. The Arabian coronavirus — now officially named MERS, for Middle East respiratory syndrome — has killed about half of those it infects, while SARS killed less than a quarter; in the lab, it replicates faster than SARS, penetrates lung cells more readily and inhibits the formation of proteins that warn the body that it is under attack.

In her closing remarks on Monday at the annual meeting of the world’s health ministers, Dr. Margaret Chan, director-general of the World Health Organization, said the virus was now her “greatest concern.”

Until experts figure out where it hides and how it infects humans, “we are empty-handed when it comes to prevention,” she said. “These are alarm bells, and we must respond.”

The H7N9 flu has been fatal in a quarter of known cases — the 1918 Spanish flu killed only 2 percent of its victims — and already has one dangerous mutation that helps it replicate at human body temperatures.

Still, better surveillance means that such threats are being caught sooner, giving time to develop countermeasures like vaccines and making it far less likely that a virus like the 1918 flu will ever again kill millions.

It also means that outbreaks that once might have faded away unnoticed now set off alarms, for better and for worse. Fifty years ago, even the dreaded H5N1 bird flu, which emerged in 2003 and kills about half its victims, might have been missed. It makes the jump to humans so rarely that even now it is basically a poultry problem: It has killed millions of chickens and occasional flocks of wild birds, but in a whole decade has claimed only 364 human lives, and that is known only because it can be distinguished from other flus by genetic typing.

The world’s ability to detect new diseases has sped up for reasons both technical and political.

First, rapid gene sequencing is now done in many laboratories.

Second, accurate symptom descriptions are instantly available. Web-based news services like ProMED, with scientist-members all over the world, issue several daily reports of outbreaks of everything from banana wilt to sheep bluetongue to human Ebola. Also, genetic sequences of new viruses are often posted on public databases, so their travels can be tracked. Scientists learned, for example, that a 2008 convention of Roman Catholic youth in Sydney, Australia, drew in influenza strains that then seeded new outbreaks all over the Northern Hemisphere.

Third, and very important, countries that used to hide their outbreaks now admit them. It would be virtually impossible now, for example, to repeat what happened in Africa in the 1980s, when presidents insisted for years that no one in their countries had AIDS.

The paragon of the new transparency cited most often is China. In 2003, it was excoriated for covering up its SARS outbreak. It later dismissed many of the officials involved. Now, with H7N9, “they’re being forthright and they’re also right at the forefront of research,” said Dr. W. Ian Lipkin, a microbe hunter at the Mailman School of Public Health at Columbia University, who just opened a partner laboratory at China’s Centers for Disease Control.

Saudi Arabia suffered a similar embarrassment in 2005, when it reacted slowly to polio spreading toward Mecca with pilgrims from northern Nigeria. Cases of paralysis ultimately reached the hills outside Mecca and from there spread briefly as far as Indonesia. Saudi Arabia now gives polio vaccines to millions of pilgrims on arrival.

Covering up an outbreak is now a violation of World Health Organization regulations adopted in the wake of SARS. The rules require members to disclose any public health event that could spread beyond their borders.

Both H7N9 and MERS fit that description. Neither is easily transmissible, though both have almost undoubtedly infected family members, nurses or hospital roommates after long exposure. Most deaths from both have been in older patients with other health problems.

More worrisome is that no one knows how these viruses first infect victims.

H7N9 is avian, a mix of genes from domestic chickens and wild waterfowl. But many Chinese H7N9 patients have had no known bird contact, and the disease has been found only rarely in birds. Unlike H5N1, it does not wipe out flocks, so it is hard to hunt. Its spread pattern is roughly circular around Shanghai, suggesting it is mostly in poultry, not migratory birds. That could change if it starts traveling in wild ducks. (Rice farmers have duck farmers drive flocks into paddies to eat the snails that eat rice shoots, and wild ducks mix with them there.)

A decade ago, H5N1 also started in China but spread west in a zigzag pattern as wild waterfowl shared Mongolian lakes in summer with species that went southwest to Eastern Europe, Egypt and Africa and were caught in storms that blew them as far as Britain.
The origins of MERS are even more baffling. Scientists assume it is from bats, because it is genetically closer to coronaviruses found in them than to SARS or to the four known human coronaviruses, which cause common colds. But while bats in Mexico, Europe and Africa have similar viruses, none have yet been found in Arabian bats or in camels, goats or other animals that might transfer it to humans.

Dr. Daszak cited Nipah virus as an example of how humans get bat diseases. It was the inspiration for the 2011 movie “Contagion,” in which Gwyneth Paltrow had vivid death and autopsy scenes. Bat feces landed on fruit eaten by pigs, and Ms. Paltrow’s character was infected when she shook the unwashed hand of a casino chef who had just cleaned out a dead pig’s mouth. (In the first real-life Nipah outbreak, in Malaysia in 1999, most victims were pig farmers and butchers.)

But another study, done in Bangladesh by a colleague of Dr. Daszak, showed that humans get Nipah directly from bats by drinking fresh date palm sap. Sap-drinking bats crawled into the collecting jugs hung in trees, drooling and urinating in them.

Small numbers of sap drinkers may have died of Nipah for decades without it being noticed, Dr. Daszak said.

Right now, doctors are relying on isolating patients and antiviral treatment with oseltamivir and zanamivir for H7N9, and ribavirin and interferon for MERS.

If either virus goes epidemic, the next step would be vaccine.

The Centers for Disease Control and Prevention began making one against H7N9 in early April. The first of several candidates may be ready for manufacturers by the end of May, a spokeswoman said. How long it then would take to make and package millions of doses is unpredictable, she said, but should take at least six additional months.

Any vaccine for MERS will take much longer, said Mark A. Pallansch, director of the C.D.C.’s viral disease division. While flu vaccines have been produced around the world for 60 years, the passion for a coronavirus vaccine has faded since the SARS epidemic. Until recently, the most interested parties were poultry farmers, since one coronavirus kills turkeys.

Coronaviruses are unusually complex, so finding potential vaccine targets has been hard, and the extensive safety testing is expensive. Also, an animal model for testing was only recently found — macaque monkeys, in which the virus causes pneumonia.