July 2012 Epidemics and AIDS Update

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La Jolla Institute scientist discovers key step in immune system-fueled inflammation

Novel mechanism plays major role in inflammation
SAN DIEGO – (July 1, 2012) – Like detectives seeking footprints and other clues on a television “whodunit,” science can also benefit from analyzing the tracks of important players in the body's molecular landscape. Klaus Ley, M.D., a scientist at the La Jolla Institute for Allergy & Immunology, has done just that and illuminated a key step in the journey of inflammation-producing immune cells. The finding provides powerful, previously unknown information about critical biological mechanisms underlying heart disease and many other disorders.

The study, published today in Nature, focuses on one of the body’s most abundant and important immune cells, known as neutrophils, which play a pivotal role in many diseases. "Neutrophils are the body's first line of defense and the main cell protecting us from bacterial infections," said Dr. Ley, a pioneer in vascular immunology and head of the La Jolla Institute's Division of Inflammation Biology. "While their protective function is very positive, neutrophils also have inflammation-producing properties that cause problems in heart disease and a host of autoimmune diseases, for example lupus. This makes understanding how to manipulate these cells extremely important in disrupting disease."
National Medal of Science winner Shu Chien, M.D., Ph.D., a UC San Diego professor renowned for his work on vascular mechanisms and atherosclerosis, praised Dr. Ley's finding as a significant advance in understanding inflammatory mechanisms in disease. "They have elucidated the molecular and mechanical bases of this type of neutrophil rolling (in the blood vessels) that have major significance in inflammation," said Dr. Chien, director of UCSD's Institute of Engineering in Medicine. "Since inflammation is at the root of a large variety of diseases, these findings not only have fundamental importance in the mechanobiology of the cell, but also in understanding the pathophysiology of many disease states."

In his Nature paper entitled "'Slings' enable neutrophil rolling at high shear," Dr. Ley revealed how neutrophils use sling-like membrane tethers to latch on to the blood vessel wall during periods when blood flow is very fast. In making the discovery, Dr. Ley and Prithu Sundd, Ph.D., a researcher at La Jolla Institute, used "dynamic footprinting," a pioneering imaging technique they developed in 2010 that uses special microscopes and total internal reflection microscopy to see and photograph the neutrophil adhesion process with unprecedented clarity. Alex Groisman, Ph.D., an associate professor in UCSD's Department of Physics, was instrumental in developing and constructing the microfluidic device in which these experiments were conducted and collaborated on the Nature paper.

Sussan Nourshargh, Ph.D., professor of Microvascular Pharmacology and head of the Center for Microvascular Research at Barts and The London Medical School, University of London, said the work provides another "major insight" from Dr. Ley whose discoveries, over the years, have repeatedly enhanced scientific understanding of the role of neutrophils in causing inflammation. In particular, she cited Dr. Ley's groundbreaking work on the discovery of the leukocyte adhesion cascade, which explained the sequential steps used by neutrophils to clamp onto the blood vessel wall as they prepare to migrate to sites of infection. His latest finding reveals another important step in that process.

"This is a completely new cellular concept that will now be added as an additional step to the leukocyte adhesion cascade that describes the sequential cellular responses involved in guiding neutrophils to sites of inflammation," she said. "This pioneering work will without doubt pave the way for other researchers to explore the occurrence of "slings" in a wide range of inflammatory scenarios."

Like other immune cells, neutrophils travel throughout the body via the blood stream pursuing their infection-fighting duties. In order to accomplish their work, neutrophils must migrate through the blood vessel walls to sites of infection, injury or inflammation.

"The activities of neutrophils are very important for our survival, so they are the subject of significant scientific study," said Dr. Ley. While some scientists study their migration out of the blood vessel, Dr. Ley's lab has focused on how neutrophils adhere to the blood vessel wall. "This is important because it provides an opportunity to develop new treatments based on modulating or blocking one of the steps in the adhesion cascade," said Dr. Ley, noting that earlier studies have shown that blocking even one of the steps can severely reduce neutrophil recruitment.

While Dr. Ley has previously shown how neutrophils adhere when blood flow is slow, his latest study reveals that neutrophils use long membrane tethers at the front of the cell, termed "slings," to slow down during high blood flow. The cells do this by separating their cytoskeleton from the cellular membrane, wrapping the sling around themselves like a lasso and then digging their hooks into the blood vessel wall, said Dr. Ley. High blood flow occurs during inflammation, when the body rushes immune cells to a site to promote healing. Inflammation is a normal part of the healing process, but is unwanted in certain diseases.

"For these cells, adhering under high shear is like being in a huge wind storm," said Dr. Ley. "The challenge in this storm is not to get blown away."

Dr. Ley's studies could prove valuable in helping scientists understand how to reduce adhesion, where inflammation is unwanted, such as in heart or autoimmune disease, or to enhance the process, where more neutrophils are desired, such as in bacterial infections like MRSA. "The body needs to have enough neutrophils to fight off bacteria faster than they can grow," he said. "Better understanding of neutrophil adhesion could be very beneficial in that process. Conversely, interrupting this process could have major impacts in autoimmune and other inflammatory diseases."

**How Secrecy in Medical Research Harms our Health**

Medical research data remain shrouded in secrecy. As a result the data is distorted and misrepresented by pharmaceutical companies launching new medicines to exaggerate their efficacy, minimize their harmful
side effects, and conceal the fact that these products are often no more effective than those already on the market. Clinical trials are unnecessarily repeated and overall, health-care and patients suffer.

“They swallowed our story, hook, line and sinker,” wrote the research and development director of the pharmaceutical company Pfizer in an e-mail after having successfully presented the new arthritis drug Celebrex. The medical director of Pfizer admitted they had given the clinical results “a data massage” because that was the only way the new medicine “could look like it was better” than existing ones. In fact, Pfizer and its partner, Pharmacia, presented the results from just the first six months of a yearlong study rather than the whole picture.

There is increasing criticism of distorted claims about new medicines that exaggerate their efficacy and minimize their secondary, often negative effects. This is especially relevant in an environment where few new drugs are significantly more effective than existing ones. Only a small percentage of all new medical products reaching the market (4 out of 97 in 2010) have a real therapeutic value over existing medicines. Most new products are “me too” drugs, which means that they are practically the same as the existing ones.

Biomedical research should strive for the truth uncontaminated by the perception of bias. The reality is that there are many examples of alleged industry bias and duplicity in the manipulation of scientific data, from Vioxx to Tamiflu. What can we do about it? One answer is transparency and openness.

Under the present model of biomedical innovation, the generation, analysis, and dissemination of clinical research data is largely controlled by the pharmaceutical industry, through opaque, closed, and “self-regulated” processes. According to many experts this has provoked a long list of medical scandals, tens of thousands of lives lost, billions in wasted public investments, and new drugs that are primarily market oriented instead of health-needs oriented.

In Europe new medicines are approved by the European Medicines Agency on the basis of a dossier containing all information related to the new drug, including clinical trial data, obtained by carefully assessing the positive and negative effects in patients. However, most of these data are kept secret, analyzed internally, and not available for the scrutiny and research of independent scientists, doctors, and patients. The reasons given for this secrecy range from “commercial confidentiality” and “patent rights” to simply “the general public might misinterpret the data.”

The issue of access to medical data poses important ethical issues. Since valuable scientific data from clinical trials and other laboratory studies related to the drug are not shared, they often need to be repeated, especially in the case of new medicine developments that have been abandoned due to scientific problems. This model of secrecy means that countless laboratory animals of pre-clinical trials and tens of thousands of human patients of clinical trials can be exposed to totally unnecessary suffering and risks.

Is it acceptable for essential medical research data to be shrouded in secrecy? Can the health of citizens be protected without adequate access to the data that are supposed to demonstrate the quality and efficacy of a drug?

At a meeting at the European Parliament in June 2012 the Director of the Nordic Cochrane Center, Professor Dr. Peter C. Gøtzsche, took a clear stance when he stated, “if commercial or academic success depends on withholding data that are important for rational decision making by physicians, patients, and governments then there is something fundamentally wrong with our priorities in health care.”

That meeting—organized by Transatlantic Consumer Dialogue and Health Action International with the sponsorship of Members of the European Parliament from three political groups—included the participation of Professor Wolf-Dieter Ludwig, chairman of the Drug Commission of the German Medical Association. He said that the duty to provide access to clinical trial data has not been met in Europe in recent years and he blamed a conflict between the goals of protecting commercial interests and promoting rational prescribing by doctors.

“The evidence base on which prescribers are obliged to make their choice of therapy was largely determined by industry-sponsored clinical trials and misleading and inaccurate publicity at the launch of the medicine,” he said.

“We do not get the information we need for prescribers and patients,” he continued, citing the case of the anti-depressant drug reboxetine which, he said, had been shown to be ineffective but only in assessments that had remained unpublished.

Both Gøtzsche and Ludwig insisted that access to the raw data is essential because we cannot rely on summaries and tables prepared by the companies, as they are often shown to misrepresent the data. According to Gotzsche the generalized lack of dependable medical data often converts the “informed consent” of patients to accept treatment into a “delusion.”
Many voices at the meeting accused the European Medical Agency (EMA) of deliberately obscuring the facts and a pro-industry bias in their strict policies of confidentiality.

The European Union (EU) Ombudsman representative Nicholas Catephores affirmed that “all citizens have the right of access to EU documents and that the EMA could not legally withhold information on the grounds of commercial confidentiality when an issue of public health is at stake.”

The head medical officer at the EMA, Hans-Georg Eichler, admitted that transparency needed to be improved at the EMA but expressed great concern about releasing “the treasure trove” of raw data from clinical trials. He stated that this data could be misused or misinterpreted and in this way lead to a negative impact on public health.

Els Torreele, of the Open Society Foundations, responded that she was surprised “about the argument of the risk of inappropriate use since today we have exactly that: the ownership of the data is in Pharma industry hands, and they have vested interests in a positive outcome of the trials. Another issue pertains to the design of clinical trials—anyone who knows anything about clinical trial methodology knows that you can design your study to make sure you will see certain results or not; clinical trials shouldn’t be left to pharmaceutical companies but be designed, conducted and analyzed by independent researchers.”

In the coming months the EU will adopt legislation that will affect access to medical data. The European Parliament is presently discussing the rules on open access to publications and data for its massive Horizon 2020 Research and Innovation program (an 80 billion Euro initiative). In addition, within a few weeks the European Commission will present a proposal for a new Directive on Clinical Trials, which presents another opportunity to regulate how clinical trial data should be treated. When it comes to EU legislation the issue of transparency regarding essential medical data will be promoted by civil society organizations, including the Transatlantic Consumer Dialogue and Health Action International, which recognize that openness is a crucial factor in the defense of our health.

**Circumcision ruling condemned by Germany's Muslim and Jewish leaders**

German court rules that procedure is bodily harm and contravenes right to choose religion in later life

Jewish and Muslim leaders were united on Wednesday in their condemnation of a German court’s decision to in effect outlaw the circumcision of boys after a judge deemed that the religious practice amounted to bodily harm.

Representatives of the two religious communities called the ruling insensitive and discriminatory, saying it was an attack on centuries of religious tradition.

A judge at a Cologne court said that the circumcision of minors went against a child's interests because it led to a physical alteration of the body, and because people other than the child were determining its religious affiliation.

Religious leaders said the court had stepped into a minefield with its decision, which undermined their religious authority and contravened Germany’s constitution.

Ali Demir, chairman of the Religious Community of Islam in Germany, said: "I find the ruling adversarial to the cause of integration and discriminatory against all the parties concerned."

Dieter Graumann, president of Germany’s Central Council of Jews, called it "an egregious and insensitive measure" which amounted to "an unprecedented and dramatic intervention in religious communities' right of determination".

The ruling followed a lengthy legal battle, sparked when a Muslim couple decided to have their son circumcised, specifically for religious reasons, by a Muslim doctor in Cologne. The doctor, identified only as Dr K, carried out the circumcision on the four-year old boy in November 2010, before giving the wound four stitches. The same evening, he visited the family at home to check up on the boy. When the boy began bleeding again two days later, his parents took him to the casualty department of Cologne’s University hospital. The hospital contacted the police, who then launched an investigation. The doctor was charged with bodily harm, and the case was taken to court.

While the court acquitted Dr. K on the grounds that he had not broken any law, it concluded that circumcision of minors for religious reasons should be outlawed, and that neither parental consent nor religious freedom justified the procedure. It ruled that in future doctors who carried out circumcisions should be punished.

The court weighed up three articles from the basic law: the rights of parents, the freedom of religious practice and the right of the child to physical integrity, before coming to the conclusion that the procedure was not in the interests of the child.
It rejected the defence that circumcision is considered hygienic in many cultures, one of the main reasons it is carried out in the US, Britain and in Germany.

After much deliberation, it concluded that a circumcision, "even when done properly by a doctor with the permission of the parents, should be considered as bodily harm if it is carried out on a boy unable to give his own consent".

It ruled the child's body would be "permanently and irreparably changed", and that this alteration went "against the interests of a child to decide for himself later on to what religion he wishes to belong".

The doctor was acquitted, the court said, because he had acted "subjectively and with a clear conscience" and because carrying out the procedure had not been punishable at the time.

Holm Putzke, a professor of penology – the study of the punishment of crime – from the University of Passau, told the German news agency DPA that the ruling would set a legal precedent and would act as a warning. "The ruling is not binding for other courts, but it will have the effect of a warning signal." He added while Dr K had been let off, from now on no doctor would be able to claim that he or she did not know it was forbidden.

He said unlike politicians who have long faced pressure to deal with the issue, "the court did not allow itself to be scared off by charges of antisemitism or religious intolerance".

Demir predicted a ban in Germany would lead to a rise in "circumcision tourism in neighbouring countries in Europe".

Condemnation also came from elsewhere in Europe, with Rabbi Aryeh Goldberg of the Brussels-based Rabbinical Centre of Europe calling the ruling "fatal to freedom of religion". He told the Jerusalem daily Haaretz that it "contravened the EU's convention on human rights, to which Germany is subservient and harms the basic freedom of religion enshrined in the German constitution".

Women's rights groups and social policy makers also condemned the decision, but for the reason that it would have the effect of putting male and female circumcision on the same footing, when they were "in no way comparable", said Katrin Altpeter, social minister in the state of Baden-Württemberg. Female circumcision she said, was a far more drastic act. It is already outlawed in Germany.

In Austria, the lay initiative Religion is a Private Matter, welcomed the Cologne decision, calling it "an important and long overdue change of direction". Its chairman, Heinz Oberhummer, said: "Bodily harm is bodily harm and children cannot be excluded from benefitting from basic rights, and certainly not for religious reasons," he said.

The World Health Organisation estimates that every third man is circumcised. Around 70% of them are Muslims, around 1% Jews.

From Twitter and Facebook to the online discussion forums of German newspapers, the decision was being hotly debated on Wednesday. An online survey of the readers of the leftwing Berlin daily Taz found two-thirds of respondents in favour of the decision.

One respondent wrote: "The issue is quite clear: the religious freedom of the parents ends precisely there where the physical harm of others begins, regardless of whether it's that of your own child or that of an unknown heathen."

But another wrote: "As a circumcised Jew, I can only add the following: did the state prosecutors in Cologne ... have nothing better to do than ... interfere in our thousands of years of Jewish religious law? No way, and that's why we need to act decisively against this horrendous decision by the Cologne regional court."

Putzke, who is a leading voice in the discussion about circumcision and the law, welcomed the decision: "After the knee-jerk indignation has subsided, hopefully a discussion will kick off about how much religiously motivated violence against children a society is ready to tolerate."

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**High Dose Vitamin D Prevents Fractures**

By Michael Smith, North American Correspondent, MedPage Today

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Reviewed by Dori F. Zaleznik, MD: Associate Clinical Professor of Medicine, Harvard Medical School, Boston and Dorothy Caputo, MA, BSN, RN, Nurse Planner
Action Points

- Explain that a new meta-analysis found a significant reduction in fracture risk—both hip and nonvertebral—for patients enrolled in trials who actually took 800 IU per day or more.
- Note that the researchers could not determine a role for calcium supplementation as those taking the higher doses of vitamin D were also all taking calcium.

High doses of vitamin D prevent fractures in older people—as long as they take the substance regularly, researchers reported.

In a meta-analysis, oral doses of at least 800 IU were associated with reductions in the risk of both hip and nonvertebral fractures, according to Heike Bischoff-Ferrari, MD, DrPH, of University Hospital in Zurich, and colleagues.

The analysis differs from previous studies and other meta-analyses in that it looked at how much vitamin D participants actually took, rather than what dose they were assigned to take, Bischoff-Ferrari and colleagues reported in the July 5 issue of the New England Journal of Medicine.

Fractures are common in older people and one strategy to prevent them might be vitamin D supplements, the researchers noted, but studies of the issue have been inconsistent. To try to clarify the matter, they looked for all controlled studies of oral vitamin D, with or without calcium, among people 65 and older.

They included 12 studies and had participant-level data on 30,011 volunteers.

The primary end points were the risks of hip fracture and any nonvertebral fracture, and the primary analyses compared the actual intake of vitamin D supplementation, in quartiles, to the controls, with actual intake calculated as the assigned dose plus any additional supplemental dose, adjusted for adherence.

The study design is important, because it takes into account the biology of vitamin supplementation, according to Robert Heaney, MD, of the Creighton University Medical Center in Omaha, Neb.

In an accompanying editorial in the journal, Heaney argued that the inconsistent outcomes of earlier work might be the result of an overfocus in meta-analyses on the methods used in individual trials.

"The question of how much vitamin D is enough is likely to remain muddled as long as meta-analyses focus on trial methodology rather than on biology," he argued.

But Heaney noted that the results of the current meta-analysis are in accordance with recommendations of the Endocrine Society. "It would appear to be prudent, and probably helpful as well, to ensure an intake at the upper end of the range" that the researchers found effective.

Bischoff-Ferrari and colleagues found that, in an intention-to-treat analysis, there was a nonsignificant 10% reduction in the risk of hip fracture and a significant (at P=0.03) 7% reduction in the risk of nonvertebral fracture.

On the other hand, when they took into account actual vitamin D intake, they found a 30% reduction in the risk of hip fracture but only for those in the highest quartile of intake—792 to 2,000 IU a day.

The relative risk in that group, compared with controls, was 0.70, with a 95% confidence interval from 0.58 to 0.86, and was significant at P<0.001.

They also found a 14% reduction in the risk of any nonvertebral fracture, but again only in the highest quartile. The relative risk was 0.86, with a 95% confidence interval from 0.66 to 0.96, and was significant at P=0.007.

A sensitivity analysis, not including any outside supplements, had similar results, they reported.

Heaney commented that the benefits of supplements might be affected by baseline levels of vitamin D but noted that such information has not been routinely collected in trials of the substance.

Indeed, baseline levels of hydroxyvitamin D were only available for 4,383 participants, Bischoff-Ferrari and colleagues reported. Despite that, the results among those participants were similar to those in the whole group, they found.

In addition to the absence of baseline hydroxyvitamin D levels, other study limitations included inability to separate calcium and vitamin D intake as those receiving high doses of vitamin D were all taking calcium and lack of trial level data for two of the 14 included trials.
New Study Maps Global Zoonotic Disease 'Hotspots'

"A global study mapping human diseases that come from animals like tuberculosis, AIDS, bird flu or Rift Valley fever has found that just 13 such diseases are responsible for 2.4 billion cases of human illness and 2.2 million deaths a year," Reuters reports (Kelland, 7/5). "The report, which was conducted by the International Livestock Research Institute (ILRI), the Institute of Zoology (U.K.) and the Hanoi School of Public Health in Vietnam, maps poverty, livestock-keeping and the diseases humans get from animals, and presents a 'top 20' list of geographical hotspots," an ILRI press release states (7/5). The study "found that Ethiopia, Nigeria, and Tanzania, as well as India have the highest zoonotic disease burdens, with widespread illness and death," Reuters writes.

"It also found the United States and Europe—especially Britain—Brazil and parts of Southeast Asia may be becoming hotspots of 'emerging zoonoses,' which are infecting humans for the first time, are especially virulent or are becoming drug resistant," the news service adds. According to Reuters, Delia Grace, a veterinary epidemiologist and food safety expert with ILRI and lead author of the study, "said targeting these diseases in the hardest-hit countries is crucial to protecting global health, and failing to tackle them would allow demand for meat products to 'fuel the spread of a wide range of human-animal infectious diseases'" (7/5).

UZH research team discovers the origins of key immune cells

Chronic inflammatory conditions are extremely common diseases in humans and in the entire animal kingdom. Both in autoimmune diseases and pathogen-caused diseases, the inflamed areas are rapidly colonized by antibody producing B lymphocytes – which organize themselves in highly structured areas called "lymphoid follicles". The scaffold of such follicles is provided by follicular dendritic cells (FDCs). FDCs have important roles in the development of immune responses, since they trap antigens for protracted periods of, thereby training B lymphocytes to recognize the invaders. However, FDCs can also play deleterious roles in disease, because they can provide sanctuaries for infectious pathogens such as the human immunodeficiency virus and prions.

But where do FDCs come from? Because they can arise so quickly, it has been discussed that FDCs might arise from circulating blood cells. Conversely, if FDCs are immobile cells, they would have to be ubiquitous in order to support formation of lymphoid follicles in any given place of the body.

In a paper which is being published in the journal Cell, Dr. Nike Kräutler in the team of Professor Adriano Aguzzi at the University of Zurich went after the latter question. Using novel markers identified in the Aguzzi laboratory in the past several years, they have identified clues suggesting that FDC precursor cells exist in the wall of blood vessels. This would explain many of the properties of FDCs, including the broad range of organs in which lymphoid follicles can arise during inflammatory conditions – because blood vessels are present in most organs of the body.

The specific morphology of the putative FDC precursor cells suggested that they be identical with mural cells, pluripotent cells which decorate vessel walls. One typical marker of mural cells is platelet derived growth factor receptor β (PDGFR-β). However, FDCs do not express PDGFR-β. Aguzzi and colleagues reasoned that this may be due to mural cells losing expression of PDGFR-β during their maturation into FDCs. In order to test this hypothesis, they used a sophisticated cell-lineage tracing approach. "Reporter" mice were generated whose FDCs would be stained by a blue marker if they had expressed PDGFR-β at any point in their life, even if PDGFR-β expression was suppressed at the time of analysis. Under these conditions, Kräutler and Aguzzi found that FDCs express the blue marker, indicating that they stem from another cell type which had previously expressed PDGFR-β.

The final piece of evidence nailing the origin of FDCs came from a transplantation experiment. Kräutler and colleagues isolated pure vascular mural cell populations from fat tissue of mice, which were then introduced into collagen sponges. The sponges were then transplanted into a special mouse strain that cannot develop FDCs. Upon induction of an inflammatory state, FDCs and lymphoid follicles were found to arise within the collagen sponges. Because the FDCs could not possibly have developed from the host animals, this experiment positively demonstrates that mural cells can give rise to FDCs.

VIDEO: Follicular dendritic cells originate in cells located in the walls of blood vessels. Click here for more information.
The work that is currently being published in Cell clarifies a question that has been controversially discussed for the last 25 years. The recognition that FDCs derive from pluripotent mural cells helps understanding autoimmune and pathogen-driven chronic inflammatory conditions, the generation of FDC-derived tumors, and certain aspects of the pathogenesis of acquired immunodeficiency syndrome (AIDS) and of prion infections. Because FDCs are an important site of prion-replication outside the brain, there is reason to hope that interfering with the differentiation of vascular FDC precursors may play a positive role in preventing prion infections.

**Diarrhoea remains a common problem in people with HIV**

Michael Carter  
Published: 06 July 2012

Diarrhoea remains common in people with HIV and usually has a non-infectious cause, according to a review article published in the online edition of Clinical Infectious Diseases. The authors stress that diarrhoea can have a severe impact on quality of life, necessitate changes to HIV therapy and contribute to poor adherence to treatment. The article sets out a matrix for the diagnosis and management of diarrhoea, considers possible therapies and sets out some priorities for future research.

Up to 60% of people living with HIV report diarrhoea. The condition is usually defined as three or more loose or liquid bowel movements per day and its prevalence is significantly higher among HIV-positive people when compared to matched controls.

A large corpus of research shows that diarrhoea has a severe impact on the quality of life of people living with HIV. In one study, 40% of participants indicated that diarrhoea adversely affected their social life. This involved restricting schedules or staying close to home because of concerns about the possibility of urgent bowel movements. Diarrhoea was also associated with feelings of shame.

Gastrointestinal opportunistic infections are a cause of diarrhoea in people with immune suppression. However, diarrhoea can affect people at all stages of HIV disease and is often unrelated to an infection. Possible non-infectious causes include the side-effects of antiretroviral drugs, the effects of HIV on the gastrointestinal tract and, more unusually, malignancies and pancreatitis.

A meta-analysis showed that approximately a fifth of people taking HIV therapy experienced moderate to severe diarrhoea. The condition has been associated with drugs in all three of the major classes of antiretrovirals. However, ritonavir-boosted protease inhibitors appear to involve the biggest risk of diarrhoea.

There are a number of possible reasons why antiretrovirals cause diarrhoea. These include damage to the intestinal epithelial barrier, leading to “leaky-flux” diarrhoea. However, much of the data for this explanation were obtained from animal models using large doses of medication. An alternative explanation is that anti-HIV drugs may alter chloride ion secretion causing so-called “secretory diarrhoea”.

HIV itself is also a potential cause of diarrhoea. The virus can infect the cells in the gastrointestinal tract and cause immune damage in this compartment, especially to gut-associated lymphoid tissue (GALT). Such damage may not be repaired with antiretroviral therapy and there is some evidence that HIV continues to replicate in gut tissue even in the presence of virologically suppressive antiretroviral therapy.

Another possible explanation is the autonomic damage that HIV can cause. Damage to autonomic nerves in the gastrointestinal tract has been observed in HIV-positive people.

More unusual causes of diarrhoea include the lesions associated with certain malignancies, as well as pancreatitis.

The authors present an algorithm for the diagnosis and management of diarrhoea in people with HIV. They note that definitions of diarrhoea can differ, and therefore propose that it should be defined as three or more daily bowel movements of unformed or liquid stool of large volume. They further propose that diarrhoea lasting four or more weeks should be defined as chronic.

Assessment of someone with diarrhoea should involve a consideration of physical examination, a detailed medical history and a review of HIV treatment history. Potential infectious causes should be considered, especially for people with a low CD4 cell count.

A stool sample should be obtained for microbiologic examination. The authors anticipate that this will yield a diagnosis for 50% of people. If no infectious cause is identified, then people with especially severe diarrhoea (ten or more bowel movements per day) should have an endoscopy.
HIV therapy should be reviewed to consider if this is the potential cause. Radiological examination is recommended if a malignancy is suspected.

Appropriate therapy should be provided for people with infection-related diarrhoea. The authors stress that there is currently no recommended therapy for non-infectious forms of diarrhoea in HIV-positive people. The use of medication such as Imodium to control symptoms should be considered purely supportive, and it should be noted that this can cause side-effects, most notably constipation. Crofelemer, an agent designed to address HIV-associated diarrhoea, is currently being reviewed by the US Food and Drug Administration and a decision is expected by September 2012.

Dietary changes, such as the use of fibre supplements, have been shown to have some impact on protease inhibitor-related diarrhoea.

Three research priorities are identified by the investigators:

- Better definition of the causes of diarrhoea in people taking HIV therapy.
- An evaluation of the safety and efficacy of anti-diarrhoea therapies.
- Exploration of how current HIV treatment can be refined so as to reduce the risk of gastrointestinal complications and improve immune responses in this compartment.

Reference

**Low bone density present in young men taking HIV therapy**

Michael Carter
Published: 04 July 2012

HIV infection in young men is associated with reduced bone mineral density, US investigators report in the online edition of *Clinical Infectious Diseases*. Antiretroviral therapy based on a protease inhibitor was especially associated with reduced bone density.

“We report evidence of low bone mass in behaviorally HIV infected young men on ART [antiretroviral therapy], particularly those on ART regimens that include a PI,” comment the authors. “This is the first report of low bone mass among youth who acquired HIV infection relatively recently, presumably after the onset of sexual debut during the late stages of puberty, and have relatively little exposure to ART.”

Low bone density is a well-recognised complication of HIV infection. There is uncertainty about the exact causes. The inflammation caused by HIV is one possible explanation, but bone loss has also been observed in people after they start antiretroviral therapy. Specific classes of anti-HIV drugs, most notably protease inhibitors, as well as some individual drugs, especially tenofovir (Viread, also in the combination pills *Truvada*, *Eviplera* and *Atripla*) have also been implicated.

Peak bone mass is achieved in adolescence and early adulthood and is the key factor governing bone mass during adult life. Little is currently known about the effects of HIV infection on bone mineral density in people who acquired the virus during this vital stage for bone metabolism.

Investigators from the US therefore designed a cross-sectional, case-controlled study comparing bone mineral density in 199 HIV-positive men aged between 14 and 25 years and 53 age-matched HIV-negative people as controls.

Just over half the HIV-positive patients were antiretroviral naive. In all, 52 people were taking therapy based on a non-nucleoside reverse transcriptase inhibitor (NNRTI) and 42 individuals were treated with a protease inhibitor-based regimen. Prevalence of tenofovir use was similar among the NNRTI and protease inhibitor groups.

The patients and controls had a median age of 21 years. The antiretroviral-naive participants had been HIV positive for a median of 1.3 years. The median duration of infection for people taking an NNRTI was 1.9 years, whereas those treated with a protease inhibitor had been HIV positive for a median of 2.2 years. Some 92% of people taking an NNRTI had an undetectable viral load (below 400 copies/ml) compared to 72% of individuals on a protease-inhibitor-based regimen.

Prevalence of risk factors associated with low bone density, such as smoking and alcohol consumption, was similar between the HIV-positive participants and the controls. However, the HIV-infected individuals were significantly more likely to have ever used cocaine (23 vs 8%; p = 0.01). Bone mineral density, content and body composition were assessed using DEXA scanning.

The HIV-negative participants were heavier (p = 0.002), and had a higher BMI (p = 0.006) and greater total lean body mass (p = 0.005), when compared to the antiretroviral-naive participants and those taking an NNRTI.
Total body bone mineral density was lower in the participants taking antiretroviral therapy (NNRTI and protease inhibitor-based regimens), compared to both the treatment-naive patients and controls ($p = 0.019$ and $p = 0.035$). Bone mineral content Z-scores were lower in the patient groups compared to the control group ($p = 0.005$).

Further analysis showed that total bone mineral density in the hip was significantly lower in both HIV treatment groups compared to the controls ($p < 0.001$) and the participants who were yet to start antiretroviral treatment ($p < 0.001$). Total hip bone density Z-scores were also lower in the patients compared to the controls ($p = 0.001$) and in the treatment-experienced participants compared to those who were treatment naive ($p < 0.001$).

In the femoral neck, both total bone density and bone density Z-scores were lower in the protease inhibitor group than in the control group ($p = 0.003$ and $p = 0.005$).

Overall, bone mineral density in the spine was marginally lower in the HIV-positive people compared to the control group. When analysis was restricted to the HIV-positive participants, the investigators found that this measure of bone density was lower in people on antiretroviral therapy – especially when based on a protease inhibitor – than the treatment-naive participants ($p = 0.025$).

Total spine bone mineral density Z-scores were lower than expected in all groups, including in the HIV-negative control group, but were markedly lower in the HIV-positive people, especially those taking a protease inhibitor.

There was some evidence that antiretroviral therapy, rather than HIV infection, was the cause of bone loss. The investigators highlight that they “saw little evidence of loss or impaired accrual of bone” in the HIV-infected participants who were still treatment naive. “In these youths who had acquired HIV infection relatively recently, the effect of HIV infection per se on bone mass appeared to be minimal.”

Although the authors were uncertain about the long-term consequences of their findings, they nevertheless believe that action to protect the bone metabolism of young HIV-positive men is required: “Risk reduction through changes in diet and lifestyle is warranted.”

Reference

Nondisclosure of HIV status should be decriminalized: report

Stephanie Law
Staff Reporter
Sex work, possession of drugs for personal use and nondisclosure of HIV should all be decriminalized, according to a report released Monday by the Global Commission on HIV and the Law.

The commission, led by the United Nations Development Program, was launched in June 2010 to make recommendations on how laws can be changed and used to protect the human rights of people living with HIV, and to help fight the global HIV epidemic.

There were 14 commissioners from different countries involved in putting together the final report, “HIV and the Law: Risks, Rights and Health,” including former heads of state and leading legal, human rights and HIV experts.

“Too many countries waste vital resources by enforcing archaic laws that ignore science and perpetuate stigma,” said former president of Brazil and commission chair Fernando Henrique Cardoso in a press release. “Now, more than ever, we have a chance to free future generations from the threat of HIV. We cannot allow injustice and intolerance to undercut this progress, especially in these tough economic times.”

The report made over 80 recommendations that are based on extensive research and first-hand accounts from more than 1,000 people in 140 countries.

“There are very significant things that need to be done in Canada, as in other countries, that the commission has quite accurately identified as necessary,” said Richard Elliott, executive director at the Canadian HIV/AIDS Legal network, one of more than 20 experts and organizations that make up the commission’s Technical Advisory Group.

Elliott identified several key recommendations that apply to Canada: abandon antiquated prostitution law; abandon the failed war on drugs; abandon the criminal law used to prosecute people living with HIV; and fix Canada’s access to medicine regime to improve access to medicines.

“All of those things are found in this report, and all are areas where Canada needs to act,” he said. “And yet on a number of them, current government policy is seemingly diametrically opposed to what actually should happen.”
The political and legal landscape around the criminalization of HIV nondisclosure, as well as Canada’s intellectual property laws that affect its ability to export generic medicines to developing countries, is about to change — although it remains to be seen whether the change would be in line with the report’s findings.

On the intellectual property front, NDP MP Hélène Laverdière introduced bill C-398 in March, which seeks to streamline Canada’s Access to Medicines Regime, a law created in 2004 to make it easier for Canadian companies to export lower-cost generic drugs to less-developed countries, including some HIV medicines. This bill had previously passed a House of Commons vote last year as bill C-393, but died in the Senate when the last parliament dissolved in March 2011. The first vote for the reintroduced bill is expected in November.

Meanwhile, the Supreme Court of Canada heard in February the case of two people living with HIV who failed to disclose their HIV status to their sexual partners. Neither of the two charged persons had transmitted HIV to their partners. The court’s decision, which is expected in fall this year, will clarify an existing criminal law that says nondisclosure of HIV status that leads to a significant risk of bodily harm constitutes an offence of aggravated sexual assault.

If convicted, an HIV-positive person can face time in prison as well as be designated as a sexual offender, possibly for life. To date, more than 130 out of 48,000 people living with HIV in Canada have been charged for not disclosing their status, a majority of whom were later convicted. These charges included cases in which a condom was used, although the bulk of these have resulted in acquittals.

“It’s a witch-hunt against people living with HIV — Canada is actually one of the worst global offenders when it comes to misusing or overusing the criminal law to deal with HIV,” said Elliott. “It’s driven by misinformation, by fear, by stigma and prejudice about HIV and against people living with HIV — rather then the sober judgment of the evidence.”

Perhaps the most controversial recommendation made by the commission is to decriminalize the possession of drugs for personal use, said Elliott. The report used Portugal, which had done exactly that in 2001, as an example of why this recommendation is valid. Since decriminalization took place, Portugal saw the number of people on methadone and buprenorphine treatment for drug dependency increase from 6,040 to 14,877, which is funded by money saved on police and prisons. It’s also had a drop in new HIV infections among people who use drugs.

The report, although applicable in many ways to Canada, in no way ensures that the government would act in alignment with its recommendation, said Elliott.

“It’s very useful road map for how the law can be helpful rather than harmful in helping people get access to treatment and preventing the further spread of HIV,” he said. “Certainly, those of us who work on legal and human rights issues will try to make sure the report has as much of an impact as possible, because the recommendations are very sensible — they’re the product of considerable international evidence and research.”

**Worst TB outbreak in 20 years kept secret**

State rushes closure of its only TB hospital in Lantana

Lilla Charline Burkhalter, 60, comes to the Clara White center for breakfast most mornings. It was here, in the soup kitchen, that a man with active, coughing TB was recently identified, leading to the discovery that Jacksonville was in the midst of the largest TB outbreak in the country. Burkhalter is coughing, but she says it’s her emphysema acting up. (Photo by Stacey Singer)

Last day of work for employees of A.G. Holley State Hospital in Lantana on Monday July 2, 2012. The Florida Legislature voted to close the state’s only tuberculosis hospital. (Gary Coronado/The Palm Beach Post)

Palm Beach Post Staff Writer

JACKSONVILLE —

The CDC officer had a serious warning for Florida health officials in April: A tuberculosis outbreak in Jacksonville was one of the worst he had investigated in 20 years. Linked to 13 deaths and 99 illnesses, including six children, it would require concerted action to stop.

That report had been penned on April 5, exactly nine days after Florida Gov. Rick Scott signed the bill that shrunk the Department of Health and required the closure of the A.G. Holley State Hospital in Lantana, where tough tuberculosis cases have been treated for more than 60 years.
As health officials in Tallahassee turned their focus to restructuring, Dr. Robert Luo’s 25-page report describing Jacksonville’s outbreak — and the measures needed to contain it — went unseen by key decision makers around the state. At the health agency, an order went out that the TB hospital must be closed six months ahead of schedule.

Had they seen the letter, decision makers would have learned that 3,000 people in the past two years may have had close contact with contagious people at Jacksonville’s homeless shelters, an outpatient mental health clinic and area jails. Yet only 253 people had been found and evaluated for TB infection, meaning Florida’s outbreak was, and is, far from contained.

The public was not to learn anything until early June, even though the same strain was appearing in other parts of the state, including Miami.

Tuberculosis is a lung disease more associated with the 18th century than the 21st, referred to as “consumption” in Dickensian times because its victims would grow gaunt and wan as their lungs disintegrated and they slowly died. The CDC investigator described a similar fate for 10 of the 13 people who died in Jacksonville.

They wasted away before ever getting treatment, or were too far gone by the time it began. Most of the sick were poor black men.

“The high number of deaths in this outbreak emphasizes the need for vigilant active case finding, improved education about TB, and ongoing screening at all sites with outbreak cases,” Luo’s report states.

Today, three months after it was sent to Tallahassee, the CDC report still has not been widely circulated.

Backer of closing hospital didn’t know

Meanwhile the champion of the health agency consolidation, Rep. Matt Hudson, R-Naples, said he had not been informed of the Jacksonville outbreak and the CDC’s role as of Friday.

Told the details, the chairman of the House Health Care Appropriations Committee vowed that there would be money for TB treatment.

“There is every bit of understanding that we cannot not take care of people who have a difficult case of TB,” Hudson said.

The governor’s office asked a reporter to forward a copy of the CDC letter on Saturday, but did not comment by press time.

Treatment for TB can be an ordeal. A person with an uncomplicated, active case of TB must take a cocktail of three to four antibiotics — dozens of pills a day — for six months or more. The drugs can cause serious side effects — stomach and liver problems chief among them. But failure to stay on the drugs for the entire treatment period can and often does cause drug resistance.

At that point, a disease that can cost $500 to overcome grows exponentially more costly. The average cost to treat a drug-resistant strain is more than $275,000, requiring up to two years on medications. For this reason, the state pays for public health nurses to go to the home of a person with TB every day to observe them taking their medications.

However, the itinerant homeless, drug-addicted, mentally ill people at the core of the Jacksonville TB cluster are almost impossible to keep on their medications. Last year, Duval County sent 11 patients to A.G. Holley under court order. Last week, with A.G. Holley now closed, one was sent to Jackson Memorial Hospital in Miami. The ones who will stay put in Jacksonville are being put up in motels, to make it easier for public health nurses to find them, Duval County health officials said.

They spoke about CDC’s report Friday, only after weeks of records requests from The Palm Beach Post. The report was released late last week only after a reporter traveled to Tallahassee to demand records in person. The records should be open to inspection to anyone upon request under Florida Statute 119, known as the Government in the Sunshine law.

TB strain spreads beyond homeless

In his report, the CDC’s Luo makes it clear that other health officials throughout the state and nation have reason to be concerned: Of the fraction of the sick people’s contacts reached, one-third tested positive for TB exposure in areas like the homeless shelter.

Furthermore, only two-thirds of the active cases could be traced to people and places in Jacksonville where the homeless and mentally ill had congregated. That suggested the TB strain had spread beyond the city’s underclass and into the general population. The Palm Beach Post requested a database showing where every related case has appeared. That database has not been released.

It was early February when Duval County Health Department officials felt so overwhelmed by the sudden spike in tuberculosis that they asked the U.S. Centers for Disease Control and Prevention to become involved. Believing the outbreak affected only their underclass, the health officials made a
conscious decision not to tell the public, repeating a decision they had made in 2008, when the same strain had appeared in an assisted living home for people with schizophrenia.

“What you don’t want is for anyone to have another reason why people should turn their backs on the homeless,” said Charles Griggs, the public information officer for the Duval County Health Department.

Even the CDC was not forthcoming about the outbreak. An agency spokesperson declined requests from The Post when asked to make an expert available to discuss a CDC-authored scholarly paper on the possible origins of the Jacksonville outbreak, offering only general fact sheets on TB.

“After checking in with the Division of TB Elimination about your specific questions, they have suggested that you reach out to your health department,” wrote Salina Cranor of the CDC’s TB prevention office. “They are really the best source for your questions.”

“With TB it’s a judgment call,” said Duval County Health Director Dr. Bob Harmon in a telephone interview Friday, after the state’s new surgeon general referred questions back to him.

“There have been TB outbreaks where we do alert the public, such as a school or a college,” Harmon added.

For weeks, there had been a dissonant message coming from the Department of Health press office in Tallahassee. It released overall numbers of Florida tuberculosis cases showing a marked decline statewide, supporting the argument that A.G. Holley had become irrelevant. Asked whether she had been aware of the severity of Jacksonville’s outbreak while delivering that message, she did not answer.

“Florida experienced a 10 percent decrease in cases for 2011 compared to 2010. For the period 2007—2011, there was a 24 percent decrease in cases,” wrote agency spokeswoman Jessica Hammonds in an emailed response to written questions on May 18. She declined, at the time, to make agency experts available for interview.

In an article published in June’s American Journal of Psychiatry, CDC experts Dr. Joseph Cavanaugh, Dr. Kiren Mitruka and colleagues described the apparent origins of the current outbreak, when a TB strain called FL 046 came to claim two lives and sicken at least 15 mentally ill residents of one assisted living facility in 2008.

A single schizophrenic patient had circulated from hospital to jail to homeless shelter to assisted living facility, living in dorm housing in many locations. Over and over, the patient’s cough was documented in his chart, but not treated. It continued for eight months, until he finally was sent under court order to A.G. Holley. That year, 2008-2009, a total of 18 people in that community developed active tuberculosis from the strain called FL 046 and two died. The CDC sent a $275,000 grant to help pay for the staff needed to contain it.

After the money ran out, Harmon said, staff were redeployed to other needs. But in 2011, suddenly, the number of active cases of FL 046 spiked, rising 16 percent to 30 cases of a specific genotype, the one seen in 2008.

“We thought after 2008 that we had it contained,” Harmon said. “It was not contained. In retrospect, it would have been better to inform the general population then.”

Harmon said the Duval County Health Department will need more resources if it is to contain the current TB outbreak. In 2008, when the TB outbreak hit, his department employed 946 staff with revenues of $61 million. “Now we’re down to 700 staff and revenue is down to $46 million,” Harmon said. “It has affected most areas of the organization.”

If he can raise at least $300,000, he will use the money to hire teams of experts — epidemiologists, nurses, outreach workers, to look under bridges, in fields — in all the places where Jacksonville’s estimated 4,000 homeless congregate, to track down the people who may still be infected unknowingly. Fortunately, only a few of the cases have developed drug resistance so far. The vast majority respond to the first-line antibiotics.

In downtown Jacksonville, in the homeless shelters and soup kitchens, the TB strain called FL 046 continues to spread.

On a recent June morning, 60-year-old Lilla Charline Burkhalter joined about 100 other poor and homeless guests being served a free hot meal of scrambled eggs, grapes, potatoes and butterless bread by a local church youth group.

The youth group was volunteering at the Clara White Mission, where a man with active tuberculosis had been identified just three weeks earlier.

Looking weary but friendly, Burkhalter described her life of late, sleeping in grassy fields and in shelter dormitories. She lived on a small Social Security disability check, she said. It had enabled her to pay for a room in an apartment, for a while. But her roommate had kicked her out for making his
girlfriend jealous, she said, and she hadn’t been able to find any other accommodations. It had been a rough few months, she acknowledged. But she had been through tough times before.

As she spoke, she coughed often. It was her emphysema acting up, she explained.

Asked if she was fearful about the TB in the community, she shrugged.

“The health department tests me for TB once a year, so I know I don’t have it,” she said. “I’m not worried.”

The Clara White Mission is now playing a key role in helping Jacksonville fight TB. Its housing case manager, Ken Covington, had spent most of his career helping bank branches assimilate after mergers. Two months ago, he joined Clara White, charged with placing homeless veterans and recently released jail inmates into homes. But the job has become much larger.

Today, Covington is the new chairman of the Duval County TB Coalition. In his hands he holds a massive binder with the intimidating title, “Core Curriculum in Tuberculosis: What the Clinician Should Know.” It was given to him by Vernard Green, the CDC’s visiting TB liaison.

Covington said he was a banker, not a clinician. But he had learned what to watch for with TB — coughing up blood, night sweats, sudden weight loss. The coalition members were looking at buying air filtration equipment, drafting intake protocols, getting to know the TB experts in the community, and educating shelter staff on what to watch for and what to do if a client appeared ill.

“We’re trying to do what we can to rein it in, and stay in front of it, and not let it get any worse,” Covington said. “I take it as a very important role for the community.”

WHAT THE POST UNCOVERED
In 2008, a schizophrenic patient contracted TB but went untreated for eight months, wandering among many places where the homeless congregate, infecting at least 17 others.

In 2012, the CDC was invited to help with a sudden spike in cases of the same rare strain the schizophrenic patient had. What they found is the worst outbreak they have investigated in 20 years, and it is not contained.

ON THE TRAIL OF TB
Hard to track: Homeless and mentally ill people and those they have come in contact with are especially hard to treat.

Long, tough treatment: Several pills a day of several virulent antibiotics for a minimum of six months, often up to two years.

What’s at stake: If treatment regimen isn’t strictly followed, antibiotic resistant strains emerge.

New silk technology preserves heat-sensitive drugs for months without refrigeration
Single silk device can store and deliver vaccine or antibiotic without costly 'cold chain'
MEDFORD/SOMERVILLE, Mass. (July 9, 2012, 3 PM EST) Researchers at Tufts University School of Engineering have discovered a way to maintain the potency of vaccines and other drugs—that otherwise require refrigeration—for months and possibly years at temperatures above 110 degrees F, by stabilizing them in a silk protein made from silkworm cocoons. Importantly, the pharmaceutical-infused silk can be made in a variety of forms such as microneedles, microvesicles and films that allow the non-refrigerated drugs to be stored and administered in a single device.

The Tufts findings address a serious obstacle to the effective use of life-saving pharmaceuticals: keeping them cold. Most vaccines, enzymes, and antibodies and many antibiotics and other drugs require constant refrigeration from manufacture to delivery to maintain their effectiveness.

International health experts estimate that nearly half of all global vaccines are lost due to breakdowns in the "cold chain." Even in industrialized nations, loss of drug efficacy at body temperature is a serious problem for advanced pharmaceutical delivery systems such as implantable drug-coated devices.

The research will be published before print in the Proceedings of the National Academy of Sciences (PNAS) Online Early Edition the week of July 9, 2012.

Tufts biomedical engineers led by David L. Kaplan, Ph.D., found that silk-stabilization preserved the efficacy of the measles, mumps and rubella (MMR) vaccine, as well as penicillin and tetracycline, at a wide range of temperatures (at least up to 60 degrees C or 140 F) significantly better than other options such as collagen encapsulants, dried powders and solutions.

"Silk protein has a unique structure and chemistry that makes it strong, resistant to moisture, stable at extreme temperatures, and biocompatible, all of which make it very useful for stabilizing antibiotics, vaccines and other drugs. The fact that we can also make silk into microneedles to deliver a vaccine is an
enormous added advantage that can potentially provide a lot of useful solutions to stabilization, distribution and delivery,” says Kaplan, who has been studying silk for two decades.

Nanoscale Bubble Wrap

Protein function depends on chains of amino acids folding into specific shapes. At higher temperatures or in the presence of water, the chains tend to unfold, then clump together, which renders them inactive. Silk fibroin is composed of interlocked crystalline sheets with numerous tiny hydrophobic pockets. The pockets trap and immobilize bioactive biomolecules—keeping them from unfolding—and also protect them from moisture. The end result is like enveloping a fragile material in a nanoscale Bubble Wrap.

According to the paper’s first author, Jeney Zhang, who is pursuing a Tufts doctorate in chemical and biological engineering, silk stabilization has "the potential to significantly change the way we store and deliver pharmaceuticals, especially in the developing world."

Measles is one of the leading killers of children worldwide. Without refrigeration, the MMR vaccine rapidly loses potency. But after six months of storage in freeze-dried silk films at body temperature (37 C) and at 113 F (45 C), all components of the vaccine retained approximately 85 percent of their initial potency.

Silk-stabilized antibiotics also retained high activity. Storage in silk films at body temperature resulted in no activity loss for tetracycline, compared with an 80 percent loss within four weeks of storage in solution. Even for films stored at 140 F (60 C), tetracycline activity loss was only 10 percent after two weeks, compared with 100 percent loss after two weeks of storage in solution. No activity loss was observed for penicillin stored in silk films at 60 C for 30 days; in contrast, total activity loss was observed within 24 hours when penicillin was stored in solution at the same temperature.

Silk stabilization also protected the tetracycline against degradation by light, a benefit that the researchers did not anticipate, according to co-author and research assistant professor Bruce Panilaitis. Panilaitis earned his Ph.D. in biology at Tufts Graduate School of Arts and Sciences before joining Kaplan’s lab in 2001 as a postdoctoral fellow.

So far, Panilaitis adds, the researchers haven’t found any pharmaceutical that they have been unable to stabilize. This could be a "universal storage and handling system."

Additional authors on the paper include Eleanor Pritchard, who earned her doctorate in biomedical engineering at Tufts and is now a postdoctoral fellow at St. Jude Children's Research Hospital in Memphis; Xiao Hu, a biomedical engineering postdoctoral fellow who will join Rowan University as an assistant professor in September; Thomas Valentin, a biomedical engineering master's degree student; and Fiorenzo Omenetto, Ph.D., professor of biomedical engineering.

Study finds 'mad cow disease' in cattle can spread widely in ANS before detectable in CNS

New pathway for infection reported in the American Journal of Pathology

Philadelphia, PA, July 9, 2012 – Bovine spongiform encephalopathy (BSE, or "mad cow disease") is a fatal disease in cattle that causes portions of the brain to turn sponge-like. This transmissible disease is caused by the propagation of a misfolded form of protein known as a prion, rather than by a bacterium or virus. The average time from infection to signs of illness is about 60 months. Little is known about the pathogenesis of BSE in the early incubation period. Previous research has reported that the autonomic nervous system (ANS) becomes affected by the disease only after the central nervous system (CNS) has been infected. In a new study published online in the August issue of The American Journal of Pathology, researchers found that the ANS can show signs of infection prior to involvement of the CNS.

"Our results clearly indicate that both pathways are involved in the early pathogenesis of BSE, but not necessarily simultaneously," reports lead investigator Martin H. Groschup, PhD, Institute for Novel and Emerging Infectious Diseases at the Friedrich-Loeffler-Institut, Riems, Germany.

To understand the pathogenesis of BSE, fifty-six calves between four and six months of age were infected orally with BSE from infected cattle. Eighteen calves were inoculated orally with BSE-negative material from calf brainstem as controls. The study also included samples collected from a calf that had died naturally of BSE. Tissue samples from the gut, the CNS, and the ANS were collected from animals every four months from 16 to 44 months after infection. The samples were examined for the presence of prions by immunohistochemistry. Samples were also used to infect experimental mice that are highly sensitive to a BSE infection.

A distinct accumulation of the pathological prion protein was observed in the gut in almost all samples. BSE prions were found in the sympathetic ANS system, located in the thoracic and lumbar spinal
cord, starting at 16 months after infection; and in the parasympathetic ANS, located in the sacral region of the spinal cord and the medulla, from 20 months post infection. There was little or no sign of infection in the CNS in these samples. The sympathetic part of the ANS was more widely involved in the early pathogenesis than its parasympathetic counterpart. More bovines showing clinical symptoms revealed signs of infection in the sympathetic nervous system structures at a higher degree than in the parasympathetic tissue samples. The earliest detection of BSE prions in the brainstem was at 24 months post infection. However, infection detected in the spinal cord of one animal at 16 months post infection suggests the existence of an additional pathway to the brain.

"The clear involvement of the sympathetic nervous system illustrates that it plays an important role in the pathogenesis of BSE in cattle," notes Dr. Groschup. "Nevertheless, our results also support earlier research that postulated an early parasympathetic route for BSE."

The results, Dr. Groschup says, indicate three possible neuronal routes for the ascension of BSE prions to the brain: sympathetic, parasympathetic, and spinal cord projections, in order of importance. "Our study sheds light on the pathogenesis of BSE in cattle during the early incubation period, with implications for diagnostic strategies and food-safety measures."

**High-level commission finds an epidemic of bad laws is stifling the global AIDS response**

NEW YORK, 9 July 2012—Punitive laws and human rights abuses are costing lives, wasting money and stifling the global AIDS response, according to a report by the Global Commission on HIV and the Law, an independent body of global leaders and experts. The Commission report, "HIV and the Law: Risks, Rights and Health," finds evidence that governments in every region of the world have wasted the potential of legal systems in the fight against HIV. The report also concludes that laws based on evidence and human rights strengthen the global AIDS response—these laws exist and must be brought to scale urgently.

"Bad laws should not be allowed to stand in the way of effective HIV responses," said Helen Clark, United Nations Development Programme Administrator. "In the 2011 Political Declaration on HIV and AIDS, Member States committed to reviewing laws and policies which impede effective HIV responses. One of the key contributions of the Commission's work has been to stimulate review processes and change in a number of countries."

The Global Commission on HIV and the Law—comprising former heads of state and leading legal, human rights and HIV experts—based its report on extensive research and first-hand accounts from more than 1,000 people in 140 countries. The Commission, supported by the United Nations Development Programme on behalf of the Joint United Nations Programme on HIV/AIDS, found that punitive laws and discriminatory practices in many countries undermine progress against HIV.

For example, laws and legally condoned customs that fail to protect women and girls from violence deepen gender inequalities and increase their vulnerability to HIV. Some intellectual property laws and policies are not consistent with international human rights law and impede access to lifesaving treatment and prevention. Laws that criminalise and dehumanise populations at highest risk of HIV—including men who have sex with men, sex workers, transgender people and injecting drug users—drive people underground, away from essential health services and heighten their risk of HIV. Laws that criminalise HIV transmission, exposure or non-disclosure of HIV status discourage people from getting tested and treated. More specifically:

- In more than 60 countries, it is a crime to expose another person to or transmit HIV. More than 600 HIV-positive people across 24 countries, including the United States, have been convicted of such crimes. These laws and practices discourage people from seeking an HIV test and disclosing their status.
- 78 countries criminalise same-sex sexual activity. Iran and Yemen impose the death penalty for sexual acts between men; Jamaica and Malaysia punishes homosexual acts with lengthy imprisonment. These laws make it difficult to prevent HIV amongst those most vulnerable to infection.
- Even though they may provide harm reduction services informally, laws in some countries criminalise some aspects of proven harm reduction services for injecting drug users, including in Cambodia, China, Myanmar, Malaysia and the Philippines. In contrast, countries that legalise harm reduction services, like Switzerland and Australia, have almost completely stopped new HIV infections among injecting drug users.
- More than 100 countries criminalise some aspect of sex work. The legal environment in many countries exposes sex workers to violence and results in their economic and social exclusion. It also prevents them from accessing essential HIV prevention and care services.
- Laws and customs that disempower women and girls, from genital mutilation to denial of property rights, undermine their ability to negotiate safe sex and to protect themselves from HIV infection. 127 countries do not have legislation against marital rape.
- Laws and policies that deny young people access to sex education, harm reduction and reproductive and HIV services help spread HIV.
- Excessive intellectual property protections that hinder the production of low-cost medicines, especially second-generation treatments, impede access to treatment and prevention.

**Enforcing bad laws squanders resources and undermines effective HIV responses**

Over the past three decades, scientific breakthroughs and billions of dollars of investments have led to the remarkable expansion of lifesaving HIV prevention and treatment, which has benefited countless individuals, families and communities. Yet, the Commission’s report finds that many countries squander resources by enacting and enforcing laws that undermine these critical investments.

"Too many countries waste vital resources by enforcing archaic laws that ignore science and perpetuate stigma," said former President of Brazil Fernando Henrique Cardoso, who chairs the Commission. "Now, more than ever, we have a chance to free future generations from the threat of HIV. We cannot allow injustice and intolerance to undercut this progress, especially in these tough economic times."

**Governments must enact laws based on evidence, human rights and public health**

The report finds that laws based on public health evidence and human rights can transform the global HIV response. According to the Commission’s report, laws and practices rooted in sound public health evidence and human rights exist and such laws and practices must be replicated. To end the epidemic of bad laws and to promote good laws that support effective HIV responses, the Commission urges governments to ban discrimination on the basis of HIV status and to repeal laws that criminalise HIV transmission or non-disclosure of HIV status. The Commission calls on governments to use the law to end the scourge of violence against women and girls and to resist international pressures to prioritise trade over the health of their citizens. The Commission also recommends decriminalising same-sex sexual activity, voluntary sex work and drug use, which will allow vulnerable populations access to HIV services.

"Women are half the world's population and young people are our future," said Nevena Ciric, a Serbian woman living with HIV. "Countries must enact laws that prevent violence against women and girls, as well as ensuring that laws support the provision of comprehensive sexual health education and services to young people."

The global community has a critical role to play. Global leaders, civil society groups and the United Nations must hold governments accountable to the highest standards of international law, public health and universal human rights, and advocate for policies and practices based on human rights and public health evidence.

"Governments across the world have a responsibility to take bold action and repeal laws that stem from ignorance and intolerance," said Maurice Tomlinson, a Jamaican lawyer and legal advisor for AIDS-Free World. "In Jamaica, where HIV prevalence among men who have sex with men is among the highest in the world, anti-sodomy law breeds fear and violence and drives these men away from the care and treatment they need."

Governments must follow the leadership of countries that have enacted laws that help advance effective HIV responses. For example, African and Caribbean countries that do not criminalise same-sex sexual activity have lower HIV prevalence among men who have sex with men. Countries that treat injecting drug users as patients instead of criminals—including New Zealand, Germany, Australia, Switzerland and Portugal—have increased access to HIV services and reduced HIV transmission rates among people who use drugs.

"We must ensure that new interventions to prevent and treat HIV reach the people who need them most," said former President of the Republic of Botswana Festus Mogae, a member of the Commission. "Laws that prohibit discrimination and violence and protect at-risk populations are a powerful, low-cost tool to ensure that HIV investments are not wasted. Undoubtedly, enforcing such laws is complex and politically challenging, but our report shows that it can and must be done."

**Technique Spots Disease Using Immune Cell DNA**

ScienceDaily (July 9, 2012) — By looking at signature chemical differences in the DNA of various immune cells called leukocytes, scientists have developed a way to determine their relative abundance in blood samples. The relative abundance turns out to correlate with specific cancers and other diseases, making
the technique, described in two recent papers, potentially valuable not only for research but also for diagnostics and treatment monitoring.

When a person is sick, there is a tell-tale sign in their blood: a different mix of the various types of immune cells called leukocytes. A group of scientists at several institutions including Brown University has discovered a way to determine that mix from the DNA in archival or fresh blood samples, potentially providing a practical new technology not only for medical research but also for clinical diagnosis and treatment monitoring of ailments including some cancers.

The key to the new technique, described in two recent papers, is that scientists have identified in each kind of leukocyte a unique chemical alteration to its DNA, called methylation. By detecting these methylation signatures in a patient’s blood sample and applying a mathematical analysis, the researchers are able to determine the relative levels of different leukocytes and correlate those with specific diseases. "You can simply look at the DNA and discern from the methylation marks the relative abundance of different type of leukocytes," said Karl Kelsey, professor of pathology and laboratory medicine in the Warren Alpert Medical School of Brown University and a senior author on both papers. "It's a way to more easily interrogate the immune system of a lot of people."

Other tests, using flow cytometry, can already sort through the abundance of different leukocytes in a blood sample, but they require the blood to be fresh and leukocyte cell membranes to be intact. Because the DNA in a blood sample remains even after cells have died and degraded, tests based on detecting methylation could help doctors or researchers analyze a patient's blood sample that has either aged or has simply not been kept fresh.

In a paper published in advance online June 19 in Cancer Epidemiology, Biomarkers, and Prevention, the researchers describe using their technique to distinguish accurately which blood samples came from patients with head and neck squamous cell carcinoma, ovarian cancer, or bladder cancer. By using methylation to determine the leukocyte populations in each sample, they could predict that the same samples were as much as 10 times more likely to have come from a patient with ovarian cancer than a healthy control patient, six times more likely to be from a head and neck cancer patient than a healthy control, or twice as likely to be from a bladder cancer patient than a control.

"Our approach represents a simple, yet powerful and important new tool for medical research and may serve as a catalyst for future blood-based disease diagnostics," wrote the authors, who hail from Dartmouth, Oregon State University, the University of Minnesota, and the University of California-San Francisco, as well as Brown. Several authors worked with Kelsey at Brown during the research. They describe the technique and its analytical methods in deep mathematical detail in another paper published in May in BMC Bioinformatics. They also report experiments that included analyses of the leukocyte mix of noncancer conditions such as Down syndrome and obesity.

The paper found many examples of differences between the immune cell mix of healthy controls and people with specific illnesses. For example, obese African Americans had an estimated increase in granulocyte leukocytes of about 12 percentage points. People with Down syndrome, had 4.8 percentage points fewer B cells. For head and neck cancer, they noted a 10.4 percentage point drop in CD4+ T-lymphocytes.

"Any disease that has an immune-cell mediated component to it would have applicability," Kelsey said.

In both papers, the authors said they expect that the technique will be applied in clinical and research efforts.

"Our approach provides a completely novel tool for the study of the immune profiles of diseases where only DNA can be accessed," the authors wrote in Cancer Epidemiology Biomarkers and Prevention. "That is, we believe this approach has utility not only in cancer diagnostics and risk-prediction, but can also be applied to future research (including stored specimens) for any disease where the immune profile holds medical information."

**Journal References:**


Keeping the Flu Away: Synthetic Protein Activates Immune System Within Two Hours

ScienceDaily (July 6, 2012) — San Diego State University researchers at the Donald P. Shiley BioScience Center may have found the secret to helping the immune system fight off the flu before it gets you sick.

A new study published July 6 in the Public Library of Science journal *PLoS ONE*, finds that EP67, a powerful synthetic protein, is able to activate the innate immune system within just two hours of being administered.

Prior to this study, EP67 had been primarily used as an adjuvant for vaccines, something added to the vaccine to help activate the immune response. But Joy Phillips, Ph.D. a lead author of the study with her colleague Sam Sanderson, Ph.D. at the University of Nebraska Medical Center, saw potential for it to work on its own.

"The flu virus is very sneaky and actively keeps the immune system from detecting it for a few days until you are getting symptoms," Phillips said. "Our research showed that by introducing EP67 into the body within 24 hours of exposure to the flu virus caused the immune system to react almost immediately to the threat, well before your body normally would."

Because EP67 doesn't work on the virus but on the immune system itself, it functions the same no matter the flu strain, unlike the influenza vaccine which has to exactly match the currently circulating strain.

Phillips said while this study focuses on the flu, EP67 has the potential to work on other respiratory diseases and fungal infections and could have huge potential for emergency therapeutics.

"When you find out you've been exposed to the flu, the only treatments available now target the virus directly but they are not reliable and often the virus develops a resistance against them," Phillips said. "EP67 could potentially be a therapeutic that someone would take when they know they've been exposed that would help the body fight off the virus before you get sick."

It could even be used in the event of a new strain of infectious disease, before the actual pathogen has been identified, as in SARS or the 2009 H1N1 influenza outbreak, Phillips said.

Right now, the testing has been done primarily in mice by infecting them with a flu virus. Those that were given a dose of EP67 within 24 hours of the infection didn't get sick (or as sick) as those that were not treated with EP67.

The level of illness in mice is measured by weight loss. Typically, mice lose approximately 20 percent of their weight when they are infected with the flu but mice treated with EP67 lost an average of just six percent. More importantly, mice who were treated a day after being infected with a lethal dose of influenza did not die, Phillips said.

She said there are also huge implications for veterinary applications, since EP67 is active in animals, including birds.

Future research will examine the effect EP67 has in the presence of a number of other pathogens and to look closer at exactly how EP67 functions within different cells in the body.

**Journal Reference:**
Questioning the HIV Cure
Sensitive tests reveal the Berlin patient believed to be cured of HIV still carries HIV RNA and antibodies.

By Hayley Dunning | June 12, 2012
Five years ago, Timothy Brown received transplants of bone marrow in Berlin to treat his leukemia. Because he also happened to be HIV-positive, he doctor chose a donor that carried a mutation in the CCR5 gene, which encodes a co-receptor that HIV uses to infect cells. Following his surgeries, Brown was able to stop conventional antiretroviral treatment and was deemed cured in 2009 when the virus failed to return. But research presented at the International Workshop on HIV & Hepatitis Virus last week (June 8) found HIV signals still lingering in Brown’s blood plasma, sparking well-known HIV researcher Alain Lafeuillade to suggest he has been re-infected, or was never cured.

Several labs looked at Brown’s blood, and some, but not all, found evidence of circulating viral RNA using sensitive PCR tests. Two strains of the virus were sequenced, which did not match each other or the original virus, suggesting Brown may have been re-infected. But no labs isolated virus that could copy itself, suggesting to collaborator Tae-Wook Chun that Brown may just have harmless pieces of the virus left over from the initial infection. But even these infection remnants, while harmless to Brown, may be infectious to other people, Lafeuillade said.

But those involved in the study have condemned Lafeuillade’s remarks, with session chair and collaborator Douglas Richman telling ScienceInsider that Lafeuillade “completely misinterpreted” the presentation. “We weren’t trying to say HIV was still there or he [Brown] hadn’t been cured,” added Steven Yukl, who gave the talk. “The point of the presentation was to raise the question of how do we define a cure and, at this level of detection, how do we know the signal is real?”

Richman is among the collaborators that found nothing, and thinks the result may be due to contamination issues. He staunchly defends the position that Brown is cured. “[Brown’s] been off ARVs for 5 years,” he told ScienceInsider. “That trumps all these as says.”

Microbial Menagerie
A massive study catalogues the microbes in the healthy human body, uncovering an unexpected level of individual variation in microbial makeup, among other surprises.

By Ed Yong | June 13, 2012
The human body is largely not human. It contains trillions of microbes that outnumber our own cells 10 to 1, affecting our health and behavior. Now, an international consortium of around 200 scientists has mapped this diverse microbial community at an unprecedented level of detail, and shown just how much it varies from person to person.

“This represents a yet another milestone that will help to expand our knowledge about the invisible world of human-associated microbes,” said Peer Bork from the European Molecular Biology Laboratory, who was not involved in the study.

The team, working together as part of the Human Microbiome Project (HMP), studied the microbes of 242 healthy volunteers, aged 18 to 40. They collected samples from 18 body parts for women and 15 for men, from the nostril to the crease behind the ear—habitats as different to bacteria as deserts or jungles are to us.

“The project has surveyed more body sites, in more individuals, with greater depth of sequencing than any previous study,” said Curtis Huttenhower from the Harvard School of Public Health, who is the lead author on one of two papers that describe the main results, published today in Nature. Many other papers, published in PLoS journals, describe the denizens of specific body parts.

In cataloguing the healthy human microbiome, the HMP has already yielded some surprises. For example, although each body part is characterised by some signature microbial groups, no species was
universally present across every volunteer. “One of the HMP’s original mandates was to define the core microbiome, or the bugs that everyone shares,” said Huttenhower. “It looks like there really aren’t any.”

And microbe species that were shared across people still differed in terms of specific strains and genetic make-up of those strains. “Even when we carry the ‘same’ microbes, they seem to have small differences between their genomes just like people do,” Huttenhower said.

However, these varied microbes carry out overlapping jobs, including creating and breaking down nutrients. “This incredible species diversity leads to an incredible conservation at the level of molecular function,” said Rob Knight from the University of Colorado in Boulder, who was part of the consortium. “This suggests different bugs are performing the same jobs in different people, just like every city has bankers and lawyers and salesmen that make the city’s ecology tick,” said Huttenhower.

While major disease-causing microbes were very rare, all the volunteers, even though they passed a rigorous health screen, carried opportunistic pathogens—microbes like *Staphylococcus aureus* that are normally harmless but occasionally go rogue and cause disease. This parallels the situation in our own genome, where genetic variants that confer a high risk of disease are rare, while those that pose a moderate risk are more common.

The HMP team revealed that our microbial communities are most diverse in the mouth and the gut, and least so in the vagina. They also showed that these communities are very stable over time, by sequencing extra samples from 131 of their volunteers after several months.

“We are similar yet different, but stable in our differences,” said Dusko Ehrlich from Institut National de la Recherche Agronomique (INRA), who was not involved in the study. “If we were all the same, there’d be no signal. If we were all different, you couldn’t do a comparison. If we changed all the time, there would be too much of a moving target. Instead, we have hope for capturing interesting differences that matter for our health.”

So far, the HMP has accumulated around 3.5 terabytes of data, all accessible through public databases. “It’s a real treasure trove,” said Ehrlich.

Still, “there is a lot of work ahead to understand the data,” Bork noted. For example, what causes the individual differences in microbial diversity? Age, gender, and body weight only explain a fraction of the total variation, and other factors such as diet, geography and host genetics probably play a role too. Despite the variation, the HMP still aims to characterize a “healthy” Western microbiome, which could be used to understand how and why the microbiome changes with disease, and how it interacts with our own cells and genes. Such studies are close to providing new diagnostic tools, and may lead to new ways of treating diseases, said Huttenhower. “The human genome’s taken a decade to be translated into clinical practice, and the same process is already beginning for the microbiome,” he said. “It will be very exciting to see the field of microbiome research also start to influence personalized medicine.”


**Cancer-Fighting Virus**

A small patient trial offers hope that cancer-killing viruses might be a viable therapy after all.

By Ruth Williams | June 13, 2012

A common virus given to patients intravenously can avoid immune detection, hitching a ride on immune cells in the blood, and find its way to tumor targets, where it replicates and destroys the cancerous cells, according to a report out today (June 13) in *Science Translational Medicine*.

“A lot of people in the field ... had suggested that giving the virus into the blood couldn’t work because the antibodies would neutralize it straight away,” explained Alan Melcher, a clinical oncologist at the University of Leeds in the UK, who led the study. His team’s work now shows it is in fact an efficient way to target a tumor.

A laboratory-grown virus that can infect humans and evade the immune system might sound like it belongs on a bioterrorist’s must-have list, but in fact it’s what some cancer researchers have been waiting for. The viruses in question, including the one used by Melcher and colleagues, are adept at killing cancer cells but leave healthy tissue alone. “Often tumors get rid of genes to become immortal, and when they do that they actually become weak when it comes to viral infection,” explained John Bell, a specialist in Cancer Therapeutics at Ottawa Hospital Research Institute in Canada, who was not involved in the work.

But because these viruses, known as oncolytic viruses, commonly infect humans, most people have antibodies against them, and researchers feared that this might put the kibosh on any plans for their
therapeutic use. Indeed in mice that were injected with reovirus—the virus used by Melcher and colleagues—preexisting antibodies prevented its delivery to tumors.

This turns out not to happen in humans, however. By injecting a large amount of reovirus into the blood, Melcher’s team was able to overcome the antibody neutralization. “I think they have demonstrated pretty nicely that one of the predicted barriers to therapeutic success isn’t in fact a barrier,” said Brain Lichty, a pathologist at McMaster University in Ontario, Canada, who also did not participate in the research. “[They’ve shown] that by simply dumping-in sufficient virus, even in people that have preexisting antibodies, they’re clearly able to deliver virus to tumors,” he said.

“It is a good example of why we have to do experiments in people and not just in animals,” added Bell. Melcher and his colleagues administered large doses of reovirus to patients suffering from colorectal cancers that had metastasized to the liver. Ten patients, who were scheduled to have the liver tumors surgically removed, agreed to be dosed in the arm with reovirus after being told that, “in theory this virus might have some activity against your cancer, but it is absolutely not proven,” said Melcher.

Reovirus normally doesn’t cause any disease in humans, only rarely resulting in mild colds or stomach upsets. The patients, who received injections between a week and a month prior to surgery, suffered mild flu-like symptoms. Blood samples were taken at various time points and, after surgery, tumor tissue and surrounding normal liver were analyzed for the presence of the virus.

The researchers found that while virus particles in the serum of patients’ blood were mostly non-viable—no doubt neutralized by the patients’ antibodies—those particles that instead associated with immune cells, survived and were capable of replication in vitro. It is not yet clear whether the viruses entered the immune cells or attached to the outside, but live replicating virus detected in the tumors indicates that these immune-cell-associated viruses were escorted unharmed to the site of cancer.

The tumor cells also showed signs of cell death, suggesting that once the viruses reached the cancer, they were effective killers. Furthermore, it was only the tumor cells, and not normal liver tissue, that showed signs of both virus infection and cell death.

The big question—whether patients actually benefited from the therapy—remains unanswered. “To be honest, the numbers are too small to say,” said Melcher. “Unfortunately, in some of them their cancer has returned... but there is absolutely no way, from 10 patients, of saying whether the virus has made things any better or worse.” Much larger clinical trials are necessary, and these are now in development, he said.

Back in the laboratory, Melcher plans to test other types of cancer-killing viruses in a variety of tumor types to see which are best at killing which cancers. However, curing cancer isn’t going to be as simple as catching a cold. “We’re putting a very high dose of virus into the circulation [of the patients],” Melcher explained. “So sadly it’s not that catching these infections just from the normal course of things would help people with cancer. It needs to be targeted and deliberate treatment.”


Corrupted Proteins Spread Disease
A protein fragment involved in Alzheimer’s can seed new clusters throughout the brain, pointing to prion-like qualities of the disease.

By Ed Yong | June 18, 2012

Many neurological diseases are caused by misfolded proteins that gather in large, destructive clumps, causing neuronal degeneration. Some of these proteins can also convert normal versions into their own twisted images, thus spreading the disease throughout the brain. The classic examples are prion diseases like mad cow disease and Creutzfeld-Jacob disease (CJD). They are caused by misshapen forms of the PrP protein, which corrupts the shapes of normal PrP.

Now, new research published today in the Proceedings of the National Academy of Sciences suggests that Alzheimer’s disease might work in a...
similar way. Its hallmarks include tangled clumps of amyloid-beta, a peptide (protein fragment) that aggregates in large plaques, which according to the new study, can seed more protein clusters, creating a wave of plaques that spreads through the brain.

“While this had been suspected, there was previously no proof,” said Kurt Giles, who studies neurodegenerative diseases at the University of California, San Francisco, and led the new study. “I think it is a really important paper,” added Anne Bertolotti from the MRC Laboratory of Molecular Biology, who also works on misfolded proteins but was not involved in the research. “It strongly supports the idea that the prion phenomenon applies to Alzheimer's disease.”

In 1982, Nobel prize-winner Stanley Prusiner first discovered prions—misfolded proteins that act as infectious agents, spreading the disease within and among individuals. Two years later, he speculated that Alzheimer’s disease might involve a similar spread of malformed proteins, at least within patients. Recent experiments have supported his idea. For example, Lary Walker from Emory University and Mathias Jucker from the University of Tübingen managed to trigger the spread of amyloid-beta plaques in the brains of mice by injecting them with brain extracts already containing clumps of the peptide.

But these extracts always contained other proteins that could have been responsible for the spread of the plaques. Now, Prusiner, along with Jan Stohr and Joel Watts from University of California, San Francisco, have shown that pure amyloid-beta can achieve the same effect on its own.

The researchers injected purified amyloid-beta into mice whose brains were engineered to fluoresce as plaques built up. By measuring the light given off by their brains, the team showed that the purified peptides were enough to induce plaques throughout the brain within 5 to 6 months. Even if the peptides were injected into just one side of the brain, both halves eventually started to glow.

Next, the team created the peptides from scratch “so there could be no doubt about other contaminants,” Giles said, and even these synthetic peptides were enough to create plaques. “It’s the first clear demonstration that amyloid-beta alone can induce amyloid-beta deposition in the living brain,” said Walker.

The synthetic peptides did appear to be somewhat less effective at seeding fresh plaques than the purified versions, however. It may be that only some “strains” of amyloid-beta can cause native proteins to clump together, or that different strains spread at different speeds, Walker said—“not all seeds are equivalent.” Identifying the strains that most accelerate Alzheimer’s disease could provide novel drug targets, Giles added.

It is becoming increasingly clear that the spread of corrupted proteins is a unifying feature in many brain diseases. Clumps of alpha-synuclein, a protein involved in Parkinson’s disease, for example, can spread from one neuron to another, transform local proteins, and gather them into clusters. SOD1, a protein involved in Lou Gehrig’s disease, can do the same.

However, unlike classical prions, it doesn’t appear that the corrupted proteins in involved these diseases can spread infections from one individual to another. “There is still no evidence that Alzheimer’s disease is infectious under everyday circumstances,” said Walker.


On the Chain Gang

More than simply helping haul out a cell’s garbage, ubiquitin, with its panoply of chain lengths and shapes, marks and regulates many unrelated cellular processes.

By Keith D. Wilkinson and David Fushman | July 1, 2012

In 1974 and 1975, a group led by Gideon Goldstein at New York University discovered and sequenced a 76-amino-acid protein from bovine thymus cells that appeared to be important in stimulating immune-cell function. But as they continued to characterize the protein, like a bad contaminant, they found it everywhere—in every tissue of the human body, and in cell cultures from worms, other animals, plants, and even bacteria. The authors surmised that the protein must be “a universal constituent of living cells,” and consequently named it ubiquitin. Later it became apparent that the ubiquitin found in bacterial cultures came from the yeast extracts on which they were cultured, leading to the realization that ubiquitin was limited to eukaryotic cells. For several years, little more was learned about the protein’s structure or function. In fact, a National Institutes of Health panel, reviewing William Cook’s proposal to determine the crystal structure of ubiquitin, concluded that the project was not interesting, since the protein was found everywhere and had no known function.

Despite such an inauspicious start, ubiquitin was soon recognized as a constituent of histone proteins (through work by Ira Goldknopf and Harris Busch) and later as a necessary cofactor in a vital cellular
process—the degradation of proteins. Work by Avram Hershko, Aaron Ciechanover, and Irwin Rose (for which they received the 2004 Nobel Prize in Chemistry) showed that covalent attachment of a small protein, which turned out to be ubiquitin, provided proteins with a tag or label that directed them to the cell’s degradation machinery. While degradation is essential for normal cellular function, such as helping clear damaged proteins, it always seemed as though a protein so well conserved and ever-present must play an even larger role in cell biology.

It always seemed as though a protein so well conserved and ever-present must play an even larger role in cell biology.

Although the biochemical studies done by the Nobel Prize winners were strongly suggestive, it was only after Alex Varshavsky began to define the genetics of the ubiquitin system in 1984 that the multifaceted cellular role of the little protein became more obvious. Varshavsky, an eminent histone biochemist who defected to the United States from the former Soviet Union, had become intrigued by this molecule that tagged both histones and damaged cellular proteins. His early genetic studies led to the discovery of a dozen or so ubiquitin-like proteins.

Soon researchers discovered new roles for ubiquitin in addition to protein degradation, and learned that the ubiquitin protein’s structure and the architecture of its polymeric forms have more to do with its function than does its mere presence on a protein substrate. In contrast to modifications such as phosphorylation, methylation, and acetylation, the attachment of one or more ubiquitin monomers provides a large interaction surface by which the modification can be encoded, and results in a vast number of potential signals by virtue of the varied architectures linking the multiple ubiquitin molecules. Recent studies have begun to define the roles of different polyubiquitin signals in physiology and disease, and it has become obvious that the manipulation of these signals and of their recognition will be important in developing new treatments.¹

**Diversity of polyubiquitin chains and linkages**

Polyubiquitin chains (polyUb) consist of ubiquitin (Ub) monomers linked to each other covalently. These ubiquitin chains are attached to protein substrates with the help of several accessory proteins called E1, E2 and E3. These accessory proteins select the appropriate protein and then recruit Ub tagging machinery to build a chain of Ub molecules on the target protein.

¹ Infographic: Ubiquitin Basics, View full size
While the attachment of a single molecule of ubiquitin to a protein resembles simple modifications such as phosphorylation or acetylation, the attachment of polyubiquitin chains more closely resembles glycosylation. Both provide a much broader functional canvas because of the immense variation in length and linkage architecture. Monomers of ubiquitin can be built into chains at multiple attachment sites to assemble a huge number of different targeting signals. Importantly, all of these polyUb forms appear to
serve diverse functions, not only tagging a protein for transport to a particular location, but also aiding in the assembly of protein complexes that modulate a protein’s function or stability.

Proteins tagged for degradation, for example, are recognized and degraded by the proteasome, a large multicatalytic protease that degrades the target protein into small peptides, which can be then broken down into free amino acids, and that also disassembles the polyubiquitin tag. This canonical mechanism is responsible for maintaining the temporal order of the cell cycle, wiping the cell clean of one type of cyclin protein after the next, allowing each subsequent wave of cyclins to push the cell further along the path to cell division. The canonical pathway was also found to be behind the cycling of the circadian clock. A master circadian protein that accumulates throughout the day is completely degraded when its levels reach a threshold, thus resetting the clock for the new cycle. Similarly, this same mechanism rapidly tags and degrades damaged proteins that arise due to aging, stress, or oxidative damage.

In the past 2 decades, researchers have found much variability in both the shape and the function of ubiquitin chains. They’ve discovered Ub chains of varying length and linkage architecture; chains that include other ubiquitin-like proteins; solitary Ub chains not attached to any proteins; and proteins with multiple Ub chains attached to them. Each new configuration of polyubiquitin hints at new functions, many of which are yet to be discovered, and confirms that these ubiquitous polymers are indeed essential for a wider variety of cellular processes than we had imagined.

Ubiquitin is often attached to proteins as a chain of various lengths, and the number of links appears, in part, to determine the ultimate destination of the protein; four links, for example, are sufficient for delivery to the proteasome. Polyubiquitin linkages can be made between the C-terminal end of one ubiquitin and any one of eight primary amino groups on the next one: an amine from one of the monomer’s seven lysines or the N-terminal methionine. Imagine ubiquitin as a dreidel with flat faces and straight edges and a hole in each of the faces. The peg of one dreidel (the C-terminus) can be inserted into any hole of another. If each face is a different color, then every combination has a unique surface architecture. Each successive ubiquitin monomer can attach at a different linkage site, and any one ubiquitin can contain more than one linkage. The variability can run the gamut from linear chains with “homogeneous” linkages (all using the same lysine) to “heterogeneous” linkages (using different lysines) to observe “branched” chains with multiple distal termini. (See illustration on opposite page.) Byzantine indeed!

Chains can also be “mixed,” made up not only of ubiquitin monomers, but also of other members of the ubiquitin-like family of proteins that are similar to ubiquitin in shape, but not in sequence. These ubiquitin-like proteins, such as the small ubiquitin-related modifier (SUMO), are themselves attached to proteins to target them to various locations in the cell, regulating such processes as apoptosis or transcriptional control.2

Very recently, researchers have also noticed chains of unanchored ubiquitin in the cell, and have found evidence that these free chains play a role in cell signalling, specifically by activating protein kinases and other pathways involved in antiviral innate immunity, although they may have other functions as well.3

In addition to the diversity generated by the manifold linkage patterns, a polyubiquitin chain can also exhibit a unique three-dimensional structure. It can fold back on itself, creating kinks or knobs at various points. These shapes alter how ubiquitin “receptor” proteins bind and thereby read the message. Receptors can be any proteins that specifically recognize and bind to mono- or polyubiquitin. Thus, the chain’s structure defines its ability to interact with specific receptors that perform various functions, from shuttling the ubiquitinated protein to a new location to hydrolyzing the ubiquitin chain to degrading the substrate protein.4

The critical question that researchers in this field are still teasing apart concerns how the three-dimensional structure of polyUb chains specifies its binding partner, i.e. how does the receptor recognize and decode the signal? Two features of the chains appear to be essential in determining binding. First, the hydrophobic patch on one face of each ubiquitin monomer can interact with the most common ubiquitin-binding domains on receptor proteins and with other ubiquitins in the chain. Second, ubiquitin’s C-terminus tail, which links monomers in the chain, is highly flexible, making possible a variety of conformations. For example, linking either two or four UbS together by attaching the C-terminal tail of one monomer to lysine at position 48 on the next monomer creates a chain that is in equilibrium between two or more forms; a tightly-packed, closed conformation that conceals the monomers’ hydrophobic patches and one or more open conformations.4 Chains linked at lysine-63 or the amino terminus, on the other hand, predominantly adopt an extended, open structure that exposes the hydrophobic patches,
making them readily available for interactions with receptors. Structural data and computer modelling indicate an even greater structural variability for polyubiquitin chains connected through other lysines.

**Polyubiquitin receptors**

While the evidence for the existence of ubiquitin receptors is strong, little is known about the molecular details of most. The two exceptions are the enzymes that disassemble polyubiquitin (deubiquitinating enzymes) and shuttling proteins that ferry polyubiquitinated proteins to the proteasome.

Specific recognition of polyubiquitin is accomplished by proteins containing one or more ubiquitin-binding domains. There are at least 20 families of these domains, and many polyubiquitin-binding proteins, or receptors, contain multiple copies, with two or three different domains connected by flexible linkers. The affinity of each of these individual domains for ubiquitin is modest (µM), but tight binding is achieved because the binding of polyubiquitin to one domain lowers the entropic barrier for binding of an adjacent ubiquitin to another domain. Some shuttling receptors have not only ubiquitin-binding domains but also ubiquitin-likedomains, so they can also bind to each other in networks that assemble into oligomers or a lattice, offering a highly selective array of available ubiquitin binding sites exhibiting specificity for certain polyubiquitin chain linkages. Indeed, studies using artificial oligomers of ubiquitin-binding domains, such as GST-UBA fusions or TUBES (tandem ubiquitin-binding entities), have emphasized that specificity is determined more by the oligomeric arrangement of these domains than by the weak specificity inherent in the individual domains.

**Specific recognition of polyubiquitin is accomplished by proteins containing one or more ubiquitin-binding domains.**

**Specific recognition of chain linkage**

The chain linkage architecture is important for determining the shape of the chain and also appears to inform the fate of the tagged protein by determining which receptors bind the chain. For instance, a linkage at lysine 6, 11, 29, or 48 directs proteins to the proteasome, while linking at lysine-63 or methionine-1 (M1) serves to mark the protein for a role in DNA-damage response or NF-kB-mediated inflammatory pathways.

Finally, receptors can distinguish between polyubiquitin chains bound to different target proteins if the receptors contain a ubiquitin-binding domain as well as a site for binding to the target protein. For instance, the A20 deubiquitinating enzyme binds both ubiquitin and RIP1, a polyubiquitinated signalling protein in the NF-kB pathway. This deubiquitinating enzyme then removes the ubiquitin tag from the signalling protein RIP1, converting RIP1 from a complex that prevents cell death to one that drives it forward, helping destroy virus-infected cells from within. There must be many of these types of receptors that recognize both ubiquitin and the tagged protein, since the cell must distinguish among the numerous proteins that have similar polyubiquitin chains attached.

**The importance of length**

Ever since Cecile Pickart at Johns Hopkins University initially observed that a four-ubiquitin chain was the minimal effective length to deliver proteins to the proteasome, the question of how chain length affects the fate of a ubiquitinated protein has been debated. Chains must achieve a length that provides sufficient binding affinity for a Ub receptor through binding at multiple sites. However, long chains can change conformation, perhaps folding together so tightly that the dissociation of catalytic intermediates, or “hand off” from one receptor to the next, is prevented.

In part, chain length can be controlled by how long the enzymes, or ligases, that link ubiquitin monomers together can remain on the chain before falling off—a property called processivity. The longer the enzyme and substrate remain associated, the more ubiquitins can be attached. Length can also be affected by deubiquitinating as they trim or disassemble chains. The modular nature of receptors containing multiple binding domains and the ability of longer polyubiquitin chains to bind multiple receptors may serve as length sensors. For instance, the deubiquitinating enzyme USP5 selectively binds a tetra-ubiquitin chain, which it then severs using an ensemble of four ubiquitin binding sites. Longer polyubiquitin chains can also be “handed off” from one receptor to another, as exemplified by the trafficking of ubiquitinated proteins through the endosomal sorting complex required for transport (ESCRT), which delivers ubiquitinated proteins into the cell’s vesicles.

**Localization of the polyubiquitin signal**

Recent observations show that both free-floating and attached methionine-1–linked polyubiquitin chains can activate signaling of the innate immune response mediated by the NF-kB pathway, protecting cells from invading viruses. (See illustration on preceding page.) These chains directly activate kinases that drive the signaling cascade. Unanchored M1-linked ubiquitin chains are also the primary gene product of several genes transcribed in response to genotoxic stress. Normally, however, levels of M1-linked ubiquitin chains in cells are very low, in part because the primary gene product is cleaved to monomeric ubiquitin as it’s being transcribed at the ribosome and because of the presence of a large amount of USP5,
the enzyme responsible for disassembling polyubiquitin intermediates that might otherwise accumulate in the cell. Thus, it is unlikely that chains with an M1 linkage are widely distributed in the cell. Rather, they may be locally generated at the site, or sites, of signaling. A similar mechanism may be at play in the case of ubiquitinated proteins that accumulate in other signaling cascades. A great deal of cellular specificity in the ubiquitin pathway seems to depend on the use of adaptors and scaffolds that colocalize polyubiquitin and the enzymes that metabolize it. For instance, deubiquitinating enzymes are very often found in the same protein complex as the ubiquitin ligases that synthesize polyubiquitin. This suggests that if a polyubiquitin chain or polyubiquitinated protein is not properly channeled to its target by ubiquitin receptors, it can be disassembled before it leaves the site of synthesis.

Future directions
The incredible diversity of polyubiquitin chains observed in vivo suggests a similar complexity in the receptors that recognize the chains. It seems likely that additional ubiquitin-binding motifs and domains remain to be discovered. More importantly, what we know about ubiquitin domain binding specificity and chain architectures suggests that a deeper understanding awaits studies of binding specificity in the context of the full-length receptors. As more engineered and synthetic polyubiquitins become available, structure determination of polyubiquitin-receptor complexes will be vital to understanding the decoding of the polyubiquitin signals. Finally, we need a more sophisticated understanding of the “hand off” of a receptor-bound polyubiquitin to the next receptor in a sequence. It is still a mystery how shuttling receptors pass ubiquitin from the ligases and chaperone complexes to the proteasome, or how sequential ESCRT complexes can direct endocytic cargos carrying the ubiquitin signal.

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References

Polypharmacy
Dietary supplements can have serious side effects when mixed with prescription drugs, but not all herb-drug interactions are bad.

By Catherine Ulbricht | July 1, 2012
For more than 5,000 years, herbs and other natural ingredients have been used for medicinal purposes. Today, people use such concentrated natural products as supplements to help combat various diseases, from depression to cancer, as well as to boost health, including immunity and memory. Based on Natural Standard research, in the United States alone more than $40 billion is spent each year on these products. An estimated 60 percent of cancer patients try natural products, and 40 percent take vitamins or other dietary supplements.

Just because herbal products are developed from plants, they cannot necessarily be deemed harmless. Like prescription drugs, herbs and supplements may cause unwanted side effects and can interact with prescription drugs, other natural products, or foods, and may even alter diagnostic and laboratory test results. Unlike regulated drugs, however, dietary supplements can be marketed without approval from the US Food and Drug Administration. As a result, herbal products are often not thoroughly evaluated by the FDA unless there is sufficient evidence to prove that they are unsafe. Partly due to this regulatory freedom, as well as to a lack of available clinical research, interactions between herbs and conventional drugs are often overlooked.

In the United States alone more than $40 billion is spent each year on natural products.
Herbs and drugs can interact pharmacodynamically by mechanisms that may be additive, synergistic, or antagonistic. For example, concurrent use of an anticoagulant/antiplatelet drug and natural ingredients that possess antiplatelet activity, such as garlic, may increase the risk of bleeding. Similarly, herbs that lower blood sugar may have additive effects with antidiabetic drugs, thereby increasing the risk of potentially dangerous hypoglycemia (low blood sugar). Some herbs, such as ephedra (ma huang in Chinese), on the other hand, are known to increase blood pressure and may counteract the beneficial effects of antihypertensive medications.

Herbs and medications can also have pharmacokinetic interactions, meaning that the herbs may change the absorption, distribution, metabolism, or excretion of a drug, resulting in altered effects. Herbs that alter gastrointestinal function, for example, can affect drug absorption, as can those that induce or inhibit metabolic enzymes and transport proteins. Many other herbs have been identified as substrates, inducers, and/or inhibitors of the liver’s cytochrome P450 enzyme system, which is extensively involved in drug metabolism, and can thus affect the clearance of drugs. St. John’s wort, popularly used for the treatment of depression, may reduce levels of antiretroviral agents and immunosuppressants, and can increase the neurotransmitter serotonin, thus making it dangerous to combine with drugs that affect serotonin levels, such as selective serotonin reuptake inhibitor (SSRI) antidepressants.

Many of these interactions could be prevented simply by taking the drug so many hours before or after the supplement, for instance. Furthermore, not all interactions are bad. Thus, combining dietary supplements and prescription drugs should not necessarily be discouraged, but clinicians need to be aware of the potential risks. Indeed, more than 75 percent of doctors and nurses now seek information on complementary and alternative medicine each year, and these numbers are growing. Given this burgeoning interest, it is important to develop and maintain credible resources on herbs and supplements, such as the Natural Standard Research Collaboration, of which I am cofounder. On our website (www.naturalstandard.com), clinicians can access databases and charts guiding appropriate dosage, as well as details about potential concerns. The collaboration also offers continuing medical-education courses dedicated to integrative medicine.

Until health-care providers begin to be more aware of potential drug-herb interactions and begin to utilize the resources available to them to avoid damaging side effects, dietary supplement-drug mixology will remain problematic.

**Catherine Ulbricht is a senior attending pharmacist at Massachusetts General Hospital, and editor-in-chief of both the Journal of Dietary Supplements and Natural Standard.**

**Comment:**

**Melissa Nguyen**

Health care providers should ALWAYS inquire about non-prescription medications when taking patient histories, and give examples of some herbal medications that a patient may neglect to mention, ie. ginseng and garlic. Because some of these herbal remedies are commonly used in various cuisines, patients may not perceive them dangerous.

**Hari Behl**

Admittedly, it is important for the doctor or pharmacist to know what dietary supplements are being taken along with prescription drug. Herbal dietary supplements are relatively safe (as compared to drugs) even if taken along with drugs (mixology as referred to in the article) is a fact that often never boldly accepted. The potency of actives of herbal dietary supplements is never life threatening. It is an interesting market where Consumer is convinced because of their traditional use despite resistance from the drug industry or those who have a bias tilt to the other side. While cancer cannot be cured by herbs, flu, cold and digestion disorders do not need drugs. If food cooked with turmeric, black pepper, basil, garlic and ginger is safe to be taken along with drugs, how can all DS be blamed for threatening life! Consumers need to be educated not threatened.

**Divya Chowdary**

It is a common practice in many countries to try an alternative medicine as their first treatment before seeking medical help. This makes it difficult for practitioners to analyze the effects of the herb or supplement with the current regimen. Tools from Natural Standard can help minimize errors and aid practitioners to follow good medical practice.

**rxman123**

Based on the flawed perception that herbal and naturals are free of side effects appears to be common hope with its users. Taking both herbs and prescription medications points to real and consistent risks of serious life threatening events. Let’s not forget the situation with Seldane and Grapefruit inhibiting the enzymatic conversion of a Seldane metabolite that caused cardiac arrhythmias and several deaths.

The information regarding serotonin enhancements with St. Johns wort if combine with SSRIs . we must remember that so many of the modern pharmaceuticals had their genesis from natural products – Aspirin, Ace inhibitors, Cardiac, Oncology drugs. As a society we are led to believe that potency comes from the pharmaceutical manufacturer.

**ChristineDavis2613**

With such an increasing number of people taking alternative supplements it is so important that people understand that what they are taking is a drug. It can have harmful effects on your body that the manufactures may not tell you or know about. In addition to doing your own research at Natural Standard or other websites, talking to a health care worker about any new supplement could save you from serious side effects or interactions. Always better safe than sorry in matters of your health!
Munching Macrophages
Making macrophages in atherosclerotic plaques digest spent organelles instead of dying may help keep plaques stable.
By Sabrina Richards | July 1, 2012

MACROPHAGE AUTOPHagy: The autophagosomes within macrophages helps keep atherosclerotic plaques from bursting and causing blood clots. Kristy Brown and Ying Wang, Columbia University

The paper

The finding
Atherosclerotic plaques can prompt heart attacks and strokes when lipid-containing macrophages inside them die and necrose, causing the plaque to rupture and clog blood vessels. Nudging macrophages to respond to stress by breaking down their own organelles—a process called autophagy—can keep plaques stable by reducing macrophage death and by making it easier for phagocytes to recognize and remove those that do die. This study is the first to connect autophagy in macrophages to protection against disease progression, says Rick Austin of the Thrombosis and Atherosclerosis Research Institute in Hamilton, Ontario, Canada, who was not involved in the research.

The hint
Although previous studies had shown that some cells in plaques were undergoing autophagy, it wasn’t clear which ones or whether it was an important process in the progression toward outcomes such as heart attack and stroke, says Ira Tabas, whose lab at Columbia University in New York City conducted the research.

The McDonald’s diet
When researchers fed mice predisposed to atherosclerosis a high-fat diet and also blocked autophagy, macrophages died at a higher rate and were not efficiently cleared by phagocytes, suggesting that autophagy may be protective against plaque rupture.

The balancing act
Surprisingly, the same plaque components that cause macrophage death, such as oxidized phospholipids and free cholesterol, can also promote autophagy, suggesting a balance between detrimental and autophagy-inducing signals, says Austin.
**Bacterial Exploitation**

Field studies reveal non-virulent bacteria take advantage of their virulent counterparts to get a free pass into their host.

*By Ruth Williams | July 5, 2012*

Toxin-producing strains of *Bacillus thuringiensis* bacteria are virulent pathogens of cabbage-eating caterpillars. But strains that don’t produce toxins are also able to infect the insects, by piggy-backing on the hard work of their toxin-producing brethren. A paper published today (July 5) in *Science* describes the first field observations of this freeloadering behavior and the relationship dynamics between the exploiting and exploited bacteria—information that could lead to more effective caterpillar-killing biopesticide approaches, and that might even have implications for human health.

“It is really interesting and important because it shows for the first time in a natural host-pathogen system that social interaction is important for understanding the evolution of pathogen virulence,” said Jeff Smith of Washington University in Saint Louis, who did not participate in the study. “It also shows that bacterial pathogens have problems with cheating.”

Bacterial pathogens release all manner of chemicals that can improve their food acquisition, virulence, immune cell evasion, and so forth. But in many cases, laboratory experiments have shown that not all of the bacteria produce the chemicals—some just reap the benefits of those produced by others in the population.

“There was some skepticism in the microbial community about how important this [phenomenon] is in a natural environment,” said Ben Raymond, an evolutionary ecologist at Royal Holloway University in London. So, Raymond and his team decided to take their experiments out into the field—literally.

They planted 204 cabbages at a farm near Oxford, United Kingdom, added 35 diamondback moth larvae to each, and sprayed the plants with fine mists of *B. thuringiensis* bacteria that differed in the proportion of virulent to non-virulent strains. For 8 weeks, the researchers analyzed the bacterial populations on the plants, and found that when the virulent strain was uncommon, the non-virulent cheaters were less capable of infecting the caterpillars, and the relative abundance of non-virulent strains decreased over time. The team also exposed caterpillars to the bacteria in the lab, observed the infections, and found that with lower proportions of virulent bacteria, fewer caterpillars died.

The results point to a clear risk of cheating: if there aren’t enough virulent bacteria around to produce the necessary toxins, the cheaters will perish without infecting a host. On the other hand, it is metabolically costly to produce toxins, explained Raymond, as evidenced by slower growth rates of virulent strains. As a result, once the bacteria get inside a host, the cheaters dominate. Indeed, when the bacterial mists contained a higher proportion of the virulent strain, the cheaters tended to thrive and out-competed the virulent bacteria.

“The way that toxin producers and non-producers compete in the field implies a stable co-existence,” said Raymond. This is precisely what the previous laboratory experiments have suggested. “The predictions of the theory, basically, held up in a field situation, with cabbages and dirt and all the noise and chaos,” he said.

*B. thuringiensis* are used by some farmers as an environmentally-friendly biological insecticide. Thus, the results suggest “that you have to be really careful about not including the non-toxin producing kind, because those could mitigate the effects of biocontrol,” said Smith. As the non-virulent bacteria outcompete the toxin-producing strains in the host, the population is less able to infect more pests. “Having just a little bit of the non-producing strain could have an outsized detrimental effect,” Smith said.

The findings may also have implications for human diseases, added Sam Brown of the University of Edinburgh in the U.K., who was not involved in the study. Many bacteria that are pathogenic in humans exhibit similar toxin sharing behavior. “If we can understand the social interactions between toxin producers and non-producers, this can open new therapeutic angles,” he said.

Brown is researching whether bacterial cheaters could be used as “a therapy of last resort” in people infected with antibiotic-resistant bacteria, for example. If researchers can genetically engineer bacterial strains to be stripped of all their virulence factors, they may be able to outcompete the virulent antibiotic-resistant strains in patients. People are dying from such infections everyday, he said, “so we need to start thinking of more off-the-wall treatment ideas.”

**ART Reduces Inflammation Overall, but Tenofovir and Abacavir Have Different Effects**

Published on Friday, 06 July 2012 00:00  
Written by Liz Highleyman

Some inflammation-associated biomarkers decreased among people with HIV who started combination antiretroviral therapy (ART) regardless of regimen, but others—including CRP and IL-6—were affected differently by different drug regimens, researchers reported in the **July 17, 2012, issue of AIDS**.

Ever since the **SMART treatment interruption trial** showed in 2006 that HIV positive people who stop ART have a higher risk of non-AIDS cardiovascular, liver, and kidney disease, scientists have extensively studied how HIV and its treatment can influence chronic inflammation.

Some researchers have suggested that inflammation might help explain the greater risk of heart attacks among people taking abacavir (Ziagen, also in the Epzicom coformulation) that has **seen in some—but far from all—studies**.

Grace McComsey from Case Western Reserve University and colleagues looked at the effects of specific antiretroviral drugs on inflammation biomarkers in **ACTG A5224**, a sub-study of the larger **A5202 trial**.

In A5202, previously untreated HIV positive participants were randomly assigned to receive abacavir/lamivudine (the drugs in Epzicom) or tenofovir/emtricitabine (the drugs in Truvada), both with either open-label efavirenz (Sustiva) or ritonavir-boosted atazanavir (Reyataz).

This sub-analysis included 244 participants. Most (85%) were men, about half were white, and median age was 39 years. At baseline the median HIV RNA level was 4.6 log copies/mL and the median CD4 T-cell count was 240 cells/mm³.

The sub-study compared changes in the abacavir/lamivudine and tenofovir/emtricitabine arms from baseline to weeks 24 and 96 in various markers associated with inflammation, coagulation (blood clotting), and immune response, including tumor necrosis factor-alpha (TNF-alpha), soluble tumor necrosis factor receptor (sTNFR) I and II, soluble vascular cell adhesion molecule 1 (sVCAM-1), solubleintercellular adhesion molecule 1 (sICAM-1), high-sensitivityC-reactive protein (hsCRP), and interleukin 6 (IL-6).

**Results**

- Levels of TNF-alpha, sTNFR-, sTNFR-II, sVCAM-1, and sICAM-1 decreased significantly at weeks 24 and 96 across the board, with no significant differences between drug regimens.
- At week 24, people taking abacavir/lamivudine had a greater mean increase in hsCRP levels compared with those taking tenofovir/emtricitabine (1.43 vs 0.88, respectively); similar results were seen at week 96.
- At week 24, people taking efavirenz had a greater mean rise in hsCRP than those taking boosted atazanavir (1.41 vs 0.88, respectively), though the difference was no longer significant at week 96.
- At week 24, IL-6 levels decreased significantly among patients taking tenofovir/emtricitabine, but not among those taking abacavir/lamivudine; by week 96, IL-6 had decreased significantly in people taking both NRTI combinations.
- IL-6 changes were not significantly different between participants taking efavirenz or boosted atazanavir at either time point.
  
  "Soluble TNF-receptors and adhesion molecules decreased following treatment initiation and did not differ by regimen," the study authors concluded. "Differences were seen on hsCRP and IL-6 changes with [abacavir/lamivudine] vs [tenofovir/emtricitabine] and on hsCRP with efavirenz vs [atazanavir/ritonavir]."

  "Our study supports the findings of earlier studies that initiation of effective ART results in an overall decrease in inflammation markers, with the exception of hsCRP, which remains unchanged or even increases," they elaborated in their discussion. "The reason hsCRP behaves differently from other inflammation markers remains elusive, with one potential explanation being HIV-associated subclinical hepatocyte [liver cell] dysfunction, as CRP is mainly produced by hepatocytes in response to IL-6."

  The study was too small to meaningfully compare clinical outcomes, but the 2 participants who had myocardial infarctions were taking tenofovir/emtricitabine plus efavirenz, which some experts assume to be the more heart-healthy regimen.  

**Reference**

H1N1 vaccine associated with small but significant risk of Guillain-Barre syndrome

Guillain-Barre syndrome (GBS) is usually characterized by rapidly developing motor weakness and areflexia (the absence of reflexes). "The disease is thought to be autoimmune and triggered by a stimulus of external origin. In 1976-1977, an unusually high rate of GBS was identified in the United States following the administration of inactivated 'swine' influenza A(H1N1) vaccines. In 2003, the Institute of Medicine (IOM) concluded that the evidence favored acceptance of a causal relationship between the 1976 swine influenza vaccines and GBS in adults. Studies of seasonal influenza vaccines administered in subsequent years have found small or no increased risk," according to background information in the article. "In a more recent assessment of epidemiologic studies on seasonal influenza vaccines, experimental studies in animals, and case reports in humans, the IOM Committee to Review Adverse Effects of Vaccines concluded that the evidence was inadequate to accept or reject a causal relationship."

Philippe De Wals, M.D., Ph.D., of Laval University, Quebec City, Canada and colleagues conducted a study to assess the risk of GBS following pandemic influenza vaccine administration. In fall 2009 in Quebec an immunization campaign was launched against the 2009 influenza A(H1N1) pandemic strain. By the end of the year, 4.4 million residents had been vaccinated. The study included follow-up over the 6-month period of October 2009 through March 2010 for suspected and confirmed GBS cases reported by physicians, mostly neurologists, during active surveillance or identified in the provincial hospital summary discharge database. Immunization status was verified.

Over the 6-month period, 83 confirmed GBS cases were identified. Twenty-five confirmed cases had been vaccinated against 2009 influenza A(H1N1) 8 or fewer weeks before disease onset, with most (19/25) vaccinated 4 or fewer weeks before onset. Analysis of data indicated a small but significant risk of GBS following influenza A(H1N1) vaccination. The number of cases attributable to vaccination was approximately 2 per 1 million doses. The excess risk was observed only in persons 50 years of age or older.

"In Quebec, the individual risk of hospitalization following a documented influenza A(H1N1) infection was 1 per 2,500 and the risk of death was 1/73,000. The H1N1 vaccine was very effective in preventing infections and complications. It is likely that the benefits of immunization outweigh the risks," the authors write.


Editorial: Influenza Pandemics—Pregnancy, Pathogenesis, and Perinatal Outcomes

In an accompanying editorial, Mark C. Steinhoff, M.D., of the Cincinnati Children's Hospital Medical Center, and Noni E. MacDonald, M.D., M.Sc., F.R.C.P.C., of Dalhousie University, Halifax, Nova Scotia, Canada, write that "taken together, these studies partially assuage concerns about safety of adjuvanted pandemic influenza vaccines during pregnancy."

"However, more studies are needed examining other types of vaccine adjuvants. In addition, observational studies of vaccines are limited by biases, including selection bias, as well as confounding by indication. Thus, future studies with improved statistical designs including prospective follow-up studies using virological end points with adjustments for selection, seasonality, and other biases are needed to confirm these data." (JAMA. 2012;308(2):184-185. Available pre-embargo to the media at http://media.jamanetwork.com)

Potential Cause of HIV-Associated Dementia Revealed

ScienceDaily (July 10, 2012) — Researchers at Georgetown University Medical Center appear to have solved the mystery of why some patients infected with HIV, who are using antiretroviral therapy and show no signs of AIDS, develop serious depression as well as profound problems with memory, learning, and motor function. The finding might also provide a way to test people with HIV to determine their risk for developing dementia.

They say the answer, published in the July 11 issue of the Journal of Neuroscience, may ultimately lead to a therapeutic solution that helps these patients as well as others suffering from brain ailments that appear to develop through the same pathway, including those that occur in the aged.

"We believe we have discovered a general mechanism of neuronal decline that even explains what happens in some elderly folks," says the study's lead investigator, Italo Mocchetti, Ph.D., professor and vice chair of the department of neuroscience at Georgetown University Medical Center. "The HIV-infected patients who develop this syndrome are usually quite young, but their brains act old."

The research team found that even though HIV does not infect neurons, it tries to stop the brain from producing a protein growth factor—mature brain derived neurotrophic factor (mature BDNF)—that Mocchetti says acts like "food" for brain neurons. Reduced mature BDNF results in the shortening of the axons and their branches that neurons use to connect to each other, and when they lose this communication, the neurons die.
"The loss of neurons and their connections is profound in these patients," Mocchetti says. HIV-associated dementia occurs in two to three percent of HIV-infected patients using retroviral therapies, all of who appear to be otherwise healthy, and in 30 percent of HIV-positive patients who are not on medication.

Mocchetti believes that HIV stops production of mature BDNF because that protein interferes with the ability of the virus to attack other brain cells. It does this through the potent gp120 envelope protein that sticks out from the viral shell—the same protein that hooks on to brain macrophages and microglial cells to infect them. "In earlier experiments, when we dumped gp120 into neuronal tissue culture, there was a 30-40 percent loss of neurons overnight. That makes gp120 a remarkable neurotoxin."

This study is the product of years of work that has resulted in a string of publications. It began when Mocchetti and his colleagues were given a grant from the National Institutes on Drug Abuse to determine whether there was a connection between the use of cocaine and morphine, and dementia. (A substantial number of HIV-positive patients have been or currently are intravenous drugs users.)

They found that it was the virus that was responsible for the dementia, not the drugs, and so they set out to discover how the virus was altering neuronal function.

Their scientific break came when the researchers were able to study the blood of 130 women who were enrolled in the 17 year-old, nationwide WIHS (Women's Interagency HIV Study, directed at Georgetown by Mary Young, M.D.), which has focused on the effects of HIV in infected females. In one seminal discovery, Mocchetti and colleagues found that when there was less BDNF in the blood, patients were at risk of developing brain abnormalities. He published this finding in 2011 in the May 15 issue of AIDS.

In this study, Mocchetti, Alessia Bachis, Ph.D., and their colleagues studied the brains of HIV-positive patients who had died, and who had developed HIV-associated dementia. They also found that neurons had shrunk, and that mature BDNF had substantially decreased.

He and his colleagues then worked out the mechanism responsible for this destruction of neurons. Normally, neurons release a long form of BDNF known as proBDNF, and then certain enzymes, including one called furin, cleave proBDNF to produce mature BDNF, which then nurtures brain neurons. When uncut, proBDNF is toxic, leading to "synaptic simplification," or the shortening of axons. It does this by binding to a receptor, p75NTR, that contains a death domain.

"HIV interferes with that normal process of cleaving proBDNF, resulting in neurons primarily secreting a toxic form of BDNF," Mocchetti says. The same imbalance between mature BDNF and proBDNF occurs as we age, he says, although no one knows how that happens. "The link between depression and lack of mature BDNF is also known, as is the link to issues of learning and memory. That's why I say HIV-associated dementia resembles the aging brain."

Loss of mature BDNF has also been suggested to be a risk factor in chronic diseases such as Parkinson's and Huntington's diseases, Mocchetti says.

The findings suggest a possible therapeutic intervention, he adds. "One way would be to use a small molecule to block the p75NTR receptor that proBDNF uses to kill neurons. A small molecule like that could get through the blood-brain barrier."

"If this works in HIV-dementia, it may also work in other brain issues caused by proBDNF, such as aging," Mocchetti adds.

The finding also suggests that measuring proBDNF in HIV-positive patients may provide a biomarker of risk for development of dementia, he adds.

"This finding is extremely important for both basic scientists and physicians, because it suggests a new avenue to understand, and treat, a fairly widespread cause of dementia," Mocchetti says.

**Should GB virus be used as a therapeutic vaccine to slow HIV progression?**

Michael Carter
Published: 12 July 2012

Acquisition of GB virus C (GBV-C) is associated with a 78% reduction in mortality risk for people with AIDS, investigators report in the online edition of *Clinical Infectious Diseases*. The beneficial effects of GBV-C infection (often incorrectly called hepatitis G virus) were significant even after controlling for CD4 cell count, HIV viral load and use of antiretroviral therapy, leading an expert in infectious diseases to ask whether GB virus C ought to be considered as a therapeutic vaccine.

Dr David Gretch, the author of an editorial accompanying the study, believes the results settle any doubts about the protective effects of GBV-C virus infection in HIV disease. He comments: “GBV-C viremia is associated with protective effects in persons with HIV, and the idea of a therapeutic GBV-C
biovaccine in persons with HIV is an important one to consider, especially in resource poor countries where AIDS death rates remain high.”

GBV-C is a non-dangerous infection that is transmitted in similar ways to HIV. An earlier meta-analysis of studies involving 1294 HIV-positive people showed that co-infection with GBV-C was associated with a 59% reduction in the relative risk of death. This finding is consistent with laboratory research which indicate that GBV-C induces cytokines that inhibit HIV, lower T-cell activation, block IL-2 mediated CD4 T-cell proliferation and reduce expression of the HIV entry receptors, CCR5 and CXCR4.

Despite these results, many doctors remain unconvinced of the beneficial effects of infection with this virus. In particular, the risk of incident GBV-C infection on disease progression in people already infected with HIV is uncertain.

Investigators from the Viral Activation Transfusion Study (VATS) believed that their cohort of people with advanced HIV disease provided an ideal population in which to examine the impact of prevalent and incident GBV-C infection on all-cause mortality.

Blood samples obtained from the study participants before and after blood transfusion, were retrospectively tested for GBV-C antibodies and RNA. The investigators were therefore able to accurately determine whether participants were infected with this virus at baseline, or acquired the infection during follow-up. The study was conducted in 1996-97, shortly after the introduction of effective antiretroviral therapy.

A total of 489 people, all of whom had AIDS, were included in the authors’ analyses. At baseline, 60% of people were negative for both GBV-C antibodies and RNA, 33% had antibodies to the infection and 7% had detectable GBV-C RNA. Participants were followed for a median of 8.4 months. A total of 67 people (55%) died and a further 9% withdrew from the study or were lost to follow-up.

Survival was significantly better for people with detectable GBV-C at baseline compared to those who did not (p = 0.02). The association between GBV-C viremia and improved survival remained significant after adjusting for several important baseline characteristics associated with outcomes in people with HIV, including CD4 cell count, HIV viral load and use of combination antiretroviral therapy (adjusted HR = 0.42; 95% CI, 0.24-0.73).

A total of 39 people (13%) acquired GBV-C infection during the course of the study. Even after adjusting for other prognostic factors, people with GBV-C RNA after incident infection had a substantial reduction in their mortality risk (adjusted HR = 0.22; 95% CI, 0.08-0.58) compared to the people who remained GBV-C RNA-negative.

“We found that GBV-C viremia is associated with lower mortality in HIV-infected patients, after adjusting for baseline HIV viral load, CD4 count and HAART status,” write the investigators. “In addition, we found a significant reduction in mortality associated with incident GBV-C infection during follow-up…even after controlling for time-updated HIV disease markers.”

Dr Gretch believes that the study’s findings are potentially of huge significance.

He stresses that GBV-C is so safe in humans that blood donations in the US are not screened for its presence, and also notes that the virus is “a natural bio-antagonist for HIV.”

“Still today,” he concludes, “the death rate from HIV remains enormous, especially in resource poor countries, and we have yet to see a trial of GBV bio-vaccination in HIV-infected populations with high death risk…it’s time for an interventional GBV biotherapy therapy study in persons with life-threatening HIV infections.”

References

HIV drug reduces graft-vs.-host disease in bone marrow transplant patients, Penn study shows
New approach redirects new immune cells from harming vital organs, without dangers of immunosuppression

PHILADELPHIA—An HIV drug that redirects immune cell traffic significantly reduces the incidence of a dangerous complication that often follows bone marrow transplants for blood cancer patients, according to research from the Perelman School of Medicine at the University of Pennsylvania that will be published today in the New England Journal of Medicine. The findings represent a new tactic for the prevention of graft-versus-host disease (GvHD), which afflicts up to 70 percent of transplant patients and is a leading cause of deaths associated with the treatment.
Allogeneic bone marrow transplantation – also known as stem cell transplantation – involve the transfusion of a matched donor's blood stem cells to rebuild the patient's bone marrow after treatment has eliminated both the defective blood cells associated with their cancer and their healthy blood cells.

"It appears that our new approach allows us to prevent some patients from developing GvHD by redirecting immune cells away from certain sensitive organs that they could harm," says lead author Ran Reshef, MD, an assistant professor in the division of Hematology-Oncology and a member of the Hematologic Malignancies Research Program at Penn's Abramson Cancer Center. "This is a novel way for us to try to decrease treatment-related complications among bone marrow transplant patients without also reducing their new immune system's ability to attack their cancer."

Typically, patients receive immunosuppressive drugs following their transplant to lower the risk of developing graft-versus-host disease (GvHD), which occurs when the newly transplanted immune cells attack healthy tissue they perceive as foreign. But since patients' own immune systems must be wiped out in order to receive their transplants, those drugs leave patients even more vulnerable to life-threatening infections and to a relapse of their cancer. The Penn team found that treatment with the HIV drug maraviroc dramatically reduced the incidence of GvHD in organs where it is most dangerous – the liver and gut—without compromising any other function of the immune system.

The findings, which involved repurposing maraviroc—approved for HIV treatment in 2007—could represent a breakthrough for prevention of GvHD. Reshef and his co-authors showed that the drug is safe in BMT patients who receive stem cells from a healthy donor, and that a brief course of the drug led to a 73 percent reduction in severe forms of GvHD in the first six months after transplant, compared with the incidence rate typically seen in similar patients who do not receive maraviroc.

"Just like in real estate, immune responses are all about location, location, location," Reshef says. "Cells of the immune system don't move around the body in a random way. There is a synchronized and well orchestrated process whereby cells express particular receptors on their surface that allow them to respond to small proteins called chemokines, which direct the immune cells to specific organs where they are needed—or in the case of GvHD, to where they cause damage. We're using maraviroc, which was initially designed to prevent certain types of HIV from entering healthy cells in the body, as a traffic signal to direct the donor's immune cells away from those places in the body where they might cause GvHD."

Thirty-eight patients with blood cancers, including acute myeloid leukemia, myelodysplastic syndrome, lymphoma, myelofibrosis and others, were enrolled in the trial. All patients received the standard GvHD prevention drugs tacrolimus and methotrexate, plus a 33-day course of maraviroc that began two days before transplant. In the first 100 days after transplant, none of the patients treated with maraviroc developed GvHD in the gut or liver, which are the most severe forms of the illness. At six months, only six percent of patients treated with maraviroc had severe graft-versus-host disease, only three percent had it in their liver, and nine percent had it in their gut. Among similar patients who receive standard drugs without maraviroc, rates of severe GvHD six months after transplant are 22 percent, with liver and gut involvement seen in 15 and 27 percent of patients, respectively. At one year, the benefit of maraviroc appeared to be partially sustained, with a cumulative incidence of severe GvHD of only 15 percent, as opposed to 29 percent in patients who receive standard therapy.

Based on these data, the research team plans to try a longer treatment regimen with maraviroc in future studies, to see if they could prolong the protective effect.

The differential impact of maraviroc on the liver and gut indicates that the drug is working as expected, by limiting the movement of immune cells called T lymphocytes to specific organs in the body. Maraviroc works by blocking the CCR5 receptor on lymphocytes, preventing the cells from trafficking to certain organs. The researchers saw no effect on skin GvHD, so they theorize that the CCR5 receptor might be more important for recruiting lymphocytes into the liver and the gut than for the skin.

Maraviroc treatment did not appear to increase treatment-related toxicities in these patients, nor did it alter the relapse rate of their underlying disease or risk of infection, and it did not slow the amount of time it took for patients' new immune systems to engraft in their bodies.

Controlling Inflammatory and Immune Responses
ScienceDaily (July 12, 2012) — Researchers at the IRCM, led by geneticist Dr. Jacques Drouin, recently defined the interaction between two essential proteins that control inflammation. This important breakthrough will be published in the July 13 print edition of the scientific journal Molecular Cell.
IRCM scientists study glucocorticoids, a class of steroid hormones that suppress the immune system and reduce inflammation. They are used in medicine to treat diseases such as allergies, asthma, and autoimmune diseases.

"In molecular biology and genetics, proteins known as transcription factors bind to DNA in order to control the expression (or transcription) of genetic information," explains Dr. Drouin, Director of the Molecular Genetics research unit at the IRCM. "Our work defined the genome-wide interaction between two transcription factors: Stat3 and the glucocorticoid receptor (GR)."

While Stat3 acts on pro-inflammatory gene targets, glucocorticoids are widely used for their anti-inflammatory properties and their receptor, GR, interacts with Stat3 to control these actions. GR can be found in almost every cell in the body and regulates genes that control development, metabolism, and inflammatory and immune response.

Transcription factors can control the flow of information alone or along with other proteins, either by promoting (as an activator) or blocking (as a repressor) the recruitment of enzymes required for the expression of specific genes. Transcription factors can bind directly to DNA or attach themselves to another DNA-bound protein.

"In some cases, the proteins will behave differently depending on how they are connected to a DNA sequence," says David Langlais, former doctoral student in Dr. Drouin’s laboratory and first author of the article. "We were interested in understanding why some transcription factors could act as activators when bound directly to DNA, but act as repressors if they are recruited by another protein. The molecular basis for this dual action remained unclear until now."

Journal Reference:

Hyperthermia Stimulates HIV-1 Replication

Abstract

HIV-infected individuals may experience fever episodes. Fever is an elevation of the body temperature accompanied by inflammation. It is usually beneficial for the host through enhancement of immunological defenses. In cultures, transient non-physiological heat shock (42–45°C) and Heat Shock Proteins (HSPs) modulate HIV-1 replication, through poorly defined mechanisms. The effect of physiological hyperthermia (38–40°C) on HIV-1 infection has not been extensively investigated. Here, we show that culturing primary CD4+ T lymphocytes and cell lines at a fever-like temperature (39.5°C) increased the efficiency of HIV-1 replication by 2 to 7 fold. Hyperthermia did not facilitate viral entry nor reverse transcription, but increased Tat transactivation of the LTR viral promoter. Hyperthermia also boosted HIV-1 reactivation in a model of latently-infected cells. By imaging HIV-1 transcription, we further show that Hsp90 co-localized with actively transcribing provirus, and this phenomenon was enhanced at 39.5°C. The Hsp90 inhibitor 17-AAG abrogated the increase of HIV-1 replication in hyperthermic cells. Altogether, our results indicate that fever may directly stimulate HIV-1 replication, in a process involving Hsp90 and facilitation of Tat-mediated LTR activity.

Author Summary

Fever is a complex reaction triggered in response to pathogen infection. It induces diverse effects on the human body and especially on the immune system. The functions of immune cells are positively affected by fever, helping them to fight infection. Fever consists in a physiological elevation of temperature and in inflammation. While the role of inflammatory molecules on HIV-1 replication has been widely studied, little is known about the direct effect of temperature on viral replication. Here, we report that hyperthermia (39.5°C) boosts HIV-1 replication in CD4+ T cells. In single-cycle infection experiments, hyperthermia increased HIV-1 infection up to 7-fold. This effect was mediated in part by an increased activation of the HIV-1 promoter by the viral protein Tat. Our results also indicate that hyperthermia may help HIV-1 to reactivate from latency. We also show that the Heat Shock Protein Hsp90, which levels are increased at 39.5°C, mediates in a large part the positive effect of hyperthermia on HIV-1 infection. Our work suggests that in HIV-1-infected patients, fever episodes may facilitate viral replication.
Discussion

We report here a positive impact of hyperthermia on HIV-1 replication. Hyperthermia is known to enhance the functions of immune cells and to confer protection against pathogen infection [1], [2], [4], [5]. Previous studies on temperature and HIV-1 mostly focused on chronically infected cell lines [31], [32] or used non-physiological heat shock treatment to study viral reactivation from latency (a few minutes at 42–45°C [33]). Here, we report that elevation of temperature to fever-like levels (39.5°C) stimulates HIV-1 replication in primary CD4+ T lymphocytes as well as in Hela and Jurkat cell lines. In single-cycle infection assays, hyperthermia increased HIV-1 infection by 2 to 7 fold. This stimulation was apparently not due to unspecific alterations of cellular metabolism, since cell growth, viability, or surface levels of various molecules were not significantly affected by hyperthermia.

To get insight into how hyperthermia stimulates HIV-1 replication, we compared the efficiency of various steps of the viral life cycle at 37°C and 39.5°C. Viral entry and fusion, measured by the Vpr-β-
lactamase assay, were similar at the two temperatures. Enzymes have a range of conditions of pH, salt concentration, and temperature, in which they display optimal activity. We did not observe an effect of hyperthermia on reverse transcriptase, as both in vitro catalytic activity of the enzyme and the levels of viral DNA synthesis in infected cells were unchanged by temperature. We then examined the influence of temperature on the viral transcription step. Hyperthermia did not induce basal LTR activity without Tat. However, in the presence of Tat, hyperthermia lead to a significantly better transactivation of the LTR. This is in line with earlier reports, demonstrating that a transient heat shock at 42°C increases HIV-1 transcription in monocytic cells lines [31], [32]. Noteworthy, the activity of the CMV promoter was not increased at 39.5°C (not shown), suggesting that hyperthermia does not trigger a global increase of cellular transcription. Accordingly, the steady state levels of several cellular proteins (actin, CD4, ICAM-1, MHC-I, etc.) were apparently similar at normal and elevated temperatures.

To characterize the molecular mechanism by which hyperthermia up-regulates HIV-1 infection and transcription, we examined the role of Hsp90. This protein exerts diverse functions in normal and stressed cells, through its ATPase activity and its protein binding domain [72], [73]. It acts as a chaperone for many cellular proteins. Hsp90 assists folding, assembly, intracellular transport, maintenance and degradation of proteins, and regulates cell signaling and cell cycle [74], [75]. Hsp90 is involved in HIV-1 infection at 37°C, regulating viral gene expression [48]. Hsp90 also impacts the replication of other viral species, such as Human Cytomegalovirus, Influenza Virus, Flock House Virus and Hepatitis C Virus [76], [77], [78], [79]. We show here that the levels of Hsp90 are augmented at 39.5°C, in primary lymphocytes and other cells (Fig. 2 and not shown). By using an immunofluorescence technique allowing the visualization of nascent viral RNA in living cells, we demonstrate that, in presence of Tat, Hsp90 can be found in the nucleus, at HIV-1 transcription sites. This localization was rather infrequent at 37°C, but was significantly increased at 39.5°C (27% and 70% co-localization, respectively). Furthermore, 17-AAG, a pharmacological inhibitor of Hsp90, reversed the stimulating effect of hyperthermia on single-cycle infection in P4C5 cells. Altogether, these results point out for a previously uncharacterized role of Hsp90, facilitating HIV-1 transcription and replication at 39.5°C. It will be worth further dissecting how Hsp90 acts on viral transcription at this temperature. One can speculate that the chaperone protein may bind more efficiently to the P-TEFb/Tat/TAR transcription complex [67] and thus increase its activity, and/or may enhance chromatin modeling and accessibility to the viral promoter [48].

Mechanisms regulating HIV-1 gene expression are also involved in viral reactivation from latency [80]. We show here that the conditioned medium from PBMCs induced viral reactivation, in the J-Lat 10.6 model of latently infected T cells. Strikingly, reactivation was more pronounced at 39.5°C than at 37°C. Futures studies will help understanding which cytokines or other molecules produced by PBMCs mediate this effect. For instance, heat shock at 42°C is known to act in synergy with IL-6 to induce viral reactivation in a latently infected monocytic cell-line [31]. It will be of interest to compare the stimulating effect of IL-6 and other cytokines, at normal and fever-like temperatures, not only in J-Lat cells, but also in other models of viral latency (PBMCs from HAART-treated patients, or latently-infected, resting primary CD4+ T cells [81]).

In this study, we have focused our analysis of the effect of temperature on a few key steps of the viral life cycle. We demonstrate that hyperthermia globally facilitates viral replication. At 39.5°C, viral entry, fusion and reverse transcription occur normally, whereas Tat-mediated transactivation of the LTR is significantly more efficient. It has been previously reported that the activity of HIV-1 integrase and protease is not increased at 39.5°C [35]. This does not rule out the possibility that other steps of HIV-1 infection (nuclear import, selection of integration sites in the cellular genome, viral translation, assembly, release, etc.) might be positively or negatively modified at a fever-like temperature.

What is the physiological relevance of our observations? Patients treated with HAART and with controlled viremia can experience transient bursts of HIV-1 replication termed viral blips [82]. Furthermore, co-infections are frequent in HIV-1-positive individuals and are often associated with fever and acute illnesses [83]. For instance, Plasmodium falciparum, the causative agent of malaria, induces recurrent, strong episodes of fever lasting 2–3 days, which correlates with increased viral loads [84]. The origin of these viral blips, or of other more pronounced viral rebounds is likely multi-factorial. Our results suggest that fever may directly stimulate viral replication or reactivation from latent reservoirs, in association with other inflammatory or immunological events.
**New Proteins to Clear the Airways in Cystic Fibrosis and COPD**

ScienceDaily (July 13, 2012) — University of North Carolina scientists have uncovered a new strategy that may one day help people with cystic fibrosis and chronic obstructive pulmonary disorder better clear the thick and sticky mucus that clogs their lungs and leads to life-threatening infections.

In a new report appearing online in The *FASEB Journal*, researchers show that the "SPLUNC1" protein and its derivative peptides may be able to help thin this thick mucus by affecting the epithelial sodium channel (ENaC). Not only does this research have implications for cystic fibrosis and COPD, but it also enhances the understanding of hypertension due to the role it also plays in controlling blood pressure.

"We hope that this study will pave the way for a new class of peptide-based channel inhibitors that can help reverse the mucus dehydration seen in Cystic Fibrosis and COPD," said Robert Tarran, Ph.D., a researcher involved in the work from the Cystic Fibrosis/Pulmonary Research and Treatment Center at the University of North Carolina in Chapel Hill. "This would help restore mucus clearance and kick-start the lung's ability to clear unwanted pathogens."

To identify which part of SPLUNC1 actually affects ENaC, scientists eliminated parts of the protein until it lost function. In fact, even after the eliminating 85 percent of SPLUNC1, it still affected ENaC, suggesting that the ENaC inhibitory domain was in the remaining 15 percent. Researchers then synthesized an 18-amino acid peptide of this region and tested its ability to bind to ENaC and to inhibit fluid absorption in human bronchial epithelial cells derived from people with and without cystic fibrosis. This peptide inhibited ENaC and fluid absorption in all systems tested, without affecting structurally-related ion channels. They also found that ENaC activity was affected for more than 24 hours in cystic fibrosis airway cultures, suggesting that this peptide may be therapeutically beneficial for the treatment of cystic fibrosis patients who suffer from over-active ENaC and consequently have too little lung fluid. "Breathing is something most healthy people take for granted," said Gerald Weissmann, M.D., Editor-in-Chief of The *FASEB Journal*. "However, people with cystic fibrosis and COPD battle for every breath because sticky mucus plugs their airways. This research should give scientists a new way of clearing the air for people with cystic fibrosis and COPD."

**Journal Reference:**

**Copper's Previously Unknown Exit Strategy from the Body**

ScienceDaily (July 13, 2012) — Scientists have long known that the body rids itself of excess copper and various other minerals by collecting them in the liver and excreting them through the liver's bile.

However, a new study led by Johns Hopkins researchers and published June 22 in *PLoS One* suggests that when this route is impaired there's another exit route just for copper: A molecule sequesters only that mineral and routes it from the body through urine.

The researchers, led by Svetlana Lutsenko, Ph.D., a professor of physiology at the Johns Hopkins University School of Medicine, found this additional copper escape hatch by studying an animal model of Wilson's disease, a rare disorder most often diagnosed in children. People with this disease accumulate abnormally large amounts of copper in the liver, eventually leading to liver damage and failure.

Micronutrients such as copper, zinc and iron are indispensable for human development. Copper is required for embryonic development, respiration, and cardiovascular function, among other processes; too little copper can be fatal whereas too much can cause neurological impairment and organ failure.

One diagnostic test for Wilson's disease is to check for high amounts of copper in the urine; copper levels could be especially high in advanced stages of this disorder. For decades doctors and scientists have blamed this high urinary copper on the breakdown of cells in the liver, which purportedly dumped their contents into the bloodstream as they died. These contents were thought to be picked up by the kidneys and eventually excreted in the urine.

However, Lutsenko says, this theory had never been tested. To verify this explanation, she and her colleagues examined mice genetically modified to have Wilson's disease. As in people, these animals' liver function gradually worsens over time due to copper accumulation. Eventually the animals' livers regenerate and liver function improves and with this the researchers expected to see less urinary copper. However, at this stage in the disease, urinary copper in the animal models continued to increase.

Additionally, the researchers found no increased urine concentrations of other minerals stored in liver cells, which would be expected if these cells were releasing all their entire contents and not just copper.
Together, these findings suggest that liver cell death isn’t the main source of urinary copper in Wilson’s disease.

Delving deeper, the researchers gave the mice radioactive copper, which they could trace as it made its way through the body. They found that when copper reached a certain threshold level in the liver, it was directed it to the kidneys instead. At the same time, they saw that levels of a protein used to transport copper to the liver decreased. Both of these observations strengthened the idea that another mechanism must exist to remove copper from the body.

To figure out what that mechanism might be, Lawrence Gray, a graduate student on Lutsenko’s team searched the animals’ urine to see what molecules copper might be bound to. Their pursuit turned up an unidentified molecule that they’ve temporarily named "small copper carrier," or SCC. Further tests showed that as liver function decreased, more SCC appeared in the animals’ blood, and that SCC could compete for copper with the proteins that normally transport this mineral to the liver.

"These findings all suggest that SCC indeed represents a previously unknown agent that the body uses to excrete excess copper,” Lutsenko explains.

She and her colleagues are now trying to learn more about SCC, both to identify this molecule and to determine whether it could be a unique marker that’s only present during Wilson’s disease. If so, it could save pediatric patients the pain of liver biopsy, a test often used to definitively diagnose this condition. In addition, SCC may also represent a treatment for this rare disorder. If scientists could develop a way to raise SCC concentration in the blood, Lutsenko says, it could increase copper export and prevent further harm to the liver.

**Journal Reference:**

**Drugs Used to Treat HIV Also Reduce Risk of HIV Infection, Review Suggests**
ScienceDaily (July 11, 2012) — People at high risk of HIV infection can reduce their risk of acquiring the disease by taking antiretroviral drugs, according to Cochrane researchers. In an update of a systematic review first published in 2009, the researchers found that uninfected people in relationships with HIV-infected partners, men who have sex with men and those in other high risk groups are at a lower risk of becoming infected with the virus if they regularly take drugs that are normally prescribed to treat people with HIV.

Antiretroviral therapy (ART) is the standard drug treatment for HIV in patients whose disease has progressed to a certain level. Antiretroviral drugs are also beginning to be used as prophylactics in people at high risk of acquiring the disease from sexual partners. The use of antiretroviral drugs in preventing as opposed to treating HIV infection is referred to as pre-exposure prophylaxis (PrEP). PrEP is often considered controversial, not only because uninfected people may develop resistance to the drugs and experience serious side effects such as kidney toxicity and bone density loss, but also because the idea that PrEP offers protection may encourage people to indulge in riskier sexual behaviour, thereby increasing their overall risk of HIV infection. It is therefore important to establish whether PrEP really works and what level of protection it affords.

The researchers analysed data from six trials that tested the protective effect of daily doses of the oral antiretroviral drug tenofovir disoproxil fumarate (TDF) with or without emtricitabine ( FTC), compared to a placebo or intermittent use. In total, the trials involved 9,849 people, including men who have sex with men, partners of HIV-infected people, sex workers and people who have multiple sexual partners. Data from four of the trials and a total of 8,813 people showed that giving TDF plus FTC reduces the risk of HIV infection by around half, from 37 in 1,000 to 19 in 1,000. Data from two trials and a total of 4,027 people showed that TDF alone reduces the risk of HIV infection by nearly two thirds, from 26 in 1,000 to 10 in 1,000.

"Our findings suggest that antiretroviral drugs can reduce the risk of HIV infection for people in high risk groups," said lead researcher, Charles Okwundu of the Centre for Evidence-Based Health Care at Stellenbosch University in Tygerberg, South Africa. "However, in the search for highly reliable HIV prevention strategies, it is important to determine how pre-exposure prophylaxis can best be combined with existing programmes, as no strategy is likely to be 100 per cent effective."

Those taking antiretroviral drugs did not suffer more adverse effects in the trials, and sexual risk behaviour was similar in both the intervention and control groups. But the researchers say further research is required to ensure that PrEP, which is still a new approach, is safe and cost-effective. "There
are still many questions that need to be answered,” said Okwundu. “For example, how do we ensure that people adhere to their ART regimens? What are the long-term effects? Is pre-exposure prophylaxis cost-effective in the long run?”

**Journal Reference:**

July 17, 2012

**Drug Approved to Fight H.I.V. Infection**

By The Associated Press

The Food and Drug Administration on Monday approved the first drug shown to reduce the risk of H.I.V. infection, a milestone in the 30-year battle against the virus that causes AIDS. The agency approved Truvada, a pill made by Gilead Sciences, as a preventive measure for people who are at high risk of acquiring H.I.V. through sexual activity, like those whose partners are infected. Public health advocates say the approval could help slow the spread of H.I.V., which has held steady at about 50,000 new infections per year for the last 15 years. An estimated 1.2 million Americans have H.I.V., which develops into AIDS unless treated with antiviral drugs. Gilead Sciences has marketed Truvada since 2004 as a treatment for people who are already infected with the virus, but starting in 2010, studies showed that the drug could prevent people from contracting H.I.V. A three-year study found that daily doses cut the risk of infection in healthy gay and bisexual men by 42 percent when accompanied by condoms and counseling. Last year, another study found that Truvada reduced infection by 75 percent in heterosexual couples in which one partner was infected.

**SNAREs at the Synapse**

Using tiny lipid discs, scientists resolve contradictory evidence about how many proteins are required for neurotransmitter release.

By Megan Scudellari | July 1, 2012
FUSION FACSIMILE: To investigate membrane fusion during synaptic transmission (top), Rothman, Pincet, and colleagues designed an artificial version of the event. They exposed lipid nanodiscs embedded with SNARE proteins to vesicles containing complementary SNARE proteins. Only one SNARE protein complex was required for fusion between the discs and vesicles (A), but three were necessary to create a stable pore to release the neurotransmitter contained within the vesicle (B).

EDITOR'S CHOICE IN NEUROSCIENCE

There is very little about membrane vesicle fusion that Yale University biochemist James Rothman doesn’t know—he codiscovered SNAREs, the proteins that orchestrate the process. But one unanswered question in the field of membrane fusion has been what happens during the first milliseconds of synaptic transmission between neurons—when a vesicle full of neurotransmitters inside a neuron fuses to the cell membrane, opening a pore to release its contents into the synapse.

A fusion pore, the opening that occurs when a vesicle binds to a cell membrane, is present for just hundreds of microseconds, a thousand times shorter than the blink of an eye. Immediately after it opens, the pore rapidly expands as the vesicle membrane melts into the surrounding cell membrane. That quick transition has made it extremely difficult to study the pore, says Rothman. “We thought that if we could find a way to artificially stabilize the fusion pore, without interfering with its opening, we might be able to gain some new insights into neurotransmission,” he said.

To do so, Rothman’s group, together with Frédéric Pincet’s team at CNRS in Paris, France, created fusion pores in nanodiscs—circular discs of lipid bilayers, held together by scaffold proteins wrapped around each lipid disc like a belt. Because of the nanodiscs’ small size and rigid
Rothman’s team added SNARE proteins, which initiate vesicle-membrane fusion, to the nanodiscs and exposed them to small vesicles embedded with different SNARE proteins, creating an artificial model of synaptic vesicle fusion. By varying the number of SNAREs in the nanodiscs, the team was able to determine that only one SNARE per disc is necessary to temporarily open a fusion pore; however, three or more are required to keep the pore open long enough for the neurotransmitter to be released through it. “This further emphasizes the importance of these proteins in the process of membrane fusion,” says Thierry Galli, who studies membrane trafficking at the Institut Jacques Monod in Paris and was not involved in the research. It also marries two previously contradictory experiments about how many SNARE proteins are required to open a fusion pore, he adds. Two in vivo studies, conducted in 2001 and 2010, found that a minimum of three SNARE complexes are necessary for neurotransmitter release, but a 2010 in vitro analysis concluded that just one SNARE complex is sufficient for membrane fusion. Since then, scientists have debated over which minimum number of SNAREs is correct. “Now the field should be able to rest in peace,” says Rothman. “Everybody’s right!”

The paper

Vitamin B12 supplements may help treat hepatitis C
Safe and inexpensive option for boosting response rate to antiviral drugs
Adding vitamin B12 to standard hepatitis C virus (HCV) treatment significantly boosts the body’s ability to keep the virus at bay, indicates a pilot study published online in the journal Gut.

The effects were particularly strong in patients whose infection was proving difficult to treat effectively, the findings showed.

Between 60% and 80% of those infected with the viral liver infection HCV will go on to develop chronic hepatitis, and roughly a third of them will progress to cirrhosis and terminal liver disease.

Standard treatment of interferon (peg IFN) and ribavirin clears the virus in about 50% of patients infected with genotype 1 HCV and 80% of those infected with genotypes 2 or 3.

But this approach fails to clear the virus in around half of all those infected with HCV or the infection returns once treatment stops.

While trials of new generation antiviral drugs show promise, they are expensive, and can make treatment more difficult. And questions still remain about how well they will work in practice, say the authors.

Experimental research dating back a decade suggests that vitamin B12 may have a role in suppressing HCV. The liver is the body’s primary storage centre for vitamin B12, but this capacity is impaired by diseases directly affecting the organ.

The researchers therefore wanted to see if adding vitamin B12 to standard treatment would make a difference.

Ninety four patients with HCV infection were randomly allocated to receive standard treatment or standard treatment plus vitamin B12 (5000 ug every 4 weeks) for between 24 (genotypes 2 and 3) and 48 weeks (genotype 1).

The body’s ability to clear the virus was assessed after 4 weeks (rapid viral response), after 12 weeks (complete early viral response), at the end of treatment and at 24 weeks after stopping treatment (sustained viral response).

There was no difference between the two treatment approaches at 4 weeks, but there were significant differences in response at all the other time points, particularly 24 weeks after stopping treatment, which is the aim of HCV treatment and the closest it can be get to a cure.

The effects were also significantly greater among those who carried the type 1 strain, which is particularly hard to treat, and those high levels of infection (high viral load) to begin with.

Overall, adding vitamin B12 to standard therapy strengthened the rate of sustained viral response by 34%, the findings showed.

The authors conclude that until clear eligibility criteria for treatment with the new generation antiviral drugs are established, standard treatment plus vitamin B12 is a safe and inexpensive alternative, particularly for those who carry a strain of the virus that is hard to treat.

They add: "This strategy would be especially useful in those countries where, owing to limited economic means, the new generation antiviral therapies cannot be given in routine practice."
Mouse with human immune system may revolutionize HIV vaccine research
Animal model replicates human immune response against HIV, could simplify vaccine trials

One of the challenges to HIV vaccine development has been the lack of an animal model that accurately reflects the human immune response to the virus and how the virus evolves to evade that response. In the July 18 issue of *Science Translational Medicine*, researchers from the Ragon Institute of Massachusetts General Hospital (MGH), MIT and Harvard report that a model created by transplanting elements of the human immune system into an immunodeficient mouse addresses these key issues and has the potential to reduce significantly the time and costs required to test candidate vaccines.

"Our study showed not only that these humanized mice mount human immune responses against HIV but also that the ability of HIV to evade these responses by mutating viral proteins targeted by CD8 'killer' T cells is accurately reflected in these mice," says Todd Allen, PhD, senior author of the report. "For the first time we have an animal model that accurately reproduces critical host-pathogen interactions, a model that will help facilitate the development an effective vaccine for HIV." Recent studies by Allen's team and others have revealed that immune control of HIV is significantly limited by the ability of the virus to evade immune responses by rapidly mutating.

The traditional animal model for HIV research is the rhesus monkey, which can be infected with the related simian immunodeficiency virus (SIV). But differences in viral sequences between SIV and HIV, along with differences between the human and monkey immune systems, limit the ability of the SIV model to replicate directly key interactions between HIV and the human immune system. Development of an effective HIV vaccine will require a greater understanding of how human immune responses succeed or fail to control HIV.

The current study was designed to test the humanized BLT mouse, a model created by transplanting human bone marrow stem cells, along with other human tissue, into mice lacking a functioning immune system. Andrew Tager, MD, a co-author of the report and director of the MGH Humanized Mouse Program, explains, "Multiple researchers have contributed to dramatic improvements in the ability of humanized mice to model human diseases. Earlier studies with BLT mice performed at the University of Texas Southwestern Medical Center, the MGH and elsewhere have demonstrated that this particular humanized mouse model reproduces many aspects of the human immune response."

Timothy Dudek, PhD, of the Ragon Institute, lead author of the current study, adds, "Unlike normal mice, these humanized mice can be infected with HIV. But there has been little evidence regarding whether they reproduce the interaction between HIV and the human immune system, particularly the development of specific immune responses that exert control over HIV by targeting critical regions of the virus."

Tager's team at the MGH Center for Immunology and Inflammatory Diseases created groups of humanized BLT mice using cells and tissues from human donors with different alleles, or versions, of the immune system's HLA molecules, which flag infected cells for destruction by CD8 T cells. Particular HLA alleles, such as HLA-B57, are more common in individuals naturally able to control HIV, and some of the mice generated by Tager's group expressed this important protective allele.

Six weeks after the mice had been infected with HIV, the researchers found that the virus was rapidly evolving in regions known to be targeted by CD8 T cells. Their observation indicated that not only were the humanized mouse immune systems responding to HIV but also that the virus was mutating to avoid those responses in a manner similar to what is seen in humans. In mice expressing the protective HLA-B57 allele, just as in human patients who control viral levels, CD8 responses were directed against an essential region of the virus, preventing viral mutation and allowing the animals to more effectively contain HIV.

"We now know that these mice appear to replicate the specificity of the human cellular response to HIV and that the virus is attempting to evade these responses just as it does in humans," says Allen, an associate professor of Medicine at Harvard Medical School. "We are currently studying whether we can induce human HIV-specific immune responses in these animals by vaccination, which would provide a rapid, cost-effective model to test the ability of different vaccine approaches to control or even block HIV infection. If we can do this, we'll have a very powerful new tool to accelerate HIV vaccine development, one that also may be useful against other pathogens."
**Closer to a Cure? Chemists Synthesize Compound That Flushes out Latent HIV**

ScienceDaily (July 17, 2012) — A new collection of compounds, called "bryologs"—derived from a tiny marine organism—activate hidden reservoirs of the virus that currently make the disease nearly impossible to eradicate.

Thanks to antiretrovirals, an AIDS diagnosis hasn’t been a death sentence for nearly two decades. But highly active antiretroviral therapy, or HAART, is also not a cure.

Patients must adhere to a demandingly regular drug regimen that carries plenty of side effects. And while the therapy may be difficult to undergo in the United States, it is nearly impossible to scale to the AIDS crisis in the developing world.

The problem with HAART is that it doesn’t address HIV's so-called proviral reservoirs—dormant forms of the virus that lurk within T-cells and other cell types. Even after all of the body's active HIV has been eliminated, a missed dose of antiretroviral drugs can allow the hibernating virus to emerge and ravage its host all over again.

"It's really a two-target problem," said Stanford chemistry Professor Paul Wender, "and no one has successfully targeted the latent virus."

But Wender's lab is getting closer, exciting many HIV patients hoping for a cure.

The lab has created a collection of "bryologs" designed after a naturally occurring, but difficult to obtain, molecule. The new compounds have been shown to activate latent HIV reservoirs with equal or greater potency than the original substance. The lab's work may give doctors a practical way to flush out the dormant virus.

The findings were published on July 15 in the journal *Nature Chemistry*.

**Nature's medicine**

The first attempts to reactivate latent HIV were inspired by observations of Samoan healers. When ethnobotanists examined the bark of Samoa's mamala tree, traditionally used by healers to treat hepatitis, they found a compound known as prostratin.

Prostratin binds to and activates protein kinase C, an enzyme that forms part of the signaling pathway that reactivates latent viruses. The discovery sparked interest in the enzyme as a potential therapeutic target, especially as it was discovered that prostratin isn't the only biomolecule to bind to the kinase.

The bryozoan *Bugula neritina*—a mossy, colonial marine organism—produces a protein kinase C-activating compound that is many times more potent than prostratin. The molecule, named bryostatin 1, was deemed to hold promise as a treatment, not only for HIV but for cancer and Alzheimer's disease as well.

The National Cancer Institute initiated a Phase II clinical trial for the compound in 2009 for the treatment of non-Hodgkin lymphoma. But the substance had a number of side effects and proved prohibitively difficult to produce.

"It took 14 tons of bryozoans to make 18 grams of bryostatin," said Wender. "They've stopped accrual in trials because, even if the trials worked, the compound cannot be currently supplied."

Patient enrollment was suspended until more accessible compounds came out of the Wender Group's lab.

**A synthetic approach**

Wender, who published the first practical synthesis of prostratin and its analogs in 2008, had set out to make a simpler, more effective synthetic analog of bryostatin.

"We can copy the molecule," he said, "or we can learn how it works and use that knowledge to create something that has never existed in nature and might be superior to it."

The seven resulting compounds, called bryologs, share two fundamental features with the original bryostatin: the recognition domain, which directly contacts protein kinase C, and the spacer domain, which allows the bryolog-protein kinase C complex to be inserted into the cell membrane.

The researchers tested the new compounds’ ability to reactivate viral reservoirs in J-Lat cell lines, which contain latent HIV and begin to fluoresce when they express the virus.

In the J-Lat line, bryologs induced virus in as many or more cells than bryostatin at a variety of concentrations, and ranged from 25 to 1,000 times more potent than prostratin. The compounds showed no toxic effects.

Bryolog testing remains in the early stages—the researchers are currently conducting *in vivo* studies in animal models. But practical bryostatin substitutes may be the first step toward true HIV-eradication therapy.
"I receive letters on a regular basis from people who are aware of our work—who are not, so far as I know, scientifically trained, but do have the disease," said Wender. "The enthusiasm they express is pretty remarkable. That's the thing that keeps me up late and gets me up early."

Journal Reference:

South Africa Reports New Success in Saving Newborns from HIV ***
Agence France Presse, (07.19.2012)
South Africa's plan to prevent mother-to-child HIV transmission spared about 117,000 babies from contracting the virus last year, Health Minister Aaron Motsoaledi said Thursday. According to the Medical Research Council, the proportion of HIV-positive mothers who passed the virus to their babies fell from 8 percent in 2008, to 3.5 percent in 2010, to 2.7 percent in 2011. "These results, if sustained, will make a major contribution to our efforts to decrease" the deaths of infants and young children, he said.

Potent New Compound Virtually Eliminates HIV in Cell Culture *****
ScienceDaily (July 19, 2012) — A new study by scientists on the Florida campus of The Scripps Research Institute shows, in cell culture, a natural compound can virtually eliminate human immunodeficiency virus (HIV) in infected cells. The compound defines a novel class of HIV anti-viral drugs endowed with the capacity to repress viral replication in acutely and chronically infected cells.

The HIV/AIDS pandemic continues to affect 34 million individuals worldwide, including more than 3 million children, according to the World Health Organization. Current treatment involves the use of several antiretroviral drugs, termed Highly Active Antiretroviral Therapy (HAART), which can extend the life expectancy of HIV-positive individuals and decrease viral load without, however, eradicating the virus.

"We know that there are reservoirs of HIV that aren't being eliminated by current treatment and that keep replenishing the infection," said Susana Valente, a Scripps Research biologist who led the study. "Viral production from these cellular reservoirs that harbor an integrated viral genome is not affected by current antiretroviral drugs, which only stop novel rounds of infection. The compound in the current study virtually eliminates all viral replication from already-infected cells where HIV hides."

The new study, published in the July 20, 2012 issue of the journal Cell Host and Microbe, focused on a medically promising compound known as Cortistatin A. This natural product was isolated in 2006 from a marine sponge, Corticium simplex, discovered more than 100 years ago. In 2008, Scripps Research chemist Phil Baran and his team won the global race to synthesize the compound, presenting an efficient and economical method.

In the new study, Valente and her colleagues collaborated with the Baran lab, using a synthetic version of the compound, didehydro-Cortistatin A, to study the compound's effect on two strains of HIV. The strains were HIV-1, the most common form of the virus, and HIV-2, which is concentrated in West Africa and some parts of Europe.

The results showed that the compound reduced viral production by 99.7 percent from primary CD4+T cells (a type of immune cell) isolated from patients without levels of the virus in their bloodstream and who had been under HAART treatment for a long period of time. When the compound was added to other antiviral treatments, it further reduced by 20 percent viral replication from CD4+T cells isolated from patients with detectable amounts of virus in their bloodstream.

The inhibitor works by binding tightly to the viral protein known as Tat, a potent activator of HIV gene expression, effectively preventing the virus from replicating even at miniscule concentrations—making it the most potent anti-Tat inhibitor described to date, Valente said.

Another interesting feature of this compound is that withdrawal of the drug from cell culture does not result in virus rebound, which is normally observed with other antiretrovirals.

While most antiretroviral compounds block only new infections, didehydro-Cortistatin A reduces viral replication from already-infected cells, potentially limiting cell-to-cell transmission.

The new inhibitor already has a drug-like structure, is effective at very low concentrations, and has no toxicity associated with it, at least at the cellular level, the study noted.

The first author of the study "Potent Suppression of Tat-dependent HIV Transcription by didehydro-Cortistatin A" is Guillaume Mousseau of Scripps Research. In addition to Valente and Baran, other authors include Mark A. Clementz, Wendy N. Bakeman,
Rapid Diagnostic Test for Pathogens, Contaminants ****

ScienceDaily (July 19, 2012) — Using nanoscale materials, researchers at the University of Georgia have developed a single-step method to rapidly and accurately detect viruses, bacteria and chemical contaminants.

In a series of studies, the scientists were able to detect compounds such as lactic acid and the protein albumin in highly diluted samples and in mixtures that included dyes and other chemicals. Their results suggest that the same system could be used to detect pathogens and contaminants in biological mixtures such as food, blood, saliva and urine.

"The results are unambiguous and quickly give you a high degree of specificity," said senior author Yiping Zhao, professor of physics in the UGA Franklin College of Arts and Sciences and director of the university's Nanoscale Science and Engineering Center.

Zhao and his co-authors—doctoral students Jing Chen and Justin Abell and professor Yao-wen Huang of the UGA College of Agricultural and Environmental Sciences—used nanotechnology to combine two well-known techniques and create their new diagnostic test. Their results appear in the early online edition of the journal Lab on a Chip and were recently presented at the SPIE Defense, Security and Sensing conference.

The first component of their two-in-one system uses a technique known as surface enhanced Raman spectroscopy, or SERS, which measures the change in frequency of a laser as it scatters off a compound. Every compound displays a series of distinctive changes in frequency, or Raman shifts, that are as unique as a fingerprint. The signal produced by Raman scattering is inherently weak, but Zhao and his colleagues have arrayed silver nanorods 1,000 times finer than the width of a human hair at a precise angle to significantly amplify the signal. In previous studies with Ralph Tripp in the UGA College of Veterinary Medicine and chemist Richard Dluhy in the Franklin College, they demonstrated that the use of SERS with silver nanorods could identify viruses such as HIV and RSV isolated from infected cells.

"In a clinical setting, the sample that you obtain from patients typically contains bacteria or viruses as well as a lot of fluid—as in blood, urine or saliva—that contains biological agents that interfere with the signal you’re trying to detect," Zhao said. "To develop a diagnostic that could be used at the point of care, we needed a way to separate those agents."

Once again, the scientists turned to nanotechnology to create a next-generation diagnostic test. Using traditional thin layer chromatography, or TLC, scientists blot a drop of sample onto a porous surface. They then apply a solvent such as methanol to the sample, and the sample components separate based on how strongly they’re attracted to the solvent and the surface.

Study co-author Justin Abell, a doctoral student in the UGA College of Engineering, explained that TLC typically requires a large sample volume because the compound of interest soaks into the surface in addition to moving along it, like a stain on a rug. The silver nanorod surface that the researchers use, in contrast, allows them to use a miniscule amount of sample in a technique known as ultra-thin layer chromatography.

"In our case, the nanorods are acting as the detection medium but also as the separation medium," Abell said, "so it's a two-in-one system."

To test their method, the researchers used mixtures of dyes, the organic chemical melamine, lactic acid and the protein albumin. In each case, they were able to directly identify the compounds of interest, even in samples diluted to concentrations below 182 nanograms per milliliter—roughly 200 billionths of a gram in a fifth of a teaspoon. And while the detection of viruses using techniques such as polymerase chain reaction can take days or even weeks and requires fluorescent labels, the on-chip method developed by the UGA researchers yields results in less than an hour without the use of molecular labels.

The researchers are currently testing their technique with biological samples from Tripp’s lab that contain viruses, and Zhao said preliminary results are promising. He adds that while his team is focused on health and food safety applications, SERS and ultra-thin layer chromatography can be used to detect compounds of all types—everything from forensic materials at a crime scene to environmental pollutants. His team also is working with colleagues across campus to create an online encyclopedia that would allow
technicians to identify viruses, bacteria, biomarkers and pharmaceuticals based on their distinctive Raman shifts.

"Every compound has a unique SERS spectrum," Zhao said, "so this is a very robust technology whose applications are practically endless."

**Journal Reference:**

**Viruses' Copying Mechanism Demystified, Opening the Door to New Vaccine Strategies**
ScienceDaily (July 19, 2012) — Certain kinds of viruses such as those that cause the common cold, SARS, hepatitis, and encephalitis, copy themselves using a unique mechanism, according to a team of Penn State scientists that includes David Boehr, an assistant professor of chemistry and a co-leader of the research team. The discovery sheds light on a previously identified, but never-before-understood region of an enzyme associated with the process of replicating genetic material. The research is an important step toward the improvement of existing vaccines, as well as toward the design of vaccines against viruses that have eluded vaccination strategies in the past.

The research will be published in the print issue of the journal *Structure* on Sept. 5.

All organisms use enzymes called polymerases to "read" and copy their genetic material. While the genetic material of viruses that cause diseases such as SARS, influenza, and polio is composed of single-stranded RNA, the genetic material of many other viruses, such as those that cause herpes and conjunctivitis, is composed of double-stranded DNA. Regardless of whether the genetic material is DNA or RNA, viruses hijack a host cell's machinery, forcing it to replicate the virus's own genetic material and, ultimately, to make copies of the virus that will spread to and infect other cells.

The polymerases of many organisms, including DNA viruses, are known to have a "cupped right hand" structure—a configuration of atoms that can be described as resembling a palm, fingers and thumb. (Credit: Boehr lab, Penn State University)

"We've known for some time that, in organisms that use DNA as their genetic material, within the 'palm' of the hand is specific helical structure where much of the enzyme action takes place. This 'fidelity' helix is where nucleotides—molecules that join to form RNA and DNA—are recognized and copied," Boehr said. "However, the polymerases of RNA viruses do not have this helix structure. Instead, the 'cupped hand' holds a different structure—a loop known as motif D. Until now, the function of motif D was a mystery."

To unravel the mystery of motif D's function, Boehr and his colleagues studied a strain of the poliovirus—an RNA virus that is similar to many other RNA viruses that affect humans. Using a technique called nuclear magnetic resonance spectroscopy, a process that probes the physical and chemical properties of atoms to determine the structure of organic compounds, they found that motif D is the
functional equivalent of the helix structure found in the polymerases of other viruses. "Previously, it was assumed that motif D had no function at all or that it provided some sort of scaffolding to support the cupped palm structure," Boehr said. "But we have found that it is responsible for identifying nucleotides and making sure that a new strand of RNA is replicated faithfully, with as few mistakes as possible."

Boehr explained that what he and his team discovered about motif D's function in the polio strain is applicable to many other RNA viruses such as the common cold. In addition, motif D may function similarly in retroviruses—viruses such as HIV that are replicated using an enzyme called reverse transcriptase to produce DNA from RNA genomes. "Additional studies will be necessary to confirm that motif D's role is of equal importance in retroviruses," Boehr said.

Boehr and his collaborators hope that motif D might provide a new direction for vaccine research. "Now that motif D has been identified as part of the mechanism by which genetic material is replicated accurately, it might be possible to use that information to create safer and more-efficient vaccines," Boehr said.

He explained that a vaccine, which is a weakened or harmless version of a virus, works by giving the vaccinated person's immune system a "picture" of the enemy. Once the immune system knows what the virus looks like, it can recognize and defend against the pathogen when it comes into contact with the wild, harmful version.

But one concern of this strategy is the possibility that a weakened, vaccine version of a virus might evolve once it has been introduced into a population, eventually reverting back to a wild type and becoming harmful again.

"Ideally, every copy a vaccine virus makes of itself inside human cells will be the original, lab-created, harmless version," Boehr said. "So by fine-tuning motif D; that is, by making this fidelity mechanism even more faithful, it might be possible to reduce the chances that the vaccine version of the virus will mutate and evolve on its own."

Boehr added that the research also might provide a new strategy to design vaccines for some of the RNA viruses for which vaccines have not yet been developed.

**Botanical Compound Could Prove Crucial to Healing Influenza**

ScienceDaily (July 18, 2012) — Building on previous work with the botanical abscisic acid,* researchers in the Nutritional Immunology and Molecular Medicine Laboratory (NIMML) have discovered that abscisic acid has anti-inflammatory effects in the lungs as well as in the gut. The results will be published in the Journal of Nutritional Biochemistry.

"While the immune effects of abscisic acid are well understood in the gut, less was known about its effects in the respiratory tract. We've shown definitively that not only does abscisic acid ameliorate disease activity and lung inflammatory pathology, it also aids recovery and survival in influenza-infected mice," said Raquel Hontecillas, Ph.D., study leader and assistant professor of immunology at Virginia Bioinformatics Institute and co-director of NIMML.

Influenza accounts for anywhere from 3,000 to 49,000 deaths per year in the United States alone, according to the Centers for Disease Control. It is difficult to treat if not caught immediately; antivirals usually become ineffective after the virus incubation period has passed and resistance to antiviral drugs poses a serious public health problem in the face of outbreaks. Abscisic acid, however, has been shown to be most effective at about seven to ten days into the infection, targeting the immune response rather than the virus itself, which many researchers feel is a safer way to reduce flu-associated fatalities.

"Most drugs for respiratory infections target the virus itself, rather than the inflammatory responses caused by the virus. Abscisic acid activates peroxisome proliferator-activated receptor-gamma, a receptor that aids in reducing inflammation, through a newly identified pathway* but it does so without the side effects of other agonists like thiazolidinediones, which are known to have strong adverse side effects. The development of complementary and alternative medicine approaches that modulate the host response has great promise in decreasing respiratory damage caused by influenza or other respiratory pathogens," said Josep Bassaganya-Riera, Ph.D., director of NIMML and professor of nutritional immunology at the Virginia Bioinformatics Institute.

From this and previous research, it's clear that abscisic acid could yield a novel way to combat inflammatory disease both in the gut and the respiratory tract. By using host-targeted strategies to ameliorate disease, alternate pathways can be established to activate immune responses without the deadly side effects of many drugs currently on the market.
**Like a Transformer? Protein Unfolds and Refolds for New Function**

ScienceDaily (July 19, 2012) — New research has shown that a protein does something that scientists once thought impossible: It unfolds itself and refolds into a completely new shape.

This protein, called RfaH, activates genes that allow bacterial cells to launch a successful attack on their host, causing disease. The researchers determined that RfaH starts out in its alpha form, composed of two spiral shapes. Later, in its beta form, it resembles spokes on a wheel and is called a barrel.

When RfaH refolds, it acquires a new function — yet another finding that researchers would not have predicted.

"We showed that RfaH refolds, which is a big enough deal already. You would think this is impossible. That's what you're told in school," said Irina Artsimovitch, professor of microbiology at Ohio State University and a lead author of the study. "But in this case, it's even better than that because we show that when RfaH refolds, it acquires a new function. It can do something that it couldn't do before."

Though the process happens in seconds, Artsimovitch likened the refolding to "having a knitted sweater that you rip out and then knit into a sweater with a different pattern."

The research is published in the July 20, 2012, issue of the journal *Cell*.

The findings have significant implications for studies of gene expression control and protein structure. This remarkable ability to refold suggests that RfaH and similar proteins might be able to bind in ways and to other molecules that had never been considered. Scientists who engineer proteins might have an entirely new step to add to their models. And now that the first case of a complete alpha to beta structural change has been demonstrated, chances are good that researchers will find other proteins that can do the same thing.

In particular, these findings are reminiscent of prion proteins, infectious agents that cause fatal brain diseases in humans and mammals. Prions refold from a harmless alpha into contagious beta forms, which initiate a chain reaction leading to the formation of large prion aggregates, the causative agents of diseases that include Creutzfeldt-Jakob Disease and bovine spongiform encephalopathy in cattle, also known as "mad cow disease."

All proteins fold into their designated shape, determined by DNA sequence, and have one job in a cell. For instance, some proteins, including RfaH, turn on gene expression — and typically that's the end of the story.

At the time of this study, the scientific community considered RfaH a transcription factor — a molecule that binds to specific DNA sequences to control the movement of genetic information from DNA to messenger RNA. Messenger RNA, also known as mRNA, carries those protein-building instructions as the gene expression process continues.

Artsimovitch and colleagues recently determined that RfaH closes a critical gap in the enzyme RNA polymerase, allowing it to maintain its grip on DNA and start the activation of genes. RNA polymerase is responsible for setting gene expression in motion in all cells by wrapping itself around the double helix of DNA, using one strand to match nucleotides and make a copy of genetic material.

But the researchers observed that when RfaH binds to DNA and RNA polymerase, its two halves separate, with one remaining bound to the enzyme and another holding on with just a string of a few amino acids. And then it refolds into a completely different shape.

This new work shows that refolding allows RfaH to participate in translation, a completely different step in gene expression. During translation, a molecule called a ribosome binds to mRNA and uses its instructions to select amino acids and join them into chains, which then fold to compose proteins, the final products of gene expression.

"You have factors that work on transcription and factors that work on translation. They are typically not the same," Artsimovitch said.

Research had long ago established that RfaH was a transcription factor, and that this function is universally conserved — meaning it is present for this role in all living organisms and has been for generations.

But this new research shows that RfaH is even more effective as a translation factor — about 100 times more powerful than it is as a transcription factor, Artsimovitch said. RfaH has more power during translation because it recruits the ribosome molecule under circumstances when there isn't enough information in the mRNA for the ribosome to do its protein construction.
"Normally the ribosome binds to a specific sequence. If there is no sequence, it doesn’t know where to bind. We think that the ribosome recognizes RfaH instead of this sequence and starts translation," Artsimovitch said. "This is what we’re going to be studying now, because it is completely unprecedented. "RfaH can interact with the ribosome once it refolds. It’s a really smart way to do it. It’s a tiny protein, yet it can bind to both the RNA polymerase and the ribosome at the same time and link them together."

Artsimovitch and colleagues actually proposed several years ago that RfaH refolds because its observed structure was so different from what they expected. However, RfaH sequence could be folded in silico—via computer simulation—into the "correct" shape.

Paul Rösch, a scientist at the Universität Bayreuth in Germany and a lead author on this paper, tested this idea using high-tech nuclear magnetic resonance imaging. His lab found that RfaH can indeed fold into two very different states. The two labs then collaborated as Artsimovitch set out to determine the functional significance of RfaH refolding.

Artsimovitch has been using Escherichia coli as a model system for these studies. In these cells, the RfaH protein is a virulence factor that gives bacteria their ability to infect and cause disease. Though that role is not emphasized in this research, it makes sense that this particular protein would have some special qualities, Artsimovitch explained.

Genes that control bacterial virulence are famous for being designed in a way that makes their translation very inefficient—hence, they need specialized proteins to help their expression. If RfaH didn’t refold and enable translation, certain genes would not be expressed, and in the wild, these bacteria could not survive.

The researchers determined that the separation of the protein’s domains, or sections, triggers the refolding because they could reproduce that event experimentally. But they haven’t yet determined exactly what allows that dissociation to happen, and are designing models in which to further investigate the refolding process.

"There is actually a big question here: How does RfaH know where to begin binding to the message to recruit the ribosome? We can tell that the ribosome knows where to start, but how it knows, we have no idea," Artsimovitch said.

Journal Reference:
Björn M. Burmann, Stefan H. Knauer, Anastasia Sevostyanova, Kristian Schweimer, Rachel A. Mooney, Robert Landick, Irina Artsimovitch, Paul Rösch. An α Helix to β Barrel Domain Switch Transforms the Transcription Factor RfaH into a Translation Factor. Cell, July 20, 2012 DOI: 10.1016/j.cell.2012.05.042

Discovery of 'Hopping' of Bacterial Enzyme Gives Insight Into Gene Expression
ScienceDaily (July 17, 2012) — UC Santa Barbara researchers' discovery of a variation of an enzyme's ability to "hop" as it moves along DNA, modifying the genetic material of a bacteria—and its physical capability and behavior—holds much promise for biomedical and other scientific applications.

The E. coli bacteria's adaptive mechanism allows it to change its phenotype—its observable characteristics—according to its environment. For example, if it senses a need to find food, to stick to the tissues of its host organism, or to reproduce, the bacteria will form pili, or hairlike structures, on its surface, to allow it to move, stick, or pass genetic material.

"We're trying to figure out what is it in the cell that's driving those changes," said Adam Pollak, first author of the paper.

The formation of these pili is driven by an epigenetic mechanism—a "tagging" done by the enzyme DNA adenine methyltransferase (Dam), which acts on a specific sequence of DNA, called GATC sites (Guanine-Adenine-Thymine-Cytosine). The tagging signals the formation of these—appends a mechanism similar to that in humans, where tagging directs the formation of tissues for different organs from the same DNA. This tagging is part of a broader field, called epigenetics, where modifications made to the genome are heritable and regulate the expression of genes.

A graphic representing the action of the Dam enzyme on E. coli DNA. The blue circles represent methylation "tags" which are added to the GATC sites on the bacteria’s DNA. The clustering of the GATC sites, as seen in the top panel, facilitates Dam’s "intrasite hopping" which causes the expression of the pili. (Credit: Image courtesy of University of California – Santa Barbara)
Where the prevailing belief used to be that the enzyme Dam slid down only one side of the bacteria's double-helixed DNA looking for these GATC sites, according to the researchers, Dam can actually "hop" to one or more such sites on both sides of the double helix.

"It moves along, finds a site, and methylates that; but it turns around, reorients itself, and methylates the other side," said Norbert Reich, UCSB professor of chemistry and biochemistry.

Using several strands of genetically engineered DNA of various lengths and differing distances between the sites of methylation, the researchers found that the hopping of Dam may occur more often, depending on the clustering of sites; e.g., it is more likely to occur when two sites are within 10 to 200 base pairs of each other. Clustered GATC sites are strongly associated with gene regulation, while an isolated GATC site on the double helix is associated with the copying of DNA. According to the authors' findings, the longer the enzyme goes without locating the GATC sequence of molecules, the less likely that it will undergo this new variation of hopping, but the introduction of a GATC sequence will stimulate the mechanism once again.

According to the paper, hopping can explain the efficiency by which DNA-modifying enzymes can find their recognition sites, despite the presence of an overwhelming amount of non-specific DNA; as well as how enzymes can modify more than one site, despite opposing strand orientations.

The research capitalizes on decades of observation of E. coli's behavior, and factors that contribute to its virulence, or its ability to persist and multiply. Studying the mechanisms that switch these abilities on and off would contribute to how humans can deal with these bacteria, which exist in warm-blooded creatures, but, in certain instances, can cause diseases.

"If we had inhibitors that could prevent the switching, we wouldn't have urinary tract infections, for instance," said Pollak.

The same research group recently reported a similar mechanism in humans, which is disrupted in certain forms of leukemia.

**Journal Reference:**

### AIDS Risk Higher for Gay, Bisexual Black Men: Called Segment of Population Most in Need of Prevention, Treatment Help

The Black AIDS Institute released a new report Wednesday called “Back of the Line: The State of AIDS Among Black Gay Men in America 2012.” Black men who have sex with men (MSM) “continue to be first in line when it comes to need, but remain at the back of the line when it comes to assistance,” said Phill Wilson, founder and executive director of BAI. The report calls for stepped-up STD campaigns and HIV testing, prevention and treatment for this population.

Black MSM account for one in four new HIV infections, even though they represent only 1 in 500 Americans, the report says. Unless they receive treatment, these men “are significantly less likely to be alive three years after testing HIV-positive” when compared with white MSM.

Black MSM “are not simply a fringe group in the fight against HIV/AIDS,” said Dr. Kevin Fenton, director of CDC's National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention. “They are, in fact, at the center of the nation’s epidemic, and we cannot achieve an AIDS-free generation, or the end of AIDS in the United States, unless we make major inroads in the fight against HIV among black gay men.”

According to Ernest Hopkins, chair of the National Black Gay Men's Advocacy Coalition and the San Francisco AIDS Foundation's director of legislative affairs, black MSM “are no more likely to engage in HIV-related risk behaviors than other MSM.” However, they are associated with risk factors such as early sexual experience, older sex partners, being molested as a child, being incarcerated, growing up in poverty, homelessness and suffering discrimination, he said.

[PNU editor’s note: To access BAI's report, visit: http://www.blackaids.org/index.php?option=com_content&view=article&id=1284&Itemid=198.]

### HIV Drug Resistance Creeps Higher: WHO
**Agence France Presse**, (07.18.2012)

HIV drug resistance in low- and middle-income nations stood at 6.8 percent in 2010, the World Health Organization said Wednesday. WHO released its first-ever report on the issue ahead of next week’s 19th International AIDS Conference.
According to WHO AIDS chief Gottfried Hirnschall, “That is a level we sort of expected. It is not dramatic but we clearly need to look very carefully on how this would evolve further.” WHO did not recommend a change in treatment guidelines based on the study.

Drug resistance can occur when HIV mutates naturally, when treatment is interrupted, or when patients take medications incorrectly or irregularly.

Approximately 8 million people in low- and middle-income countries received antiretroviral drugs last year, up 20 percent from 2010, according to a separate UNAIDS report released Wednesday. High-income countries have higher rates of resistance, from 8 percent to 14 percent. Many of these nations launched widespread treatment programs years ago, often using single- or dual-drug therapies, which can encourage resistance. However, these higher rates have largely leveled off or decreased over time.

In 12 of the low- and middle-income countries in the WHO study, health care facilities lost count of up to 38 percent of people who began treatment. When people interrupt or stop treatment, “this not only means that they are themselves more likely to become sick, it also increases the likelihood that drug resistance will emerge and the resistant virus could be transmitted to others,” the report said.

WHO called for clinics to monitor for early warning indicators of resistance, including poor treatment adherence, supply breaks, and signs of treatment failure, such as rising viral levels in the blood.

Hirnschall added, “Simpler regimens using fixed-dose combinations have made it much easier for people to adhere to antiretroviral treatment, limiting the spread of drug resistance in recent years.”

What did we learn from the 2010 California whooping cough epidemic?
Cincinnati, OH, July 19, 2012 – Because whooping cough (pertussis) is almost as contagious as measles (affecting ~12-17 individuals with each case), clinicians are required to report cases of this bacterial respiratory tract infection to the state’s department of public health. In 2010, California had the highest number of cases of whooping cough in 60 years. A new study scheduled for publication in The Journal of Pediatrics describes the 2010 whooping cough epidemic and details strategies to decrease the incidence of this infection.

Kathleen Winter, MPH, and colleagues from the California Department of Public Health (CDPH) evaluated 9,154 cases of whooping cough with onset between January 1 and December 31, 2010; 809 cases were hospitalized and 10 resulted in death. All deaths and most of the hospitalized cases (62%) were in infants less than 3 months of age, and infants less than 6 months of age had the highest disease rates. In the population aged less than 6 months, Hispanics had the highest incidence of whooping cough. However, in children and adolescents 1-18 years of age, Whites had the highest incidence.

It is recommended that infants should receive 4 doses of DTaP (diphtheria, tetanus, and pertussis) vaccine by 18 months of age, and children should receive whooping cough "booster" doses at 4-6 years of age (DTaP) and 11-18 years of age (Tdap). Adults are also encouraged to receive the Tdap booster because immunity from both the disease and the vaccine wanes over time. The number of cases of whooping cough was elevated in pre-adolescents, even when they are fully vaccinated, indicating that protection from the 5-dose DTaP series may wane before the Tdap booster is given. However, the authors believe that the decrease in cases of 11-14 year olds suggests that Tdap is effective for adolescents.

In response to the sharp increase of cases in 2010, CDPH implemented a public health campaign to distribute educational materials to health care providers and the public to stress the importance of rapid diagnosis and treatment, especially in young infants, recommend vaccination for adults older than 64 years of age, under-immunized children 7-9 years old, and pregnant women, and provide free Tdap booster vaccines to hospitals, community health centers, and Native American health centers for pregnant and postpartum women and other infant contacts. To decrease the occurrence of whooping cough in infants who are too young to be vaccinated, it is important to immunize household and family members who will be in close contact with the baby (a strategy known as "cocooning") and increase the immunity of the population as a whole to decrease infants' exposure to pertussis.

Prior to the epidemic in 2010, only 23% of California birth hospitals had policies to offer Tdap to postpartum women. In 2011, the Centers for Disease Control and Prevention recognized the extreme vulnerability of young infants and recommended universal Tdap immunization for pregnant women (after the 20th week of gestation) who previously had not received Tdap. According to Ms. Winter, "In the absence of better vaccines, it is imperative that strategies to protect young infants directly, such as maternal vaccination during pregnancy, be evaluated for effectiveness. In addition, it is critical that providers continue to be vigilant and promptly diagnose and treat young infants with whooping cough."
Mild HIV type slows development of AIDS and makes new preventive treatments possible

19 July 2012

A new study from Lund University in Sweden has opened the way for new approaches to slowing the development of AIDS in HIV-1-infected patients. It is hoped that this could lead to better treatment methods and preventive measures to combat HIV and AIDS.

The findings have just been published in the distinguished scientific journal New England Journal of Medicine.

The most common type of the virus that causes AIDS – HIV-1 – is less aggressive when it infects a person already carrying the milder HIV-2. The study looked at how the disease developed in those who had been infected with HIV-1 and those who were infected with both HIV-1 and HIV-2.

“The moderating effect of HIV-2 was extremely strong. The time it took to develop AIDS was around 50 per cent longer for those infected with both strains than for those only carrying the HIV-1 virus. The unusually large difference makes me, as a researcher, very optimistic that it will be possible to identify new and significant approaches that can be taken to combating the development of AIDS”, says Joakim Esbjörnsson, a virologist at Lund University.

“The unique thing about our study, which has been carried out over 20 years, is that we have been able to follow healthy individuals from when they were infected with HIV-1 only or both HIV-1 and HIV-2 through the entire course of the disease, and to make comparisons of how the infection has developed over time”, says Hans Norrgren, doctor in infectious diseases and researcher at Lund University and Skåne University Hospital.

An observation that was linked to an early stage of HIV infection was a difference in the genetic diversity of the HIV. It is well-known that different strains of HIV co-exist during the course of the infection and that the genetic difference between them increases the closer to AIDS the infection comes. This genetic difference was lower early on in the disease among people with a dual infection than among those with only HIV-1 infection, which gave them a better starting point from which the development of AIDS was delayed.

The researchers have also studied the levels of CD4+ T cells – helper cells with a key role in the immune system that are attacked and destroyed by the HIV virus. Patients with dual infection were also seen to be at an advantage in this. They had a higher number of CD4+ T cells throughout the period of infection. It took longer to reach critically low levels of CD4+ T cells, and thus also longer before the infection developed into AIDS.

“Our results suggest that HIV-2 can activate cellular reactions which naturally check the development of AIDS. If we can map these, I think we can also uncover entirely new mechanisms that are key to the slower development of the disease. In the long run, this could lead to better preventive measures and treatments”, says Patrik Medstrand, Professor of Virology at Lund University.

Besides the findings lies a unique 20-year follow-up of 4 700 people in Guinea-Bissau in West Africa.

“Our work is the result of many people’s work over many years, in particular the staff of the National Public Health Laboratory in Guinea-Bissau and the police health station in the capital Bissau, who have carried out the practical work of examining the study participants, taking samples and conducting laboratory analyses”, says Fredrik Månsson, a doctor in infectious diseases in Malmö and one of the researchers behind the study.

Publication:
Article: ‘Inhibition of HIV-1 Disease Progression by Contemporaneous HIV-2 Infection’
Authors from Lund University: Joakim Esbjörnsson, Fredrik Månsson, Anders Kvist, Marianne Jansson, Eva Maria Fenyo, Hans Norrgren and Patrik Medstrand.

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About HIV-2
West Africa is the only region where the milder strain of HIV, HIV-2, is found on a large scale. Like the globally dominant and more aggressive HIV-1, HIV-2 is an infection that can lead to AIDS. However,
fewer of those infected with HIV-2 develop AIDS, only around 25–30 per cent of those who do not receive treatment.

**Beneficial Bacteria May Help Ward Off Infection**
ScienceDaily (July 19, 2012) — While many bacteria exist as aggressive pathogens, causing diseases ranging from tuberculosis and cholera, to plague, diphtheria and toxic shock syndrome, others play a less malevolent role and are critical for human health.

In a new study, Cheryl Nickerson and her group at ASU’s Biodesign Institute, in collaboration with an international team including Tom Van de Wiele and lead author Rosemarie De Weirld at Ghent University, Belgium, explore the role of Lactobacillus reuteri—a natural resident of the human gut—to protect against foodborne infection.

Their results demonstrate that this beneficial or probiotic organism, which produces an antimicrobial substance known as reuterin, may protect intestinal epithelial cells from infection by the foodborne bacterial pathogen *Salmonella*.

The study examines for the first time the effect of reuterin during the infection process of mammalian intestinal cells and suggests the efficacy of using probiotic bacteria or their derivatives in future therapies aimed at thwarting *Salmonella* infection.

Members of the Nickerson lab at the Biodesign Institute’s Center for Infectious Diseases and Vaccinology involved in this study were Shameema Sarker and Aurélie Crabbé.

Results of the new study recently appeared in the journal *PLoS ONE*.

**Cell cultures: Now in 3-D**

Over the past decade, the Nickerson group and their colleagues have developed organotypic three-dimensional (3-D) tissue culture models of the small and large intestine, lung, placenta, bladder, neuronal tissue and vaginal epithelium that mimic key characteristics of the parental tissue, and applied them to study the infectious disease process. Such models offer exciting new insights into host-pathogen interactions, cell proliferation, differentiation and immune function, and are providing a platform to understand normal tissue homeostasis and transition to disease.

For the current study, 3-D colon epithelial cells were used. Nickerson explains that cells derived for study through this technique more faithfully approximate key in vivo responses to *S. Typhimurium* infection, compared with the traditional monolayer methods, making such cells an ideal model to observe infection processes.

3-D cell culture models are cultured in a special environment within a device known as a Rotating Wall Vessel bioreactor—a cylindrical, rotating apparatus, filled with a culture medium supplying essential nutrients, oxygen and physical forces to the cells. Within the reactor, the natural sedimentation of cells due to gravity is balanced by the bioreactor’s rotation, resulting in a gentle tumbling of cells within the media in the chamber.

During the culturing phase, cells attach themselves to tiny porous beads, termed microcarriers, or other scaffolding. Under these conditions, cells are able to respond to molecular and chemical gradients in three-dimensions in a way that approximates their behavior under in vivo conditions, causing the cells to aggregate based on natural cellular affinities and form 3-D tissue-like structures.

"In previous studies, we applied our 3-D intestinal cell cultures as human surrogates to further our understanding of how *Salmonella* interacts with the intestinal epithelium to cause gastrointestinal disease," Nickerson explains. "We found that these models were able to respond to infection in key ways that mimicked the parental tissue in vivo and which conventional models could not recapitulate. We are excited to advance the use of our 3-D models in the current work to study how commensal intestinal microbes and their products can protect against *Salmonella*-induced foodborne infection. The results of this study may provide fundamental knowledge for development of new probiotics and other functional food based strategies."

**Bacterial Blizzard**

A swarm of some hundred trillion bacteria occupies the human body, outnumbering human cells by about 10 to 1. Among these are members of the genus *Lactobacilli*, some of which have been associated with therapeutic, probiotic properties, including anti-inflammatory and anti-cancer activity.

The current study zeros in on *Lactobacillus reuteri*—one of the more than 180 species of *Lactobacilli*. The group investigated the potential of this bacterium to inhibit the early stages of *Salmonella* infection, seeking to identify plausible mechanisms for such inhibitory effects.
Intestinal infections by non-typhoidal Salmonella strains induce diarrhea and gastroenteritis, and remain a leading source of foodborne illness worldwide. Such infections are acutely unpleasant but self-limiting in healthy individuals. For those with compromised immunity however, they can be deadly and the alarming incidence of multi-drug resistant Salmonella strains has underlined the necessity of more effective therapeutics.

The use of benign microorganisms offers a promising new approach to treating infection from pathogens like Salmonella and indeed, L. reuteri has been shown to help protect against gastrointestinal infection and reduce diarrhea in children.

Safeguarding cells
The origin of L. reuteri’s protective role still remains unclear, and the present study investigated whether reuterin, a metabolite produced by L. reuteri during the process of reducing glycerol in the gut, could be one of the keys to protection. While it has been speculated that reuterin acts by regulating immune responses or competing with Salmonella for key binding sites, the current study represents the first in vitro examination of host-pathogen interactions using human intestinal epithelium in the presence of reuterin-producing L. reuteri.

Two approaches were used to study host-pathogen interactions. In the first, 3-D intestinal epithelial cell aggregates were seeded into 24-well plates. Salmonella was added to these intestinal cells along with supernatant of L. reuteri—that is, cell-free culture medium in which the Lactobacillus grew and produced reuterin (obtained by filtering out the bacteria).

In the second approach, L. reuteri was first allowed to produce reuterin in the presence of the 3-D colon cells (seeded into the wells), after which the cells were exposed to Salmonella. Here, the L. reuteri bacteria (in the presence of glycerol) produced reuterin in situ. In both approaches, reuterin exposed controls were also tested, and the effect of reuterin on a Salmonella population in the absence of host cells was assessed as well.

L. reuteri regulates response to infection
The results showed a reduction in the Salmonella population (without host cells) after one hour of exposure to a diluted supernatant containing reuterin. Further, the reuterin-containing ferment of L. reuteri was shown to significantly reduce adhesion, invasion and intracellular survival of Salmonella to 3-D colon cells, compared with an untreated control.

In an unexpected twist, the application of L. reuteri supernatant lacking glycerol actually stimulated adhesion, invasion and intracellular survival of Salmonella. The authors speculate that the stimulatory effect observed may have been due to low concentrations of acetic acid, previously shown to stimulate expression of Salmonella virulence-related genes.

Applying the second approach, live L. reuteri were incubated with 3-D epithelial cells and the medium supplemented with glycerol, allowing for in situ production of reuterin. The presence of L. reuteri was shown to reduce the population of Salmonella by diminishing their capacity for adhesion, invasion and intracellular survival and this effect increased when L. reuteri were producing reuterin.

Another interesting detail uncovered in the study is that the effects of reuterin on Salmonella's infectious capacity are increased in the presence of host cells, suggesting that some type of synergistic protection occurs during epithelial infection, potentially involving the combined activity of reuterin and host cell gene-related responses.

Prolonged exposure (of 24 hours or more) to the reuterin-containing supernatant solutions caused a loss of viability in host cells, though shorter exposure times did not appear to adversely affect them. Importantly, the introduction of L. reuteri strains in vivo have been safely carried out in infants and even immuno-compromised adults, indicating that other cell types, host factors or the complex gut microbiota in vivo could counteract the observed cytotoxic effects of reuterin in vitro.

While the authors stress that much work remains, particularly in terms of understanding reuterin’s role in the context of a complex gut microbiome, the results are encouraging and suggest a new avenue for fighting Salmonella infection, through the process of glycerol conversion to reuterin by L. reuteri.

Journal Reference:
Unique Mechanism Identified in Bacteria as Potential Target for Developing New Antibiotics

ScienceDaily (July 20, 2012) — Researchers from Florida Atlantic University’s Charles E. Schmidt College of Medicine have identified a unique mechanism in bacteria that has the potential to serve as a target for developing new antibiotics for diseases such as AIDS and soft tissue infections including respiratory and urogenital tracts, which are currently difficult to treat.

The results of these findings were published in an article titled "Novel One-step Mechanism for tRNA 3'-End Maturation by the Exoribonuclease RNase of Mycoplasma genitalium" in the current issue of the Journal of Biological Chemistry.

Co-authors of the article are Ravi K. Alluri, a pre-doctoral student in the department of biomedical science and Dr. Zhongwei Li, Ph.D., associate professor of biomedical science in FAU’s Charles E. Schmidt College of Medicine.

Li and Alluri explain that every organism lives on the same principle that genes direct the production of proteins. This process depends on a set of small RNAs called tRNAs that carry the building blocks of proteins. A tRNA is produced from its gene initially as a precursor that contains extra parts at each end (5' and 3' ends) and sometimes in the middle. These extra parts must be removed through RNA processing before tRNA can work during protein production. The processing of tRNA 5' end has been known for quite some time and work on this enzyme has received a Nobel Prize. Processing of the 3' end is much more complicated and has only been revealed in some organisms more recently. Organisms that have nucleus in their cells, including humans, appear to process the 3' end of tRNA in a similar way. A tRNA must be precisely processed before it can carry a building block for proteins.

"Intriguingly, bacteria appear to process the 3' end of tRNA very differently," said Alluri. "And we are still trying to reveal the various enzymes called RNases, which remove the 3' extra parts of tRNA precursors."

Some of the RNases cut the RNA in the middle, while others trim the RNA from the 3' end. Most of the bacterial pathways involve multiple RNases to complete tRNA 3' processing.

"Knowing how tRNA is processed in different types of bacteria is important not only for understanding how bacteria live, but also for developing novel antibiotics that specifically control bacterial pathogens," said Li.

One such pathogen is the bacterium Mycoplasma genitalium, which is the second smallest known free-living organism that is thought to cause infertility. Alluri and Li's current work focuses on this bacterium—its genome only contains about 10 percent of the genes found in other common bacteria. Surprisingly, this bacterium contains none of the known RNases for tRNA 3' processing and hence it has to use a different RNase to do so.

"What we have discovered with Mycoplasma genitalium is that it uses a completely different RNase called RNase R to process the 3' end of tRNA," said Alluri. "RNase R can trim the 3' extra part of a tRNA precursor to make a 'functional' tRNA. It is even smart enough to recognize some structural features in the tRNA and tell where the trimming has to stop without harming the mature tRNA."

The ability of RNase R to completely remove the 3' extra RNA bases in a single-step trimming reaction represents a novel mechanism of tRNA 3' processing. Other mycoplasmas generally have small genomes and likely process tRNA in the same way. Using only one enzyme for this complicated task saves genetic resources for mycoplasmas.

"Importantly, blocking the function of RNase R in mycoplasmas can stop protein production and kill the bacteria, making RNase R an excellent target of new antibiotics for treatment of mycoplasma infection," said Li.
World’s Toughest Bacterium Holds Promise for Rapid Vaccine Development Against Deadly Diseases

ScienceDaily (July 18, 2012) — Scientists from the Uniformed Services University of the Health Sciences (USU) have developed a new preparation method that renders a virus or bacterium non-infectious while preserving its immune-boosting ability after exposure to gamma radiation. A vaccine exposed to megadoses of gamma radiation was successfully tested in mice against drug-resistant Staphylococcus aureus bacteria by colleagues at the National Institutes of Health (NIH), and holds promise for other such deadly diseases.

The results of the breakthrough study were published in the July edition of *Cell Host and Microbe*.

High doses of radiation typically destroy a pathogen's genome, rendering it unable to cause infection when used in a vaccine. However, radiation also damages a microbe's protein epitopes, which the immune system must recognize for a vaccine to be protective. Organisms inactivated, or killed, by radiation trigger better immune responses than those inactivated by traditional heat or chemical methods.

Although live vaccines may provide better immune protection than irradiated vaccines, live vaccines are frequently not an option as they can carry an unacceptable risk of infection with an otherwise untreatable disease (e.g., HIV). Lethally irradiated vaccines could also help the developing world, where the need for cold storage limits the availability of live vaccines.

To separate genome destruction from epitope survival, the researchers borrowed some complex chemistry from the world’s toughest bacterium Deinococcus radiodurans, nicknamed “Conan the Bacterium,” which can withstand 3,000 times the radiation levels that would kill a human being. In 2000, *Deinococcus* was engineered for cleanup of highly radioactive wastes left over from the production of atomic bombs. Now, unusual Mn(II)-antioxidants discovered in this extremophile have been successfully applied to preparing irradiated vaccines.

*Deinococcus* accumulates high concentrations of manganese and peptides, which the scientists combined in the laboratory—forming a potent antioxidant complex which specifically protects proteins from radiation. They found that the complex preserves immune-related epitopes when applied to viruses and bacteria during exposure to gamma radiation, but did not protect their genomes.

Michael J. Daly, Ph.D., professor of Pathology at USU, and his research team, collaborated on the work with Sandip K. Datta, M.D., and colleagues at NIH’s National Institute of Allergy and Infectious Diseases (NIAID). Daly devoted 20 years to studying *Deinococcus radiodurans*, which has led to three patents for his work.

The scientists used the Mn-peptide complex in a laboratory setting to successfully protect from radiation damage the protein epitopes of Venezuelan equine encephalitis virus, a microbe that causes a mosquito-borne disease of the nervous system. They also used the preparation method to develop an effective vaccine against methicillin-resistant S. aureus (MRSA) infections in mice. (Credit: Image courtesy of USU)
Learning to Care for Those in Harm’s Way
The researchers believe the whole-microbe vaccine approach could extend to any infectious organism that can be cultivated, whether fungi, parasites, protozoa, viruses or bacteria—including agents that mutate rapidly, such as pandemic influenza and HIV. The groups aim to demonstrate this method of irradiation as a rapid, cost-effective approach to vaccine development.

Journal Reference:

Hair samples from infants show exposure to anti-HIV drugs in the womb and during breast-feeding July 21, 2012 in HIV & AIDS

Hair samples from infants show exposure to anti-HIV drugs in the womb and during breast-feeding
Susan Merrell/University of California, San Francisco Researchers from the University of California, San Francisco (UCSF) and Makerere University in Uganda have used hair and blood samples from three-month old infants born to HIV-positive mothers to measure the uninfected babies' exposure—both in the womb and from breast-feeding—to antiretroviral medications their mothers were taking. The results, they said, are surprising. Ads by Google Hair Follicle Drug Tests—Paymer Associates will come to you! Accredited US Lab, fast and private—www.paymerassociates.com Generic drug list—Easy-to-use tool finds generics for brand-name prescription drugs.—smart-health.com "We found high levels of exposure to three antiretroviral medications in the hair samples of HIV uninfected infants at twelve weeks of life," said study senior author, Monica Gandhi, MD, MPH, associate professor of medicine at the UCSF Division of HIV/AIDS at San Francisco General Hospital and Trauma Center (SFGH). "From looking at plasma level data at the same time point, we believe that transfer of two of the medicines from mother to baby occurs exclusively in the womb and transfer of the third medication occurs both in the womb and through breastfeeding." The findings could lead to new ways to protect infants from HIV transmission and to better understand the development of toxicities and resistance to the drugs, the researchers said. A single plasma level of a medication reflects drug exposure over approximately 24 hours. Measuring the concentrations of antiretrovirals in a small hair sample reveals exposure over the past month. The team therefore measured both plasma and hair levels of medications in babies whose mothers were taking HIV medications to get a better idea of when drugs are being passed from mother to baby. "Since fetuses start growing hair in the womb, hair sampling gives us an opportunity to examine exposures to drug before birth," said Gandhi. UCSF researchers have pioneered the use of hair sampling for measuring antiretroviral levels. The procedure is now a standard measure in many research studies, equivalent in HIV clinical care to measuring hemoglobin A1C to monitor average blood glucose levels in patients with diabetes. In the study, the team took hair and blood samples from two groups of HIV-positive mothers, all of whom breast-fed their infants. For 45 mother/infant pairs, the mothers' antiretroviral regimens included a protease inhibitor, lopinavir, boosted by ritonavir, another antiretroviral medication. The other 64 mothers were on an efavirenz-based regimen. Ads by Google Doula & Lactation Support—Birth & Postpartum Doula Services Lactation Support & Counseling—www.bethbrownstein.com California Drug Treatment—Drug and Alcohol Rehab Programs. 30, 60 or 90 Days. 866-513-1710.—AboveItAllTreatment.com/California Infants in the lopinavir group had levels of the drug in their hair that measured 87 percent of the levels found in their mothers' hair. The levels of ritonavir were about 45 percent of the levels found in their mothers' hair. When the researchers looked at the drug levels in the blood drawn from the mothers and infants at 12 weeks, they found the expected levels of lopinavir and ritonavir in the mothers, but none of either in the blood of the infants. "The inability to find drug in the infants' blood at 12 weeks tells us that the lopinavir and ritonavir in their hair is not due to recent exposure, so breast-feeding did not transfer these drugs to the infants. Our conclusion is that the lopinavir and ritonavir were transferred to the babies in the womb, and lopinavir at quite a high level," said Gandhi. In the efavirenz group, researchers found infant drug levels in hair samples that were about 40 percent of the levels found in their mothers. Additionally, they found that infants had levels in their blood that were about 15 percent of what was found in their mothers. These findings indicate a moderate transfer of efavirenz both in the womb and during breastfeeding said Gandhi. "Our findings, as we verify them, will have important implications. One, being able to measure drug exposures of fetuses in the womb and during breast-feeding can help us understand how to better protect infants from HIV transmission from HIV-positive mothers during pregnancy, birth and after birth. Antiretroviral medications are delivered
prophylactically to HIV-positive mothers and newborns to prevent transmission, and fetuses derive protection from transmission if their HIV-positive mothers are on an antiretroviral regimen," she said. "Second, the development of resistance to antiretroviral medications in infants is an important issue. HIV develops resistant mutations after fairly low levels of exposure to the class of medications to which efavirenz belongs, non-nucleoside transcriptase inhibitors (NNRTIs). Additionally, hair sampling for antiretroviral exposure levels will ultimately help us monitor toxicities associated with these medications in infants." Using hair to measure exposure to antiretrovirals has advantages in that it is a painless, bloodless, biohazard-free method of collecting a stable specimen from HIV patients. It measures drug exposure over time and has been shown to be more predictive of treatment response than the "snapshot" of exposure provided by a single plasma level of medication. Gandhi said that researchers are finding hair sampling to be a very useful tool in several settings. One use is in resource-limited settings where collecting, storing and handling blood draws is difficult and expensive. Hair is snipped, wrapped in foil and needs no refrigeration. Another setting is in monitoring drug exposures in uninfected people. Researchers have been using the technique to measure adherence/drug levels in some of the pre-exposure prophylaxis trials, where high-risk uninfected patients take antiretrovirals to prevent getting infected with HIV. Non HIV-infected individuals cannot be monitored for adherence to antiretrovirals like HIV-infected individuals (where levels of HIV in the blood are measured routinely to indicate how well they are taking their pills) so hair levels provide a novel and reliable indicator of adherence. A third setting is for monitoring prenatal exposures. Hair sampling is the only way currently to measure how much antiretroviral exposure fetuses are getting in the womb long-term. Cord blood measurements of antiretrovirals at birth, which are expensive and cumbersome to collect, still only reflect exposure to the babies over the short-term. And collecting hair levels is a much easier technique for monitoring drug exposure levels in infants, especially when compared to blood draws. This work will be presented on Saturday July 21, 2012 during the 4th International Workshop on HIV Pediatrics, which takes in Washington, D.C. preceding the XIX International AIDS Conference. A poster presentation of the same data, titled, "Lopinavir and efavirenz concentrations in paired hair samples as a marker of cumulative exposure among postpartum women and breastfeeding infants in Tororo, Uganda" will be unveiled at the XIX International AIDS Conference on Sunday, July 22, 2012.

University of Alberta's designer compounds inhibit prion infection
(Edmonton) A team of University of Alberta researchers has identified a new class of compounds that inhibit the spread of prions, misfolded proteins in the brain that trigger lethal neurodegenerative diseases in humans and animals.

U of A chemistry researcher Frederick West and his team have developed compounds that clear prions from infected cells derived from the brain.

"When these designer molecules were put into infected cells in our lab experiments, the numbers of misfolded proteins diminished—and in some cases we couldn't detect any remaining misfolded prions," said West.

West and his collaborators at the U of A's Centre for Prions and Protein Folding Diseases say this research is not yet a cure, but does open a doorway for developing treatments.

"We're not ready to inject these compounds in prion-infected cattle," said David Westaway, director of the prion centre. "These initial compounds weren't created for that end-run scenario but they have passed initial tests in a most promising manner."

West notes that the most promising experimental compounds at this stage are simply too big to be used therapeutically in humans or animals.

Human exposure to prion-triggered brain disorder is limited to rare cases of Creutzfeldt-Jakob or mad cow disease. The researchers say the human form of mad cow disease shows up in one in a million people in industrialized nations, but investigating the disease is nonetheless well worth the time and expense.

"There is a strong likelihood that prion diseases operate in a similar way to neurodegenerative diseases such as Alzheimer's, which are distressingly common around the world," said West.

HIV Injection Could Someday Replace Daily Pill Regimen
ScienceDaily (July 18, 2012) — This has been a good week for breakthroughs in HIV/AIDS.
Earlier this week, the Food and Drug Administration approved a daily pill, Truvada, which reduces the risk of HIV infection. Now, a University of Nebraska Medical Center research team's progress toward developing weekly or twice-monthly injectable antiretroviral therapy (ART) nanomedicines for patients with human immunodeficiency virus (HIV) infection will be highlighted as the cover story in the Journal of Infectious Diseases.

A long-acting, nanoformulated ART (nanoART) would be a substantive improvement over daily and sometimes more complex regimen of pills, said Howard Gendelman, M.D., the lead investigator on the development of nanoART for HIV/AIDS and professor and chairman of the department of pharmacology and experimental neuroscience (PEN) at UNMC.

The journal article hails the successful testing of UNMC's ART injectables as treatment of HIV-infected mice and in preventing new infections.

"We actually followed the process exactly as we would with a person—and it worked," Dr. Gendelman said. "This is all very exciting. Although there are clear pitfalls ahead and the medicines are not yet ready for human use, the progress is undeniable."

Dr. Gendelman said one of the project’s real advantages is in the nanoformulations. "NanoART is cell directed," he said. "So when you take a pill, the pill travels throughout the body indiscriminately. In these nanomedicines, you can use the body’s own cells to direct the medicine where you want it to go."

The UNMC project directs the medicine to the monocyte-macrophage, cells which carry the drug particle to sites of the body specifically where HIV grows.

"You’re using the cell that is the target for the virus to deliver the drug against the virus," Dr. Gendelman said.

Dr. Gendelman calls the progress made "a Nebraska invention," as it involved so many of the state’s scientists in different disciplines working together. He said this marks the third article his team has had published in major biomedical journals in recent months with all the articles related to the nanomedicine injectable therapy.

The advance to use the new nanomedicines to specifically target reservoirs for viral infection was explained by Georgette Kanmogne, Ph.D., associate professor in PEN, lead investigator, and published in the International Journal of Nanomedicine. This paper describes how these drug inventions can enter the brain and ameliorate nervous system disease.

The third study, published in Trends in Neurosciences, is a research area led by Larisa Poluektova, M.D., Ph.D., associate professor in PEN, and the developer of the mouse models of HIV/AIDS used in study.

"We work as a team and work effectively as a team with different scientists with very different disciplines involved in different aspects of the work," Dr. Gendelman said.

This research team is made up of two UNMC colleges, four departments and two universities. The drugs were developed in UNMC labs using nanoformulation approaches.

The ART tests in mouse models were made possible by UNMC's development of specially designed mice. Because mice cannot catch human HIV, UNMC has developed mice with the equivalent of a human immune system. Such a mouse model enables advanced tests on HIV and its treatment.

UNMC is one of a handful of research institutions in the world to have developed such a mouse model, Dr. Poluektova said.

Dr. Kanmogne said an ART injection would be an improvement for HIV patients because it would produce all the medicine needed without the complications of remembering to take pills daily, drug toxicities, buildup of viral resistance, intestinal problems, or the social stigma of pills.

An injectable nanoformulated drug could also be more available for worldwide distribution as the toxicity profiles tested so far are limited, Dr. Gendelman said.

The work was a team effort amongst many and notably Upal Roy, Ph.D., and JoEllyn McMillan, Ph.D., both PEN researchers.

Journal Reference:

Children With Trisomy 13 and 18 and Their Families Appear Happy
ScienceDaily (July 23, 2012) — Children with trisomy 13 or 18, who are for the most part severely disabled and have a very short life expectancy, and their families lead a life that is happy and rewarding overall,
contrary to the usually gloomy predictions made by the medical community at the time of diagnosis, according to a study of parents who are members of support groups published July 23 in Pediatrics. The study was conducted by Dr. Annie Janvier of the Sainte-Justine University Hospital Center and the University of Montreal with the special collaboration of the mother of a child who died from trisomy 13, Barbara Farlow, Eng, MSc as the second author.

The study interviewed 332 parents who live or have lived with 272 children with trisomy 13 or 18. It turns out that their experience diverges substantially from what healthcare providers said it would be, according to which their child would have been "incompatible with life" (87 %), would have been "a vegetable" (50 %), would have led "a life of suffering" (57 %) or would have "ruin their family or life as a couple" (23 %).

It should be noted that trisomies 13 and 18 are rare chromosome disorders that are most often diagnosed before birth and sometimes after. Children who have received these diagnoses generally do not survive beyond their first year of life, while some who do have severe disabilities and a short life. When trisomy 13 or 18 is diagnosed before birth, many parents decide to interrupt the pregnancy, whereas others choose to carry it to term and in such cases miscarriages are common.

As children with trisomies 13 or 18 generally receive palliative care at birth, some parents who opt to continue the pregnancy or desire life-prolonging interventions for their child encounter the prejudices of the medical system. In this regard, the parents interviewed in the study consider that caregivers often view their child in terms of a diagnosis ("a T13," "a lethal trisomy") rather than a unique baby.

"Our study points out that physicians and parents can have different views of what constitutes quality of life," states Dr. Annie Janvier, a neonatologist and co-founder of the Master's program in Pediatric Clinical Ethics at the University of Montreal. In fact, over 97% of the parents interviewed considered that their child was happy and its presence enriched the life of their family and their life as a couple regardless of longevity. "In the medical literature on all handicaps, disabled patients—or their families—rated their quality of life as being higher than caregivers did," adds Dr. Annie Janvier.

Parents who receive a new diagnosis of trisomy 13 and 18 and join a parental support group often acquire a more positive image of these diagnoses than the predictions made by the medical profession. In fact, according to the parents interviewed, belonging to a support group helped them view their experience positively. "Our research reveals that some parents who chose a path to accept and to love a disabled child with a short life expectancy have experienced happiness and enrichment. My hope is that this knowledge improves the ability of physicians to understand, communicate and make decisions with these parents," concludes Barbara Farlow.

Given the rarity of trisomy 13 or 18 cases (one case out of approximately every 10,500 births), the parents were recruited through online support groups that parents often join after receiving the physicians’ diagnosis. Dr. Annie Janvier and Barbara Farlow sometimes give joint talks on the subject of trisomies 13 and 18.


Co-administration of rifampicin and efavirenz does not reduce efavirenz concentrations or efficacy
Lesley Odendal
Published: 24 July 2012
Standard dosing of efavirenz, that was not adjusted for patient weight, resulted in therapeutic efavirenz concentrations and excellent virological outcomes in patients coinfected with TB and HIV who were also taking a rifampicin-containing TB treatment, according to results from the ACTG 5221 STRIDE study presented at the at the 19th International AIDS Conference (AIDS 2012) in Washington DC.

These findings have important implications for guidelines on rifampicin and efavirenz co-administration in TB/HIV coinfected patients. Rifampicin is known to cause drug-drug interactions especially with efavirenz, which is recommended in first-line ARV treatment. Rifampicin co-administration is associated with an approximately 30% decrease in efavirenz trough concentrations ($C_{min}$). Previous studies have showed that efavirenz dosing should be increased from the standard dose of 600mg to 800mg in TB/HIV patients who weigh more than 50kg when taking rifampicin; a recommendation which the Food and Drug Administration of the United States of America made in January this year.
Efavirenz concentrations were measured using high-performance liquid chromatography where the lower limits of quantification was defined as 1mg/l. C_{min} samples were obtained 20–28 hours after efavirenz administration. Efavirenz levels were evaluated at ARV treatment weeks 4, 8, 16 and 24 for those patients on rifampicin, and weeks 4 and 8 for those not on rifampicin. Samples were only collected in participants with no self-reported missed efavirenz or rifampicin doses in the previous three days.

According to Dr Annie Luetkemeyer, higher weight did not jeopardise efavirenz efficacy in patients taking rifampicin-containing TB treatment. In 505 patients who were taking both efavirenz and rifampicin, none reached below the minimum recommended plasma concentration of <1 mg/l of efavirenz. The median efavirenz C_{min} was found to be 1.96 mg/l (IQR: 1.24 – 3.7).

Heavier patients did have significantly lower efavirenz concentrations, but only when heavier patients were defined as weighing 60kg or above. The median efavirenz C_{min} in patients weighing below 50kg was 2.08 (IQR:1.33-4.33) compared to 1.86 (IQR:1.18-3.64) in those patients weighing 50kg and above (p=0.09). In contrast, the median efavirenz C_{min} in patients weighing below 60kg was higher at 2.02 (IQR: 1.29-4.09) compared to 1.68 (IQR: 1.07-3.06) in those patients weighing 60kg and above (p=0.02).

When examining the efavirenz C_{min} in patients also taking rifampicin compared to those who were not, there was no significant difference found across patient weight levels, except when disaggregated by race. There was a significant difference in efavirenz C_{min} in black patients taking efavirenz with or without rifampicin. For the black patients taking rifampicin (n=367), the efavirenz C_{min} level was 2.1 compared to those not taking rifampicin (n=269) whose efavirenz C_{min} level was 1.8 (p=0.01). The researchers suggested that this paradoxical finding is likely due to genetic distinctions in metabolism in black patients. The study did not include enough members of other races to show significant differences.

Subtherapeutic efavirenz C_{min} of less than 1 mg/l was not associated with rifampicin coadministration, according to the study results. 27.3% on rifampicin versus 26.2% not taking rifampicin had any efavirenz C_{min} less than 1mg/l and this finding was not found to be statistically significant (p=0.72).

Rates of HIV virological suppression were also found not to be reduced in patients above 50 kg or 60 kg taking efavirenz and rifampicin. The investigators concluded that these data do not support weight-based increase of efavirenz during rifampicin-containing TB treatment.

### Efavirenz and rifampicin coadministration in pregnant women

Rifampicin was also shown to have no significant effect on efavirenz C_{min} levels in pregnant women and their infants, according to the preliminary results from the TSHEPISO efavirenz pharmacokinetic substudy presented at the 19th International AIDS Conference (AIDS 2012) in Washington DC.

The combined effect of pregnancy and rifampicin-containing TB treatment on efavirenz C_{min}, virologic suppression and prevention of maternal-to-child transmission of HIV (PMTCT) has not been studied before.

TSHEPISO is a prospective cohort study among HIV-infected pregnant women with TB (n=250 cases) and without TB (n=500 controls), currently enrolling in Soweto, South Africa. Women (n=150) with and without TB, on efavirenz-containing ARV will enrol in the substudy, along with their infants.

The preliminary results from 76 women and 70 infants in the substudy to date show that the estimated efavirenz C_{min} among women pre/intrapartum and post-partum were not significantly different. The model which also took weight and CYP2B6 genotype (the enzyme central to the metabolism of efavirenz) into account, was unable to show a significant effect of rifampicin on efavirenz C_{min} (except among slow efavirenz metabolizers). The median efavirenz C_{min} of the 40 pre/intrapartum women taking rifampicin was 1.76 (IQR: 0.89-3.13), with 29.6% with an efavirenz C_{min} of less than 1mg/l. In the 46 women not taking rifampicin six weeks post-partum, the median efavirenz C_{min} was 1.52 (IQR: 1.14-2.02) with 17.1% with an efavirenz C_{min} of less than 1mg/l.

Despite the 29.6% of women on rifampicin with C_{min} less than 1mg/l, the viral load was suppressed in most women taking efavirenz for three months or more at the time of delivery. There were no cases of mother to child transmission of HIV.

The effect of genotype CYP2B6 showed that 56.3% with extensive, 5.7% with intermediate, 16.7% with slow and 0% with very slow efavirenz metabolism had an efavirenz C_{min} of less than 1mg/l.

Difference in weight did not prove a significant factor for EFV C_{min} levels in those taking rifampicin or not taking rifampicin. The median C_{min} levels were both 1.91 for women below and above 60kg in those taking rifampicin, but 20% of those less than 60kg had a C_{min} less than 1mg/l compared to 31.6% in the women weighing more than 60kg. The median C_{min} for women less than 60kg was 1.33 (IQR: 1.12-1.64, 11.1% with a C_{min} less than 1mg/l) and 1.55 (IQR:1.13-2.07,16.7% with a C_{min} less than 1mg/l) in women not on rifampicin treatment.
Preliminary results also showed that 70% of TB/HIV co-infected women and 83% of HIV-only infected women had undetectable viral load counts at delivery, although this difference was not statistically significant (p=0.24). Of those taking efavirenz for at least 12 weeks at delivery, 82% of TB/HIV co-infected women and 93% of HIV-only infected women had undetectable viral loads, although this difference was again statistically insignificant (p=0.26).

Blood samples taken from infant umbilical cords in 45 infants showed the median efavirenz \( C_{\text{min}} \) to be 1.15 (IQR: 0.628-1.91, 8.9% with a \( C_{\text{min}} \) less than 1mg/l. Cord and maternal pre-partum concentrations were highly correlated (r=0.93). However, the median efavirenz \( C_{\text{min}} \) in infant blood at seven days after birth was 0.079 (61.4% with a \( C_{\text{min}} \) less than 1mg/l). However, quantifiable values were related to larger cord blood concentrations.

**References**


**US evangelical Christians accused of promoting homophobia in Africa**

Liberal thinktank says rightwingers are aggressively targeting the continent with an anti-abortion and anti-gay agenda

*David Smith* in Johannesburg

Christian evangelical groups in the US are attempting a "cultural colonisation" of Africa, opening offices in numerous countries to promote attacks on homosexuality and abortion, according to an investigation by a liberal thinktank.

American religious organisations are expanding their operations across the continent, lobbying for conservative policies and laws and fanning homophobia, argues the Boston-based Political Research Associates (PRA).

The groups include the American Center for Law and Justice (ACLJ), founded by the televangelist Pat Robertson, which has established bases in Kenya and Zimbabwe.

"The religious right [in effect] claims that human rights activists are neocolonialists out to destroy Africa," the report states. Groups named in it vehemently rejected the claims.

Entitled Colonising African Values: How the US Christian Right is Transforming Sexual Politics in Africa, the study analysed data from seven African countries and employed researchers for several months in Kenya, Malawi, Zambia and Zimbabwe.

It identified three organisations it believes are aggressively targeting the continent: Robertson’s ACLJ, the Catholic group Human Life International and Family Watch International, led by the Mormon activist Sharon Slater.

Each of these "frame their agendas as authentically African, in an effort to brand human rights advocacy as a new colonialism bent on destroying cultural traditions and values", the report says.

In the past five years, the report alleges, all "have launched or expanded their work in Africa dedicated to promoting their Christian right worldview". A loose network of rightwing charismatic Christians called the transformation movement joins them in fanning the flames of the culture wars over homosexuality and abortion by backing prominent African campaigners and political leaders."

Dr Kapya Kaoma, an Anglican priest from Zambia and author of the report, said rightwing Christian groups encourage perceptions that same-sex relations are "un-African", and imposed by the west, a view that is in fact based on the Bible that arrived with colonialism rather traditional African culture.

He gave the example of a young lesbian in Zimbabwe who was taken to several churches to have "the devil driven out of her", but later honoured when her grandmother said she was in fact possessed by the spirit of her dead uncle, who had never married.

"The foreignness of homosexuality is not true, but it is when presented in Christian-right language," Kaoma said.

Certain countries are more hospitable to US Christian-right campaigners than others, the research found, in part because of support from government officials.

"The presidents of Zambia, Zimbabwe, and Uganda themselves accused opposition parties of promoting homosexuality to undercut their influence and cater to powerful African religious conservatives."
The ACLJ was invited by Zimbabwe's president, Robert Mugabe, for example, to open offices to train lawyers to work on a constitution that would reflect "Christian values".

A similar effort was made to influence the writing of Kenya's and Zambia's constitutions with the inclusion of phrases such as "life begins at conception".

The report accuses Slater, from Family Watch International, of indulging in alarmist rhetoric that the UN's population control strategy will destroy the African family.

She has claimed homosexuals are significantly more promiscuous and "more likely to engage in paedophilia", it says.

Kaoma said: "[Slater] claims the UN has been taken over by homosexuals. She makes up nonsense and presents it as facts to Africans. She argues that terms such as 'gender rights' and 'sexual identity' are code for homosexuality."

Kaoma believes the American groups are in retreat in the US and so turning to Africa for quick gains. "They seem to know they are losing the battle in the US, so the best they can do is to be seen to be winning somewhere.

"This gives them a reason to be fundraising in the US. Africa is a pawn in the battle they are fighting at home."

The report was welcomed by gay rights campaigners. Frank Mugisha, executive director of Sexual Minorities in Uganda, said: "I'm grateful for the documentation in the report that confirms that it is homophobia (not homosexuality) that is exported from the west.

"I hope this report serves as a wakeup call for faith communities in Uganda and the west alike to realise that the American culture wars imposed on us by the Christian right threaten not only African culture, but the very lives of LGBTQI Africans like me."

Human Life International acknowledged that it has several affiliates in Africa, some of which receive grants, educational materials and other support.

"We feel that it is important for us to be there because the assault on the natural African pro-life and pro-family values is coming from the United States, so we feel obliged to help them understand the threat and respond to it based on their own values and culture," spokesman Stephen Phelan said.

"That is why we can operate with a tiny fraction of the budget that the true colonialists – the extremely well financed population controllers and western governments – operate with.

"We speak to the deep and natural values of our brothers and sisters in Africa, and help them resist the encroachment of very powerful western interests who think that there are too many children in Africa."

He dismissed Political Research Associates' claim that his organisation was practising a new colonialism.

"We expect your more thoughtful readers to note the irony in PRA's argument. Powerful western governments and very wealthy NGOs spend billions annually to stop Africans from having children, to change African laws to be more accommodating to this population control, all in an effort to make them culturally more like the west.

"And the PRA, a proponent of this effort, is accusing a small group of Christian organisations, who together spend a tiny fraction of the development industry's annual budget to preserve pro-life and pro-family natural African values, of 'colonialism'. Where does one begin?"

Slater also attacked the report. "We have no offices in Africa as Mr Kaoma falsely claims," she said.

"To make such a fundamental error is alone an indication of the unreliability of his entire report."

She added: "We are not the religious Christian right as Mr Kaoma has insisted on portraying us, despite what I told him and despite the content of our materials published and on our website."

"The only mention of religion on our website or in any of our materials is our concern that religious freedom be protected, regardless of the faith that might be under attack.

"Our position here is based on the clear data that shows a high correlation between religious observance and stable families, and not due to any particular belief or doctrine."

Joy Mdivo, executive director of the East African Centre for Law and Justice, said the US division pays for office space and salaries, but the EACLJ raises its own funds for activities.

"Someone was saying we were given money by the Americans to spread homophobia, and I was telling them, 'I don't have to spread homophobia. Just take a walk down the street, hold another man and look like you are being romantic. I don't have to tell anyone what to do.' That's just the reality of where we are."
Voices on HIV/AIDS and the Black Church


As the 19th International AIDS Conference began in Washington, several faith leaders and others were interviewed about the black church’s handling of the HIV/AIDS epidemic.

The Rev. Anthony Evans, president of the D.C.-based National Black Church Initiative, is about to unveil a controversial recommendation that people “take a year off of sex and deal with who they are.”

Evans acknowledged that talking about AIDS “goes against historic and deep-seated folkways and norms of the black community, that you are to be silent about your personal life.” However, he wants people to get tested and share the results.

The Rev. Tony Lee launched Community of Hope, a nightclub-turned-church in Hillcrest Heights, Md. that does HIV testing during services four times a year. Lee himself has been tested at the pulpit and said, “What better place to be than in the House of the Lord, to find out where you stand? And who you can stand on?” When people get their results during the service, they receive health and spiritual support.

Pernessa Seele’s group, the Balm in Gilead, is co-sponsoring a conference on faith and AIDS at Howard University this weekend. She said, “The role of the church is unique for the African-American community. It’s where we disseminate information on anything. ...The role of faith is to dismantle stigma. We do that by speaking truth around people’s lives.”

Alton B. Pollard III, dean of Howard Divinity School, said HIV/AIDS awareness is growing in the black church. “Instead of pointing fingers at behaviors, it should be an ethical challenge. ...It’s a turn, to look at yourself, the man in the mirror.”

Joe Madison, a Washington radio talk show host on WOL-AM, said HIV/AIDS issues remain “basically ignored” in the black community because of homophobia, ignorance, and embarrassment.

Fewer Americans Suppressing HIV Virus, Study Finds

*Baltimore Sun*, (07.22.2012) Meredith Cohn

According to a new study, many US HIV patients are not effectively controlling their infection, mostly due to a lack of drug adherence. Young adults, African-Americans, injection drug users, and the uninsured are particularly affected.

The researchers looked at 100,000 blood tests from more than 30,000 patients over a decade—believed to be the longest review of its kind. They found 72 percent were controlling their viral loads well, which was lower than the 87 percent previously found. Still, these numbers are significantly better than 2001, when only about 45 percent had well-controlled viral loads, noted Dr. Kelly Gebo, senior study investigator and infectious-disease specialist at Johns Hopkins University.

The researchers pointed to concerns of drug resistance and putting others at risk. Dr. Baligh Yehia, a postdoctoral fellow in Pennsylvania’s School of Medicine, said, “An individual who misses one day’s worth of drugs is at risk of becoming resistant.” Also, “When you consider that over a large population, that’s how people spread the virus. ... And they may be spreading the resistant kind. It’s a dangerous spiral.”

Most people can now take one daily, multi-drug pill; however, if they become resistant to one of the drugs, they must take different medications in multiple pills, causing potential drug adherence problems. More efforts are needed to ensure drug adherence; the researchers plan additional research.

The study may increase concerns about using an antiretroviral drug for prevention among HIV-negative people, despite FDA’s recent approval for that purpose, according to Gebo. Yehia noted, “We’ve made progress, but being able to take a pill every day is a lot harder than previously thought.”


HIV Test Wins Award

*Australian Associated Press*, (07.20.2012)

A CD4 test developed by researchers at Melbourne’s Burnet Institute has received an international award for innovation. The test, to be manufactured by Omega Diagnostics Group, uses a blood drop from a finger-prick to track the damage caused by HIV; it delivers results in less than an hour and is much cheaper and more efficient than existing tests. The money accompanying the award will be used by Professor Stanley Luchters of the Institute’s Center for International Health to validate the test among HIV-positive pregnant women in sub-Saharan Africa. The test was one of 15 projects funded through Saving Lives at Birth, an initiative support by USAID, Norway, the Bill & Melinda Gates Foundation, Grand Challenges Canada, and the UK Department for International Development.
Under the right conditions, peptide blocks HIV infection at multiple points along the way

Researchers: Findings about innate peptide may offer new avenue of research for combating HIV, other viruses

Human defensins, aptly named antimicrobial peptides, are made in immune system cells and epithelial cells (such as skin cells and cells that line the gut). One of these peptides, human neutrophil peptide 1, under certain circumstances hinders HIV infection, but exactly how it works remains unclear.

HIV entry into mature T-helper cells (cells essential to the immune system) proceeds by attachment of the virus to specific targets on T-helper cells, uptake of the virus, fusion of its envelope with the cell membranes, and release of the virus into the cells. In a forthcoming *Journal of Biological Chemistry* Paper of the Week, Gregory Melikyan at Emory University and colleagues investigated the ability of human neutrophil peptide 1 to impede each step of this process.

Using model cell lines, Melikyan’s group showed that human neutrophil peptide 1 effectively prevented HIV entry into cells in multiple ways. First, human neutrophil peptide 1 reduced the number of specific targets on the cells available for HIV attachment. Second, this defensin also bound to specific targets on both the HIV envelope and the cells, preventing early and late stages of HIV-cell fusion. Finally, human neutrophil peptide 1 prevented HIV uptake into the cells without compromising the general ability of the cells to engulf other molecules.

While human neutrophil peptide 1 hinders HIV entry into cells under these lab conditions, it does not do so as effectively in the presence of serum—meaning that it may not be as successful at blocking HIV in our bodies. But Melikyan’s team showed that human neutrophil peptide 1 remained attached to its specific targets in the presence of serum, despite its reduced efficacy. Their work suggests that the structure of human neutrophil peptide 1 is important for its anti-HIV activity, and they propose that serum may interfere with the ability of this defensin to form complexes, reducing its ability to block HIV.

“Our work provides new insights into the ability of defensins to recognize and neutralize diverse pathogens, including HIV,” Melikyan says. This research reveals that human neutrophil peptide 1 can bind various viral and cellular targets and that a previously unappreciated feature is essential for its anti-HIV activity, possibly its propensity to form large complexes, Melikyan explains.

The team’s findings suggest a new avenue of research for combatting HIV and viruses that infiltrate cells in a similar manner.

*From the article:* “Multifaceted mechanisms of HIV-1 entry inhibition by human alpha-defensin” by Lusine H. Demirkhanyan, Mariana Marin, Sergi Padilla-Parra, Changyou Zhan, Kosuke Miyachi, Maikha Jean-Baptiste, Gennadiy Novitskiy, Wuyuan Lu, and Gregory B. Melikyan (to be published in the Aug. 17 issue of the *Journal of Biological Chemistry* and currently online as a Paper in Press at [http://www.jbc.org/content/early/2012/06/25/jbc.M112.375949.full.pdf](http://www.jbc.org/content/early/2012/06/25/jbc.M112.375949.full.pdf))

Human papillomavirus types do not replace others after large-scale vaccination

by Mary Ruth — last modified Jul 23, 2012 04:55 PM

Vaccines against human papillomavirus (HPV) are now recommended by the Centers for Disease Control and Prevention for both teenage boys and girls. The vaccine protects against the two most common types of the virus that cause cervical cancer: HPV 16 and 18. Is there a chance that the increased number of people vaccinated might result in an increase of other types of HPV that cause cancer?

A UNC-led international team of scientists studied this question in a group of 2228 Kenyan men as a “nested” trial in a larger trial. Their first paper in the *Journal of Infectious Diseases* showed that little evidence exists for potential HPV type competition in a cross-sectional study. Viral type competition occurs when different types of a particular virus compete for dominance.

Their new work is reported in the June 18, 2012 early online issue of the *Journal of Infectious Diseases*. Using prospective data, their study presents the first epidemiological data in men on the type-specific associations between prevalent HPV infections and future acquisition of other HPV types.

Jennifer Smith, PhD, MPH, study senior author explains, “We found no evidence for competition between different HPV types over time in high-risk men from Kenya. While these data are based only on non-vaccinated men, our findings are of potential importance because they suggest that HPV types are generally acting independently from one another, and thus it is unlikely that HPV type-replacement will occur following large scale vaccination programs of young male adolescents.”

Dr. Smith is an associate professor of epidemiology in the UNC Gillings School of Global Public Health and a member of UNC Lineberger Comprehensive Cancer Center.
With the recent approval of prophylactic HPV vaccination of young men, data are needed to understand if patterns of HPV acquisition differ among men with specific HPV type infections as compared to men without these HPV infections. The effect of current vaccine-relevant HPV infections on the subsequent acquisition of different HPV types could impact the long-term potential for HPV type replacement following population-based HPV vaccination.

Bats, a Reservoir of Resurgent Viruses
ScienceDaily (July 24, 2012) — Measles, mumps, pneumonia, influenza and encephalitis in man, Carré's disease in dogs, Ovine Rinderpest (PPR)... all of these diseases are caused by viruses from the same family: Paramyxoviridae. A vast international study(1), carried out in collaboration with IRD researchers and published in Nature Communications has led to the discovery of more than 60 new species of these dangerous infectious agents, almost double the number previously recorded. This family of highly diverse pathogens affects all animals, from canines to fowl, cattle and humans. As a result, it is not always easy to determine which host is responsible for these viruses. Thanks to testing carried across the globe, the research team has recently discovered their source: bats.

Virologists have collected over 10,000 animal samples, including more than 90 Chiroptera(2) species from Africa, Latin America, Asia and Europe. As a result of blood and organ analysis, researchers have observed a large genetic diversity of paramyxoviruses in these small mammals. This suggests that these infectious agents have had enough time to evolve in bats over the course of history. They have thus been present for a very long time in this order of animals. In addition, scientists have found them in all known species of bat worldwide. This planetary spread signifies that it is the result of movement from continent to continent from a common ancestor and that these flying hosts have been carriers for millennia. Lastly, biologists have found nearly all genera from the paramyxovirus in bats, which has not been the case with any other animal. Such viral representation confirms that they are at the origin of all infection across the animal kingdom. To provide the final proof, researchers investigated the probability that each order—bats, rodents, birds, humans, canines or bovines—could be the source of contamination. Using paramyxovirus phylogeny—the family tree, so to speak—the probability of transfer is highest from bats to other animals.

The threat is still hovering
Researchers have also made a worrying discovery. Chiroptera might also be a reservoir of certain paramyxoviruses that were thought to be specific to humans. Scientists have found evidence among these small animals of paramyxoviruses that are genetically very similar to those observed in man and which could cause infection in humans once again. Childhood diseases such as measles or mumps, which the WHO considers as having been practically eradicated, in developed countries at least, could re-emerge. Any eradication hypothesis(3) requires all animal reservoirs to be eliminated.

Continents on the brink
Another worrying finding from the study is that certain highly dangerous viruses have been discovered in regions of the world where they were thought to be absent. This is the case for the Hendra and Nipah viruses, two emerging pathogens which have recently been the cause of fatal encephalitis(4) epidemics in Asia and Australia. No other cases have been detected in the world until now. And yet, researchers have found the viruses in the organs of African bats. In Gabon and Ghana, where the study has focused, two infectious agents seem to be highly present, which raises fears for possible emergence on the African continent.

Bats are already recognised as carrying diseases such as Ebola and rabies, notorious for devastating outbreaks, although these are rare and geographically contained. We are now learning that they are reservoirs of a multitude of infections that affect humans and animals worldwide. All epidemiological study on paramyxoviruses should now take into account the ecological data available for these airborne animals.

Notes:
(1) This research has been carried out in collaboration with the universities of Bonn, Hanover, Marburg, Cologne and Ulm, the Noctalis centre, the Bernhard Nocht Institute for Tropical Medicine, the Charité Medical School and the Institute for Novel and Emerging Infectious Diseases in Germany, CIRMF in Gabon, the Czech Republic Academy of Sciences, Strandja national park in Bulgaria, Kumasi University in Ghana, Lubumbashi University in DRC, Bahia University in Brazil and Stellenbosch University in South Africa, Chumakov Institute of Poliomyelitis and Viral Encephalitides in Russia, the Smithsonian Tropical Research Institute in Panama, KCCR in Ghana, the Institut Pasteur in Bangui, Central African Republic,
the Netherlands Center for Infectious Disease Control, the Muséum National d’Histoire Naturelle and the CNRS.

(2) Bats belong to the order Chiroptera.
(3) The WHO announced a new strategic plan in April 2012 aiming to eliminate measles in at least six WHO Regions by 2020.
(4) Encephalitis is a swelling of the brain

Journal Reference:

Review | July 25, 2012

Antiretroviral Treatment of Adult HIV Infection 2012 Recommendations of the International Antiviral Society–USA Panel ***** (very long)
Melanie A. Thompson, MD; Judith A. Aberg, MD; Jennifer F. Hoy, MBBS, FRACP; Amalio Telenti, MD, PhD; Constance Benson, MD; Pedro Cahn, MD, PhD; Joseph J. Eron, MD; Huldrych F. Günthard, MD; Scott M. Hammer, MD; Peter Reiss, MD, PhD; Douglas D. Richman, MD; Giuliano Rizzardini, MD; David L. Thomas, MD; Donna M. Jacobsen, BS; Paul A. Volberding, MD

ABSTRACT
Context New trial data and drug regimens that have become available in the last 2 years warrant an update to guidelines for antiretroviral therapy (ART) in human immunodeficiency virus (HIV)—infected adults in resource-rich settings.

Objective To provide current recommendations for the treatment of adult HIV infection with ART and use of laboratory-monitoring tools. Guidelines include when to start therapy and with what drugs, monitoring for response and toxic effects, special considerations in therapy, and managing antiretroviral failure.

Data Sources, Study Selection, and Data Extraction Data that had been published or presented in abstract form at scientific conferences in the past 2 years were systematically searched and reviewed by an International Antiviral Society–USA panel. The panel reviewed available evidence and formed recommendations by full panel consensus.

Data Synthesis Treatment is recommended for all adults with HIV infection; the strength of the recommendation and the quality of the evidence increase with decreasing CD4 cell count and the presence of certain concurrent conditions. Recommended initial regimens include 2 nucleoside reverse transcriptase inhibitors (tenofovir/emtricitabine or abacavir/lamivudine) plus a nonnucleoside reverse transcriptase inhibitor (efavirenz), a ritonavir-boosted protease inhibitor (atazanavir or darunavir), or an integrase strand transfer inhibitor (raltegravir). Alternatives in each class are recommended for patients with or at risk of certain concurrent conditions. CD4 cell count and HIV-1 RNA level should be monitored, as should engagement in care, ART adherence, HIV drug resistance, and quality-of-care indicators. Reasons for regimen switching include virologic, immunologic, or clinical failure and drug toxicity or intolerance. Confirmed treatment failure should be addressed promptly and multiple factors considered.

Conclusion New recommendations for HIV patient care include offering ART to all patients regardless of CD4 cell count, changes in therapeutic options, and modifications in the timing and choice of ART in the setting of opportunistic illnesses such as cryptococcal disease and tuberculosis.

Since the first antiretroviral drug was approved 25 years ago, improvements in the potency, tolerability, simplicity, and availability of antiretroviral therapy (ART) have resulted in dramatically reduced numbers of opportunistic diseases and deaths where ART is accessible. New data show that viral suppression due to ART results in decreased human immunodeficiency virus (HIV) transmission on individual and population levels and that, when used consistently by HIV-uninfected persons, ART also may provide protection against HIV infection. Together, these developments have translated into newly articulated visions of the “beginning of the end of AIDS.” This revision of the International Antiviral (formerly AIDS) Society–USA (IAS-USA) guidelines reflects new data informing consideration of when to initiate ART, new options for initial and subsequent therapy, ART management in the setting of special conditions, and new approaches to monitoring treatment success and quality. Discussion of the emerging area of antiretroviral preexposure prophylaxis for high-risk HIV-seronegative persons is included.
METHODS
A systematic literature review using PubMed and EMBASE was conducted to identify relevant evidence published since the last report. Data presented at scientific conferences in abstract form or released as safety reports by regulatory agencies or data and safety monitoring boards also were considered. Specific search terms included "HIV and antiretroviral and treatment (or prevention or toxicity or monitoring)" and filters included dates (July 1, 2010, to May 25, 2012), English, humans, adults, clinical trial OR meta-analysis OR guidelines OR editorials OR review, and full text OR free text OR abstracts. More than 600 potentially related articles were identified, of which 141 were determined to be relevant. Panel members conducted hand searches for newly published reports, abstracts from scientific conferences, and safety reports throughout the guideline development process; manufacturers of antiretroviral drugs provided lists of published, presented, and safety data, which were cross-checked with search results. Data that were not published or presented in a peer-reviewed setting were not considered.

Recommendations were developed by an international panel established initially by the IAS-USA in 1995 with planned member rotations. Members are experts in HIV research and clinical care and serve in a volunteer (noncompensated) capacity. Members do not participate in industry promotional activities such as speaker bureaus, lectures, or other marketing activities during their membership on the panel. The current panel convened in January 2012 and met twice weekly by teleconference. Section leaders (J.A.A., J.F.H., A.T., and P.A.V.) and teams were appointed to evaluate evidence and summarize panel discussions for each section. Prior to selection of teams and leaders, panel members declared and discussed potential conflicts of interests and recused themselves from serving as section leaders or team members, accordingly.

The panel limited recommendations to HIV-infected adults in international resource-rich settings with ART that was available (approved by regulatory bodies or in expanded access) or in late-stage development (New Drug Application filed). Recommendations were made by full panel consensus and rated according to the strength of the recommendation and the quality of the supporting evidence (eBox). For areas in which recommendations have not changed substantially or no or few new data are available, the previous report is referenced.2

WHEN TO START
All adults with HIV infection should be offered ART regardless of CD4 cell count, based on recent observational cohort data that all patients may benefit from ART and data from a randomized controlled trial showing that ART reduces the likelihood of HIV transmission while providing clinical benefit to treated individuals. When prescribing ART, the following should be considered: (1) a patient must be ready and willing to adhere to ART, and adherence education and support should be offered; (2) the benefit of ART is unknown in elite controllers (HIV-1 RNA below the level of quantification without ART) and long-term nonprogressors (those with stable CD4 cell counts >500/μL and HIV-1 RNA <1000 copies/mL while not taking ART); (3) the benefit of ART in asymptomatic acute HIV infection is not as well studied as in symptomatic acute HIV infection; and (4) there is no CD4 cell count threshold at which starting therapy is contraindicated, but the strength of the recommendation and the quality of the evidence supporting initiation of therapy increase as the CD4 cell count decreases and when certain concurrent conditions are present (Box 1).

Box 1. Recommendations for When to Initiate Antiretroviral Therapy (ART)a
- Patient readiness for treatment should be considered when deciding to initiate ART. Clinicians should engage supportive services as needed to assist with ART education and to address barriers to adherence (AIII).
- ART is recommended and should be offered regardless of CD4 cell count (AIIa-CIII). The strength of the recommendation increases as CD4 cell count decreases and in the presence of certain conditions, with the following ratings:
  - For CD4 cell count of 500/μL and below: AIIa
  - For CD4 cell count above 500/μL: BIII
  - Ratings for specific conditions are as follows:
    - Pregnancy: AIIa
    - Chronic hepatitis B virus (HBV) coinfection: AIIa
    - Hepatitis C virus (HCV) coinfection: CIII (however, coinfection with CD4 cell count >500/μL may delay ART until after completion of HCV treatment)
    - Age older than 60 years: BIIa
    - Human immunodeficiency virus (HIV)-associated nephropathy: AIIa
• ART is recommended and should be offered to persons during the acute phase of primary HIV infection, regardless of symptoms (BIII).

• ART should be started as soon as possible, preferably within the first 2 weeks of diagnosis, in patients with opportunistic infections (A1a). The optimal timing for patients with cryptococcal meningitis is less certain, but initiating ART early during cryptococal treatment may be associated with higher mortality; therefore, ART initiation in these patients should be managed in consultation with experts (BIII).

• ART is recommended in all HIV-infected persons with tuberculosis (TB) and should be started within 2 weeks of TB treatment when the CD4 cell count is below 50/μL and by 8 to 12 weeks for those with higher CD4 cell counts (A1a). The optimal timing for patients with TB meningitis is less certain, but ART should be started within the first 2 to 8 weeks of diagnosis and managed in consultation with experts (BIII).

aRatings of the strength of the recommendations and quality of evidence are described in the eBox.

bThese recommendations differ from some HBV treatment guidelines that require both high-level HBV replication and necroinflammation. However, HBV liver disease progresses more rapidly in HIV-infected persons and the safety of the low necroinflammatory state is less well established than in persons without HIV.

**Established HIV Infection**

In addition to the previously described data, recent evidence increasingly supports earlier initiation of ART. Although no randomized controlled trial defines the optimal time of initiation, available data are consistent with and further strengthen the recommendation for early ART.

In the HIV-CAUSAL collaboration, there was a significant and steady decrease in AIDS-free survival as the CD4 cell count threshold for initiation of therapy decreased. There was an estimated 38% increase in the hazard of AIDS or death when therapy was initiated below a CD4 cell count of 350/μL compared with 500/μL. The CASCADE seroconversion cohort, with more than 9000 study participants, confirmed the benefits of starting ART below 500 CD4 cells/μL. The COHERE study of 75,336 individuals examined the prognostic value of the CD4 cell count after virologic suppression by ART and noted that higher CD4 cell count was associated with incremental decreases in the risk of new AIDS events, all-cause mortality, and non-AIDS mortality across all CD4 cell strata up to 500/μL and a slightly reduced risk of disease progression above 500/μL.

Similarly, other cohort studies noted that the higher the CD4 cell count achieved after ART, the greater the survival benefit, implying that starting ART earlier may lead to improved outcomes. In the Athena cohort, older age, lower CD4 cell nadir, and lower plasma HIV-1 RNA at the start of ART were independent predictors of poor immunologic recovery, leading to increased morbidity and mortality. Furthermore, the HIV Prevention Trials Network (HPTN) 052 study of 1763 HIV-serodiscordant couples with CD4 cell counts between 350/μL and 550/μL showed that immediate initiation of therapy resulted in a 41% reduction in serious World Health Organization stage 4 events, pulmonary tuberculosis (TB), serious bacterial infections, and death. Because the study was conducted largely in low- and middle-income countries, the clinical end-point analysis was driven predominantly by TB.

In a registry of 20,775 HIV-infected and 215,158 uninfected persons, the incidence of most cancers was either no longer elevated in HIV-infected persons with CD4 cell counts at or above 500/μL compared with HIV-uninfected persons or was greatly decreased, also supporting earlier initiation of ART. Several cross-sectional studies examining the effect of CD4 cell count nadir on surrogate markers of cardiovascular risk suggest benefit for early therapy, although studies proving that ART can decrease this risk are lacking at this time.

The concentration of HIV in both blood and seminal plasma correlates with the probability of transmission of HIV to a sexual partner. Reducing levels of HIV with ART decreases the probability of transmission, as confirmed in the HPTN 052 study, in which ART was 96% effective in reducing HIV transmission. Reduction of transmission has also been shown in high-risk men who have sex with men, although viral suppression in plasma does not guarantee suppression in semen, especially in the presence of inflammation. Additionally, other sexually transmitted infections such as hepatitis C virus (HCV) and syphilis continue to be reported at high rates, especially in men who have sex with men, underscoring the importance of continued condom use.

Several communities with high ART use have observed an association between reduced “community viral loads” and lower rates of new infections. The use of HIV treatment as prevention addresses an important public health objective, especially in the absence of a vaccine or additional inexpensive, highly
effective prevention strategies other than condom use and male circumcision. Fortunately, the expanding recommendations for nearly universal treatment of HIV-infected persons in resource-rich countries and some middle-income countries render the recommendations for treatment of the individual concordant with public health goals. Challenges include limited financial and workforce resources, the need to implement broader testing, and the need for improved strategies to enhance engagement in HIV care and adherence to ART.

Special Considerations

Pregnancy. ART is indicated for all pregnant women to prevent HIV transmission to the infant and for the mother’s health. Those not yet taking ART should start fully suppressive therapy as soon as possible. The potential for nonadherence due to morning sickness should not be an impediment to starting therapy. Women who conceive while already taking ART, including efavirenz or tenofovir, should continue the same therapy unless there is a need for change due to failure or intolerance. Therapy should not be discontinued post partum.

Opportunistic Infections. Early initiation of ART is recommended after starting active treatment of opportunistic infections. However, implementation may require focused educational and logistical support and consideration of the potential for drug interactions requiring dosage alterations.

Recent data have raised concerns about the timing of ART initiation during cryptococcal meningitis. In a randomized clinical trial conducted in Zimbabwe, ART was begun within 72 hours after diagnosis of cryptococcal meningitis or delayed until completion of 10 weeks of antifungal treatment with 800 mg/d of fluconazole alone. The risk of death was 2.85 times higher in the early ART group. Immune reconstitution inflammatory syndrome (IRIS) occurred in patients in both groups and did not explain the increased mortality. The increased mortality seen in the early ART treatment group is concordant with the recent announcement of the cessation of randomization in the COATS trial following data and safety monitoring board review. Antifungal therapy consisted of fluconazole alone in the former study and amphotericin B plus fluconazole during induction followed by fluconazole alone in the COATS study. These data suggest that persons with HIV and cryptococcal meningitis should be closely monitored after starting ART and managed in consultation with experts, particularly if CD4 cell counts are below 50/μL.

Three randomized trials evaluating when to start ART during TB treatment demonstrated that early ART improved AIDS-free survival compared with initiation after completion of TB treatment. The greatest benefit was achieved in persons with CD4 cell counts below 50/μL, and for this subgroup, the optimal time of ART initiation was within the first 2 weeks of TB treatment. Those with higher CD4 cell counts who deferred ART until 8 to 12 weeks after starting TB treatment had lower rates of IRIS and other adverse events. In all 3 studies, trends toward improved AIDS-free survival were observed across all CD4 cell count strata. Benefit was greatest in those with most advanced immunosuppression, as were rates of IRIS. Deaths attributable to IRIS were few. In a randomized trial of 253 patients with HIV and TB meningitis, initiation of ART within 2 vs 8 weeks of TB treatment was not associated with improved survival, and those in the immediate ART group had significantly more severe adverse events. Whether these results also can be generalized is unclear because the patient population included a high proportion of injection drug users with underlying viral hepatitis; most deaths occurred in the first month of treatment, before an effect of ART could be observed; and risk of death was related to severity of TB meningitis. Therefore, early initiation of ART should be considered in persons with HIV and TB meningitis, but with close monitoring and management in consultation with experts, particularly if CD4 cell count is below 50/μL.

Hepatitis B Virus. The risk of liver-related morbidity and mortality is increased in persons dually infected with HIV and hepatitis B virus (HBV). Although there are conflicting data as to whether HBV adversely affects the natural history of HIV, the potential to treat both infections with the same medications provides a compelling argument for treatment of all HIV- and HBV-coinfected persons who otherwise have no contraindications to therapy.

Hepatitis C Virus. Infection with HIV also increases the risk of liver-related morbidity and mortality in persons dually infected with HCV. In some but not all studies, treatment of HIV reduces progression of HCV-related liver disease. It is also possible that ART improves the response to HCV treatment by improving immune function. However, most of the evidence that HCV treatment might be more effective in persons receiving ART is based on lower responses to HCV therapy in persons with CD4 cell counts below 500/μL. That observation and interactions between ARV drugs and the currently available HCV drugs might provide a justification to delay ART until after completion of HCV treatment in patients with CD4 cell counts greater than 500/μL.
**Older Age and HIV-Associated Nephropathy.** As previously recommended, age older than 60 years is an indication to start ART regardless of CD4 cell count. Persons with HIV-associated nephropathy should begin therapy as soon as the diagnosis is made because ART improves survival and kidney function in these patients.  

**Acute HIV Infection.** ART initiation has been recommended for those with symptomatic acute HIV infection. In the absence of definitive data from randomized controlled trials on the risks and benefits of treating asymptomatic primary infection, several arguments can be made for initiating ART during acute and early infection.

Early treatment has been associated with reduced lymphoid tissue pathology, conserved lymphocyte function, lowered cell-associated HIV-1 DNA, and a transient reduction of viral set point after treatment interruption. Randomized clinical trials of immediate vs deferred ART for recently infected individuals have shown a delayed rate of CD4 cell decline after treatment interruptions of 6 to 15 months compared with deferred treatment.

A substantial proportion of ongoing HIV transmission is attributable to individuals with acute infection. These individuals may have markedly higher HIV-1 RNA levels in plasma and genital secretions, which increases the risk of transmission per sexual encounter. Thus, offering persons with acute HIV infection early treatment represents a high priority in ART-prevention strategies.

**WHAT TO START**

The options for initial therapy for treatment-naive adults with confirmed drug-susceptible virus continue to expand, with new drugs and coformulations (Table 1 and Table 2). Because therapy is expected to be sustained indefinitely, regimen choice must consider patient convenience, potential toxicities, and tolerability that may affect adherence. The aim of therapy continues to be maximal, lifelong, and continuous suppression of HIV replication to prevent emergence of resistance, facilitate optimal immune recovery, and improve health. Interactions among ART drugs and with other medications are a growing challenge as persons with HIV age and require additional medications for comorbid conditions. The cost of therapy is expected to be an increasingly important issue as part of a larger movement to control health care expenditures. Generic ART drugs may reduce program costs and allow for treatment of more individuals, but it will be crucial to ensure that any resulting medication choices do not revert to older and more toxic drugs no longer recommended in these guidelines. Also, more complex regimens without coformulated drugs raise adherence concerns and may increase out-of-pocket costs in regions where patients have co-payments for each prescription.

**Table 1. Recommended and Alternative Initial Antiretroviral Regimens, Including Strength of Recommendations and Quality of Evidencea**

<table>
<thead>
<tr>
<th>NNRTI plus NRTIs</th>
<th>Recommended Regimens</th>
<th>Alternative Regimens</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efavirenz/tenofovir/emtricitabine (A)</td>
<td>Nevirapine plus tenofovir/emtricitabine or abacavir/3TC (A)</td>
<td>Severe hepatotoxicity and rash with nevirapine are more common in initial therapy when CD4 cell count is &gt;250/μL in women and &gt;400/μL in men.</td>
<td></td>
</tr>
<tr>
<td>Efavirenz plus abacavir/3TC (A) in HLA-B*5701-negative patients with baseline plasma HIV-1 RNA &lt;100,000 copies/mL</td>
<td>Ripivirine/tenofovir/emtricitabine (Bla) (or rilpivirine plus abacavir/3TC)</td>
<td></td>
<td></td>
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</tbody>
</table>

**Pr/rr plus NRTIsa**

<table>
<thead>
<tr>
<th>Recommended Regimens</th>
<th>Alternative Regimens</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Darunavir/ritonavir plus tenofovir/emtricitabine (A)</td>
<td>Darunavir/ritonavir plus abacavir/3TC (Bla)</td>
<td>Other alternative PIs include fosamprenavir/ritonavir and saquinavir/ritonavir but indications to use these options for initial treatment are rare.</td>
</tr>
<tr>
<td>Atazanavir/ritonavir plus tenofovir/emtricitabine (A)</td>
<td>Lopinavir/ritonavir plus tenofovir/emtricitabine (Bla) (or abacavir/3TC)</td>
<td></td>
</tr>
<tr>
<td>Atazanavir/ritonavir plus abacavir/3TC in patients with plasma HIV-1 RNA &lt;100,000 copies/mL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**INSTI plus NRTIsb**

<table>
<thead>
<tr>
<th>Recommended Regimens</th>
<th>Alternative Regimens</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raltegravir plus tenofovir/emtricitabine (A)</td>
<td>Raltegravir plus abacavir/3TC (Bla)</td>
<td>Raltegravir is given twice daily; experience with elvitegravir/cobicistat/tenofovir/emtricitabine is limited to 48-week data.</td>
</tr>
<tr>
<td></td>
<td>Elvitegravir/cobicistat/tenofovir/emtricitabine (Bli)</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** INSTI: integrase strand transfer inhibitor; NRTI: nucleoside reverse transcriptase inhibitor; NNRTI: nonnucleoside reverse transcriptase inhibitor; PI: protease inhibitor; r, ritonavir-boosted.

*a* Ratings of the strength of the recommendations and quality of evidence are described in the eBox. Fixed-dose combinations are recommended when available and appropriate.

*b* Zidovudine/3TC is an alternative NRTI component of NNRTI-, PI-, and raltegravir-based regimens, but the toxicity profile of zidovudine reduces its utility.

*c* HLA-B*5701 screening is recommended before abacavir administration to reduce the risk of hypersensitivity reaction.

Avoiding the use of abacavir or lopinavir/ritonavir might be considered for patients with or at high risk of cardiovascular disease.

New Drug Application for this combined formulation has been filed with regulatory authorities. Approval decisions pending.
Table 2. CCR5 Antagonist–Based and NRTI-Sparing Initial Regimens That Can Be Considered Only in Special Circumstances, Including Strength of Recommendations and Quality of Evidence

<table>
<thead>
<tr>
<th>Regimens</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR5 antagonist plus NRTIs (NNRTI-, PI-, and InSTI-sparing)</td>
<td>Tropism assay to confirm R5 virus should be done before prescribing maraviroc. Maraviroc is not effective in persons who have X4 or dual/mixed X4/R5 virus infection. Few data are available for maraviroc with tenofovir/emtricitabine or abacavir/lamivudine.</td>
</tr>
<tr>
<td>Maraviroc plus tenofovir/emtricitabine or abacavir/lamivudine (CII)</td>
<td></td>
</tr>
<tr>
<td>PI/r plus InSTI (NRTI-sparing)</td>
<td>Data emerging for these regimens. Clinical trial evidence needed before formal recommendation can be made.</td>
</tr>
<tr>
<td>Darunavir/ritonavir plus raltegravir (Blia)</td>
<td></td>
</tr>
<tr>
<td>Lopinavir/ritonavir plus raltegravir (Blia)</td>
<td></td>
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</tbody>
</table>

**Table 2.** CCR5 Antagonist–Based and NRTI-Sparing Initial Regimens That Can Be Considered Only in Special Circumstances, Including Strength of Recommendations and Quality of Evidence

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**Nucleos(t)ide Reverse Transcriptase Inhibitors**

Three 2-drug NRTI FDCs are currently available. In some cases, these FDCs are coformulated with another potent drug, adding to the overall regimen convenience.

**Recommended.** Tenofovir disoproxil fumarate and emtricitabine are available in a once-daily FDC with no food restrictions. Tenofovir is well tolerated but has been associated with kidney injury, which appears to increase in incidence with long-term administration and concurrent PI/r use. Renal function should be assessed before use and monitored over time, dosing adjusted according to the package insert in the case of renal impairment (estimated glomerular filtration rate [eGFR] <50 mL/min), and tenofovir discontinued when eGFR is below 30 mL/min. Tenofovir causes a decrease in bone mineral density in the spine and hip, the long-term progression of which currently remains ill defined. Emtricitabine is similar to lamivudine in mechanism of action, potency, toxicity, and patterns of resistance.

An abacavir and lamivudine FDC offers once-daily administration, no food restriction, and minimal subjective toxicity. Screening for HLA-B*5701 markedly reduces the risk of potentially life-threatening hypersensitivity reaction to abacavir. In some studies but not in others, abacavir has been associated with a higher risk of acute myocardial infarction.

Initial regimens containing abacavir/lamivudine had lower rates of viral suppression in persons with baseline HIV-1 RNA levels above 100 000 copies/mL than regimens containing tenofovir/emtricitabine.
However, in a second randomized trial, this difference was not observed. Lamivudine is extremely well tolerated.

**Alternatives.** A zidovudine and lamivudine FDC must be used twice daily. Zidovudine commonly causes headache, nausea, anemia, neutropenia, and progressive and persistent peripheral lipoatrophy. Its use should be reserved for individuals unable to use abacavir or tenofovir.

**Nonnucleoside Reverse Transcriptase Inhibitors**

Nevirapine, efavirenz, and rilpivirine are each available as a single pill for once-daily use; the 2 latter drugs are available in FDCs with tenofovir and emtricitabine.

**Recommended.** Efavirenz is used once daily, preferably without food at bedtime. Central nervous system adverse effects include sleep disturbance, abnormal dreams, and, less commonly, depressed mood. Efavirenz can cause a rash, which usually but not always resolves despite continued treatment.

**Alternatives.** Nevirapine is now available in a 400-mg once-daily formulation. Nevirapine requires a 2-week lead-in of 200 mg once daily. Rash is more common and usually more severe than with efavirenz. Severe hepatotoxicity is occasionally seen with initial use. Both severe rash and hepatotoxicity are more common with baseline CD4 cell counts above 250/μL in women and 400/μL in men.

Rilpivirine is administered once daily. In 2 studies, rilpivirine was noninferior to efavirenz, although rates of virologic failure were higher with rilpivirine while rates of adverse events were higher with efavirenz. **Virologic failure was more common in patients with baseline HIV-1 RNA above 100 000 copies/mL, and rilpivirine should be avoided in this population.** Rilpivirine has substantial food interactions and should be taken with at least a 400-kcal meal. Concomitant use of rilpivirine and proton-pump inhibitors is contraindicated.

**Protease Inhibitors**

Protease inhibitors are used in combination with 2 NRTIs as part of initial ART. The bioavailability of PIs requires coadministration with a drug such as ritonavir that augments or “boosts” levels of the PI through inhibition of the CYP34A enzyme. Another drug with this property, cobicistat, is being developed. As a class, PIs are associated with mild to moderate nausea, diarrhea, and dyslipidemia. All PIs may be associated with cardiac conduction abnormalities, particularly PR interval prolongation. A baseline electrocardiogram and avoidance of other agents causing prolonged PR or QT intervals should be considered.

**Recommended.** Ritonavir-boosted atazanavir is used in initial therapy once daily. It blocks bilirubin conjugation, resulting in a nearly universal elevation in unconjugated (indirect) bilirubin. Usually modest, this can cause visible jaundice in some individuals but does not represent hepatotoxicity. Atazanavir requires gastric acidity for absorption and should be taken with meals and with avoidance of proton-pump inhibitors; if used, proton-pump inhibitors should be taken distant from the time of atazanavir/r administration. Unboosted atazanavir has reduced potency and is not recommended. Atazanavir may be associated with nephrolithiasis and in 1 study was associated with renal dysfunction. Atazanavir is the only PI/r shown to be noninferior to efavirenz-based therapy in a large randomized trial.

Darunavir must be boosted to be active. Ritonavir-boosted darunavir is used once daily in initial regimens and should be taken with a meal to improve bioavailability. Darunavir contains sulfa and may produce hypersensitivity reactions, especially in those with sulfa allergy.

**Alternatives.** Lopinavir is available only as an FDC with ritonavir. Fewer individuals randomized to lopinavir/r in combination with tenofovir/emtricitabine maintained HIV-1 RNA below 50 copies/mL at 48 and 96 weeks vs those randomized to darunavir/r or atazanavir/r. **Ritonavir-boosted lopinavir causes more frequent gastrointestinal adverse effects than other PIs. It can be used once daily and does not require administration with food.**

Fosamprenavir or saquinavir boosted with ritonavir may be used once daily, taken with a meal, in initial therapy. Fosamprenavir contains a sulfa moiety and may cause rash. In 1 randomized trial, once-daily saquinavir/r was noninferior to atazanavir/r and had comparably mild adverse effects.

**Integrase Strand Transfer Inhibitors**

The newest drug class of potent antiretroviral drugs used with a dual NRTI backbone, the InSTIs are well tolerated. Similar to NNRTIs, current InSTIs have a low genetic resistance barrier.

**Recommended.** Raltegravir should be used twice daily, as once-daily dosing diminishes efficacy. Raltegravir does not require concomitant food consumption.

**Alternative.** A once-daily coformulation of tenofovir, emtricitabine, elvitegravir, and cobicistat is pending regulatory approval in the United States for treatment-naive patients. Elvitegravir is an investigational InSTI pending regulatory approval in the United States for treatment-experienced patients. It requires boosting to achieve sufficient potency. Cobicistat is an investigational
pharmacokinetic booster pending regulatory approval in the United States that can cause substantial drug-drug interactions. Cobicistat causes an immediate and reversible small increase in serum creatinine and eGFR without actually affecting measured creatinine clearance because it competes with excretion of creatinine by the kidney. When substantial or progressive increase in serum creatinine occurs, evaluation of kidney function and adjustment of the regimen should be considered.

**Attachment Inhibitors**

Drugs that block CCR5 have durable antiretroviral activity only if the individual is infected with HIV that uses CCR5 exclusively and not CXCR4. The use of these drugs thus requires receptor tropism screening. The phenotypic assay that measures tropism is expensive and time-consuming, but genotypic tropism testing is faster, cheaper, and may facilitate the use of such drugs. Maraviroc is the only currently approved CCR5 attachment inhibitor. It is used twice daily and has no food restrictions.

**Special Considerations**

**Pregnancy.** The choice of ART in pregnant women should take into consideration the same benefits and risks as in HIV-infected adults as well as any special considerations associated with the pregnancy. The Antiretroviral Pregnancy Registry of more than 15,000 HIV exposures (January 1989–July 2011) notes no increase in rates of congenital birth defects with exposure to ART, including efavirenz, even in the first trimester.

**Comorbid Diseases.** Preexisting risks or existence of particular comorbidities influence the choices among otherwise equally effective recommended initial regimens. Comorbidities may be exacerbated by the potential toxicity of individual ART drugs and may be subject to drug-drug interactions with treatments needed for such conditions.

**Cardiovascular, Renal, and Bone Diseases.** Abacavir, lopinavir/r, and fosamprenavir/r each have been associated with an increased risk of cardiovascular disease (CVD) in some but not all studies. Such associations have not been found for tenofovir, efavirenz, nevirapine, or atazanavir/r.

Data on CVD risks are not yet available for darunavir/r, raltegravir, rilpivirine, or elvitegravir. In persons at high risk of CVD, avoiding abacavir, lopinavir/r, and fosamprenavir/r might be considered. In patients with reduced renal function, prolonged use of tenofovir is associated with cumulative nephrotoxicity and should be avoided. Prolonged use of atazanavir/r and lopinavir/r is also associated with cumulative loss of renal function.

Compared with uninfected individuals, persons with HIV infection are at increased risk of osteoporotic fragility fractures. In addition to traditional factors associated with bone loss, use of tenofovir and lopinavir/r are independent risk factors for fractures in some but not all studies. Although all initial ART regimens are associated with a reduction in bone mineral density during the first year of treatment, the effect is more pronounced with tenofovir-containing regimens. Notably, in postmenopausal women, both HIV infection and tenofovir use are independently associated with higher rates of bone loss. Given their increased risk of fragility fractures, it may be prudent to consider avoiding tenofovir as part of initial therapy in postmenopausal women.

** Opportunistic Infections.** Drug interactions and tolerability are key considerations in the context of acute opportunistic infections. Drug interactions with triazole antifungal drugs and those associated with rifamycins are among the most important. The recommended initial ART regimen in the setting of rifampin-based TB therapy is efavirenz plus NRTIs. Data are conflicting about the effect of rifampin coadministration on efavirenz concentrations. Early studies reported a 26% reduction in efavirenz exposure, but more recent studies in patients with HIV and TB coinfection have not shown a clinically significant effect of rifampin on efavirenz exposure.

Although the prescribing information for efavirenz indicates the dosage should be increased to 800 mg/d for patients weighing more than 50 kg who are being treated with rifampin, the current FDC with 600 mg of efavirenz is associated with good HIV and TB outcomes regardless of weight. If efavirenz cannot be used, rifabutin-based TB therapy with a PI/r plus NRTIs is recommended. Rifabutin reportedly has little effect on atazanavir/r or lopinavir/r, results in only modest increases in darunavir, and has no clinically meaningful effect on raltegravir. However, serum concentrations of rifabutin and its major metabolite are markedly increased by all PI/r, requiring dosage adjustment of rifabutin in this setting. Rifabutin, 150 mg every other day, resulted in increased rates of acquired rifamycin resistance when used with a PI/r regimen and lower-than-expected concentrations of rifabutin. Additional clinical trials are under way, but in the interim, rifabutin, 150 mg/d, is suggested when used with a PI/r regimen, and patients should be closely monitored. Raltegravir concentrations are decreased when coadministered with rifampin; if a raltegravir-based ART regimen is used, the raltegravir dosage should be increased to 800 mg twice daily or rifabutin should be substituted for rifampin, but neither approach has been evaluated in patients with HIV and TB.
coinfection. The recent recommendation for use of a 3-month, once-weekly regimen of isoniazid with rifapentine for treatment of latent TB infection is not recommended for HIV-infected patients receiving ART. 27

**Cirrhosis.** In persons with cirrhosis but without encephalopathy, coagulation disorders, or liver synthetic abnormalities, there are no restrictions on ART. In persons with hepatic failure, HIV PIs and selected other antiretroviral drugs should be avoided or used with caution.

**Hepatitis B Virus.** The optimal ART regimen for HIV- and HBV-coinfected persons should include tenofovir and emtricitabine (or lamivudine) as the NRTI background. If renal insufficiency occurs in HBV- and HIV-coinfected persons, a reduced dose of tenofovir, but not of the other components in the regimen, can be used. Entecavir has been used safely in coinfected patients but has impaired activity against lamivudine-resistant HBV and can select for M184V in HIV reverse transcriptase. 88 In persons without lamivudine-resistant HBV, entecavir is an alternative to tenofovir if used with a fully suppressive antiretroviral regimen. Treatment of coinfected patients with regimens containing lamivudine or emtricitabine as the only antivirals with activity against HBV provides suboptimal efficacy and usually results in NRTI-resistant HBV. 93- 99 Interferon alfa is approved for treatment of chronic HBV infection but has not been rigorously tested in HIV-coinfected persons.

**Hepatitis C Virus.** Peginterferon alfa and ribavirin have been routinely used in HIV- and HCV-coinfected persons. Ribavirin cannot be used with didanosine and has overlapping toxicity with zidovudine. It is not clear whether peginterferon alfa plus ribavirin is less effective when used with abacavir than with tenofovir. The addition of the HCV PIs telaprevir or boceprevir to peginterferon alfa and ribavirin improves treatment responses for genotype 1 chronic HCV infection. 103- 105 Likewise, preliminary phase 2 data in HIV-/HCV-coinfected persons showed superior responses in those randomized to peginterferon alfa, ribavirin, and boceprevir or telaprevir compared with peginterferon alfa, ribavirin, and placebo. 105- 104 As phase 3 studies are ongoing and US Food and Drug Administration (FDA) approval is pending for coinfected patients, the superior responses suggest either telaprevir or boceprevir should be added to peginterferon alfa/ribavirin when treating genotype 1 chronic HCV infection.

Drug-drug interactions between telaprevir or boceprevir and antiretroviral drugs may alter the optimal choice of ART when their use is anticipated. Data from clinical trials continue to evolve but are currently insufficient to guide firm recommendations about recommended regimens. Available data suggest that tenofovir, emtricitabine, raltegravir, and etravirine may be safely used with boceprevir, and these drugs and rilpivirine, atazanavir/r, and efavirenz (with increased telaprevir dose) may be used with telaprevir. However, HIV and HCV RNA levels should be carefully monitored when coadministering these drugs, and evolving data on drug-drug interactions should be considered. 105

**Malignancy.** Concomitant use of anticancer drugs and ART is associated with overlapping toxicities and the potential for substantial drug interactions due to elimination using CYP450 routes of metabolism. Raltegravir-based regimens may be considered in this setting because of their favorable drug interaction profile. 106 Recommendations for initial regimen in the above specific circumstances are summarized in **Box 2.**

**Box 2. Recommendations for Initial Treatment in the Setting of Specific Conditions, With Strength of Recommendations and Quality of Evidence**a

- In patients with or at high risk of cardiovascular disease, avoiding use of abacavir, ritonavir-boosted lopinavir, or ritonavir-boosted fosamprenavir might be considered (BIIa).
- In patients with reduced renal function, tenofovir should be avoided, or if treatment for hepatitis B virus (HBV) coinfection is needed, dosing should be adjusted according to the prescribing information (AIIa).
- Given the increased risk of fragility fractures, it may be prudent to avoid tenofovir as part of initial therapy in postmenopausal women (BIIa).
- The recommended initial ART regimen in the setting of rifampin-based tuberculosis treatment is efavirenz plus 2 nucleos(t)ide reverse transcriptase inhibitors (NRTIs) (AIIa).
- The recent recommendation for use of a 3-month, once-weekly regimen of isoniazid with rifapentine for treatment of latent TB infection is not recommended for human immunodeficiency virus (HIV)–infected patients receiving ART (BIII).
- The ART regimen for HIV- and HBV-coinfected persons should include tenofovir and emtricitabine (or lamivudine) as the NRTI background (AIIa).

*a* Ratings of the strength of the recommendations and quality of evidence are described in the **eBox**.
MONITORING
Suppression of plasma HIV-1 RNA to less than 50 copies/mL by 24 weeks should occur with effective therapy, regardless of prior treatment experience. No recent work has defined the optimal frequency of monitoring in resource-rich economies, despite the perception that such research could lead to substantial cost savings. Therefore, previous recommendations for frequency of CD4 cell count and HIV-1 RNA monitoring have not changed.7

Recently introduced third-generation HIV-1 RNA assays show a lower limit of quantification of 40 or 20 copies/mL and can report qualitative RNA detection below these cutoffs. In addition, many patients receiving stable suppressive treatment show residual viremia of 1 to 10 copies/mL using research-based assays. The source, significance, and optimal management of detectable viremia of less than 50 copies/mL during treatment are poorly defined. Recent studies indicate that detectable HIV-1 RNA below the 50-copies/mL threshold predicted rebound; however, the lower the viral load, the less likely it is to result in confirmed rebound.8-10 Evolution of viral resistance can occur in the setting of low-level viremia. In 2 clinical trials and a cohort analysis, new resistance mutations were detected in 37% and 65%, respectively, of participants who developed persistent low-level viremia.11-13 There is lack of consensus on management of patients with HIV-1 RNA levels between 50 and 200 copies/mL. The AIDS Clinical Trials Group definition of virologic failure (confirmed detectable HIV-1 RNA >200 copies/mL after virologic suppression) is commonly used.14 However, the optimal management of these patients has not been determined.

There is limited evidence that ART modifications have an appreciable impact for patients with residual HIV-1 RNA levels between 1 and 10 copies/mL.15 In practice, it is recommended that a detectable HIV-1 RNA level during therapy should be confirmed in a subsequent sample, usually drawn within 2 to 4 weeks, prior to making management decisions. However, the optimal interval before repeating the HIV-1 RNA test after low-level viremia occurs has not been determined, and guidance about management strategies awaits further evidence.16

Published data suggest that the prevalence of transmitted drug resistance has remained stable worldwide and averages 11% in Europe and 15% in North America.17 The presence of transmitted drug resistance may be underestimated if a resistance test is not performed early in infection. Although some mutations may persist in the long term (such as resistance mutations to NNRTIs), others (such as M184V) that confer impaired fitness are quickly replaced by wild-type HIV variants. Patients with resistance mutations detected prior to initiation of ART have a 3- to 5-fold greater risk of virologic failure if a drug to which the virus is resistant is included in the regimen, underscoring the importance of pretherapy resistance testing.18 For confirmed virologic failure, resistance testing is essential and should, when possible, be performed while the patient is still receiving the failing regimen.7

Therapeutic drug monitoring is not recommended for general care. However, it may be useful in pregnant women, children, and patients with renal or liver impairment to minimize overexposure and adverse effects. Therapeutic drug monitoring also may serve to assess adherence or to evaluate virologic failure in the absence of resistance. Therapeutic drug monitoring may be useful if HCV PIs (telaprevir or boceprevir) must be used with ART for which the drug interactions are either not clarified or are known to cause substantially increased or decreased exposure of 1 of the drugs. Awareness of the potential for drug interactions with these agents is important.19-21,22

Increasing attention has been focused on determinants, measurements, and interventions to improve entry into and retention in care and monitoring of and interventions to improve ART adherence. Recent recommendations have covered these issues. National initiatives have generated quality-of-care indicators, including in the area of follow-up of patients receiving treatment. An important quality-of-care factor is management by physicians experienced in HIV medicine.119-121 Recommendations for monitoring are summarized in Box 3.

Box 3. Recommendations for Monitoring, With Strength of Recommendations and Quality of Evidence
- Plasma human immunodeficiency virus (HIV) 1 RNA levels should be monitored at least every 3 months after treatment is initiated or changed for virologic failure to confirm suppression of viremia below 50 copies/mL (A1a).
- CD4 cell count should be monitored at least every 3 months after initiation of therapy, especially among patients with less than 200/μL, to determine the need for primary opportunistic infection prophylaxis (BIII).
- Once viral load is suppressed for 1 year and CD4 cell count is stable at 350/μL or greater, HIV-1 RNA and CD4 cell count can be monitored at intervals of up to 6 months in patients with dependable adherence (CIII).
- Detectable HIV-1 RNA (>50 copies/mL) during therapy should be confirmed in a subsequent sample between 2 and 4 weeks afterward and prior to making management decisions (BIII).
- Sustained elevation of HIV-1 RNA between 50 and 200 copies/mL should prompt evaluation of factors leading to failure and consideration of switching of antiretroviral therapy (ART) (BIII).
- Baseline genotypic testing for resistance should be performed in all treatment-naive patients (AIIa) and in cases of confirmed virologic failure (AIIa).
- Therapeutic drug monitoring is not recommended in routine care; however, selected patients might benefit from this intervention (BIII).
- Health care practitioners and health systems should initiate strategies to monitor and improve entry into and retention in care and ART adherence and to incorporate and analyze quality-of-care indicators (CIII).

*a*Ratings of the strength of the recommendations and quality of evidence are described in the eBox.

**TREATMENT-EXPERIENCED PATIENTS**

New regimens for ART-experienced patients should include the most active drugs available based on genotypic analysis, treatment and adverse effect history, and availability of additional classes of drugs.

**Initial Virologic Failure**

Management of virologic failure of an initial regimen is usually straightforward, and a new regimen with 3 active drugs can generally be constructed. The regimen should be changed promptly on confirmation of virologic failure.

**Initial NNRTI-Based Regimens.** Delaying a treatment change allows the accumulation of additional NNRTI resistance mutations that may limit future treatment options with etravirine and rilpivirine. Generating a new regimen with 3 active agents is attainable using a PI/r and active NRTIs. If choice is limited by resistance, HLA-B*5701 positivity, or adverse reactions, use of agents from other classes such as InSTIs and CCR5 inhibitors are options.

**Initial PI/r-Based Regimens.** The difference between initial virologic failure of an NNRTI-based vs a PI/r-based regimen is that the presence of NNRTI resistance mutations is likely in the former; protease mutations are rarely observed at the time of treatment failure with recommended initial PI/r regimens. If the NNRTI backbone is compromised, NNRTIs, raltegravir, or elvitegravir should be used with caution. Darunavir/r is associated with a lower incidence of virologic failure than lopinavir/r in treatment-experienced patients. There are no trials directly comparing darunavir/r and atazanavir/r in treatment-experienced patients.

**Initial Raltegravir-Based Regimens.** There are several available treatment options with 3 fully active drugs from classes not used in an initial raltegravir-based regimen. Standard genotypic tests do not include the integrase region, and there are cost and access issues for integrase resistance assays. Raltegravir and elvitegravir are almost completely cross-resistant. With high-level raltegravir resistance, there is no clinical benefit from continuing raltegravir. Prompt discontinuation of these drugs in a failing regimen increases the potential utility of the investigational drug dolutegravir (see below).

**Multidrug-Resistant Virologic Failure**

Following virologic failure of second and later regimens, the presence of multidrug-resistant (MDR) HIV is likely. Occasionally, patients with transmitted drug resistance to 3 classes require initiation of therapy with drugs not included in the above recommended initial regimens. Effective regimens usually include a PI/r with activity against resistant strains, usually darunavir/r. This can be combined with etravirine depending on the NNRTI resistance mutations detected. Raltegravir has substantial benefit in patients with MDR HIV. Fewer data are available for elvitegravir. The entry inhibitor enfuvirtide also was used successfully in salvage regimens but is poorly tolerated because of injection site reactions. Maraviroc was used effectively in those with CCR5-tropic HIV in combination with other active or partially active drugs in salvage regimens. In patients with MDR HIV and no treatment option with a regimen containing 2 active drugs, continuation of some NRTIs, such as lamivudine or emtricitabine and/or tenofovir, might be considered for continuation in a regimen, even if resistance is present, because residual activity of these compounds has been demonstrated in this setting. Expert advice should be sought in the setting of MDR virus.

Dolutegravir, an InSTI currently in development, appears to have good activity against raltegravir- and elvitegravir-resistant virus, but reduced susceptibility has been reported for virus with the Q148 or
G140 signature mutations. It is administered once daily in the absence of integrase mutations and twice daily when integrase mutations are present. It does not require boosting. An expanded access program for dolutegravir provides access to drugs for patients with documented resistance to raltegravir and elvitegravir and who are unable to construct a viable new background regimen with commercially available medications (http://www.dolutegravir-eap.com/).

Treatment interruption is not recommended outside of clinical trials, apart from very short interruptions due to surgery, severe illness, or serious drug toxicity. Studies have shown either no benefit or inferior clinical and virologic outcomes. For planned short treatment interruptions, the different half-lives of the individual components of ART regimens may require a staggered cessation of treatment.

**Immunologic Failure**

There is no consensus definition of immunologic failure, which encompasses patients who are unable to achieve adequately protective CD4 cell count increases despite durable virologic suppression with ART. Higher risk of morbidity (due to AIDS and serious non-AIDS events) and mortality are reported in those with poor immunologic recovery despite virologic suppression. A number of strategies to improve CD4 cell count responses have been evaluated with no consistent benefit, including switching of NRTIs or class of drugs and treatment intensification. Currently, there is no immune-based therapy that has shown a clinical benefit.

**Switching for Toxicity or Improved Tolerability and Adherence**

Switching regimens to reduce toxicity, improve adherence and tolerability, and avoid drug interactions in virologically suppressed patients can be done by switching 1 or more agents in the regimen. Switches of single agents for acute or chronic toxicity are possible in patients with virologic suppression, as long as regimen potency is maintained. Although switching from enfuvirtide to raltegravir in virologically suppressed patients with MDR was not associated with virologic rebound, switching a PI/r to raltegravir has shown conflicting results.

In virologically suppressed patients with efavirenz intolerance or toxicity, substitution with nevirapine or rilpivirine is possible. There was no increased risk of nevirapine-induced hepatotoxicity or rash at high CD4 cell count at the time of the switch from efavirenz to nevirapine. The rilpivirine switch can be accomplished with a rilpivirine/tenofovir/emtricitabine FDC. Changing efavirenz to a PI/r or InSTI is another approach. There are fewer supporting data for switching to a maraviroc-based regimen in virologically suppressed individuals. Some virologically suppressed patients may require switching of regimen components owing to anticipated drug interactions such as with chemotherapy, treatment for TB, or need for proton-pump inhibitors or HCV PI therapy. If dose modification and therapeutic drug monitoring are not possible (see “Monitoring” section), then switching the antiretroviral drug anticipated to cause the problem is appropriate.

Preemptive or reactive changes for short- and long-term toxic effects such as metabolic abnormalities and prevention or management of lipodystrophy, cardiovascular risk, and renal impairment have been used successfully with maintenance of virologic suppression.

Regimens that avoid NRTIs are currently being investigated and may be considered in circumstances where recommended or alternate regimens are contraindicated. Selection of components should be guided by resistance testing.

**Simplification**

A number of strategies have been explored for regimen simplification in virologically suppressed patients. Reduction in pill burden using FDCs or decreasing regimen dosing frequency to improve or maintain adherence has been used successfully, and a meta-analysis has confirmed better adherence for once-daily vs twice-daily regimens. Not all dose frequency reductions effectively maintain virologic suppression in treatment-experienced patients; raltegravir once-daily dosing was inferior to twice-daily dosing in a study of simplification from PI/r based regimens. Once-daily dosing of darunavir/r is effective in treatment-experienced patients with either no prior exposure to PIs or no darunavir-associated resistance mutations.

The induction/maintenance strategy of initiating therapy with 2 NRTIs and a PI/r until virologic suppression is achieved, with subsequent continuation with PI/r monotherapy alone, has been evaluated for lopinavir/r and darunavir/r. A darunavir/r monotherapy maintenance strategy reported good efficacy, but concern about poor central nervous system penetration persists, with reports of discordant plasma and cerebrospinal fluid viral loads. This also was observed in a randomized trial of lopinavir/r monotherapy maintenance. At this point, there are insufficient data to support PI/r monotherapy owing to higher rates of virologic failure than for combination therapy. Recommendations for treatment-
experienced patients are summarized in Box 4. Selected new recommendations since the last report are summarized in Box 5.

**Box 4. Recommendations for Management of Treatment-Experienced Patients, With Strength of Recommendations and Quality of Evidence**

- In the setting of confirmed virologic failure, changing to a new regimen should occur promptly, with consideration of potential contributory factors to prevent further evolution of drug resistance (AIIa).
- A new regimen should be constructed using resistance testing (both past and present), treatment history, and consideration of tolerability and adherence issues (A1a).
- Initial failed regimens should be changed to regimens including a minimum of 2 and ideally 3 fully active drugs (AIIa).
- Management of multidrug resistance is complex and expert advice should be sought (BIII).
- In virologically suppressed patients, switching single agents for toxicity or prevention of anticipated adverse reactions or drug interactions is generally safe and effective (A1a).
- Intensification of or switching therapy has not been successful in improving suboptimal CD4 cell count responses in the setting of durable virologic suppression and is not recommended (A1a).
- Treatment interruptions (outside of clinical trials) should be avoided because of increased risk of death, AIDS, and serious non-AIDS morbidity associated with untreated human immunodeficiency virus (HIV) infection (A1a).
- Ritonavir-boosted protease inhibitor monotherapy is associated with an increased risk of virologic failure and is not recommended when other options are available (A1a).

*Ratings of the strength of the recommendations and quality of evidence are described in the eBox.*

**Box 5. Summary of Selected New Recommendations and Those for Which Strength or Quality of Evidence Has Changed Substantially**

- Antiretroviral therapy (ART) is recommended and should be offered regardless of CD4 cell count (A1a-CIII depending on CD4 cell count and existing conditions).
- ART is recommended and should be offered to persons during the acute phase of primary human immunodeficiency virus (HIV) infection, regardless of symptoms (BIII).
- ART should be started as soon as possible, preferably within the first 2 weeks of diagnosis, in patients with opportunistic infections (other than cryptococcal and tuberculous meningitis), with attention to drug interactions and the potential for immune reconstitution inflammatory syndrome (A1a).
- The optimal timing of ART initiation in patients with cryptococcal meningitis is less certain, but initiating ART early during cryptococcal treatment may be associated with higher mortality; therefore, ART initiation in patients with cryptococcal meningitis should be managed in consultation with experts (BIII).
- ART is recommended in all HIV-infected persons with tuberculosis (TB) and should be started within 2 weeks of TB treatment when CD4 cell count is below 50/μL and by 8 to 12 weeks for those with higher CD4 cell counts (AIIa). The optimal timing for patients with TB meningitis is less certain, but ART should be started within the first 2 to 8 weeks of TB treatment and managed in consultation with experts (BII).
- Abacavir/lamivudine (in patients with HIV-1 RNA levels <100 000 copies/mL) is now a recommended rather than alternative dual nucleoside reverse transcriptase inhibitor (NRTI) component of initial ART (A1a).
- Rilpivirine has been added as an alternative NNRTI component of the initial regimen (BIIa).
- Coformulated elvitegravir/cobicistat/tenofovir/emtricitabine has been added as an initial regimen component, pending regulatory approval (BIIb). Elvitegravir is an investigational integrase strand transfer inhibitor and cobicistat is an investigational pharmacokinetic booster.
- Given increased risk of fragility fractures in postmenopausal women, it may be prudent to consider avoiding tenofovir as part of initial therapy in this group (BIIa).
- The recommended initial ART regimen in the setting of rifampin-based TB therapy is efavirenz plus 2 NRTIs (AIIa).
- The recent recommendation for use of a 3-month, once-weekly regimen of isoniazid with rifapentine for treatment of latent TB infection is not recommended for HIV-infected patients receiving ART (BIII).
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Sustained elevation of plasma HIV-1 RNA between 50 and 200 copies/mL should prompt evaluation of factors leading to failure and consideration for switching of ART (BIII).

Health care practitioners and health systems should initiate strategies to monitor and improve entry into and retention in care and ART adherence and to incorporate and analyze quality-of-care indicators (CIII).

Management of multidrug resistance is complex and expert advice should be sought (BII).

Ratings of the strength of the recommendations and quality of evidence are described in the eBox. The recommendations in Box 1 were chosen because (1) the recommendation was entirely new compared with the 2010 International AIDS Society–USA guidelines or (2) the recommendation had changed in some substantial way, including strength of grading, compared with the 2010 guidelines. The section leaders reviewed and approved inclusion of appropriate recommendations and the entire committee reviewed and approved Box 1.

EMERGING ISSUES: PREEXPOSURE PROPHYLAXIS

The field of HIV transmission prevention has dramatically changed since the last published guidelines. In addition to crucial modes including behavioral change, condoms for men and women, male circumcision, and access to safe injecting methods, strategies based on antiretroviral drugs have gained ground based on important clinical trials. ART can prevent mother-to-child transmission and has a role in postexposure prophylaxis. Antiretroviral-containing vaginal and anal gels and other formulations are also being studied, though no commercially available products are available. Recently, ART used as oral preexposure prophylaxis (PrEP) has been shown to be effective in 3 large trials using daily tenofovir/emtricitabine or tenofovir in gay and bisexual men and transgender women (iPrEx), heterosexual HIV-serodiscordant couples (Partners PrEP), and heterosexual men and women (TDF2). A PrEP trial in high-risk women (FEM-PrEP) failed and one with an oral daily tenofovir group (VOICE) failed to show benefit (although the tenofovir/emtricitabine treatment group of VOICE is continuing). The degree of efficacy of PrEP in these trials had an overall positive correlation with medication adherence, particularly as measured by drug levels. Pharmacokinetic and pharmacodynamic variability and the presence of vaginal or rectal inflammation also may affect outcome. Following publication of the iPrEx results, the Centers for Disease Control and Prevention issued interim guidance for management of HIV-seronegative men who have sex with men who elect to take tenofovir/emtricitabine for prophylaxis. An update to this document is expected should the FDA approve the application for this expanded indication.

CONCLUSIONS AND FUTURE DIRECTIONS

When HIV is allowed to replicate uninhibited by ART, resultant immune activation and inflammation are associated not only with immune destruction and opportunistic infections but also increased rates of cardiovascular, renal, hepatic, and neurologic diseases; malignancies; and other serious non-AIDS diseases. Evidence from clinical trials, observational cohorts, and pathogenesis studies all point toward the health benefits of earlier ART. Potent and tolerable treatment regimens now make durable viral suppression possible for most persons throughout the course of HIV infection. Clinical trial and ecological data likewise underscore the role of treatment in the prevention of new HIV infections.

Although it is crucial to intensify efforts to find a cure for persons who are already infected and an effective vaccine for those who are not, many of the tools needed to control the HIV/AIDS pandemic are already at hand. Critical components of the toolkit to eradicate AIDS include expanded HIV testing, increased focus on engagement in HIV care, early and persistent access to ART, and attention to improving ART adherence. These must occur in the context of strategies to address social determinants of health, including the elimination of stigma and discrimination. Although preventing and treating HIV are cost-effective, current economic realities demand bold steps to ensure that ART and quality medical care are globally accessible for all persons with HIV and that advances in prevention also become broadly available as their efficacies are proven.

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The paradox of HIV in black MSM in the US — very high infection rates despite no more risky sex and more precautions

Roger Pebody
Published: 25 July 2012

A new meta-analysis, presented to the 19th International AIDS Conference (AIDS) 2012 on Tuesday, shows that the exceptionally high rates of HIV infection seen in black men who have sex with men (MSM) cannot be explained by the factors very often thought to drive HIV epidemics — frequency of having sex without a condom, number of sexual partners, drug use and so forth.

In comparison with MSM of other ethnic groups, black men have either comparable rates of risky behaviour, or less. But they are much more likely to report socio-economic problems and barriers to accessing care, suggesting that the explanation may lie at the structural rather than individual level.

Greg Millett of the Centers of Disease Prevention Control presented the data at a session organised by the medical journal The Lancet, which has just published a special issue on HIV in men who have sex with men, distributed to every conference delegate.

In the United States, HIV is concentrated in men who have sex with men (around 2% of the population but 61% of new infections) and in black people (14% of the population but 44% of new infections).

And there is a particular concentration in men who belong to both categories — compared to the general black population, black MSM have 22 times the odds of being HIV positive. Furthermore, the conference heard on Monday that in one cohort of black MSM, 3% of men acquire HIV every year.

In order to better understand the problem, Millett and colleagues conducted a meta-analysis which identified and pulled together all the relevant studies that had already been conducted. They were
specifically interested in research which made comparisons between black MSM and MSM of other ethnic groups, and which examined factors that might be associated with HIV infection.

A total of 174 studies that met pre-determined criteria were identified, either from peer-reviewed journals or from conference proceedings. Their data were pooled in order to produce more reliable estimates that would not be so dependent on the particularities of the samples recruited for the individual studies.

In total, 69 different factors were examined. In the paragraphs that follow, the figures in the brackets are odds ratios – when an odds ratio is above 1, it means black men have an increased likelihood of reporting the factor. When it is below 1, black men are less likely to report the factor than men of other racial groups. All figures reported are statistically significant.

Results

Firstly the analysis confirmed that black men who have sex with men in the United States are at increased risk of HIV infection. Black men had a three-fold greater odds of testing HIV positive (3.0) and a six-fold greater odds of having undiagnosed HIV infection (6.38) compared with other MSM.

But it is notable that black MSM reported fewer sexual risk behaviours than MSM of other ethnic groups. They were less likely to report unprotected anal intercourse (though this was not statistically significant), they were less likely to have a high number of sexual partners (0.58) and they were more likely to use condoms (2.06). They were more likely to have tested for HIV in the past year (1.51).

There were no differences between the ethnic groups in terms of having multiple partners at the same point in time, being HIV negative and knowingly having sex with an HIV-positive man, or having unprotected sex with a man of a different HIV status. Black HIV-negative men were less likely to report unprotected sex with men they thought were HIV negative (0.51).

Perceived risk of HIV infection, HIV treatment optimism beliefs and circumcision status did not differ between black MSM and other MSM. They were however more likely to report selling sex (1.54). Black MSM were less likely to engage in any substance use (0.67), including of crystal methamphetamine (0.39) although there was a non-significant trend to greater use of crack cocaine.

Although black men were as likely as other men to have sexual partners from the same ethnic group, they were more likely to have black partners (11.47).

However pronounced disparities were seen between black MSM and other MSM in relation to experiencing socio-economic difficulties and structural barriers (2.28). Specifically, black men were more likely to have finished education before the end of high school (3.50), to earn less than $20,000 a year (2.29), to have been incarcerated (2.17) or to be unemployed (1.5).

The authors suggest that these interrelated factors will affect the availability and choice of sexual partners and may be associated with isolation in a person’s own neighbourhood, in which HIV is likely to be highly prevalent.

The researchers are looked specifically at the 33 studies which compared young black MSM with young MSM of other ethnic groups. ‘Young’ here could be anywhere between 13 and 29 years.

Young black MSM were as likely as other young MSM to have ever had unprotected anal sex, but were less likely to have done so in the past 6 months (0.73). They had a similar number of sexual partners to their peers of other racial groups and had similar rates of HIV testing. They were particularly unlikely to report any substance use (0.22).

Despite this, young black men were five times more likely to have HIV (4.95), seven times more likely to have undiagnosed HIV (7.14), and had a greater chance of being diagnosed with an STI than other young MSM (1.45).

Notably, young black MSM were more likely than others to begin having sex at a younger age (1.65), which is associated with having a greater number of sexual partners and HIV infection. They were also more likely to have older sexual partners (1.52), which increases the chances of having an HIV-positive partner.

Moreover more young black men reported a history of childhood sexual abuse (1.82). They were also more likely to have a low income (3.05).
Black MSM and the HIV treatment cascade

A key concept for anyone considering the success of anti-HIV treatment in improving the health of individuals and in preventing onward transmission in their communities is the ‘treatment cascade’ or ‘care continuum’. This shows how, at every stage, patients are not retained in the healthcare system or are unable to access the medical care they need. (The graphic relates to all Americans with HIV, not black MSM specifically).

Millett’s meta-analysis identified a number of risk factors for HIV-positive black MSM in relation to the care continuum. As noted above, black men had a far greater likelihood of having undiagnosed HIV (6.38).

Furthermore, the following odds ratios are derived from the 24 studies which compared black MSM living with HIV with their peers of other racial groups. Black men were less likely to:
- Have health insurance (0.47)
- Attend clinic visits (0.61)
- Start treatment before CD4 cell count fell below 200 copies/ml (0.40)
- Take antiretroviral therapy (0.56)
- Be adherent to antiretroviral therapy (0.50)
- Have an undetectable viral load (0.51)

Commenting on these findings, Chris Beyrer of Johns Hopkins University said that structural change was essential, but that the data set out a map for what needs to be done. “We have to address each one of those steps with interventions that make sense for men, that are led by the community, that protect human rights and that are grounded in biological insights,” he said.

Policy shifts

Greg Millett, the lead researcher on this meta-analysis, began as a community activist and became an epidemiologist who has purposefully and persistently drawn attention to racial disparities in HIV infection.

In 2009 he was seconded to the White House to work on the country’s first ever National HIV/AIDS Strategy.

It is notable that inequalities are a key focus of the strategy – reducing HIV-related health disparities and increasing access to healthcare are two of the three primary goals. And in order to achieve the other primary goal, reducing new HIV infections, the first step is to intensify HIV prevention efforts in the communities where HIV is most heavily concentrated.

It remains to be seen whether the strategy will affect real change in prevention work and health outcomes, but many government officials and advocates argue that President Obama’s healthcare reform law will make a substantial difference by providing health coverage to the 49 million Americans who currently lack health insurance and are not covered by Medicaid (which provides some healthcare to some people with a low income). Black people and young adults on lower incomes are especially likely to be uninsured.
Some important questions remain unresolved – including whether insurers will be obliged to cover the costs of HIV testing – but a key aspect of the Affordable Care Act is that insurance companies won't be able to refuse to cover people because they have HIV.

In a plenary speech, Phill Wilson of the Black AIDS Institute described the law as the “most important piece of domestic legislation in the last 40 years.”

“Because of this law, no insurance company can deny insurance because of pre-existing conditions, jack up your rates, or drop you because you get sick or because your care costs too much,” he said. “For people with AIDS, these provisions are absolutely life-saving.”

Reference

Researchers to test monthly vaginal ring for HIV prevention
Wed, Jul 25 2012
By Julie Steenhuyzen
WASHINGTON (Reuters) – Two large clinical trials in Africa are ramping up to test the effectiveness of a vaginal ring that releases an HIV-fighting drug for a month or more, offering women at high risk a discreet way to protect themselves from the virus that causes AIDS.

The studies will test the effectiveness of a vaginal ring containing the antiretroviral drug dapivirine in thousands of women in several African countries to evaluate its ability to prevent new HIV infections and its long-term safety.

If effective, the ring will add “a long-acting, female-initiated technology to the existing toolkit of HIV prevention options,” said Dr. Zeda Rosenberg, chief executive officer of International Partnership for Microbicides (IPM), a nonprofit group founded by Rosenberg which is developing the ring.

Because it only needs to be replaced once a month, the ring may help address some of the problems with getting women to consistently use vaginal gels each time they have sex, Rosenberg said during a briefing at the International AIDS Conference in Washington.

Irregular use is thought to be the reason a large study of the microbicidal gel containing the anti-HIV drug tenofovir failed to prevent infections in women in sub-Saharan Africa.

IPM has a royalty-free licensing agreement with Johnson & Johnson’s Janssen unit in Ireland to use its dapivirine antiretroviral product in gel and ring forms to prevent HIV infections in low and middle income countries.

Dapivirine is part of a class of antiretroviral drugs that have long been used to treat HIV and prevent mother-to-child transmission of the virus.

NIH PARTNERSHIP
The IPM study will enroll 1,650 women aged 18 to 45, who will be randomly assigned to use the ring or a placebo in four sites in South Africa, with plans to expand to sites in Rwanda and Malawi.

It is being conducted in partnership with the U.S. National Institutes of Health-backed Microbicide Trials Network, which just started enrolling women in a separate trial called ASPIRE.

"Developing scientifically proven forms of HIV prevention that women can control is essential," said Dr. Anthony Fauci, director of the NIH’s National Institute of Allergy and Infectious Disease.

"Because the vaginal ring is a long-acting intervention, it has a potential added benefit in that women may find it relatively easy to use."

The ASPIRE study will test the ring in 3,476 women aged 18 to 45 in Malawi, South Africa, Uganda, Zambia and Zimbabwe.

Women in the studies will be offered condoms and counseling on HIV prevention, and taught how to insert the vaginal ring. At monthly visits, researchers will keep track of whether women are still using the ring and give them a replacement.

Those women who become pregnant during the study will discontinue use of the ring, and their safety and that of their child will continue to be monitored.

Dr. Saidi Kapiga of the London School of Hygiene and Tropical Medicine, who is coordinating the ring study in Africa and has also conducted tests of vaginal gels for HIV protection, said there are already signs that women prefer the new option.

"It is acceptable," Kapiga told the briefing. "The fact that they use it only once in four weeks was a major advantage."

Both trials are designed to detect at least a 60 percent reduction in HIV risk, but researchers said they hope for even better results, which are expected in 2015.
"If proven to be effective, I think this will really revolutionize prevention for women," Dr. Sharon Hillier, who heads the Microbicide Trials Network at the University of Pittsburgh School of Medicine, told the briefing.

**Pioneering Study Shows Drug Can Purge Dormant HIV ****

ScienceDaily (July 25, 2012) — Researchers from the University of North Carolina at Chapel Hill have published pioneering research showing that a drug used to treat certain types of lymphoma was able to dislodge hidden virus in patients receiving treatment for HIV.

The existence of persistent reservoirs of dormant HIV in the immune system that are not attacked by anti-AIDS drugs is believed to be a major reason why infection reemerges once patients stop taking their medication. The disruption and clearance of these reservoirs is critical to finding a cure for AIDS.

The study was published in the July 25 issue of the scientific journal, *Nature*.

Researchers at UNC, working in collaboration with scientists from the Harvard School of Public Health, National Cancer Institute, Merck, and the University of California at San Diego, undertook a series of experiments designed to evaluate the potential of the drug vorinostat, a deacetylase inhibitor that is used to treat some types of lymphoma, to activate and disrupt the dormant virus.

Initially, laboratory experiments measuring active HIV levels in CD4+ T cells, which are specialized white blood cells that the virus uses to replicate, showed that vorinostat unmasked the hidden virus in these cells. Subsequently, vorinostat was administered to eight HIV-infected men who were medically stable on antiretroviral therapy and the levels of active HIV virus were measured and compared to the levels prior to administration.

Those patients receiving vorinostat showed an average 4.5-fold increase in the levels of HIV RNA in CD4+ T cells, evidence that the virus was being unmasked. This is the first published study to show the potential for deacetylase inhibitors to attack latency within dormant virus pools in a translational clinical study.

"This work provides compelling evidence for a new strategy to directly attack and eradicate latent HIV infection," said David Margolis, MD, professor of medicine, microbiology and immunology, and epidemiology at the University of North Carolina at Chapel Hill. Targeting latency is the first step on a path that may lead to a cure.

"Long-term, widespread use of antiretrovirals has personal and public health consequences, including side effects, financial costs, and community resistance," said Margolis, who led the study. "We must seek other ways to end the epidemic, and this research provides new hope for a strategy to eradicate HIV completely from the body."

Early results of this study were first presented and reported in March 2012 at the Conference on Retroviruses and Opportunistic Infections in Seattle, Washington.

**Journal Reference:**

**More Black Teenagers Practicing Safe Sex**


According to the most recent findings from CDC’s health survey of high school students, far fewer black students are engaging in risky sexual behavior than they were 20 years ago. However, they still participate in risky behavior more often than white and Hispanic students, according to findings released Tuesday at the 19th International AIDS Conference.

Rates of risky behavior among all teens have declined only slightly over the past two decades. In 2011, 46 percent reported ever having sex, compared with 54 percent in 1991. Fourteen percent reported having four or more sex partners, down from 19 percent in 1991. For Hispanic students, the rates in both categories barely changed.

According to CDC, one possible reason for the good news about black students could be sex education and HIV prevention efforts: The proportion of black students taking these classes rose to 87 percent in 2011 from 84 percent in 1991. Black students were the only group to show a steady increase in those taking the classes. Overall, fewer US high-school students have received instruction about HIV and sex due to budget cutbacks in the past decade.
At 65 percent, sexually active black students were the most likely to use a condom in their most recent sexual encounter, though this rate dropped from 70 percent in 1999.

"The overall plateau [among all students] is troubling," Laura Kann, senior scientist at CDC, said at the AIDS conference.

**Intellectual property "still a threat," to antiretroviral access, says panel**

Mara Kardas-Nelson
Published: 26 July 2012

Patents and intellectual property restrictions continue to affect access to antiretroviral drugs, particularly in middle-income settings, according to research presented at the 19th International AIDS Conference (AIDS 2012) in Washington DC on July 25.

While generic competition has been essential to reducing the price of first-line ARVs, because of patent protection, the price of second- and third-line drugs have not seen a comparable drop. Presenting on a UNITAID-funded study on ARV price determinants, Jean-Paul Moatti said: "When activists say there is still a major problem for second- and third-line drugs, they are right." Globally, first-line drugs are 65.3% cheaper than second-line drugs.

The price of branded second-line drugs has actually been increasing since 2008/2009, says Moatti, part of a general trend among pharmaceutical companies to increase the price of their product at the end of a patent life. While there is an average dip in price a few years into the 20-year period – due primarily to pressure from generic competitors – the price actually increases before the drug goes off patent, and increases even more after the patent has expired.

"When the patent is over, branded drugs don't try to follow the generic companies and engage in competition," said Moatti. Instead, in both developing and developed world markets, "they try to differentiate their product with packaging and so on", keeping the price high.

Chan Park, the interim executive director of the Medicines Patents Pool, presented an analysis of current trends in voluntary licensing practices globally. Park noted that engaging in voluntary licenses was "hugely common throughout the industry", with seven of the eight originator ARV companies signing such agreements. Yet, "there's very little known about the various provisions that can be included in these licenses....there is wide variance within the industry," he said.

Considering access to medicine, Park is specifically concerned about the number of licensed generic companies included in each agreement, and whether licensees can produce their own active pharmaceutical ingredients (APIs), and/or purchase APIs from other generic suppliers.

"Limiting the number of licensees may hinder the robust generic competition that can bring prices down," Park said. "As a general rule, the more competitors there are, the lower the price of the ARV." Given that the cost of APIs comprise a "significant proportion" of a final generic production cost, "there ought to be minimal restrictions on manufacture and sale of APIs by generic producers".

Remarking on the methodology of the study, Chan noted that "a full evaluation of the terms and conditions was impossible as a result of the absence of transparency in voluntary licensing practice", as the full terms of voluntary licence agreements are rarely made public. "Today, I call for increased transparency in voluntary licensing practices, and for companies to adopt access-maximising terms and conditions."

Park also noted that while low-income countries are generally "well covered" in the scope of voluntary licences, "we still have a long way to go with covering upper-middle-income countries."

Speaking on the panel, Kajal Bhardwaj of the Lawyers Collective noted that this was part of a trend by pharmaceutical companies to increasingly "cut out" middle-income countries from so-called "access policies", in which pharmaceutical companies engage in voluntary licences and/or differential pricing, where lower-income and/or high burden countries pay less than their richer counterparts. This is despite the fact that, according to the Human Development Index, "more than half of the world’s poor actually live in middle-income countries," said Bhardwaj, with huge economic discrepancies in places like India and South Africa.

As a result of the exclusion, Bhardwaj explained, middle-income countries have to "negotiate separately on a case-by-case basis with these companies, which makes it harder for them to get lower prices."

While middle-income countries could utilise TRIPS flexibilities, specifically compulsory licences, many do not have the legislative framework to do so, and are being pressured by the world's superpowers to ramp up intellectual property protection. (TRIPS – The Agreement on Trade-Related Aspects of
“What's happening with the global aid and the global trade structure and how that shapes our countries' decisions going forward should not be under-estimated,” said Bhardwaj. “The reality is that we're actually going back on ten years of progress. The minute you have exclusions, you will be leaving many, many people out of treatment and access.”

But countries can flex their muscles. Francisco Viegas Neves da Silva of the Brazilian Ministry of Health spoke on the country’s compulsory licence for efavirenz. Initially granted in 2007 for a period of five-years, and recently renewed for another five, da Silva noted that the compulsory license – which allowed for local generic production of the drug while still under patent – significantly brought down the price of a 200mg tablet from $2544 per patient per year to $259 per patient per year (the current price is even lower at $107 per patient per year). Between 2007 and 2011 Brazil achieved savings of 58% in efavirenz drug costs as a consequence of compulsory licensing, da Silva explained.

“Increasing access is more important than economics,” said da Silva. “This is about how we can save money to put more patients on treatment.”

da Silva said that Brazil’s president, Dilma Roussef, would consider compulsory licences for more antiretrovirals and other drugs, such as for non-communicable diseases. He noted that simply having the threat of utilising a compulsory licence is an important tool in negotiations with pharmaceutical companies.

But according to Brazilian activists, the country can do more to promote access to medicines. Pedro Villardi of the Brazilian Interdisciplinary AIDS Association, or ABIA, presented the outcomes of a survey done by the Working Group on Intellectual Property to consider whether and how pharmaceutical patents were blocking access to medicine.

By conducting a thorough patent search on ARVs, the group found multiple patents on a single medicine, which could block access to generic versions. For example, while the patent for darunavir is committed and down. We will fight for the resources necessary to achieve this historic milestone.”

The 2012 international Aids Conference in Washington DC has so far been a feel-good event framed by encouraging developments in HIV/AIDS treatment and research. The theme of the conference, an "Aids-free generation", refers to the elimination of new Aids infections among children by 2015 – a commendable objective that not so long ago would have been received as a farce. Such optimism is warranted and welcome, as there has been surplus of good news in recent years – the most encouraging is a recent push by scientists to discover and develop a "functional cure" for HIV.

While President Barack Obama skipped the conference, Hillary Clinton showed up to confirm the US's dedication to the cause, stating: "I'm here to make it absolutely clear that the US is committed and will remain committed to achieving an Aids-free generation. We will not back off and we will not back down. We will fight for the resources necessary to achieve this historic milestone."

References

The end of HIV/Aids may not be possible if the war on drugs continues
The danger in ignoring rising HIV rates among drug users is it could reignite the virus when it enters manageable decline

The 2012 international Aids Conference in Washington DC has so far been a feel-good event framed by encouraging developments in HIV/AIDS treatment and research. The theme of the conference, an "Aids-free generation", refers to the elimination of new Aids infections among children by 2015 – a commendable objective that not so long ago would have been received as a farce. Such optimism is warranted and welcome, as there has been surplus of good news in recent years – the most encouraging is a recent push by scientists to discover and develop a "functional cure" for HIV.

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However, beyond all of this optimism lies a few deeply disturbing trends and omissions. While the Obama administration did lift a decades-old travel ban on people with HIV in 2009, the US still denies visa entrance to two of the communities most affected by HIV/Aids: drug users and sex workers.

Their absence has angered many, and lead to the construction of protest satellites in Kiev and Kolkata, as well as rolling demonstrations throughout the week. But perhaps the strangest transgression has been the inability of attending politicians to confront the reality of HIV transmission in 2012: that injecting drug use accounts for one third of new HIV infections outside of sub-Saharan Africa, and that while new infections have been falling since the 1990s, HIV rates have increased 25% in seven countries, largely as a result of needle and syringe sharing.

The reason why such an essential element of the fight against HIV has been overlooked is painfully obvious: talking intelligently about drug use is still taboo among those who are actively engaged in the global war on drugs. And so we find ourselves in a very dangerous quagmire: even though the end of HIV/Aids may be achievable in the near future, such possibilities will be nullified without a determined effort to halt the transmission of HIV through injection drug use. Which, in turn, requires a global reassessment of the war on drugs.

One of the most credible critics of the drug war’s role in the HIV epidemic has been Dr Evan Wood of the BC Centre for Excellence in HIV/Aids Vancouver, Canada (also home to North America’s only supervised injection site, Insite). In an interview with the CBC on Tuesday, Wood stated:

"We don’t have a cure yet, but the tools in the tool box to control this epidemic are essentially at our fingertips. We need the political will to look at some of the political strategies that are hobbling this effort – and I include in that the war on drugs – and to start employing these strategies that really can bring about an end of Aids."

The danger inherent in ignoring rising HIV rates among injection drug users is that it could reignite the virus at the very moment it enters into manageable decline. As the Global Commission on Drug Policy’s most recent report demonstrates, HIV rates are closely linked with the flux of drug markets and drug enforcement. Namely, with increased enforcement comes lower prices, higher purity and amplified incarceration rates – an ideal environment for transmission to take place, unless proper harm reduction services are available.

Nowhere in the world provides a better example of a disastrous drug policy than Russia, which pursues a zero-tolerance approach to drug use and has seen infection rates quadruple since 2000. And as the drug war expands into Africa, with the Drug Enforcement Administration following the cartel lead, we find ourselves pinned in between the invisible hand of narcotic markets and reckless government routine.

By not effectively and swiftly addressing such an vital aspect of the fight against HIV/Aids, our leaders are letting the epidemic simmer and jeopardising much of the progress that has been made in the past 30 years. If there is to be a truly Aids-free generation, it will depend on depoliticising all aspects of the virus, not just those most convenient during our current election cycle.

**Women with HIV Too Often Unseen: US Advocate**

*Agence France Presse*, (07.25.2012)

Heterosexual African-American women are being disproportionately affected by the HIV/AIDS epidemic, attendees were told on Wednesday at the 19th International AIDS Conference (IAC) in Washington. These women comprise 60 percent of new cases among US women and face infection rates 15 times the rate of white women, according to C. Virginia Fields, president of the National Black Leadership Commission on AIDS.

CDC data for 2009 show that black women represented the next-largest group of new infections, after men who have sex with men (MSM) of all races, with 5,400 cases. One in 32 black US women can expect an HIV diagnosis in her lifetime, according to CDC.

At the IAC, Linda Scruggs, an African-American woman, explained how she was first diagnosed with HIV 22 years ago when she became pregnant. Her doctors expressed little hope, but her son was born HIV-negative and recently turned 21. Scruggs recounted being molested and raped multiple times; she does not know which attack may have caused her infection.

In Washington, the HIV prevalence rate of 2.7 percent exceeds that of many developing countries. Among the city’s black residents, who make up about half of the population, the prevalence rate is 4.3 percent.
AIDS advocates say healthcare reform could turn the tide on the AIDS epidemic by extending coverage to more people, especially those who are poor or minorities. “This is an epidemic of communities of color,” said Daniel Montoya, deputy executive director of the National Minority AIDS Council.

**Lawmakers Call For Bipartisan Support For AIDS Funding At AIDS 2012 Panel Discussion**

Rep. Barbara Lee (D-Calif.) and Sens. Chris Coons (D-Del.), Marco Rubio (R-Fla.) and Mike Enzi (R-Wyo.) on Wednesday at the XIX International AIDS Conference (AIDS 2012) in Washington, D.C., joined former Senate Majority Leader Bill Frist (R-Tenn.) for a panel discussion on bipartisanship "focusing on sustaining the engagement of the U.S. Congress in order to demonstrate and encourage continued U.S. leadership in the fight against global AIDS," the Center for Global Health Policy's "Science Speaks" blog reports (Mazzotta, 7/25). During the session, Rubio "expressed strong support for foreign aid, especially funding to combat HIV/AIDS," and Lee "recounted with Frist the history of U.S. efforts to provide global funding," CQ HealthBeat reports (Adams, 7/25). "We have to keep our eyes on the prize,' and focus on moving forward and figuring out how to work together in a bipartisan way. It happened in the past, [Lee] said, and we can do it again," "Science Speaks" writes (7/25). Coons "recommended the United States 'double down' on investments for AIDS," in order to "innovate and cure our way out of this. That, I think, is in keeping with the optimism and the entrepreneurship of the American character,' added Mr. Coons," the Washington Times notes. The discussion was "punctuated by protesters with red umbrellas and signs calling for an end to the 'criminalization' of sex workers, drug addicts and other marginalized groups with AIDS," the Washington Times writes (Wetzstein, 7/25).

In an article examining funding for AIDS programs, National Journal notes "AIDS programs enjoy wide bipartisan support in Congress, but funding for them might still be crowded out by other priorities in the budget fights ahead." In a statement last week, Enzi said, "It is crucial that we continue to work nationally and internationally to prevent new infections and ensure people living with HIV/AIDS have access to the best treatment and care they need," according to the news service (Sanger-Katz/Brownstein, 7/25). At the conference, GlobalPost correspondent John Donnelly interviewed Frist, who said, "The times are challenging. The backdrop for the next five years will be focused on fiscal restraint," according to the news service. Noting that most Americans incorrectly believe the U.S. spends 10 percent—instead of the actual one percent—of its budget on foreign aid, Frist said, "That can be rectified by more communication, better education and establishing the facts, reaching out directly to Americans such that they understand and don’t put pressure on their legislators to indiscriminately cut," according to GlobalPost (7/26).

**Faith-Based Organizations Meet To Discuss HIV/AIDS Response On Sidelines Of AIDS 2012**

On the sidelines of the XIX International AIDS Conference (AIDS 2012), Georgetown University hosted a conference for faith-based organizations and leaders to come together to discuss their efforts to respond to the HIV/AIDS epidemic, the Washington Post reports. Speaking at the conference, Saddleback Church pastor Rick Warren, "considered one of the country's leading evangelicals on fighting AIDS, said he was willing to work with anyone 'who wants to end AIDS,' but blamed the government for trying to get traditional faith workers to what he called 'change' their anti-abortion views in order to partner," according to the newspaper. "Multiple speakers agreed that Christian churches are indispensable in the AIDS fight," the newspaper notes (Boorstein, 7/25). In a separate article, the Washington Post notes that Warren’s wife, Kay Warren, spoke at the conference on a panel of religious leaders from all over the globe who "discussed the evolution of faith-based organizations' thinking on AIDS and HIV since the epidemic began" (Bahrampour, 7/24).

**NPR Examines Whether Goal Of Treating All HIV-Positive People With ARVs Is Feasible**

Noting new guidelines released at the XIX International AIDS Conference (AIDS 2012) in Washington, D.C., this week "call for everybody with HIV to be started on antiretroviral drugs [ARVs] as soon as they test positive for the virus," NPR's "Shots" blog examines "whether the goal is achievable, and who would pay for this enormous expansion in treatment." "Right now about eight million people across the world are getting treated for HIV at a cost of around $17 billion a year," the blog writes, adding, "Universal treatment would cost another $22 billion, by some estimates." The blog notes Bernhard Schwartlander,
director for evidence, strategy and results at UNAIDS, in a plenary speech at the conference on Tuesday "offered up several possible ways to raise the money," including a tax on shipping and aviation fuel (Knox, 7/26).

**New HPTN 052 study results reveal additional benefits of early HIV treatment**

Study results released today by the HIV Prevention Trials Network (HPTN) show additional benefits of early antiretroviral therapy (ART) in HIV clinical outcomes. Expanded analysis of HPTN 052 study data, presented today at the XIX International AIDS Conference in Washington, D.C., demonstrated that early versus delayed ART showed a trend toward delaying the time to both AIDS and non-AIDS primary events and significantly delayed the time to AIDS events, death and tuberculosis. The overall incidence of clinical events was significantly lower in participants treated in the early therapy arm. The new findings show that immediate ART significantly decreased the incidence of clinical events likely due to reversal of immune suppression.

Commenting on the findings, Myron Cohen, MD, Co-Principal Investigator of HPTN, and the HPTN 052 Protocol Chair said, "These new findings provide further confirmation of the health benefits of early antiretroviral therapy. The combined prevention and treatment benefits of antiretroviral therapy make broader testing and treatment urgent and imperative."

HPTN 052 is a landmark study which has received worldwide attention for demonstrating that early ART reduces HIV transmission by 96%, in serodiscordant couples and has been used to revise World Health Organization (WHO) and U.S. treatment guidelines.

HPTN 052 is an ongoing randomized clinical trial. A total of 1763 HIV serodiscordant couples were enrolled in HPTN 052 between April 2005 and May 2010. The study is being conducted at 13 sites in Africa, Asia, and North and South America. The majority of couples (97%) are heterosexual. All participants receive couples risk-reduction counseling, free condoms, and testing and treatment for sexually transmitted infections. Primary HIV care is also provided to the HIV-infected partner. Following the public announcement of results in May 2011, all HIV infected participants in the study were offered ART. All participants will continue to be followed until the planned study end in April 2015 to assess the durability of the prevention and clinical benefits.

**Deadly E. coli strain decoded**

Published: July 26, 2012

EAST LANSING, Mich. — The secret to the deadly 2011 E. coli outbreak in Germany has been decoded, thanks to research conducted at Michigan State University.

The deadliest E. coli outbreak ever, which caused 54 deaths and sickened more than 3,800 people, was traced to a particularly virulent strain that researchers had never seen in an outbreak before. In the current issue of the academic journal PLoS ONE, a team of researchers led by Shannon Manning, MSU molecular biologist and epidemiologist, suggests a way to potentially tame the killer bacteria.

The strain, E. coli O104:H4, shares some characteristics as other deadly E. coli bacteria, but its combination is novel. Researchers haven’t determined the mechanism it uses to cause disease, although Manning and her team were able to find the strain’s Achilles heel – its biofilm.

By focusing on the bacteria’s biofilm, the grouping of many E. coli bacteria that stick to a cell’s surface and grow encased in a self-produced protective coat, Manning and colleagues were able to determine why it was so deadly. When the bacterium found in Germany forms a biofilm, it begins to make more toxic genes like the Shiga toxin.

Increased production of the Shiga toxin is the probable culprit that contributed to so many incidents of kidney damage and death during the 2011 outbreak, Manning said.

“What made the German outbreak so different is that many victims suffering from kidney failure were adults,” she said. “Rather than attacking adults, other types of E. coli that produce Shiga toxins typically damage kidneys of children under 10.”

In addition, the incubation period was considerably longer among individuals infected with the German outbreak strain compared to individuals infected with E. coli O157, a similar bacterium that can also cause illness and death. Manning believes this is because the German strain needs a longer period of time to form a biofilm, whereas biofilms are not important for O157 infections.

“Our research demonstrates that biofilm formation is critical for toxin production and kidney damage,” she said. “If we can block the bacteria from forming a stable biofilm, then it is likely that we can prevent future E. coli O104:H4 infections.”
The next phase of Manning’s research is already focusing on creating mutant strains in an effort to prevent the bacterium from forming a biofilm. This would prevent the disease completely since the conditions would not be favorable for bacterial growth.

Chris Waters, MSU assistant professor of microbiology and molecular genetics, and scientists from the University of Michigan and the Michigan Department of Community Health contributed to the research.

**Bone marrow transplant eliminates signs of HIV infection**

2 Brigham and Women’s Hospital patients have no detectable traces of HIV following transplantation

Boston, MA – Two men with longstanding HIV infections no longer have detectable HIV in their blood cells following bone marrow transplants. The virus was easily detected in blood lymphocytes of both men prior to their transplants but became undetectable by eight months post-transplant. The men, who were treated at Brigham and Women’s Hospital (BWH), have remained on anti-retroviral therapy. Their cases will be presented on July 26, 2012 at the International AIDS Conference by Timothy Henrich, MD and Daniel Kuritzkes, MD, physician-researchers in the Division of Infectious Diseases at BWH.

"This gives us some important information", said Dr. Kuritzkes. "It suggests that under the cover of anti-retroviral therapy, the cells that repopulated the patient's immune system appear to be protected from becoming re-infected with HIV."

One patient's bone marrow transplant was two years ago, the other was four years ago. Both were performed at the Dana-Farber/Brigham and Women’s Cancer Center. Over time, as the patients’ cells were replaced by donor cells, traces of HIV were lost. Currently, both patients have no detectable HIV DNA or RNA in their blood. The level of HIV antibody, a measure of exposure to HIV, also declined in both men.

"We expected HIV to vanish from the patients' plasma, but it is surprising that we can't find any traces of HIV in their cells", said Dr. Henrich. "The next step is to determine if there are any traces of HIV in their tissue."

The research team is currently designing studies that would enable them to look for HIV in the tissues. Researchers also plan to study additional HIV-positive patients who have undergone a bone marrow transplant.

Researchers point out that there are two key differences between the Brigham patients and the "Berlin patient", a man who was functionally cured of HIV after a stem cell transplant. In the Berlin patient's case, his donor was specifically chosen because the donor had a genetic mutation that resisted HIV. The Brigham patients' bone marrow transplants were done without any thought to selecting an HIV-resistant donor. Second, the Berlin patient ceased anti-retroviral therapy after his transplant, while the Brigham patients have remained on anti-retroviral therapy.

**NIH team describes protective role of skin microbiota**

Commensal bacteria and immune cells work together to fight harmful microbes

WHAT: A research team at the National Institutes of Health has found that bacteria that normally live in the skin may help protect the body from infection. As the largest organ of the body, the skin represents a major site of interaction with microbes in the environment.

Although immune cells in the skin protect against harmful organisms, until now, it has not been known if the millions of naturally occurring commensal bacteria in the skin—collectively known as the skin microbiota—also have a beneficial role. Using mouse models, the NIH team observed that commensals contribute to protective immunity by interacting with the immune cells in the skin. Their findings appear online on July 26th in *Science*.

The investigators colonized germ-free mice (mice bred with no naturally occurring microbes in the gut or skin) with the human skin commensal Staphylococcus epidermidis. The team observed that colonizing the mice with this one species of good bacteria enabled an immune cell in the mouse skin to produce a cell-signaling molecule needed to protect against harmful microbes. The researchers subsequently infected both colonized and non-colonized germ-free mice with a parasite. Mice that were not colonized with the bacteria did not mount an effective immune response to the parasite; mice that were colonized did.

In separate experiments, the team sought to determine if the presence or absence of commensals in the gut played a role in skin immunity. They observed that adding or eliminating beneficial bacteria in the
gut did not affect the immune response at the skin. These findings indicate that microbiota found in different tissues—skin, gut, lung—have unique roles at each site and that maintaining good health requires the presence of several different sets of commensal communities.

This study provides new insights into the protective role of skin commensals, and demonstrates that skin health relies on the interaction of commensals and immune cells. Further research is needed, say the authors, to determine whether skin disorders such as eczema and psoriasis may be caused or exacerbated by an imbalance of skin commensals and potentially harmful microbes that influence the skin and its immune cells.


**Swaziland HIV incidence results announced at AIDS 2012**

*Study indicates that the rate of new infection is beginning to level off in the nation with the world's highest prevalence of HIV*

The results from a nationally representative HIV incidence study in Swaziland indicate that the national rate of new HIV infections is 2.38% among adults ages 18-49. This figure, comparable to the 2009 UNAIDS estimate of 2.66% for Swaziland adults ages 15-49, suggests that the HIV epidemic in Swaziland may have begun to stabilize in the past few years. The findings of the Swaziland HIV Incidence Measurement Survey (SHIMS) were presented today at the XIX International AIDS Conference in Washington DC.

"The country continues to have very high HIV incidence rates. Since HIV services in Swaziland are more widely available now and we understand that ART treatment prevents the spread of new infections, the Ministry of Health will use these new results to plan HIV prevention, care, and treatment programs in Swaziland," said Rejoice Nkambule, deputy director of health services – public health at the Ministry of Health. SHIMS was led by the Swaziland Ministry of Health and supported by the U.S. Centers for Disease Control and Prevention (CDC) and ICAP at Columbia University through the U.S. President's Emergency Plan for AIDS Relief (PEPFAR).

SHIMS is a multi-phase study designed to evaluate the impact of HIV prevention and treatment services in the country. The first phase of the SHIMS survey included approximately 13,000 households in Swaziland, representing a cross-section of the national population and consisting of 18,154 men and women, ages 18-49. Findings from the first phase indicated that the national prevalence of HIV in Swaziland is the highest in the world, with 31% of adults infected.

HIV-negative adults from the survey who gave permission were retested six months later and the number of new HIV infections were assessed using state-of-the-art laboratory methods. Retention rates were high, with 94% of participants completing follow-up at six months. "This is the gold standard method for measuring HIV incidence and it hasn't been attempted before at a national level," said Jessica Justman, MD, ICAP's senior technical director, and Associate Professor of Clinical Epidemiology at Columbia University's Mailman School of Public Health. While overall HIV incidence is 2.4 %, HIV incidence among women in Swaziland peaks among those 20-24 and 35-39 years old, with rates of 4.2 % and 4.1 %. Among men, HIV incidence peaks among those 30-34 years old, at 3.0 %. Not knowing the HIV status of the current partner predicts new HIV infection for both men and women and suggests prevention and treatment programs need to target this specific problem.

"SHIMS provides a remarkable epidemiologic look into the most severe national HIV epidemic in the world. Clearly, the strategic scale-up of effective HIV interventions in combination is warranted. SHIMS has established an unequivocal baseline incidence rate against which to judge the effectiveness of such strategies for an entire national population. This may not be feasible anywhere else," said Dr. Jason Reed of the CDC.

While SHIMS is primarily assessing new HIV infections, the study has several other goals such as enhancing laboratory infrastructure and strengthening research capacity in Swaziland.

**BCG vaccination causes immune activation of CD4 T-cells in infants**

Lesley Odendal
Published: 31 July 2012

BCG vaccination, routinely given at birth to protect from tuberculosis (TB), causes an immune-activation of CD4 T cells, the HIV target cells, according to a South African study presented at the Nineteenth International AIDS Conference in Washington DC.
This immune activation may increase the risk of infant HIV infection through breastfeeding, particularly in cases where the infant is not receiving antiretroviral prophylaxis or where the mother is not taking fully suppressive antiretroviral therapy.

The BCG vaccine is given routinely to infants in settings where TB is endemic and is recommended for HIV-positive children in South African treatment guidelines. The BCG vaccine has been shown to be a safe and effective vaccination against disseminated TB in children without HIV. Although it is associated with a 1% risk of disseminated disease in HIV-positive children, it is still recommended to be administered to all infants at birth since their HIV status is often only definitively known once they are 4 to 6 weeks old.

The study randomised 118 HIV-exposed, uninfected infants from Khayelitsha, a large township outside of Cape Town, where the antenatal HIV-prevalence is 30%. 62 infants were given the BCG vaccination at birth as per recommended guidelines, while 56 infants were given their BCG vaccinations at the age of 8 weeks. Blood was collected at birth, 2 weeks, 6 weeks, and 8 weeks for analysis.

The results at 6 weeks showed significantly higher HLA-DR expression on CD4 T cells among the infants who had received BCG at birth, compared with those in the delayed group (p=0.024). The expression of HLA-DR on regulatory T cells correlates positively with immune activation in HIV. In addition, the results at 6 weeks showed significantly higher CCR5, HLA-DR and CD38 co-expression on CD4 T cells in infants who received BCG at birth compared with those in the delayed group (p=0.01). The CCR5 agonist, MIP-1-beta, was significantly higher at 6 weeks in the plasma of unvaccinated infants (p=0.02).

There was no difference in the activation of CD8 T cell activation between activation between BCG-vaccinated and unvaccinated infants.

The immune activation of CD4 T cells places breastfed infants especially at an increased risk of HIV infection and disease progression. 39% of mother-to-child HIV transmission is caused by breastfeeding from HIV-infected mothers. However, formula feeding is associated with morbidity and mortality in sub-Saharan Africa and breastfeeding is therefore recommended.

There was no significant difference between the groups in the median maternal CD4 count, the mean infant birth weight, infant gender or whether or not the infants were breastfed or formula fed.

Although other vaccinations given at 6 weeks are associated with increase in activation, the immune activation effect of BCG is sustained at 8 weeks.

“We think these data have important implications both for the timing of infant BCG vaccination in HIV-exposed infants and for perhaps the use of live replication competent bacteria as HIV and other vaccine vectors in these infants,” said Dr Heather Jaspan, one of the authors of the study.

Further research regarding the risks and benefits of BCG vaccination in HIV-exposed infants is needed to inform policy and practice.

Reference

Female sex workers have 14 times the risk of having HIV as other women
Roger Pebody
Published: 31 July 2012
Although researchers and public health organisations in most low and middle income countries have not collected any recent data on the prevalence of HIV in female sex workers, the data that do exist are alarming, the 19th International AIDS Conference (AIDS 2012) heard on Thursday.

Pooling the data available for 50 countries, female sex workers have a 14-fold higher risk of infection as women of a similar age in the general population.

Rates are especially elevated in some countries, including Bangladesh, Benin, Cambodia, China, Guinea, Guyana, India, Indonesia, Malaysia, Mauritius, Mexico, Nepal and Senegal.

The conference also heard advocates describe what is required to change this situation.

“The epidemic is not driven by the lack of a pill or a gadget, the epidemic is driven by repression,” Cheryl Overs told a plenary session. “Sex workers from Sweden to Singapore to Swaziland all say that the greatest threat to their health and human rights is the law that makes it impossible to find safe places to work, and prevents them from having the in protections as other workers and other citizens.”

In many countries, the needs of sex workers remain ignored and under-researched. Stefan Baral, Deanna Kerrigan and colleagues from the Johns Hopkins Bloomberg School of Public Health – the same group that has taken a lead in highlighting elevated HIV rates in men who have sex with men around the
world – conducted a systematic review and meta-analysis to pull together estimates of HIV rates in low and middle income countries.

They were able to include 102 reports which met pre-determined quality criteria, encompassing 12,197 sex workers. All reports came from 2007 to 2011.

But reports were only available for 50 of 145 countries. “We must look critically at the global policy environment which limits comprehensive assessments of HIV prevention and service delivery needs of sex workers across settings,” commented Deanna Kerrigan.

The available data do show that women who sell sex are at particularly high risk of infection. Kerrigan noted that their increased vulnerability is not just due to behavioural factors (large numbers of sexual partners, etc.) but also structural factors (criminalisation, human rights violations, etc.).

These data are a call for action to invest in and address the needs of sex workers

Data were available for 14 Asian countries. Whereas HIV prevalence in women aged 15-49 in these countries is 0.18%, for sex workers it is 5.2%, meaning their risk of having HIV is 29 times greater.

In sub-Saharan Africa, data were available for 16 countries. Whereas HIV prevalence for women in the general population is 7. 4%, it is 36.9% for women who sell sex, meaning their risk is 12 times greater.

In Latin America and the Caribbean, there were data for 12 countries. With a background female HIV prevalence of 0.4% and 6.1% for women who sell sex, their risk is also 12 times greater.

In the Middle East and North Africa, 1.7% of female sex workers had HIV. In Eastern Europe, prevalence was 10.9%. However because data were only available for a handful of countries in these regions, further analysis was not felt to be reliable.

The data show wide variations from country to country, and sometimes between different regions of the same country. Some of this is likely due to different sampling and research methods.

Deanna Kerrigan concluded that “these data represent a call for action to invest in and address the needs of sex workers to prevent HIV, including evidence-based comprehensive HIV prevention strategies which protect and promote their human rights”.

Moreover, the same group of researchers also reported the results of modelling work which estimated the impact of providing such prevention strategies. Two strategies were examined:

- Improving sex workers’ access to antiretroviral therapy so that coverage is the same as for other adults in their country.
- A comprehensive community-empowerment programme, in which structural barriers are addressed collectively. The programme typically includes community organising and mobilising, peer education, condom distribution and more accessible clinical services for sexually transmitted infections.

A soon to be published systematic review conducted for the World Health Organization has found that community empowerment programmes typically reduce inconsistent condom use by half. The Johns Hopkins researchers applied this finding to the epidemics of Brazil, Kenya, Thailand and Ukraine.

For example, in Kenya, just improving ART access would reduce infections in sex workers by 25% over five years. Only making the empowerment intervention available to two thirds of sex workers would reduce infections by 11.5%. Doing both would reduce infections by 33%.

There would also be a significant impact on the epidemic in the wider population, with 30% fewer infections if the interventions were combined.

Although it may appear that the ART intervention makes the greater difference, the researchers note that empowerment and reduced structural barriers are probably a necessary requirement for expanded ART access. Indeed, the interaction between ART use and empowerment had not been fully accounted for in the model.

“Is there really a product or a medicine that can change the balance of power between sex workers and their clients?” Cheryl Overs

The sex worker activist and researcher Cheryl Overs spoke to these issues at a plenary that morning. In particular, she commented on the conference’s ‘turning the tide together’ slogan.

While a video showed waves washing up on a beach, she said that the tide is made up of many waves – including social exclusion, lack of legal rights, family rejection, poverty, bad working conditions, violence, condoms used as evidence and corruption.

“The waves are interconnected, so there’s no selecting which waves to turn back,” she said. “Involving and empowering sex workers is crucial to turning that tide.” However many sex workers have been prevented from participating in the Washington DC conference, due to bans on them entering the United States.
Overs pointed to the Global Commission on HIV & the Law’s recommendations for governments on treating sex workers in a way that is consistent with human rights obligations.

And she commented on what treatment as prevention, microbicides and pre-exposure prophylaxis (PrEP) could offer sex workers. “Is there really a product or a medicine that can change the balance of power between sex workers and their clients?” she asked.

She warned that the cost and responsibility of using the new methods will continue to fall on sex workers, who will still need protection from sexually transmitted infections and pregnancy. HIV testing is the gateway to new prevention methods, but forced testing and breaches of confidentiality are already commonplace for sex workers.

“I haven’t raised these issues about new prevention technologies to suggest that they can’t work for sex workers,” Overs said. “I raised them to illustrate that they create challenges that can’t be solved without strong inputs from sex worker advocates.”

To those rolling out treatment as prevention and PrEP she said: “You need to focus more on the challenges in the broader environment of sex work, not just on getting the products to sex workers.”

References
2. The data were simultaneously published in a journal:

Cobicistat matches ritonavir as atazanavir booster
Liz Highleyman
Produced in collaboration with hivandhepatitis.com
Published: 30 July 2012
The new boosting agent cobicistat works as well as ritonavir (Norvir) as a pharmaco-enhancer or booster for the first-line protease inhibitor atazanavir (Reyataz) at 48 weeks, according to a report presented last week at the 19th International AIDS Conference in Washington, DC.

Some antiretroviral drugs, including most protease inhibitors and the experimental integrase inhibitor elvitegravir, have trouble reaching effective levels in the body. Boosters like ritonavir and cobicistat inhibit the activity of the CYP3A enzyme in the liver, which slows processing and raises blood levels of other drugs metabolised by the same pathway. Unlike ritonavir, however, cobicistat is not itself active against HIV.

Joel Gallant from Johns Hopkins School of Medicine presented findings from a phase III randomised controlled trial comparing the safety and efficacy of cobicistat against ritonavir when used as part of a first-line regimen with atazanavir and the nucleoside reverse transcriptase inhibitor duo tenofovir/emtricitabine (the drugs in Truvada).

Study 114 included 692 treatment-naïve participants. About 80% were men, about 60% were white and the average age was about 38 years. The mean CD4 T-cell count was about 350 cells/mm³, with 17% having less than 200 cells/mm³.

People with poor kidney function were not included in this trial, as earlier studies suggested cobicistat might cause kidney toxicity. Further research indicated that the observed increase in serum creatinine was likely to have been due to the drug's effect on tubular secretion, not impaired filtration, meaning it altered estimated but not actual glomerular filtration rate (GFR). In Study 114, the median estimated GFR at baseline was about 114 mL/min (using the Cockcroft-Gault method) and people with estimated GFR below 70 mL/min were excluded.

By 48 weeks 15% of cobicistat recipients and 11% of ritonavir recipients had stopped the study. Half of all discontinuations in the cobicistat arm and two-thirds in the ritonavir arm were due to adverse events, with small numbers stopping for various other reasons including loss to follow-up, protocol violations or pregnancy.

The primary analysis at 48 weeks showed that 85% of people taking cobicistat and 87% taking ritonavir suppressed HIV viral load below 50 copies/mL in an intent-to-treat ‘snapshot’ analysis, showing
that cobicistat is non-inferior to ritonavir. Virological non-suppression was seen in 6% of cobicistat recipients and 4% of ritonavir recipients.

In a more traditional intent-to-treat missing equals failure analysis, response rates were 89% for cobicistat and 90% for ritonavir. When people with missing data were excluded, the as-treated response rates were 97% and 96%, respectively.

Response rates with cobicistat and ritonavir were similar when comparing people with high (>100,000 copies/mL) or low viral load, as well as those with CD4 counts above or below 350 cells/mm³. CD4 cell gains at 48 were similar, at 213 and 219 cells/mm³, respectively. Gallant noted that CD4 counts had not yet plateaued and were still rising at the time of this analysis.

Serious adverse event rates were statistically similar in both arms, 11% with cobicistat vs 7% with ritonavir. In both arms, 7% stopped treatment due to adverse events, including similar numbers due to bilirubin-related events and kidney problems. Elevated bilirubin (hyperbilirubinemia)¾ a known side-effect of atazanavir¾ was more common among cobicistat recipients (65% vs 57%, respectively).

Serum creatinine rose a bit more (0.13 vs 0.09 mg/min) and estimated GFR fell more (-13 vs -9 mL/min) in the cobicistat arm. Five of six people who stopped taking cobicistat and two of five who stopped ritonavir due to kidney problems experienced proximal tubule dysfunction.

Turning to blood lipid elevations—a side-effect of ritonavir—levels of total cholesterol, LDL "bad" cholesterol and triglycerides rose more in the ritonavir arm, while HDL "good" cholesterol rose more in the cobicistat arm, but these differences did not reach statistical significance.

The researchers concluded that cobicistat plus atazanavir and tenofovir/emtricitabine "demonstrated non-inferior efficacy" to a corresponding regimen with ritonavir, and that cobicistat was well-tolerated. Rates of discontinuation due to kidney side-effects were "low and comparable", they added, and rates of bilirubin-related discontinuation were similar.

Cobicistat as a single agent has been submitted for regulatory approval in Europe and the US. Cobicistat is part of the single-tablet regimen known as the Quad (with elvitegravir, tenofovir and emtricitabine), which is under review for treatment-naive people. Gilead is also looking at other cobicistat coformulations that include atazanavir, darunavir and the tenofovir pro-drug GS-7340.

Reference

July 30, 2012

**Porn Trial: This Time it's Extreme**

Today the Crown Prosecution Service will attempt to persuade a jury that images of fisting should be classified as “extreme pornography” with the risk to the defendant of three years in custody, inclusion on the sex offenders' register and damage to his personal and professional standing.

All for a type of image which is commonly viewed, of an activity which is itself is legal to perform and is even discussed in the book Fifty Shades of Grey. Nonetheless the defendant, Simon Walsh, has been charged with being in possession of extreme pornographic images under section 63 of the Criminal Justice and Immigration Act 2008: so the Prosecution must prove that the act of fisting is “likely to result in serious injury to a person’s anus”.

**The Defendant – Simon Walsh**

Simon, who is represented by my firm (Hodge Jones & Allen) has given his express permission for this information to be published.

Before being arrested and charged with these offences, Simon was a successful professional and politician in the City who, amongst other things, prosecuted police officers accused of disciplinary offences.

After being charged, Simon lost both professional and political positions, despite the fact that no pornography was found on any of his work computers. In fact, no pornography was found on Simon’s home computers either.

Instead, the police had to “interrogate” Simon's personal email account (server) in order to discover a few images they deemed questionable. This included an image of a man wearing a gas mask. Their expert stated that this was likely to cause serious harm, even death by asphyxiation: despite being a piece of equipment designed to assist breathing. This charge was eventually dropped.
Unfortunately, by performing the “interrogation” of Simon’s email account in the fashion they did, the police contaminated the only source of evidence; making it impossible to identify whether images attached to emails had in fact been opened and viewed.

The Peacock Trial

Readers familiar with the jury decision in Micheal Peacock's obscenity trial earlier this year, where the defendant was unanimously acquitted of publishing fisting DVDs under the Obscene Publications Act 1959 (OPA), may be surprised to hear that the CPS are having another bite at the cherry when it comes to fisting.

As the Peacock obscenity trial was under the OPA the CPS needed to show that fisting pornography was likely to “deprave and corrupt” the viewer. Since this Trial is under section 63 of the CJIA 2008 the CPS must show that act of fisting is “likely to result in serious injury to a person’s anus” in order to persuade a jury that the mere representation (pictures) of this activity is a criminal offence, despite the fact that the act itself is legal to perform.

The Prosecution Case

What follows is a summary of elements of what the Prosecution must prove to substantiate an offence has been committed under the CJJA 2008:

According to Section 63 images are “extreme” if they are “grossly offensive, disgusting or otherwise of an obscene character” and according to subsection 7:

“(7) An image falls within this subsection if it portrays, in an explicit and realistic way, any of the following—

(b) an act which results, or is likely to result, in serious injury to a person’s anus, breasts or genitals”

Unfortunately, what is “likely” to result in “serious injury” is not specifically defined in the Act itself. There were Ministry of Justice Guidelines specifically on the extreme pornography legislation, but they seem to have disappeared from the internet, possibly disavowed.

Conclusion

As with the Peacock obscenity case, it will be instructive to see whether the police and prosecution are out of step with current cultural and moral values towards sexuality; and instead whether a jury of reasonable people simply deem that fisting pornography is neither extreme nor criminal.

Updates

Simon’s Trial is listed to start today, Monday the 30th July 2012, at Kingston Upon Thames Crown Court from 12pm and is listed to last for up to seven days.

With the Trial Judge’s permission, the Trial will be live-tweeted under the hash tag #porntrial.

Hence it should be possible to follow the Trial as it unfolds; and discover whether a jury will swallow such an intrusive Prosecution.

Most HIV-Positive Americans Lack Regular Care

Wall Street Journal, (07.27.2012) Betsy McKay, Health blog

Only 25 percent of Americans with HIV have their virus under control, according to a CDC report released Friday at the 19th International AIDS Conference in Washington. African Americans and younger people are least likely to be in regular care and treatment, CDC said.

[PNU editor’s note: Among African Americans with HIV, 81 percent have been diagnosed; 34 percent are retained in care; 29 percent have been prescribed antiretroviral therapy; and 21 percent are virally suppressed, CDC reported.]

Among Americans ages 25-34 who have HIV, 72 percent have been diagnosed; but only 28 percent receive regular care and 15 percent are virally suppressed.

“We’ve got to do better,” said Dr. Jonathan Mermin, director of CDC’s Division of HIV/AIDS Prevention. More widespread HIV testing is critical, and it needs to be easier to link those infected directly to care and make sure they stay there, he said: “I want to make the healthy choice the easy choice.”

Programs to retain patients are not working or are not plentiful enough, the report said.

“We need to reverse-engineer; we understand how many other countries are doing this better and often with US tax dollars,” said Nancy Mahon, chair of President Barack Obama’s Advisory Council on HIV/AIDS and global executive director of the MAC AIDS Fund (MAF).

Toward that end, MAF announced its support of joint efforts with the US Department of Health and Human Services to improve retention in HIV care. These efforts include UCARE4LIFE, a two-year mobile
texting pilot program in the South sending disease-management reminders and tips. Another is a forum to explore successful programs, including US overseas efforts.

**AIDS Rise in Uganda Shows Need for More Resources**

Almost 260,000 people with HIV/AIDS in Uganda are being treated by the US President’s Emergency Plan for AIDS Relief program launched by President George W. Bush in 2003—treating nearly half of the 600,000 in need of antiretrovirals there. PEPFAR, said Dr. Stella Talisuna, has enabled thousands of Ugandans to “get back on their feet.”

Concurrently, however, new government data show HIV prevalence in Uganda has risen from 6.4 percent in 2004 to 7.3 percent in 2011. The number of Ugandans with HIV has increased two-fold since 2004, from 1.2 million to 2.4 million.

Critics say Uganda’s past success in reducing the HIV infection rate has been undermined by a shift in focus from prevention to treatment. Ugandans “now see AIDS as much more like diabetes, one of these chronic diseases you can live with indefinitely,” said well-known social critic Timothy Kalyegira. Official figures show Uganda’s rate is rising primarily because people are having multiple sex partners.

Years ago, Uganda rolled out the “ABC” HIV prevention policy: abstain, be faithful, or use condoms. A generation of students watched videos on how AIDS ravages the human body, and they were encouraged to postpone first intercourse.

“There is need for continuous dissemination of information,” said Joshua Musinguzi, head of the country’s AIDS control program. “Individuals have the power to make the correct decision if they want to. The menu is there: ABC.”

Meanwhile, US officials are pressing Uganda to devote more of its own resources to AIDS and other health issues, noting that dependency on foreign donors is unsustainable in the long term. Country coordinator Michael Strong said PEPFAR is scheduled to transition from service delivery to technical assistance, and Ugandan officials must step up their role going forward.

**Call for Syringe Programs in Prisons to Curb Swell of Virus**

A man is preparing to sue Victoria for failing to protect him from hepatitis C while he was incarcerated in one of the state’s prisons.

More than 40 percent of prisoners have hepatitis C, and drug use continues behind bars. Philip Lynch, director of the Human Rights Law Center, said it is only a matter of time before an inmate prevails in a suit saying prisons and the government exposed him or her to infectious diseases. Inmates could argue that a failure to provide clean needles and syringes violates the state’s common-law duty of care and obligations under the Victorian Charter of Human Rights and Responsibilities, he said.

“These are not frivolous arguments,” Lynch said. “We’ve been advised by leading senior barristers and law firms on the merits of this.”

“It’s a big problem ... prisons are basically a hot-bed for viral transmission,” said John Ryan, chief executive of Anex, a group working on harm reduction with prominent scientists Sir Gustav Nossal and Professor Peter Doherty.

Victorian prison officials provide bleach for inmates to clean syringes. But Dr. Mark Stoove, head of HIV, AIDS and STI research at the Burnet Institute, called this inadequate, noting studies show bleach only reduces the risk of hepatitis C transmission by 65 percent.

“The provision of bleach acknowledges drugs are entering prisons and that injecting is occurring ... so the question is do you provide this half-measure to protect people’s health, or do you provide the evidence-based response, which is clearly prison-based needle and syringe programs,” said Stoove.

**Credible Online Health Information Eludes Collegians**
*Chicago Tribune*, (07.18.2012) Leslie Mann

Northwestern University researchers say results from their new study show first-year college students struggle to find accurate information on birth control online despite being heavy Internet users.

Lead author Eszter Hargittai, an associate professor of communication studies, and colleagues gave 210 first-year students from an urban and a suburban Midwest college a hypothetical scenario: A girlfriend calls at midnight on a Friday during the summer, saying the condom broke during sex with her boyfriend the previous night. What can she do to prevent pregnancy? “We intentionally told them it was
during the summer so they would not refer their friends to their colleges’ medical clinics,” explained Hargittai.

Two-thirds of the freshman said their friend should use emergency contraception, but only 40 percent knew the pills are sold over the counter. Three percent were unable to give the friend any advice.

Most participants used a search engine to seek out online advice. Sites visited by the freshmen included: Planned Parenthood (31 percent); morningafterpill.org, a site opposed to the use of emergency contraception (10 percent); Wikipedia (6 percent); and the maker of Plan B One-Step (4 percent), a popular emergency contraceptive.

“Despite being on the Internet so much, we were surprised to see how few of them had the navigating skills to find credible health information for their friends,” Hargittai said. “And we were surprised how many of them gave their friends inappropriate advice, such as ‘wash your genitals,’ ‘wait it out’ or ‘buy a new condom.’”

“This study tells us we need to incorporate more [Internet] navigating lessons in the curricul—in college or earlier,” Hargittai said.

[PNU editor’s note: The study, “Searching for a ‘Plan B’: Young Adults’ Strategies for Finding Information About Emergency Contraception Online,” was published in the journal Policy & Internet (2012;4(2):Article 4).]