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IAVIReport

The Publication on AIDS Vaccine Research

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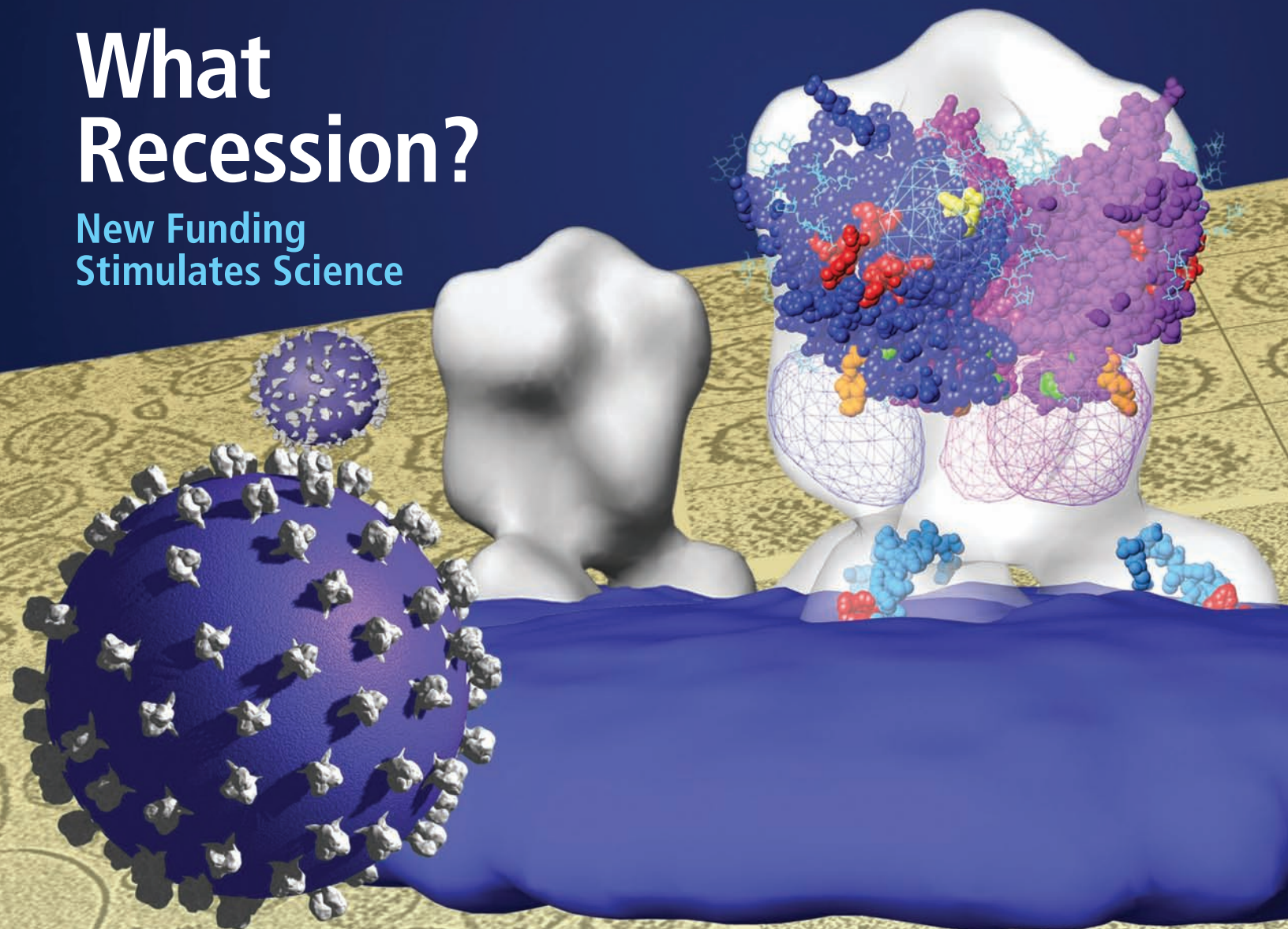
Crowdsourcing Vaccine Challenges

What Recession?

New Funding Stimulates Science



EDITOR'S LETTER

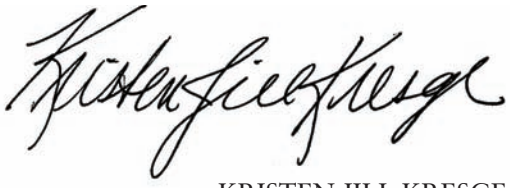
WHAT RECESSION? Instead of mortgage-backed securities, the US government is investing more heavily in science.

Although the world is still in the throes of a major economic crisis, US government spending on scientific research is actually on the rise. This is most welcome news for the multitude of researchers who depend on US government funding to conduct their work. It is also reassuring to the larger scientific community that research is once again a top priority in Washington, D.C. On top of an increase in the annual operating budget of the National Institutes of Health (NIH) that was put in place by the outgoing Bush administration, President Barack Obama has pledged an unprecedented amount of money in his proposed 2010 budget to support scientific research. And through a one-time economic stimulus package passed by the US Congress in February, the NIH received US$10.4 billion to fund both infrastructure and so-called beaker-ready projects. Research grants from the stimulus dollars just started rolling out. In this issue, we examine how much of that money will be spent on HIV prevention research and AIDS vaccine-related projects in particular.

Fears have been percolating in the international community about how the global recession will impact international AIDS spending in the coming years. The US, as the biggest backer of HIV/AIDS prevention, treatment, and care services in the world, has been under the most scrutiny. *Despite Recession, New Funding Stimulates Scientific Research*, reviews the AIDS-related spending in Obama's budget, which is now under review by US lawmakers. Although many changes will likely occur before a final budget is approved later this year, this article outlines the administration's priorities and commitments.

With more money f owing to science, innovation is likely to follow. At the same time, a cheaper path to progress could be just a few clicks away. *Not Sure? Ask Everyone* explores how crowdsourcing is using the wisdom of the masses to try to solve some of the challenges that plague researchers, including those working to design improved AIDS vaccine candidates.

Let's hope both paths to new discoveries spur progress.



KRISTEN JILL KRESGE

The International AIDS Vaccine Initiative (IAVI) is a global not-for-profit organization whose mission is to ensure the development of safe, effective, accessible, preventive HIV vaccines for use throughout the world. Founded in 1996 and operational in 24 countries, IAVI and its network of collaborators research and develop vaccine candidates. IAVI's financial and in-kind supporters include the Alfred P. Sloan Foundation, the Bill & Melinda Gates Foundation, the Foundation for the National Institutes of Health, The John D. Evans Foundation, The New York Community Trust, the James B. Pendleton Charitable Trust, The Rockefeller Foundation, The Starr Foundation, The William and Flora Hewlett Foundation; the Governments of Canada, Denmark, India, Ireland, The Netherlands, Norway, Spain, Sweden, the United Kingdom, and the United States, the Basque Autonomous Government, the European Union as well as The City of New York, Economic Development Corporation; multilateral organizations such as The World Bank; corporate donors including BD (Becton, Dickinson & Co.), Bristol-Myers Squibb, Continental Airlines, Google Inc., Henry Schein, Inc., Merck & Co., Inc., Pfizer Inc, and Thermo Fisher Scientific Inc.; leading AIDS charities such as Broadway Cares/Equity Fights AIDS and Until There's A Cure Foundation; other private donors such as The Haas Trusts; and many generous individuals from around the world. For more information, see www.iavi.org.

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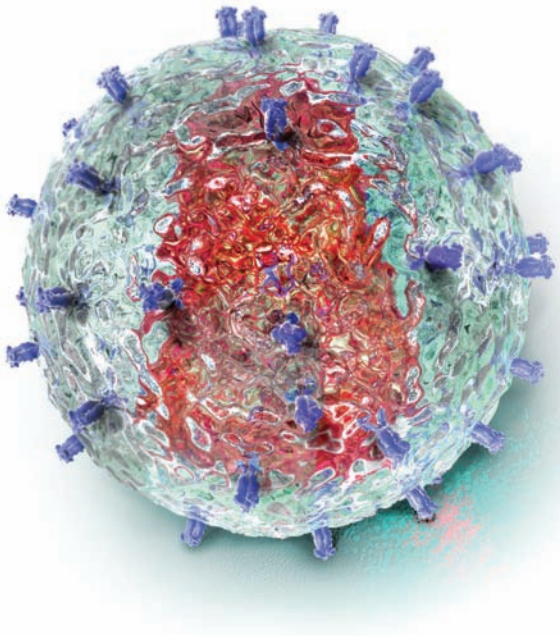

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[ON THE COVER]

Composite of simian immunodeficiency virus (SIV) cryo-electron microscopy tomogram data images and models. Tomography slices (background), overlaid with modeled virion (small blue sphere) with envelope spikes (white) and idealized modeled virion (large blue sphere). A section of the viral membrane (blue) with averaged envelope spike model (white) and the spike model fitted with gp120 and gp41 atomic structures appears to the right.

Image courtesy of Zhu, P., Liu, J., Bess Jr., J., Chertova, E., Lifson, J.D., Grise, H., Ofek, G., Taylor, K.A., and Roux, K.H. Nature, *441:847-852, 2006.*

Not Sure? Ask Everyone

Crowdsourcing is becoming an increasingly common tool to solve scientific challenges both big and small. It is even being put to the test in AIDS vaccine research

By Andreas von Bubnoff

1974 Josephus. These were the two words that had to be retyped exactly as they appeared on the screen in a recent attempt to sign up for a Facebook account. These images of scanned text are called captchas and are used by many web sites to make sure that whoever signs up is human, and not a computer program written to fill in the form.

But, unbeknownst to them, internet users who retype captchas also help digitize scanned text taken from books or sources such as the *New York Times*, which were created prior to the computer age. Although the scanned text from these sources can often be recognized automatically by computers, human help is necessary about 30% of the time to decipher words computers can't identify, according to Luis von Ahn, a computer scientist at Carnegie Mellon University. Von Ahn, who co-invented captchas with his PhD advisor in 2000, started a project over a year ago to use these captchas to help fill in the words from books and periodicals that computers couldn't identify. He realized that 200 million captchas were filled out every day for internet security, amounting to about 500,000 hours of work that could be used for another purpose. The project now utilizes about 100,000 web sites with captchas, including Facebook, Twitter, and Ticketmaster, which means people help decipher about 30 million words per day, says von Ahn.

This project is just one example of what some call "crowdsourcing." Jeff Howe, a contributing editor at *Wired*, dubbed the term crowdsourcing in a 2006 article for the magazine. It describes a phenomenon where an undefined, generally large group of people, or a crowd, takes on tasks once performed by a designated person, usually an employee, in response to an open call. "The labor isn't always free, but it costs a lot less than paying traditional employees," he wrote. "It's not outsourcing; it's crowdsourcing." With captchas, millions of people are helping to digitize books and articles for free. Another example, Howe says, is Amazon Mechanical Turk (MTurk.com). With MTurk, people offer small amounts of money to the public for solving simple tasks. Howe has been using an online service, which uses MTurk, to get interviews transcribed for his book on crowdsourcing that was published last year.

In contrast to such simple tasks, crowdsourcing is also used to solve more complex scientific problems. For example, researchers recently started to use an online game to get help from the general public in solving protein structures. Also, companies such as InnoCentive or NineSigma

have sprung up to issue open calls to the public to get their help in tackling scientific or engineering challenges. These companies post challenges on their web sites on behalf of clients, often companies, who then offer a reward to anyone in the general public who can come up with a solution to the problem. The solutions can come from anyone, anywhere, and they often do—the success rate for the challenges posted through these sites is surprisingly high. This approach has even been used recently to address a challenge in AIDS vaccine research. In 2008, IAVI posted a challenge on the InnoCentive website to create a stable version of the HIV Env protein.

Although using the internet to post challenges is a fairly recent phenomenon, the strategy of putting out an open call to the general public in exchange for a reward or prize has existed for a long time. In 1927, Charles Lindbergh won the Orteig prize for f ying non-stop across the Atlantic Ocean. Today, some organizations still offer large amounts of money for the solution of quite ambitious goals. Google's "Project 10^{100}" offers US$10 million to fund up to five "ideas to change the world," and the X-Prize Foundation has announced a $10 million award to anyone who can sequence the human genome faster than ever before. The X-Prize Foundation may soon get involved in infectious disease research as well—the organization has received a grant from the Bill & Melinda Gates Foundation to explore a future prize for a better tool to diagnose tuberculosis.

Gaming for science

It doesn't take as much knowledge to retype a captcha as it does to develop a faster way to sequence the human genome. But not all solutions to scientific problems require specialized knowledge. Last year, University of Washington researchers launched the online game Foldit, which allows players to earn points by finding the lowest possible energy structure of proteins. Players use their mouse to move around parts of proteins, which are displayed on the screen, and score points for getting the protein in a conformation closer to its lowest energy state, which usually represents its natural structure. Using a computer to find the lowest-energy state requires a significant amount of time because there are many possible low-energy structures for any protein.

Originally, David Baker, a University of Washington professor of biochemistry, and his team developed a downloadable program called rosetta@home that used the computer's downtime to sort through protein structures. As a reward, it showed the results of the calculations as a screensaver. The online game Foldit was created because users of rosetta@home wanted to participate, not just watch, Baker says. "They thought they could do better," he says. And it seems that they can. People see which particular options to try in a more efficient way than computers would, says Zoran Popović, a computer scientist at the University of Washington who developed Foldit with Baker and others. As of May 2009, about a year after Foldit was launched, it had over 100,000 players, Popović says, adding that people seem to be at least as competitive as massive computational efforts in finding low energy protein structures. "They can find solutions that the computers have not found," he says. Recently, Foldit announced a new feature of the game that allows players to design the HIV Env protein to expose areas that are vulnerable to neutralizing antibodies.

Paying the crowd

Successful Foldit players are rewarded with peer recognition and being able to affect the direction of research, Popović says. "The best performing proteins will be synthesized in the lab." But whoever solves scientific or engineering challenges posted by companies like InnoCentive or NineSigma is usually eligible for a financial reward.

At InnoCentive, a seeker looking for a solution to a problem pays a fee to InnoCentive to post a specific challenge on its website. Anyone can then submit a solution. Some challenges only require a written proposal of ideas as to how to solve a challenge, others require additional evidence that the solution actually works, such as original data from experiments or even a physical sample. The seeker then pays a cash award to the solver who provides the solution that best meets the requirements of the challenge.

Ed Melcarek, a 60-year-old Canadian engineer and scientist, says he has made over $115,000 for solving seven challenges since 2003. InnoCentive named him one of the most successful solvers of 2007. "[Seven solved challenges] is a lot given the complexity of those problems," Howe says. Melcarek has submitted solutions to 31 additional challenges which did not get awarded, and he currently has five others pending.

As a postdoctoral student at the University of Chicago, Laurie Parker spent 30 minutes in 2006 to solve a $5,000 challenge. She found a new way

to synthesize large collections of random peptides without using a traditional biological approach like polymerase chain reaction or cloning, says Parker, now an assistant professor of medicinal chemistry and molecular pharmacology at Purdue University. It didn't take her long to solve that challenge because she was already working on a chemical reaction that also applied to this challenge, she says. In addition, the seeker only asked for ideas about how to solve the problem without necessarily proving that it worked, says Parker.

On average, it takes two weeks, or 80 hours, for solvers to come up with a solution to an InnoCentive challenge, according to a study of 166 challenges solved through the company's website between 2001 and 2004. The study also found that the further removed the background of the solver was from the area the challenge pertained to, the more likely it was that the problem got solved, says one of the study's authors, Karim Lakhani, an assistant professor in the technology and operations management unit at Harvard Business School. "In our analysis, the problem solvers said that the problem that they tried to create a solution to was typically outside their own field of expertise," he says.

For example, John Davis solved a challenge to help with oil spill recovery. The challenge from the non-profit Oil Spill Recovery Institute was to find a way to liquefy the oil/water slush collected on barges from arctic waters in the case of an oil spill so that it could be pumped from the barges to larger storage tanks on land. Davis says he had once helped a friend whose family owns a small concrete business and remembered that construction workers used a vibrating device to keep the concrete from solidifying at construction sites. He thought the same approach might work on the oil/water slush. After a day of work, and a call to the company asking if they could modify the vibrating device for this purpose, he filed the solution. A few months later, he received $20,000.

InnoCentive says that about a third of its challenges get solved, but Lakhani says it's hard to know how this compares with the in-house success rate of companies, because most don't keep track of that or share it publicly. Still, Lakhani says that in his conversations, research and development chiefs at various organizations seem "very surprised" by the high success rate of InnoCentive, especially considering that these challenges likely get posted on the InnoCentive web site because the firms couldn't solve them in-house.

NineSigma, another company that connects clients with potential solvers, was founded in 2000 by Mehran Mehregany, a professor of electrical engineering and computer science at Case Western Reserve University. Mehregany says he founded the company once he realized that the elaborate system the government uses to issue open calls to academic researchers wasn't available to industry. "Industry does not have a similar systematic infrastructure to broadcast its science and technology needs to the broader science and technology community," Mehregany says. Anyone can submit a solution for a NineSigma challenge, but for all challenges, the company also uses a proprietary system to proactively find experts that are likely to be able to solve a challenge. "If you are out there and we think you relate to our challenge, we will do our best to find you," Mehregany says. That's why NineSigma calls its approach "expert sourcing" instead of crowdsourcing. For some of its challenges, InnoCentive also tries to identify potential solvers outside of its network of registered solvers.

One issue for the two companies is how to handle intellectual property (IP) rights. At NineSigma, solvers negotiate their IP rights directly with the seeker. At InnoCentive, solvers sometimes transfer their IP rights when they accept the award money. Melcarek doesn't have a problem with that. "I would much sooner have the cash in the bank than a piece of paper saying that I own property rights," he says. "[Then] you have to find somebody that's in that field to buy the patent from you." But Parker says she might not want to sign away her IP rights if it kept her from patenting future work in her own field. "I would never want to sign away the rights to something that I am interested in pursuing," she says.

Crowdsourcing for non-profits

InnoCentive typically has companies as clients, but non-profits also post challenges. Prize-4Life, for example, a non-profit organization trying to accelerate the discovery of treatments and cures for Amyotrophic Lateral Sclerosis (ALS), posted a challenge to identify a biomarker to measure disease progression in ALS. "We try to make it very appealing for non-profits because we think non-profits have not had access to the same innovation channels that the commercial interests have," says Dwayne Spradlin, president and CEO of InnoCentive. "We will typically either lower the prices or increase

[FROM CROWDSOURCING TO CROWDFUNDING]

While many organizations are using the principle of crowdsourcing to find solutions to problems, some are using the same principle to find funding. IAVI is sponsoring three projects on globalgiving.com, a web portal where people interested in making a donation can look through hundreds of causes or projects and choose which one to help fund, says GlobalGiving Program Officer Saima Zaman. One project aims to increase the number of HIV testing and counseling outreach teams around AIDS vaccine clinical research centers in Entebbe, Uganda. The goal is to raise $27,000 for the project. So far, $2,554 has been raised from 75 donors, according to the GlobalGiving web site. —*AVB*

the amount of services we provide to non-profits."

Also, the Rockefeller Foundation collaborated with InnoCentive from 2006 until 2008 to encourage non-profits to participate. The foundation would typically pay the fee required to post a challenge as well as half of the award money on behalf of the non-profit, according to Amanda Sevareid, a research associate at the Rockefeller Foundation. Once a problem was solved, the foundation would pay the rest of the award money if there was evidence that the solution was successfully implemented. Six non-profits took part in the program, and most of their challenges were solved. In late 2008, the TB Alliance announced two awards of $20,000 each for improving the synthesis of a tuberculosis drug candidate.

IAVI posted a $150,000 challenge late last year as part of the Rockefeller Foundation program. The challenge was to create a protein that mimics the trimeric HIV Env protein and would remain stable in laboratory testing. In its natural state, the Env trimer is unstable and breaks down easily when entering the body, according to Kalpana Gupta, director for new alliances and initiatives at IAVI, who was involved in developing the challenge. As a result, it has been difficult to trigger antibody responses against the trimer.

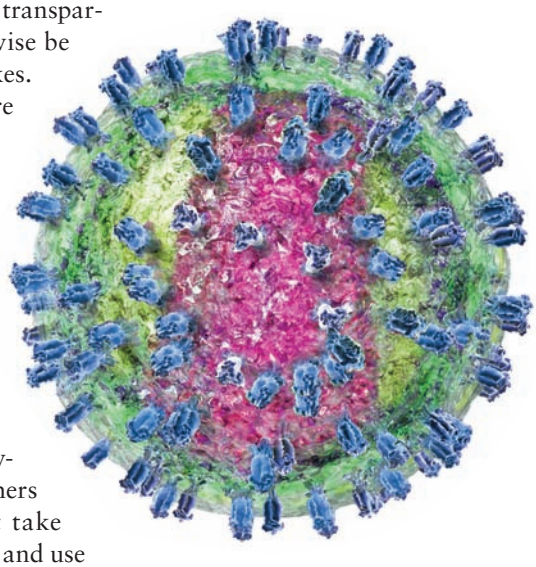
The solution required showing that neutralizing monoclonal antibodies to Env can bind the new protein *in vitro*. If the Envelope structure also turned out to be sufficiently immunogenic in animal testing, the solver would be eligible for a bonus of up to $500,000 and/or the opportunity to pursue their research further with support from IAVI, Gupta says. Because the award money was quite high in this case, Rockefeller agreed to pay just a third of the initial $150,000 of the award money. However, none of the solutions submitted by the deadline met the requirements of the challenge.

Making science more transparent

In addition to solving problems, scientists are also increasingly using the principle of crowdsourcing to share and collect information and data. In one such effort, scientists use the online encyclopedia Wikipedia to compile information about scientific topics. In late 2007, Andrew Su, group leader of bioinformatics and computational biology at the Genomics Institute of the Novartis Research Foundation in San Diego, started the Gene Wiki project, by creating thousands of gene-related entries on Wikipedia, initially using information from existing databases (*PLoS Biol.* **6**, e175, 2008). The Gene Wiki project by now has added about 9,000 gene pages to the approximately 650 gene-related pages that existed on Wikipedia before the project started. Su says that once the entries are created, people are more likely to add information to them than when they have to create a new entry first.

In his daily work, Su also sometimes asks his fellow scientists for advice using a site called FriendFeed. That's where he went when asked for this article about how scientists use crowdsourcing. Within a few hours, the answers started to arrive. "I like the recursive nature of crowdsourcing an answer to a question about crowdsourcing," read one reply.

One example Su mentioned of how scientists use crowdsourcing is Jean-Claude Bradley, an associate professor of chemistry at Drexel University. Bradley coined the term "open notebook science," which aims to make the scientific process more transparent by making a researcher's lab notebook public, in real time. Bradley, the members of his lab, and students from around the world post the results of their measurements of the solubility of chemical compounds for antimalaria drugs on the web. He says such transparency can save time that would otherwise be wasted repeating other people's mistakes. "You have to see how other people are failing," Bradley says, adding that transparency also enables collaboration with other researchers.



Still, seekers or solvers of challenges through companies like InnoCentive might need to keep the way a challenge was solved confidential to protect intellectual property rights. For academic researchers, it might be less of a concern as long as they don't want to patent a finding. However, there is the concern that researchers from large, well-funded labs might take research ideas they find in open access and use their resources to do experiments to turn that into a grant or publication, even though the original research idea wasn't theirs, says Parker. But Bradley says it should be easy to identify plagiarism because everything is on the web, providing a track record as to who came up with the findings when. "I think it would be really embarrassing if somebody came in and copied stuff that anybody can Google," Bradley says. "I think we are safer because it is so public." ■

DESPITE RECESSION,

New Funding Stimulates Scientific Research

New research dollars will energize scientific projects, including many focused on HIV prevention strategies, but uncertainty remains about the sustainability of long-term HIV/AIDS funding

By Regina McEnery

In recent years, budgets of the major US government research engines, including the US National Institutes of Health (NIH), have not increased. Tighter budgets have meant more competition for research dollars, much to the chagrin of scientists who rely on government funding to get their projects off the ground. During difficult economic times, all types of discretionary spending, including scientific research budgets, can get squeezed even further. Yet, even in this time of great economic uncertainty, science funding in the US is now actually on the rise. Ironically, the current recession has spurred dramatic levels of new funding for research projects through the US economic stimulus package. This new money, along with a strong commitment to science from US President Barack Obama, has many researchers excited.

Some of the projects that will receive stimulus funding involve HIV prevention strategies, some of which are related to AIDS vaccine research. The Office of AIDS Research (OAR), which coordinates the US government's AIDS research budget, views HIV prevention as the highest priority. "Disappointing results from recent clinical studies of HIV vaccine and microbicide candidates underscore the need for additional discovery (basic) research of HIV," the OAR noted when it presented the president's budget request to lawmakers, adding that, "biomedical and behavioral interventions are urgently needed."

Apart from the stimulus money, there are also some much smaller increases being doled out to HIV/AIDS research. Obama's 2010 budget, which is being considered by lawmakers, seeks a US$45 million, or 1.5%, overall increase in HIV/AIDS research, for a total of $3 billion (see Figure 1, page 9). This includes less than a $1 million, or 1%, increase for AIDS vaccine research and a 3.2% increase for research on microbicides.

As the HIV pandemic approaches its third decade, the Obama administration maintains that HIV/AIDS prevention and treatment is a priority, both globally and domestically, and will remain so despite the recession. "In a time of tightening budgets and economic constraints, the 2010 budget request demonstrates commitment to the global fight against HIV/AIDS," Acting Deputy US Global AIDS Coordinator Thomas Walsh said when he appeared before Congress.

Stimulating science

Since promising to return science to its rightful place in government policies, Obama has unveiled several plans to increase research budgets. He has pledged to devote 3% of the US gross

domestic product (GDP) to strengthen science and technology—the current level of spending is 2.6% of GDP. This amount of money exceeds the nation's peak level of spending on science (2.9% of GDP), which occurred in 1964 following President Kennedy's pledge to put a man on the moon by the end of the decade. If Obama's man-on-the-moon-style budget pledge pans out, the money will double funding for the National Science Foundation within 10 years and provide more money for the NIH, including nearly $6 billion for cancer research.

"Science is more essential for our prosperity, our security, our health, our environment, and our quality of life than it has ever been before," Obama said when he laid out his scientific spending agenda during an address in April at the National Academy of Sciences.

This isn't the only new money being funneled into the NIH. After many years of f at funding, the NIH received a 3% annual budget increase already this year, based on the budget of the previous administration. And in February 2009, under a one-time economic stimulus package known as the American Recovery and Reinvestment Act, the NIH received $10.4 billion more in new funding. The National Institute of Allergy and Infectious Diseases (NIAID) at the NIH, the largest funder of HIV/AIDS research in the world, will receive $1.2 billion of the economic stimulus money. About a third of it will likely go toward funding two-year research grants focused on HIV prevention, says NIAID Director Anthony Fauci. NIAID will use the stimulus money to fund already submitted grant applications, which were previously unfunded because of budget constraints (see Figure 2, page 10). Some of the stimulus money is going to fund AIDS vaccine-related projects, including research on the use of a synthetic activator of natural killer cells as an adjuvant for mucosal vaccine candidates, and another project studying the impact of helminth parasites on immune responses to HIV vaccine candidates. Another project involves designing and evaluating new immunogens based on the HIV Env trimer.

But Fauci says he is focusing primarily on three categories of prevention research for the stimulus dollars. Two involve broader applications of antiretrovirals (ARVs). One is studying the delivery of ARVs prior to HIV exposure to prevent infection—a strategy known as pre-exposure prophylaxis (PrEP). The other is looking at the feasibility of providing ARVs to every HIV-infected individual who meets the World Health Organization's treatment guidelines, a concept known as treatment as prevention, which aims to reduce the spread of HIV. Individuals on ARVs have much lower viral loads and are therefore thought to be less infectious.

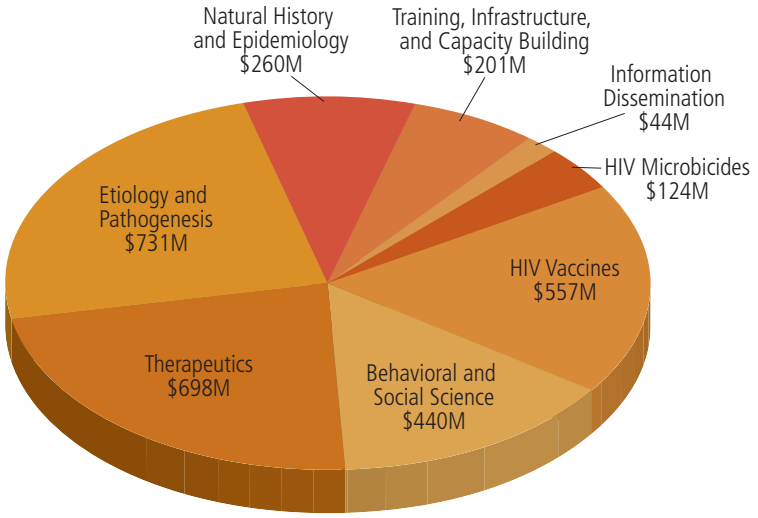
The third category involves the tantalizing prospect of eradicating HIV from infected individuals. Although complete eradication of HIV—essentially curing an infected individual—is considered a long shot, some scientists think it may be possible to achieve a "functional cure" with strategies that could help root out and eliminate HIV from some of the reservoirs of latently infected cells. Although this likely wouldn't clear out the viral reservoirs completely, it might diminish the virus enough to make it possible for a person's immune system to keep the residual

FIGURE 1

Obama's 2010 Proposed Budget for AIDS Research by Program

Prevention of HIV infection is the National Institute of Health's (NIH) highest priority for HIV-related research, according to the Office of AIDS Research. Shown below is a breakdown of the US$3.1 billion NIH budget for AIDS research, by category, in President Obama's proposed 2010 budget.

Source: Office of AIDS Research



Total: $3.055 Billion

HIV in check without continually taking ARVs.

University of North Carolina virologist David Margolis is one researcher studying viral eradication. He says the two-year infusion of economic stimulus money will arrive just as scientists are gaining important new insights into latent reservoirs of HIV. In his view, recent setbacks in AIDS vaccine and microbicide research make the question of viral eradication a reasonable one to pose right now. "Given that the area of viral eradication is minimally invested in—there are very few labs looking at this—it is an obvious area to focus on," says Margolis.

Margolis co-authored an article in *Science* earlier this year with Martin Delaney, founder of the AIDS service organization Project Inform in San Francisco, as well as other researchers, calling for a collaborative approach to find novel HIV therapeutics that would "purge latent HIV cells" and eliminate the need for ARVs to control replication of the virus (*Science* **323**, 1304, 2009).

Long-time AIDS activist Peter Staley, the founder of AIDSMeds.com, wrote in a recent blog entry that between the economic stimulus dollars for NIAID and newfound energy among activists and researchers willing to look beyond treatment, the US could be entering the "golden age" of AIDS research. "After many years of basic research and vaccine research we are putting more pieces together on how the immune system interacts with the virus," says Staley. "At least we have a better idea where to look."

The projects receiving funding from the economic stimulus plan can be tracked at http://grants.nih.gov/recovery. One drawback to stimulus funding is that researchers lucky enough to receive the extra cash, which must be spent by 2011, may find it difficult to receive funding to continue their projects once these stimulus grants expire. Unless more money is added to NIAID's annual budget in 2011 to support these additional grants, "it will be a very difficult year for [these] people to secure funding," says Fauci.

Turning the focus on home

In addition to funneling money to research, Obama administration officials are also focusing extra attention on the static HIV epidemic on their home turf. In April, the Obama administration rolled out a five-year, $45 million multimedia HIV prevention campaign, "Act Against AIDS," that targets African Americans and Latinos in high-risk neighborhoods in cities such as Washington, D.C. In the US Capital, at least 3% of residents are infected with HIV, according to the city's health department. The US Centers for Disease Control and Prevention (CDC) oversees this campaign. Former New York City Health Commissioner Thomas Frieden was picked by Obama to oversee the CDC, which until January was led by AIDS infectious disease specialist Julie Gerberding. During his tenure in New York City, Frieden lobbied strongly to make HIV testing a routine part of medical exams and fought to minimize the spread of drug-resistant tuberculosis in the city.

Two other AIDS-related posts have also recently been filled. Jeffrey Crowley, a Georgetown University health policy analyst and former director of the National Association of People with AIDS is heading up the Obama administration's Office of National AIDS Policy, and Eric Goosby, the chief medical officer of the Pangaea Global AIDS Foundation based in San Francisco is now the US Global AIDS Coordinator in charge of all international HIV/AIDS efforts.

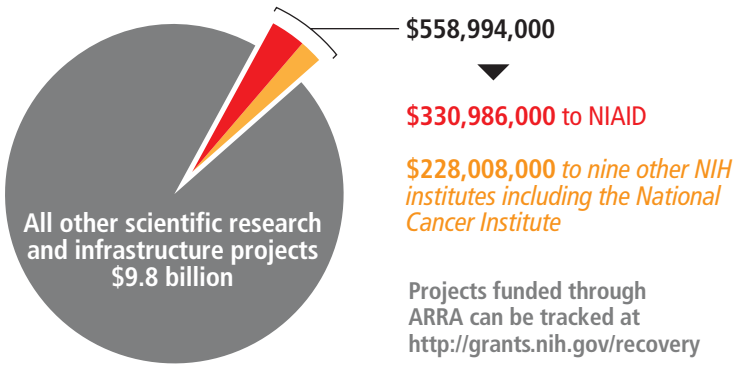
Crowley is playing a key role in developing a National AIDS Strategy, which should provide clear national guidelines on a range of prevention strategies such as circumcision, syringe exchange,

FIGURE 2

Stimulus Funds for AIDS Research at the NIH

The National Institutes of Health (NIH), the largest government funder of AIDS research, received US$10.4 billion in economic stimulus money from the American Recovery and Reinvestment Act (ARRA) of 2009. An estimated $559 million is being considered for AIDS-related research across 10 NIH institutes. The National Institute of Allergy and Infectious Diseases (NIAID) will receive approximately $331 million in AIDS-related funding.

Source: National Institutes of Health and Office of AIDS Research



counseling and testing, and abstinence-until-marriage. "We are at a crossroads in HIV prevention and we need to be focused on making sure we are doing everything we can to prevent new cases of HIV/AIDS," says Crowley. "My experience has been that we have spent a lot of money not targeted to populations at the greatest risk for HIV." Other countries such as South Africa, which has the highest HIV prevalence in the world, already have such national plans, and AIDS advocates in the US have long argued that a government-backed blueprint on how to tackle the epidemic is needed for AIDS organizations to reach their goals.

Judy Auerbach, deputy executive director of science and public policy at the San Francisco AIDS Foundation, says that a National AIDS Strategy should push for more evidence-based AIDS prevention. Obama has already signaled his support for evidence-based policies by lifting the ban on federally funded embryonic stem cell research, and he is considering doing the same for syringe-exchange programs to quell HIV transmission among injection-drug users.

"We can't keep funding things willy-nilly, which is to some degree how things have gone," says Auerbach. "More money is always better because it means more science can be conducted. But it shouldn't just be more science; it has to be the right kind. We need to be more responsible with how we are spending money."

Global programs

While the new research money is a boon for scientists, another battle is being waged over US spending on international HIV/AIDS programs. US funding to fight AIDS internationally has grown significantly in recent years due to the President's Emergency Plan for AIDS Relief (PEPFAR), a program started in 2003 under then-President George W. Bush. PEPFAR started as a $15 billion, five-year program, and was reauthorized last year by the US Congress for $48 billion over five years (or $9.6 billion a year) to fund HIV/AIDS prevention, treatment, and care, as well as tuberculosis and malaria programs.

Obama's budget proposal includes a $6.6 billion allocation for PEPFAR in 2010, an increase of $165 million over the amount being spent this year. This is significantly less than the $1 billion increase per year that Obama promised during his campaign. As a presidential candidate he also promised to spend $50 billion over five years on PEPFAR, and though he says he still intends to spend that amount, he says it will be over six years rather than five.

This drew criticism from AIDS advocates. "To not put the resources in will create a worse epidemic down the road," countered Ken Mayer, a professor of medicine at Brown University and co-chairman of the scientific advisory committee of the Infectious Diseases Center for Global Health Policy and Advocacy. "And there are a lot of other ways in which an uncontrolled AIDS epidemic will jeopardize health in very serious ways, whether it's creating more AIDS orphans or spreading multi-drug resistant tuberculosis. HIV is clearly more than just treating HIV."

> More money is always better because it means more science can be conducted. But it shouldn't just be more science; it has to be the right kind. We need to be more responsible with how we are spending money.
>
> — *Judy Auerbach*

Others say that Obama's failure to fund PEPFAR at the level he previously promised will also hamper HIV prevention efforts. "People now are coming in for testing in huge numbers because there is the hope of getting treated if they are infected. If that hope is no longer there, people will not seek testing," says Peter Mugyenyi, director of the HIV/AIDS Joint Clinical Research Centre in Uganda, which is a PEPFAR recipient. "Without testing, you can't have effective prevention."

According to the Global AIDS Alliance, about 2.9 million people in developing countries are currently receiving ARV therapy—only a third of the 9.7 million who need it. PEPFAR has brought ARVs to more than two million people in 15 target countries—most of them in Africa—but has not reduced the rate of new HIV infections, according to a recent study (*Ann. Intern. Med.* **150**, 688, 2009). By plugging UNAIDS data into mathematical models, researchers at Stanford University compared epidemiological data from 12 PEPFAR-recipient countries in sub-Saharan Africa with 29 countries from the same region that have a generalized epidemic—an

HIV prevalence higher than 1% in antenatal clinics and where the primary mode of transmission was heterosexual sex. Though HIV-related deaths declined in countries receiving PEPFAR funding, there were no changes in HIV prevalence trends between the 12 PEPFAR-funded countries and the 29 others.

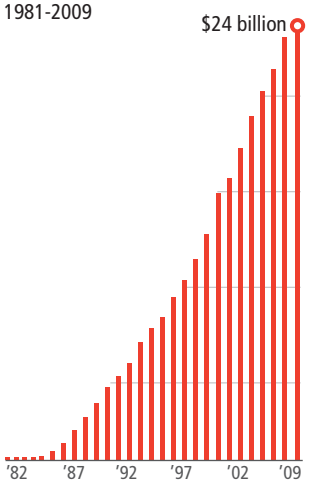
"Projections suggest that the gap between the available funds and those needed will continue to increase unless the incidence of HIV in Africa is substantially reduced," said Eran Bendavid and Jayanta Bhattacharya, the Stanford University researchers who conducted the study.

In testimony before US lawmakers, Walsh said, "PEPFAR will redouble the focus on prevention." He called this one of the highest priorities for the program, adding that, "While treatment is incredibly important, we cannot beat this epidemic with treatment alone."

Obama has praised PEPFAR's goals but is also interested in a more integrated approach to tackling global health problems. In May, he introduced plans to meld PEPFAR into a $63 billion Global Health Initiative, which will support global health more broadly, including programs related to maternal and infant health and immunization. "We cannot simply confront individual preventable illnesses in isolation," said Obama when he announced the initiative. "The world is interconnected, and that demands an integrated approach to global health."

In a commentary published last year Ezekiel Emanuel, brother to Obama's chief-of-staff Rahm Emanuel and currently an advisor to Obama on health care reform, said doubling or tripling PEPFAR's allocation is not the best use of international health funds (*JAMA* **300**, 2048, 2008). "In focusing so heavily on HIV/AIDS treatments, the United States misses huge opportunities. By extending funds to simple but more deadly diseases, such as respiratory and diarrheal illnesses, the US government could save more lives—especially young lives—at substantially lower cost," wrote Emanuel and co-author Colleen Denny, both bioethics researchers at the NIH.

Federal Funding for HIV/AIDS
1981-2009



Forecasting the future

In addition to the less-than-expected allotment in Obama's budget for PEPFAR, AIDS activists and public health experts have raised concerns that the sour economy could pose a threat to the sustainability of global AIDS funding in the future for many programs. In a recent address, Secretary General of the United Nations Ban Ki-moon called on governments to not use the economic crisis as a reason for cutting AIDS funding.

> …failure to continue a scale-up [of] investments in health will betray the trust of millions of people who have been given hope of survival from deadly diseases by the promises of the international community.
>
> — *Michel Kazatchkine*

"Despite lack of resources being a major challenge, failure to continue a scale-up [of] investments in health will betray the trust of millions of people who have been given hope of survival from deadly diseases by the promises of the international community," says Michel Kazatchkine, executive director of The Global Fund to Fight AIDS, Tuberculosis and Malaria. The Global Fund depends on annual contributions from many wealthy countries to fund its treatment and prevention programs. Obama's budget calls for $900 million for The Global Fund in 2010, a $400 million increase over this year.

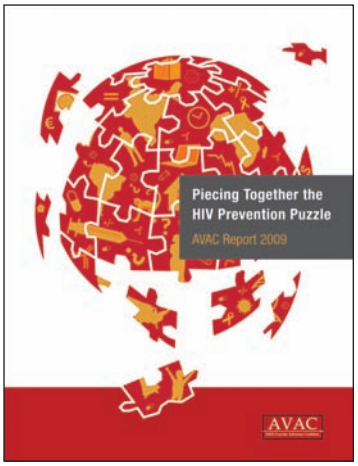
Although lower prices have been negotiated for many ARVs, overall treatment costs continue escalating as more and more HIV-infected people require treatment and more people become newly infected. The Joint United Nations Programme on HIV/AIDS (UNAIDS) estimates that by 2015 it will cost approximately $54 billion a year to provide ARVs to all people in need living in low- and middle-income countries. The world is currently spending $5.5 billion on HIV treatment, according to the latest UNAIDS estimates.

Laurie Garrett, a senior fellow for global health at the Council on Foreign Relations, says there is already fatigue among some international donors and that two decades from now the money to sustain these AIDS treatment programs may not be there. "Either you have a fantasy in which somehow a series of drug [regimens] are available for pennies, or you have to start really focusing on prevention." ■

Vaccine BRIEFS

AVAC Reports on HIV Prevention Puzzle

In its 13th annual report, "Piecing Together the HIV Prevention Puzzle," the AIDS Vaccine Advocacy Coalition (AVAC) said there is an "energized focus on discovery, innovation and basic science" in the field of AIDS vaccines, but noted that successful HIV prevention will likely depend on a combination of approaches and strategies. AVAC, which was formed in 1995, uses public education and policy analysis to advocate for the development of an AIDS vaccine. The organization has also taken a central role in advocating for other HIV prevention strategies, primarily pre-exposure prophylaxis (PrEP)—the delivery of antiretrovirals to uninfected individuals to prevent HIV infection. The report, written by AVAC staff, urged the HIV prevention field to prepare for the potential efficacy of prevention strategies such as PrEP, and called for governments in the countries hardest hit by HIV to "add specificity" around health-care infrastructure, as well as financial and human rights implications regarding the use of this modality.



To access a copy of AVAC's report, go to www.avac.org

"To maintain the ground gained, the rate of new infections must be slowed down," said AVAC Executive Director Mitchell Warren in the report, which was released on May 18th, coinciding with World AIDS Vaccine Day (see *World AIDS Vaccine Day Observed*, page 15).

Casting comprehensive HIV prevention as a puzzle still missing vital pieces—most notably a safe and effective vaccine against the virus—AVAC listed eight recommendations in its report. These recommendations include development of better communication tools to explain upcoming vaccine trials to a lay audience, as well as to communicate the results of the soon-to-be-completed Phase III prime-boost vaccine trial in Thailand. Another focus of the report is on the role of the Global HIV Vaccine Enterprise, an international alliance of researchers, funders, and advocates committed to accelerating the development of an AIDS vaccine. Based on interviews with various stakeholders, AVAC concluded that the "added value" of the Enterprise is "not yet completely convincing." The AVAC Report recommended that the Enterprise should demonstrate greater leadership, particularly through publication of an updated scientific plan in 2010.

AVAC also highlighted advances in the field—more initiatives aimed at bringing young investigators into HIV prevention research was a notable area of progress. And with the initial results from the now infamous STEP trial nearly two years old, AVAC noted that the failure of Merck's vaccine candidate has helped propel new and exciting directions in research. With a global recession threatening to strain budgets for HIV prevention, Warren says "funding decisions must be wise, non-duplicative and evidence-based, yet bold, expansive and innovative." —*Regina McEnery*

New iavireport.org Launches

IAVI Report recently launched an updated and improved website, www.iavireport.org, which offers several new features, in addition to the content from all print editions of *IAVI Report* and *VAX*. Some of the new features include an Events and Meetings page with details on relevant scientific conferences and a searchable database of all preventive AIDS vaccine trials that allows users to access information by trial status or strategy. Soon, users will also be able to navigate the clinical trials database through an interactive map that displays all countries with ongoing AIDS vaccine trials.

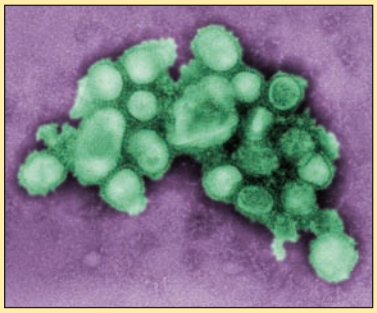
The new site also features the first *IAVI Report* podcast series, called "A Living History of AIDS Vaccine Research," which offers historical analysis from some of the leading voices in the field. The series opens with Anthony Fauci, director of the National Institute of Allergy and Infectious Diseases. Additional installments in this series will be added to the site throughout the year, as well as other video projects.

Thoughts about the new site? The IAVI Report team would greatly appreciate any feedback or comments, which can be submitted through the Letters to the Editor or Contact Us links. If you do not currently receive *IAVI Report* or *VAX*, please visit the website to sign up for a free subscription. —*Regina McEnery*

Vaccine Considerations for H1N1

With H1N1 now evident in at least 73 countries, the World Health Organization declared the flu outbreak a pandemic—which it defines as a sustained community-level outbreak of a new virus in two or more regions of the world—the first in 41 years. At a May 28 conference, "Human Swine Flu (H1N1) and Novel Influenza Pandemics," influenza scientists from public health agencies, academia, and the pharmaceutical sector gathered to discuss strategies for combatting H1N1. The conference, sponsored by The New York Academy of Sciences (NYAS), was billed as the first scientific conference devoted to the newly emergent strain.

Overall, public health agencies have become better able to recognize and respond to influenza outbreaks, but surveillance in the southern hemisphere—notably in South America and Africa—is hampered by poor infrastructure and lack of skilled personnel. "You can't run a lab if you can't get clean water and electricity," said Michael Shaw, associate director for laboratory science in the Influenza Division at the US Centers for Disease Control and Prevention (CDC).



▲ **H1N1 VIRUS** Image courtesy of CDC / C.S. Goldsmith and A. Balish

John Treanor, an infectious disease professor at the University of Rochester School of Medicine and Dentistry and a long-time influenza researcher, said at the conference that it is technically possible to incorporate the H1 component found in the H1N1 strain into the annually produced seasonal flu vaccines. But to do so would slow down production of the seasonal flu vaccine—which is usually available by mid-summer and administered in the fall—because the formula would have to be changed.

The CDC has already shared seed virus with industrial vaccine manufacturers (Sanofi Pasteur and GlaxoSmithKline have acknowledged their work in this area) to begin development of a vaccine specifically against the H1N1 flu virus, and they hope to begin clinical trials this summer, said Shaw. Researchers will need to determine the optimal antigen to use in the vaccine and the required dose. Safety of the new construct must also be evaluated. The US National Institutes of Health (NIH) will be working closely with vaccine developers in testing the candidate vaccines for safety and immunogenicity, although the size and scope of the studies is still unclear at this point, said Dr. Anthony Fauci, director of the NIH's National Institute of Allergy and Infectious Diseases. "We will go as quickly as we possibly can," said Fauci, who was not at the May 28 conference.

Because production of the vaccine may be slow, at least in the beginning, countries, including the US, are considering using adjuvants to stretch the vaccine supply in the event that mass vaccination against H1N1 is recommended. One adjuvant, alum, which contains aluminum hydroxide compounds, is licensed in the US but hasn't been found to work with influenza. Philip Dormitzer, senior director of viral vaccine research at Novartis Vaccines and Diagnostics, said Europe has been using a seasonal flu vaccine boosted with his company's MF59 adjuvant—an oil in water emulsion—with good results.

Additionally, Dormitzer says some vaccine candidates against H5N1—a highly pathogenic strain of avian influenza that kills about half the people it infects—also utilizes adjuvants. "The utility of adjuvant vaccines is that you can make more doses," said Dormitzer.

But even before considering adjuvants, public health officials must decide whether to vaccinate at all. Some influenza researchers warn that vaccinating prematurely can cause unnecessary complications like those that occurred three decades ago in the US.

In the winter of 1976, two epidemics of influenza—one an unknown strain thought to be similar to the swine flu that sparked the catastrophic 1918 Spanish flu—struck Fort Dix, a military installation in New Jersey. A few hundred soldiers became infected and one soldier died. The US government launched a massive vaccination campaign the following fall. More than 40 million Americans received the shots. Fears of a pandemic proved unfounded, but more than 500 people who received the swine flu vaccine developed Guillain-Barré syndrome, a rare neurological condition that causes paralysis and is sometimes fatal. The syndrome has been known to occur sometimes following vaccination.

Edwin Kilbourne, a professor emeritus at New York Medical College who helped develop the 1976 swine flu vaccine, said the decision to mass vaccinate was a tough one at the time, considering what else was unfolding. Along with the swine flu scare, US government officials were also soon dealing with what came to be known as Legionnaire's disease.

Kilbourne noted that the current wave of H1N1 cases may seem relatively mild—most infected individuals have recovered spontaneously or with treatment—but he warned that the same was true for the first wave of the 1918 pandemic. It was the second, deadly wave several months later that triggered most of the illness and deaths. —*Regina McEnery*

New South Africa Institute to Tackle HIV and TB

The University of KwaZulu-Natal (UKZN) in South Africa, which claims the highest AIDS prevalence in the world, has teamed up with the Howard Hughes Medical Institute (HHMI) in Maryland to develop a research center focused on the twin scourges of tuberculosis (TB) and HIV. The toll from HIV and TB is huge. In 2007, there were 2.7 million new HIV infections and two million deaths attributed to the virus, according to the Joint United Nations Programme on HIV/AIDS (UNAIDS). Based on the latest figures from The Global Fund to Fight AIDS, Tuberculosis and Malaria, about two billion people—a third of the global population—were estimated to be infected with TB last year, while 9.3 million people became sick with active TB. When HIV and TB infections coexist, it intensifies the effect of both, often with dire consequences—TB is the leading killer of people with HIV/AIDS, according to UNAIDS.

The KwaZulu-Natal Research Institute for Tuberculosis and HIV (K-RITH) will receive US$60 million over 10 years from HHMI and will be housed within the Nelson Mandela School of Medicine in Durban. HHMI has committed about $20 million to construct K-RITH and $4 million a year for 10 years to support research. The UKZN is committing about $11 million for infrastructure costs. The new institute will be adjoined to the Doris Duke Medical Research Institute, which houses several AIDS research groups, including the Human Pathogenesis Programme headed by Bruce Walker, an HHMI investigator and head of the Ragon Institute in Boston, and the Centre for the AIDS Programme of Research in South Africa, led by Salim Abdool Karim.

K-RITH will initially focus on four research areas: the development of rapid and more effective diagnostic tests for TB; characterizing the genotypic and phenotypic properties of drug-resistant strains of TB; analyzing and characterizing complex immune responses to TB, particularly those seen in people also infected with HIV; and the study of recurrent TB infections in HIV-infected individuals.

K-RITH will also be used as a platform to test candidate vaccines, both for TB and HIV. "The institute is working on understanding spontaneous control of HIV replication and is focused on T-cell based vaccines, but the point is that the infrastructure will be there to facilitate the most promising new vaccine ideas forward," says Walker, who was instrumental in establishing K-RITH.

Along with conducting research, K-RITH is also hoping to become a magnet for young African scientists who want to base their laboratory work in Africa but are hindered by the lack of research facilities and funding. —*Regina McEnery*

World AIDS Vaccine Day Observed

Twelve years ago on May 18, during a commencement address at Morgan State University, then-US President Bill Clinton called for a renewed commitment to the development of an AIDS vaccine. Several organizations and communities marked the 12th anniversary of Clinton's speech—celebrated each year as World AIDS Vaccine Day—with candlelight vigils, charity walks, and educational fora to recognize recent developments in the field and to educate the world about the importance of AIDS vaccine research.

IAVI marked the day by focusing on recent accomplishments in the quest for a vaccine. In the past year, IAVI opened the world's first laboratory devoted exclusively to AIDS vaccine research, known as the AIDS Vaccine Design and Development Laboratory, and partnered with The Scripps Research Institute to establish the HIV Neutralizing Antibody Center, dedicated to developing AIDS vaccine candidates that can elicit broadly neutralizing antibodies. In advance of World AIDS Vaccine Day, IAVI, the Global Health Council, and the Joint United Nations Programme on HIV/AIDS (UNAIDS) co-sponsored a Congressional briefing to spotlight advances in AIDS research in Africa.

In South Africa, the Emavundleni Community Outreach team, in partnership with the community advisory board and an adolescent outreach group called Future Fighters, sponsored an event at which volunteers distributed condoms along with information about AIDS vaccine research.

Other events included an informational forum in Jamaica focused on both circumcision and HIV vaccine research, and a program at a Baptist church in Georgia, "Hope in Our Souls," to dispel common myths and increase AIDS awareness within the black community. The US Military HIV Research Program in Kenya sponsored research talks for students throughout the month. And the Treatment Action Group in New York City, along with the AIDS Vaccine Advocacy Coalition and the Global HIV Vaccine Enterprise, convened a discussion on the future of AIDS research. —*Regina McEnery*

Research BRIEFS

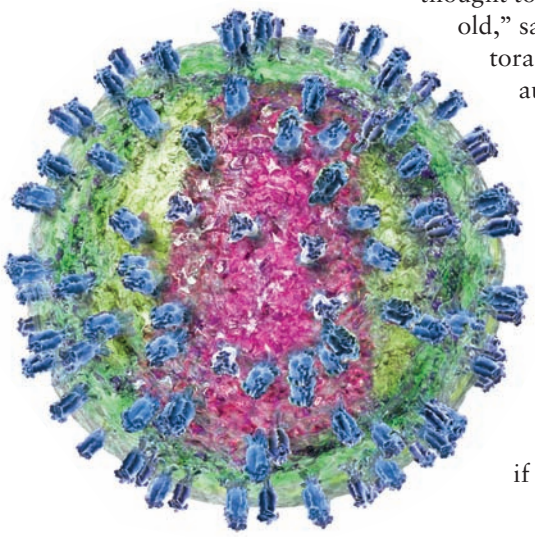
SIV May Be Much Younger Than Previously Thought

Simian Immunodeficiency Virus (SIV) may have existed in chimpanzees and sooty mangabeys for just hundreds of years before it jumped to humans, giving rise to the HIV/AIDS pandemic, a study has found (*PLoS Comput. Biol.* **5**, e1000377, 2009). This is substantially less time than previously thought—SIV was thought to have coexisted in its natural hosts for perhaps millions of years, long enough to render it nonpathogenic. SIV typically does not cause disease in its natural hosts, including sooty mangabeys.

Joel Wertheim and Michael Worobey of the University of Arizona in Tucson, the authors of the study, estimated the rate of virus evolution from sequences of conserved regions of the *gag*, *pol,* and *env* genes from samples collected from humans, chimpanzees, and sooty mangabeys between 1975 and 2005. Based on that rate of viral evolution, they then determined how long ago the common ancestor of the SIVs in chimpanzees or in sooty mangabeys must have existed.

They found that the common ancestor of chimpanzee SIV dates back to about 1492. This is just a little over 400 years before this SIV is thought to have jumped to humans, in 1908, to give rise to HIV-1 group M, which makes up the vast majority of HIV-1 infections. They also found that the common ancestor of sooty mangabey SIV dates back to about 1809, only about 120 years before this virus is thought to have jumped to humans to give rise to HIV-2. The analysis also found that SIV in sooty mangabeys is evolving at the same rate as HIV-2.

"These results were surprising because SIV has long been thought to be millions of years old," says Wertheim, a doctoral candidate and first author of the study. Still, he notes that it is possible that SIV really coexisted longer in its natural hosts than these estimates suggest. There could be an unknown bias that could mask an older age of SIV, and if so, then that same unknown bias may also affect the estimates as to how long ago HIV-1 and HIV-2 jumped from nonhuman primates to humans. "This is a serious issue that needs to be addressed if these biases exist," Wertheim says.



David Robertson of the University of Manchester, who was not connected to the study, also says that SIV could have existed in primates longer than was shown in the study. "What we have circulating now are the descendents of some successful virus that existed some number of years ago," Robertson says. "That's not necessarily the point when they first entered primates—that's just the common ancestor of the ones that circulate now."

> These results were surprising because SIV has long been thought to be millions of years old. – *Joel Wertheim*

However, if the findings from this study are true, it could mean that SIV evolved avirulence or nonpathogenicity in its animal hosts over a much shorter period of time than previously thought, Wertheim says, or that, alternatively, SIV may have been nonpathogenic to begin with. "The disease in humans and chimps [could be] an aberration," Wertheim says. Chimpanzees have recently been shown to get sick from infection with SIV (http://www.retroconference.org/2009/Abstracts/34339.htm).

Another argument for SIV being old is that closely related SIV strains are found in closely related host species, suggesting that SIV had previously infected the common ancestor of these species, according to Wertheim. But two years ago, Wertheim and Worobey showed evidence that the evolution of SIV in African green monkeys doesn't exactly mirror the evolution of its host (*PLoS Pathog.* **3**, e95, 2007), suggesting that SIV might have been transmitted more recently between closely related host species, instead of having infected their common ancestor.

Robertson has also developed a model, which showed that the observation that closely related viruses infect closely related species can be explained by a tendency for SIV to successfully jump between more closely related species. The new study by Wertheim and Worobey "very much confirms that message," Robertson says. —*Andreas von Bubnoff*

New Research Suggests HIV Enters Target Cells by Endocytosis

HIV has long been thought to enter cells by direct fusion with the outer plasma membrane. But a recent study suggests that instead, it enters target cells by endocytosis, and fuses with the target cell membrane only once it is inside the endosome (*Cell* **137**, 433, 2009). As a result, HIV particles may be harder to reach for antibodies that target HIV while it is fusing with target cells.

"[This] goes against dogma," says Gregory Melikyan, an associate professor of microbiology and immunology at the University of Maryland in Baltimore, who led the study. "The dogma in the field was that HIV fuses directly with the cell plasma membrane."

Melikyan and colleagues infected cultured cells with HIV particles that were stained with two different dyes: One for the viral lipid membrane, the other for the content of HIV particles. They found that at the plasma membrane, HIV particles fused only partially, without emptying their contents into the cell. In contrast, once HIV particles were endocytosed, they emptied their contents into the target cell (see Figure 3). "We never expected that to happen," Melikyan says, "and then several thousand particles later we convinced ourselves that the fusion of the plasma membrane simply doesn't happen."

In addition, the researchers used a small peptide that cannot cross the plasma membrane and inhibits HIV fusion only at the outer plasma membrane, but not once an HIV particle is inside an endosome. The later this fusion inhibitor was added, the more virus was able to fuse with the membrane, presumably because it was inside the endosome and therefore protected from the inhibitor. Low temperature, however, which inhibits all types of fusion, even inside endosomes, inhibited HIV's fusion to the target cell membrane for much longer. This suggests that the virus was first endocytosed and then fused with the target cell membrane only later, once inside the endosomes, Melikyan says.

The study also found that dynasore, an agent that inhibits dynamin—a protein important for endocytosis—inhibited HIV endocytosis and also its fusion with the membrane inside endosomes. This suggests that HIV may need target cell factors like dynamin to fuse with the membrane inside endosomes, Melikyan says, adding that such host cell factors could be future drug targets.

Dynamin has been suggested before to be required for HIV entry (*J. Virol.* **79**, 1581, 2005), but Melikyan says it wasn't clear as to whether dynamin was directly involved. He says that while such previous evidence was "not sufficiently strong to convince the majority of scientists that this is serious," his study now demonstrates that dynamin is required for both endocytosis of HIV as well as the delivery of viral content into the cytoplasm.

Overall, Melikyan says, his findings are the strongest evidence so far that HIV infects cells via endocytosis and not via direct fusion to the plasma membrane. However, he acknowledges that the study used cultured cell lines as target cells for HIV infection, and that the findings should be tested in primary T cells. "This has to be done in natural target cells that are more relevant," he says. "[In] cell lines we may not get an adequate picture."

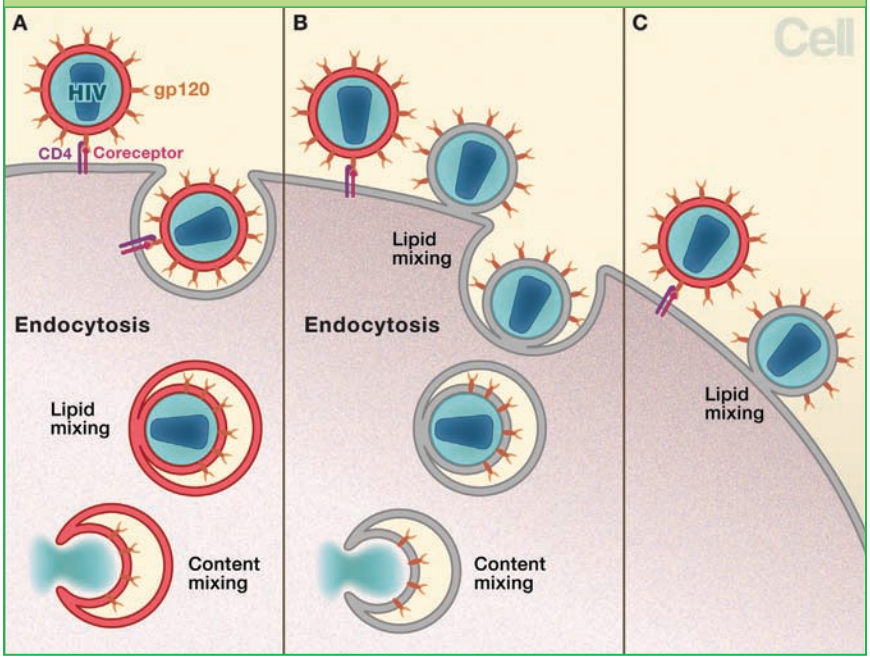
If true, the findings mean that HIV is endocytosed into the target cell before undergoing fusion, making it difficult to inhibit HIV with certain drugs or antibodies that cannot cross plasma membranes and target intermediate conformations of Env that only form while HIV fuses with the target cell.

FIGURE 3

Entry Pathways for HIV

By visualizing the mixing of viral lipids (red) and contents (blue) with host cell membranes and cytosol, respectively, Miyauchi et al. (2009) observe three distinct routes for entry of host cells by HIV. These include endocytic events in which two-colored HIV particles are internalized, undergo lipid mixing with the vesicle membrane, and deliver their contents into the cytoplasm (A). There are fusion events that occur at the plasma membrane and proceed at least to the stage of hemifusion (B). These are followed by subsequent endocytosis and content mixing. There are also fusion events at the plasma membrane that do not result in any subsequent content mixing (C).

Image and legend courtesy of Cell Press, Elsevier Inc., Uchil and Mothes, 2009. Cell *Volume 137 n3, pages 402-404*



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While Melikyan and colleagues did their study on cell-free HIV, their findings add to recent observations, in living cells, that HIV transmission between cells may also involve the endosomal pathway in the target cell (*Science* **323**, 1743, 2009; see *Research Briefs*, *IAVI Report*, March-April 2009). Benjamin Chen, an assistant professor at the Mount Sinai School of Medicine and lead author of that study, says the study by Melikyan and colleagues is timely and related. "We are coming at it from very different directions but we are coming to the same conclusions, so in a way it's a bit of a convergent discovery."

In a commentary on the study in the same issue of *Cell*, Pradeep Uchil and Walther Mothes of Yale University wrote that the study "presents the most comprehensive analysis of HIV entry to date and demonstrates that it does depend on endocytosis." While not everyone in the field is ready to accept the findings, Mothes adds, many research groups will now take a closer look. "Without the paper being in *Cell*, people would not address these issues." —*Andreas von Bubnoff*

Identifying Broadly Neutralizing Antibody Activity in HIV-infected Individuals

So far, only a handful of broadly neutralizing monoclonal antibodies against HIV have been isolated from HIV-infected individuals. To identify others, researchers are actively screening HIV-infected individuals throughout the world. In an effort led by IAVI's Neutralizing Antibody Consortium (NAC), researchers have conducted the largest screening and evaluation, to date, of virus neutralization patterns for sera collected from non-clade B HIV-infected individuals. This study identified some HIV-infected individuals, referred to as "elite neutralizers," whose sera have broadly neutralizing activity (*J. Virol.* **83**, 7337, 2009). The study's authors define an elite neutralizer as having neutralizing antibody activity against more than one pseudovirus in a panel of five—representing clades A, B, and C and one circulating recombinant form referred to as CRF01_AE—with a 50% inhibitory concentration neutralization titer of at least 300 within a single clade, as well as across at least four clades. Out of approximately 1,800 individuals screened in this study, 1% of them were classified as elite neutralizers.

Initially, researchers created an algorithm to assess neutralization activity based on 463 sera samples. They then used this algorithm to score and rank the neutralization capabilities of an additional 1,234 sera samples collected from individuals in Ivory Coast, Kenya, South Africa, Thailand, and the US. "Our results suggest that neutralizing activity across multiple geographic regions, with different spectra of circulating HIV-1, can be reliably assessed using a small panel of pseudoviruses," the study's authors write. All individuals were HIV infected for at least three years, did not meet a clinical AIDS diagnosis—either according to the World Health Organization's criteria or by having $CD4^+$ T-cell levels above 200 cells/ml, and had not been taking antiretrovirals in the year prior to sample collection.

These results confirm previous observations, indicating that chronically HIV-infected individuals have broadly neutralizing antibody activity, according to the study's authors. Studying elite neutralizers may lead to the identification of additional broadly neutralizing antibodies against HIV, creating more targets for AIDS vaccine researchers. —*Kristen Jill Kresge*

Broadly Neutralizing Antibodies Bind HIV Mostly With Just One Arm

The broadly neutralizing HIV antibodies b12 and 4E10 appear to mostly bind the Env spike with only one of their two antigen-binding arms at a time, a recent study suggests (*Proc. Natl. Acad. Sci.* **106**, 7385, 2009). The reason could be that HIV has only about 14 spikes on its surface. These spikes are too few and often too far apart from each other for both antibody arms to reach two spikes at the same time (*Nature* **441**, 847, 2006; see Figure 4, page 19). By comparison, the f u virus, while of a similar size as HIV, has about 450 spikes on its surface, making it more likely for both arms to be able to bind two spikes at the same time.

These findings could explain why HIV can easily escape from antibody neutralization by accumulating a few mutations on the Env protein, says Joshua Klein, the first author of the study who was a doctoral student at the California Institute of Technology (Caltech) at the time the study was published. "If only one arm is able to bind," Klein says, "then it becomes much easier [for HIV] to acquire mutations to render those antibodies harmless."

That's because mutations that result in less efficient binding will have much less of an effect if both arms of the antibody can bind. "When HIV mutates, it really matters because you don't have the buffering effect of [crosslinking]," Klein says.

The results of this study also give researchers clues about how to engineer larger versions of antibodies that can bind with both arms and could be administered in the form of gene therapy. "Our results suggest that the traditional vaccine approach—i.e., injecting an antigen in order to elicit an immune response to a virus—may never produce effective anti-HIV antibodies due to the inability of most anti-HIV antibodies to bind bivalently to the virus," says Pamela Bjorkman, a professor at Caltech who led the study.

In the study, Klein and colleagues broke two broadly neutralizing antibodies, b12 and 4E10, down into their component parts, and tested how these different antibody parts could bind and neutralize HIV *in vitro*. As expected, the two-armed immunoglobulin G (IgG) versions of both antibodies could better neutralize HIV than the one-armed Fab versions. However, the improvement of two-armed over one-armed versions was much smaller than for antibodies to other

pathogens like the f u virus, Klein says. Two-armed antibody neutralization was 17-fold better for b12 and 4.4-fold better for 4E10, compared to one-armed neutralization. By comparison, others have shown that two-armed IgG antibodies to the f u virus neutralize the virus about 1,000 times better than one-armed versions, Klein says.

Klein and colleagues also observed that two-armed antibodies with a shorter reach had less of a neutralization advantage compared with their one-armed versions than antibodies with a longer reach compared with their one-armed versions. This suggests that if one arm of an antibody was bound to a spike, then the second arm was less likely to bind to another spike if the arms were shorter than if the arms were longer. The researchers concluded, based on this, that the spikes on the surface of HIV probably don't move freely.

Another finding of the study was that smaller versions of 4E10—which bind to the inner part of the Env spike called gp41—could neutralize HIV better than larger versions. This suggests that the site on the Env spike where the 4E10 antibody binds is not easy for the antibody to reach. "The bottom line is smaller is better," Klein says, adding that it could also explain why previous studies have found that 4E10 is generally not a very potent antibody, and less potent at neutralizing different HIV strains than b12 (*J. Virol.* **78**, 13232, 2004).

Although an earlier study showed that larger IgM versions of 4E10—a ring of five IgGs—are weaker than IgG versions (*AIDS Res. Hum. Retroviruses* **20**, 755, 2004), Klein says the current study is "by far the most thorough analysis" to date, to show evidence for a steric occlusion of 4E10.

Pascal Poignard, an adjunct professor at the Scripps Research Institute and a principal investigator at IAVI's Neutralizing Antibody Center who was not connected to the study, says he is not too surprised by the study's findings since previous studies have shown similar improvements between the neutralization abilities of the two-armed IgG versus the one armed Fab versions of b12 and 4E10.

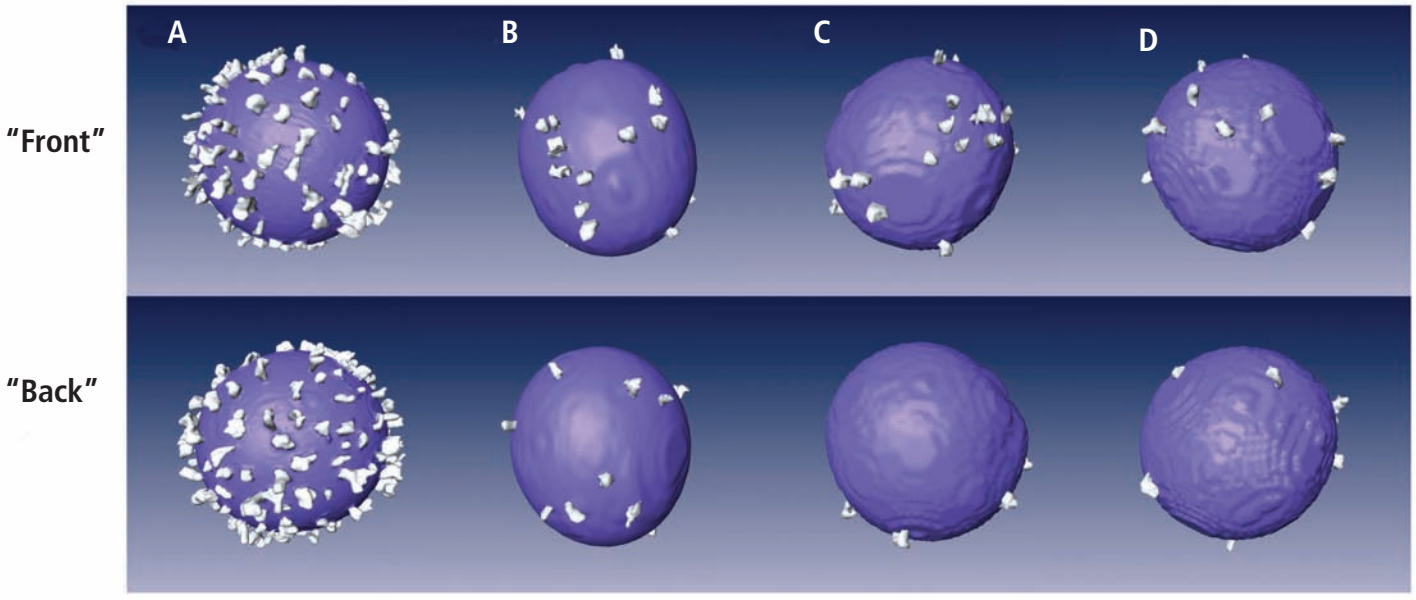
Next, Klein plans to use the insights from this study to engineer antibodies that can be used in gene therapy experiments, in a collaborative project with Bjorkman's group and David Baltimore's group at Caltech (see *Engineering Immunity*, *IAVI Report* Jul.-Aug. 2008). "Now that I've figured out what this thing needs to look like to get bivalent binding," Klein says, "my job is to try to make that molecule."

Bjorkman says such an engineered antibody would have to be able to bind two sites on the same spike or two spikes at the same time. "By increasing the distance between their [binding] sites, it might be possible to create anti-HIV reagents that can take advantage of avidity effects," she says. —*Andreas von Bubnoff*

FIGURE 4

Env Spikes on SIV and HIV Virions

SIVmac239 virion (A) and three representative HIV-1 virions (B-D) showing the "front" (top panel) and "back" (bottom panel) of the virions, and the distribution of the Env spikes (white). The images come from an analysis of individual virions by cryoelectron microscopy that showed that SIVmac239 virions have about 73 Env spikes per particle, and HIV-1 virions have about 14 spikes per particle. A version of the image originally appeared in *Nature* **441**, 847, 2006. Reprinted with permission.



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