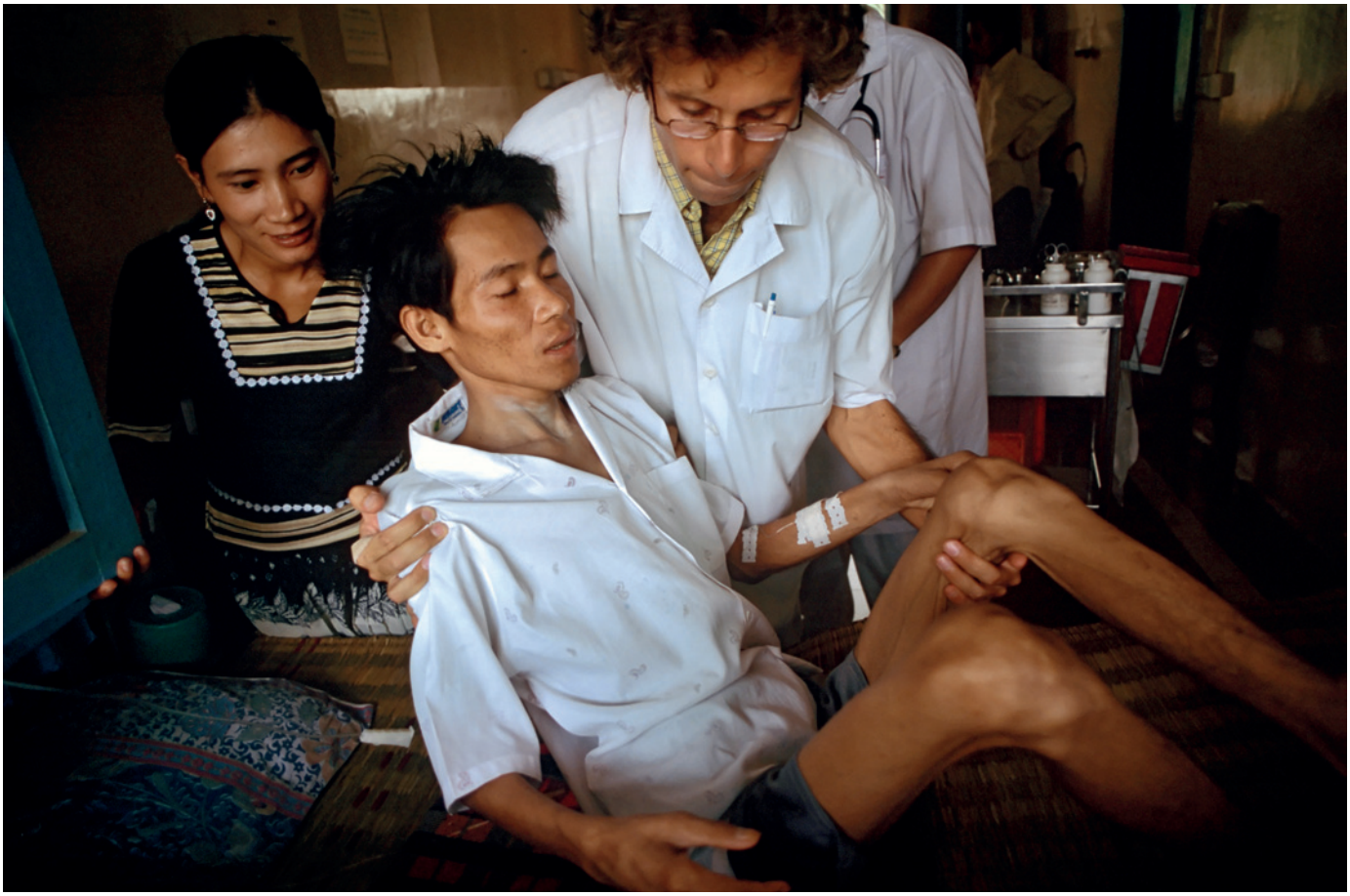

KAREN KASMAUSKI/SCIENCE FACTION/CORBIS

A doctor lifts an AIDS patient in Cambodia. A therapeutic HIV vaccine could be in clinical trials in 2016.

INFECTIOUS DISEASE

Beating the big three

Malaria, HIV/AIDS and tuberculosis are humanity's deadliest foes, and have stymied vaccinologists for centuries. New technology and ideas could finally make a difference.

KATHERINE BOURZAC

Vaccines have conquered so many pernicious diseases that their success has started to seem inevitable. But three of the deadliest infectious diseases — two of them ancient and one that emerged as a threat just 30 years ago — have so far defied vaccine developers.

There are no examples of natural immunity to any of these three diseases — malaria, tuberculosis (TB) and HIV/AIDS. Those who don't die of AIDS or active tuberculosis must live with HIV or latent TB. And those who survive malaria don't develop long-term immunity: they can be infected by the parasite again and again. Without natural models of immunity to work from, vaccinologists have had to develop new strategies. Now these efforts are starting to pay off, leading to cautious optimism that the bacterium, parasite and virus responsible for these diseases might not be as invincible as they seem.

The technology and methods that underpin vaccine research for each of the three pathogens involved have all benefited from the intensive global-health efforts that followed the emergence of AIDS. Funding organizations such as the Bill & Melinda Gates Foundation and the Global Fund to Fight AIDS, Tuberculosis and Malaria began pumping money into research on these killer diseases around the year 2000.

Although new drugs and more ambitious prevention efforts have lessened their toll, the long-term solution to these diseases is prevention. "A vaccine is the ultimate medicine: you don't have to get sick, and they're cheap," says Dennis Burton, an HIV researcher at the Scripps Research Institute in La Jolla, California. Indeed, the economics of vaccines are compelling. For example, about US$2 billion is spent each year on preventing and treating malaria; but if a vaccine were available for US$10 a dose, it would cost only about US$300 million each year to vaccinate every newborn in the countries where the disease is endemic, says Adrian Hill, who studies malaria vaccines at the Jenner Institute in Oxford, UK.

Malaria vaccines under development will target the parasite at every stage of its complex lifecycle, and researchers are optimistic about prospects for eradicating the disease. New genetic analysis methods are finally shining a light on people's different responses to TB and might help to bring down the costs of clinical trials. Meanwhile,

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basic science has shown up some of HIV's weak points, and a vaccine that cures monkeys of simian AIDS is being developed for the clinic.

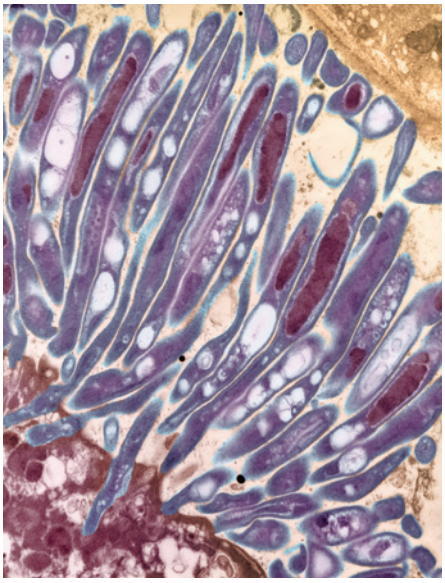
MALARIA: TARGETING A PARASITE

In 2013, there was very good news on the malaria vaccine front. In October, London-based pharmaceutical giant GlaxoSmithKline (GSK) announced that it will apply to the European Medicines Agency for approval to begin selling its malaria vaccine in 2015. GSK developed the vaccine in partnership with the PATH Malaria Vaccine Initiative, funded by the Bill & Melinda Gates Foundation. The vaccine, called RTS,S, was tested in a late-stage clinical trial in more than 15,000 infants and children at 11 sites in sub-Saharan Africa. After 18 months of follow-up, there were 47% fewer cases of malaria in children aged 5 to 17 months at first vaccination, and 27% fewer cases in infants who were 6–12 weeks old when first vaccinated, than in unvaccinated children. RTS,S is the first vaccine to produce protection against any parasitic disease.

The GSK vaccine works like many existing vaccines. It induces the body to make antibodies, in this case against a protein made by the infectious stage of the malaria parasite. If measured solely in terms of the levels of anti-malarial antibodies that it is can induce, RTS,S is "the world's most potent vaccine," says Hill. It induces antibody concentrations ten times greater than does the hepatitis vaccine.

So why does it protect only 50% of children, at best? The answer lies in the parasite's multi-stage life cycle. "From a vaccine point of view, malaria isn't one disease — it's four," Hill says. Malaria parasites reproduce in mosquito salivary glands in one form; travel through the human blood stream in another; replicate in the liver as a third; then infect red blood cells and reproduce again. RTS,S targets only the infectious stage — the single-celled sporozoites that are injected into the body by a feeding mosquito. To prevent disease, there needs to be enough antibody in the blood to eliminate every single sporozoite before they reach the liver. "Once it's in the liver, a single parasite can expand by 10,000 to 40,000 times," says Robert Seder, a vaccinologist at the National Institute of Allergy and Infectious Disease in Bethesda, Maryland.

Because a single parasite can do so much damage, vaccines have to have redundancy built in, says Hill. The ultimate malaria vaccine will not just induce the immune system to attack every stage of the parasite in the human body — critically, it will also block reproduction in mosquitoes. A transmission-blocking vaccine will induce the human immune system to make antibodies against the plasmodium's mosquito-borne stage. When people are bitten, mosquitoes will take up these antibodies, which will prevent the parasite from reproducing within the insect.



LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE/SCIENCE PHOTO LIBRARY

Malaria parasites growing in mosquito salivary glands can be used for an experimental vaccine.

Transmission-blocking vaccines have long shown promise in theoretical models; in 2013, their potential was confirmed in the lab. An intervention that inhibited transmission of a similar plasmodium parasite from mouse to mosquito by just 32% was able to eliminate the disease entirely in populations with low transmission rates.

For other ideas about malaria vaccination, some researchers are turning to the results of a bizarre 45-year-old experiment that shows that when people are bitten by at least 1,000 sporozoite-carrying mosquitoes that have been irradiated to inactivate the parasites, they are protected against malaria for 20 years. Although a vaccine such as RTS,S works by introducing a single malaria antigen, exposing people to the entire sporozoite instead has the advantage of letting the body pick the targets, says Seder.

Using mosquitoes as a vaccine vector is not practical, as it would require researchers to capture and breed millions of the insects and to release them in thousands of places. So entrepreneur Stephen Hoffman has been working since 2003 to bottle the process. His Rockville, Maryland-based company, Sanaria, has developed a process for growing sporozoites in mosquitoes in the lab. Sanaria breeds mosquitoes in clean conditions, feeding them on banked human red blood cells infected with the parasite. After about three weeks, sporozoites develop in the insects' salivary glands. The sporozoites are then irradiated and purified.

Solving the production problem wasn't enough. Injecting the Sanaria vaccine into muscle, which is how most vaccines are given, doesn't work. Looking at Sanaria's work, Seder had the idea of giving the company's vaccine candidate intravenously. A clinical trial in 40 people yielded promising results that were published in August 2013. A group given six doses was completely protected against malaria infection. The Sanaria vaccine went into larger clinical trials in Tanzania and Mali in December 2013. But even if these studies establish the vaccine's effectiveness, that might not be enough. The vaccine has to be given multiple times, intravenously, and Hill is sceptical that this will ever be practical for resource-poor areas. Even in rich countries, he points out, no vaccines are given intravenously.

In one important respect, malaria is easier to study than HIV or TB: vaccine ideas can be tested quickly and inexpensively. People inoculated with an experimental malaria vaccine can be "challenged" with infection by malaria, because it is possible to kill all the parasites. With the help of existing antimalarial drugs, a person will completely recover from malaria. This vaccinate-then-challenge strategy means that malaria trials can enrol fewer people and still get statistically significant results. It's unethical to challenge people with HIV or TB because there is no way to completely eliminate infection by these pathogens. HIV and TB vaccine trials have to be large: success is gauged by calculating whether the natural infection rate in a population has been lowered.

Partly because of the relative ease of trying out new ideas, malaria researchers are optimistic about prospects not just for RTS,S but also for experimental vaccines coming down the pipeline. Seder says he expects there to be a highly effective malaria vaccine within the next ten years.

TUBERCULOSIS: PREDICTING PROTECTION

So far, there are no vaccines for malaria or HIV, but there is one for TB, and it's the most widely used vaccine in the world. The Bacillus Calmette-Guérin (BCG) vaccine — named after the French researchers who developed it by attenuating one of TB's bacterial cousins — was first used in people in 1921.

BCG, though time tested, has major limitations. For reasons that are poorly understood, BCG protects only infants; it is ineffective in older children and adults. Its efficacy also depends on latitude. BCG works better, and protects people at later ages, farther from the equator. Both effects may result from interfering exposure to noninfectious mycobacteria that are closely related to both BCG and TB. These bacteria are more abundant closer to the equator, and while infants are unlikely to have encountered them, children and adults have had more exposure over time.

And so, in spite of the vaccine, TB ranks second only to AIDS in the number of people it kills every year — 1.3 million in 2012. One-third of the world population is infected with the bacterium, though mostly in a latent form that will never cause a problem for most people. So there is a pressing need for a vaccine that will protect older children and adults against TB.

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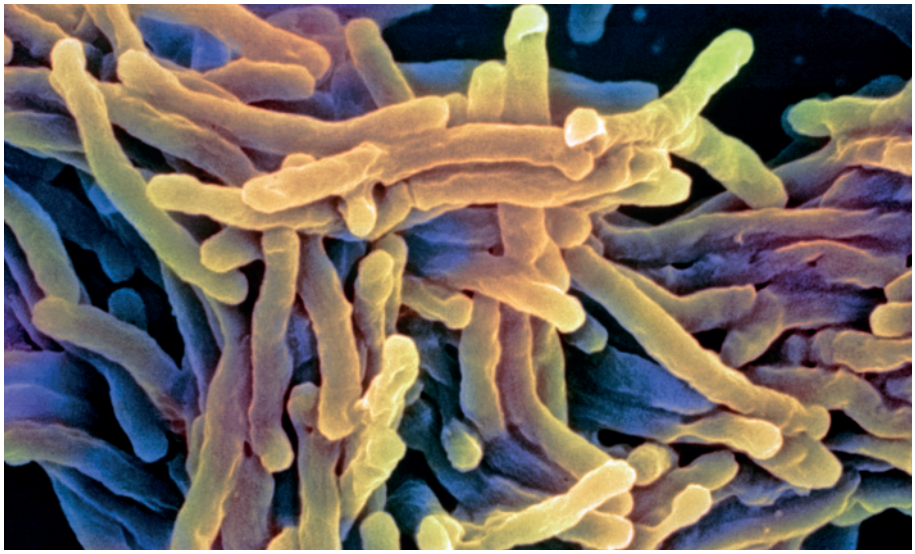
In 2013, researchers completed the first efficacy trial of a novel TB vaccine in infants since 1968, when the last infant BCG trials were done. The trial investigated the vaccine as a post-BCG booster in babies, chosen because they have higher rates of TB infection than adolescents and therefore the trial could include fewer subjects, says Helen McShane, a vaccinologist at the Jenner Institute who was one of the leaders of the trial. But by choosing infants, they may have set themselves too high a bar. "BCG is doing a lot, and it is hard to improve on that," she says. And in fact the new vaccine showed no improvement over BCG alone. But McShane is not discouraged. "It took malaria and HIV researchers a few clinical trials to get to vaccines that show modest efficacy," she says. "We need to keep moving forward, and make sure we learn from things that don't work."

Even more important than improving the infant vaccine is to extend TB protection to the adolescents and young adults who are most likely to be killed by pulmonary TB. The problem is becoming more urgent with the emergence of strains that are resistant to multiple antibiotics, particularly in post-Soviet countries such as Belarus, Ukraine and Kazakhstan. And TB has deadly synergy with AIDS: TB causes one-quarter of all deaths in people with HIV.

The biggest challenge in TB vaccine research is that researchers don't know what immunity looks like. While modestly effective vaccines for HIV and malaria are providing researchers in those fields with promising avenues to pursue, those developing new TB vaccines have almost nothing to go on. The response of the undeveloped newborn immune system to BCG is not a guide to what adult immunity would look like, and the animal models of TB infection are poor. One very basic question is this: how is it that some people can carry the bacteria in latent form for years without getting sick, while others get pulmonary TB? "We're shooting in the dark right now," says Christopher Dye, a TB expert at the World Health Organization (WHO).

Biologists now believe they know how to turn on the lights. To make sure they test only the most promising TB vaccines in future trials, researchers are coming up with new ways of predicting success. One strategy is to challenge people given an experimental vaccine with infection by BCG bacteria, as it's not ethical to infect people with TB. The closely related BCG can act as a surrogate — it's not the same as TB, but watching the immune response to this bacterium could help to guide vaccine development in early clinical trials. The idea, says McShane, is to "challenge vaccinated people with BCG, do a biopsy to study the immune response, redesign the vaccine, then test again."

As interest in TB has grown, so has the ability of biologists to study large networks of genes in both people and bacteria, in order to identify previously invisible markers of immunity and vulnerability. For example, people who live with latent TB — or who are exposed to it but never infected — might have some kind of protective signature that a vaccine could induce in others. This kind of approach — using bioinformatics to parse huge amounts of genetic data for patterns and interconnections — is called systems biology (see "Searching for patterns", page S10). "If ever there was a disease readymade for a systems biology approach, TB is it," says Anthony Fauci, director of the National Institute of Allergy and Infectious Disease.



A. DOWSETT, PUBLIC HEALTH ENGLAND/SCIENCE PHOTO LIBRARY

Large-scale analysis of biomarkers may show up weaknesses in *Mycobacterium tuberculosis* bacteria.

"We need to bring much more effort into biomarker discovery," says Stefan Kaufmann, a TB researcher at the Max Planck Institute for Infection Biology in Berlin. Kaufmann is participating in an international, multi-institution biomarker-discovery study. One branch of the study has enrolled 850 people in South Africa, Ethiopia and The Gambia at the time of their TB diagnosis. The group will also follow about 4,500 of their household contacts — people who were TB negative at the start of the study but are at great risk of infection. Every six months, the researchers are looking closely at gene expression and metabolism in people who are exposed to TB in the home; they hope to find differences between those who go on to develop TB and those who do not. Researchers at Stellenbosch and Cape Town Universities in South Africa are collecting clinical data and storing the samples, which will be analysed by Kaufmann's group and by a team at the Seattle Biomedical Research Institute in Washington.

One goal of this study, says Kaufmann, is to find a biological signature associated with a higher risk of developing the disease so that future clinical trials of TB vaccines could enrol only people from this smaller population who are at higher risk. "This could bring the cost of an early stage trial down from more than US$5 million to less than US$2 million," Kaufman says. He expects the results of this study to be available in two to three years.

HIV: PORTRAIT OF A PATHOGEN

HIV is responsible for more deaths than any other infectious disease — an estimated 1.6 million in 2012. The virus that causes AIDS was discovered in 1983 at the Pasteur Institute in Paris. It took 20 years for the first HIV vaccine to enter clinical testing, and the news was not good: the first two trials, completed in 2003, showed no effect. Results from three other trials have also been disappointing. In 2007, Merck's Step trial was halted when it was found that the vaccine actually increased the risk of infection in some men (a problem that was traced to the viral carrier used to deliver the HIV antigens); a separate trial of the same vaccine was also halted because of these results. In spring 2013, a fifth trial, using different viral carriers and antigens, was halted early because the vaccine showed no sign of efficacy.

"Without natural models of immunity to work from, vaccinologists have had to develop new strategies."

Against this background of miscues, a 2009 trial that showed modest efficacy is a bright spot. This 16,402-person trial, conducted in Thailand, protected 31% of men and women at 42 months after vaccination. That's not good enough to market the vaccine but the results of the Thai trial provide, at last, a sketch of what immunity against HIV might look like. Now, researchers are trying to learn why the vaccine seemed to work in some people and not others.

A picture is emerging from the data, according to Jerome Kim, an immunologist at the United States Military HIV Research Program in Bethesda, Maryland, and a

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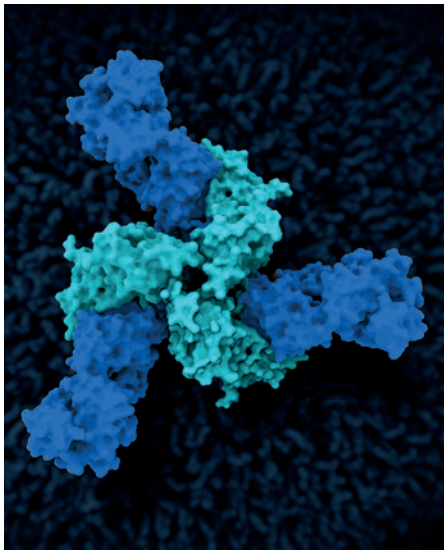


GRAHAM JOHNSON, UCSF

New imaging methods show a key HIV protein, the Env trimer (centre), bound to antibodies.

leader of the Thailand trial. For example, vaccinated participants who did not become infected with HIV were more likely than those who did become infected to have antibodies to a particular region of HIV's coating, or envelope, called V2, which seems to help the virus to enter the human immune cells it infects. Other parts of the envelope act as decoys — the body makes antibodies against them, but they do no good. "V2 is tucked away, camouflaged in sugars," says Kim. He also notes that, in the 2013 trial that was halted, the people who were vaccinated did not make antibodies to V2 — a sign that it may be an important part of the virus to target with vaccines.

Some researchers are sceptical about the Thailand trial. The antibodies made by vaccinated people were specific to the strain of the virus targeted in the study, but HIV is a tremendously mutable, variable virus, and any successful vaccine will have to offer broad protection. A successful HIV vaccine will have to induce what are called 'broadly neutralizing' antibodies — those that will react with any strain of the virus, says Burton.

A small fraction of people living with HIV do produce antibodies that neutralize a broad variety of HIV strains. But they don't start to do so until they have been infected for two or three years, at which point the virus is entrenched, and the antibodies cannot eliminate it. People who make these antibodies can still die of AIDS. However, researchers expect that if a vaccinated person is already making broadly neutralizing antibodies when he or she is exposed to HIV, the antibodies could prevent infection.

Inducing these antibodies is a major challenge, made more difficult by the lack of understanding of HIV's structure. Key parts of the HIV envelope — including V2 and other regions that are important for initiating infection — won't crystallize. This means that X-ray crystallography, the primary method for figuring out protein structures, can't be used to create images of them. To solve this problem, Burton and others are enlisting new imaging techniques and modelling software to learn more about which part of the virus the antibodies bind to and how, so that they can be engineered from the ground up.

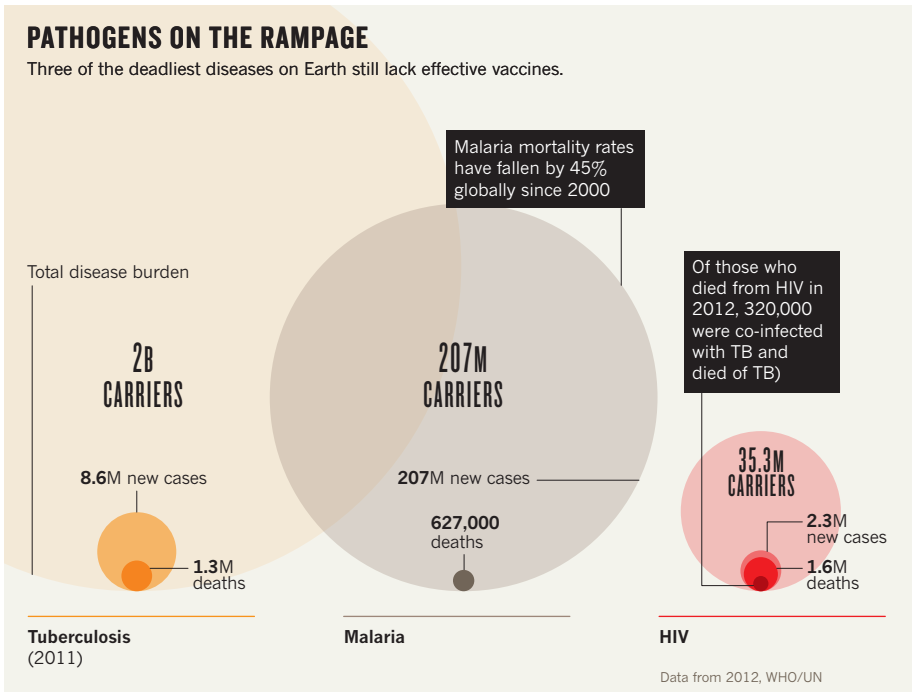
Burton has pioneered the discovery of broadly neutralizing antibodies and the development of the technology needed to study them and their binding sites. In November 2013, using a high-resolution imaging method involving an electron beam and very low temperatures (cryo-electron microscopy), researchers were able to take a picture of one of the most important parts of the virus that until then had been blurry: the three-part envelope protein called the Env trimer.

These pictures give protein researchers somewhere to start. The idea is to engineer a protein that contains the vulnerable region of the HIV envelope, packaged with protein sequences that will help it to hold its proper shape. When exposed to this artificial antigen, a person's immune system would make broadly neutralizing antibodies. Designing such a structure requires sophisticated computer models that can predict what sequence of amino acids will fold into the proper shape — a technology that now exists. Nevertheless, Burton suspects this endeavour will take time; the first such structural vaccines probably won't work, but researchers will keep testing and improving them in animals until they're ready for the clinic.

These types of engineered vaccine could be used to overcome one of HIV's greatest challenges: its incredible diversity. The key is to move beyond simply making antibodies; there is another branch of the human immune system that needs to be engaged, says Louis Picker, who is developing an HIV vaccine at the Oregon Health & Science University in Portland. Picker specifically cites the need to activate memory T cells, which remember and kill pathogens. No existing vaccine works through T-cell memory, says Picker, but researchers doubt whether any HIV vaccine can work without engaging it.

Picker's idea was recently tested against the virus that causes AIDS in monkeys — simian immunodeficiency virus (SIV). The vaccine used in the study uses another pathogen, called cytomegalovirus (CMV), to attack the immunodeficiency virus. CMV itself leads to a lifelong, but usually harmless, infection. The Oregon researchers engineered a CMV carrier that makes SIV antigens. In monkeys, the vaccine version of CMV activated the immune system not just to make antibodies but also to generate memory T cells that recognized SIV. Picker gave the vaccine to monkeys with persistent SIV, and the infection was cleared, an effect that he attributes to memory T cells. The protective effect should last as long as the CMV infection persists — that is, a lifetime. Picker is now developing a version of the vaccine designed to target HIV, and he hopes to start clinical trials in 2016.

According to Picker, a successful AIDS vaccine will probably involve combining his approach with structural antibodies such as those Burton is trying to make. "It will take time," says Burton, "but an HIV vaccine is going to come." ■



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