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Spotlight

Cooking up vaccines

Safety is key when manufacturing candidate AIDS vaccines for clinical trials

Before every launch of a space shuttle, all systems are carefully checked to prevent anything from going wrong. Failure is unacceptable. The same applies to the manufacture of candidate AIDS vaccines that will be tested in clinical trials. Each vaccine is unique and during every step of production the candidates must be inspected, and adjusted if necessary, to ensure they are safe and that they retain their activity.

For a vaccine candidate to be safe, it has to be pure, and the process of eliminating any potentially harmful substances takes substantial time and money. Take, for example, a type of experimental vaccine that consists of DNA. Making that in a research laboratory usually takes just a few days. But regulatory agencies like the US Food and Drug Administration (FDA) will not allow vaccine candidates that are made in a research lab to be tested in humans, even for early Phase I clinical trials, says Eddy Sayeed of IAVI.

And making a DNA vaccine that is safe enough for such trials can take months, because quality and purity need to be carefully evaluated. It's also much more expensive. While making enough for a Phase I trial costs about US$100 in a regular lab, the same amount made by a specialty manufacturer costs several hundred thousand dollars, says Tomas Hanke of the University of Oxford. The Vaccine Research Center (VRC) in Bethesda, Maryland, part of the National Institute of Allergy and Infectious Diseases, paid $12 million to have the company Vical manufacture six different DNA plasmids for the PAVE 100 trial that, as originally planned, would involve 8,500 volunteers, according to Alan Engbring of Vical.

Much of the manufacturing costs for candidate vaccines used in human trials are due to the conditions required by a set of standards called Good Manufacturing Practice (GMP), which are required by regulatory agencies like the FDA or the European Medicines Agency (EMEA) for products that are tested in humans. GMP conditions require, among other things, clean and highly-purified air and water. All material and people in the GMP-certified facility must also uphold high standards of cleanliness. In addition, everything a person does is double-checked. "One person does the work and another person watches them and they both sign off and follow the protocols exactly," says Jerald Sadoff who heads the AERAS Global TB Vaccine Foundation.

And keeping up to snuff on GMP isn't cheap. Running a GMP facility costs more than $100,000 a week, according to Sadoff of the AERAS Foundation, which runs its own facility to manufacture vaccines against tuberculosis (TB). In fact, 80% of the expense to manufacture a vaccine is due to maintaining GMP conditions, estimates Andreas Neubert, head of vaccine production at IDT Biologika GmbH, a German company that manufactures vaccines for IAVI and Oxford University, among others.

No pathogens, please

But there is more to vaccine production than GMP. Each type of vaccine also needs to be free of disease-causing agents known as pathogens, or any other potentially harmful substances. And which pathogens to watch out for depends on the way a vaccine candidate is made.

DNA vaccine candidates, for example, are produced using bacteria. The outer membrane of these bacteria can contain endotoxins, which are a concern because they are toxic to humans and therefore must be carefully removed from vaccine candidates. To remove endotoxins, the DNA vaccines are filtered and then tested to detect any remaining impurities.

Other vaccines use weakened or disabled viruses as vectors to carry genes that encode fragments of HIV, or immunogens. Some types of viral vaccine vectors are grown in cells from chicken eggs, which need to be free from pathogens like avian viruses or bacteria. This is important because live viruses grown in the chicken cells can't be chemically treated to kill contaminants, as is done for inactivated influenza vaccines that are also grown in eggs, because that would render the viral vector inactive. These pathogen-free eggs aren't cheap. Germany-based vaccine manufacturer IDT buys them at about 20 times the cost of regular eggs, says Neubert.

Vaccines that use adenovirus as a vector are typically grown in cells derived from humans and these also need to fulfill certain safety criteria before they get approved by regulatory

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agencies. For one thing, they need to be checked for many contaminating viruses and pathogens, Sayeed says. Prions, for example, are infectious protein particles that are believed to cause diseases in animals, including mad cow disease, and a fatal variant called Creutzfeldt-Jakob disease in humans. There are also many other potential safety concerns for vaccines that are grown in animal cells, so the FDA has strict requirements regarding their production. For this reason there are only a handful of cell lines available to grow adenovirus vector-based vaccines.

Keeping it consistent

Consistency between vaccine batches is another challenge for vaccine manufacturers. "It's unethical to do a trial with something that would never be reproducible," Sadoff says.

But that's easier said than done. For example, adenoviruses used as vaccine vectors are altered so they can no longer replicate (see *Primer*, this issue). Researchers remove certain genes the virus needs to copy itself, such as one called E1. They then add the gene to the cells that are used to produce the vector—this allows the cells to replicate indefinitely and makes it much easier to produce adenovirus particles. But sometimes during the process of manufacturing, the gene moves back from the host cells into the adenovirus cells, restoring the adenovirus' ability to replicate. "If you have too many of such [replicating] viruses, you have to throw away the whole batch," Hanke says.

Another challenge is that during vaccine production, viral vectors can lose part of the HIV genes they carry. One reason this happens is that some immunogens can be toxic to cells and can therefore render the vectors carrying them genetically unstable, Sayeed says. That's why manufacturers have to repeatedly test the vector to verify the HIV inserts are still there, adds Sadoff.

Optimizing the process

Safety and consistency are not the only things to monitor when manufacturing candidate vaccines. The production also needs to be optimized before larger quantities are made.

With DNA vaccines, for example, manufacturers identify the best bacteria to use for manufacturing the DNA and find the ideal time to stop bacterial growth before harvesting the DNA. Simple steps like these help optimize the process and can make a big difference in the efficiency of vaccine production.

Manufacturers also have to formulate the ideal growth conditions for vaccines made in animal cells. Some chicken embryo cells that are used to grow a viral vector called modified vaccinia Ankara (MVA), for example, prefer growing while adhered to surfaces, while others grow best in liquid suspension, according to Neubert. And growth efficiency drops as soon as HIV immunogens are introduced into the vector, Sayeed says.

But once a vaccine is manufactured at a large scale, the price is likely to come down. Making large batches is easier for some vaccines than others, depending on how they are made. It is relatively easy for DNA vaccines that are manufactured in bacteria. Growing the bacteria in larger batches could bring the per dose price of a DNA vaccine down to about $4, Sayeed says. That's just a fraction of the estimated $1,000 it costs initially. Vaccines that use adenovirus as a vector can also be made on a large scale rather easily. However, producing larger batches of vaccine becomes more difficult for MVA-based candidates. Since the chicken embryo cells they are grown in do not multiply indefinitely, they need to be harvested from fresh eggs. As a result, manufacturing an MVA-based vaccine for millions of people could require a hundred-thousand eggs per week, Sayeed says, adding that companies are developing new avian cell lines for large scale production to circumvent the dependency on fresh eggs.

Beyond more efficient processes, there are also other mechanisms that could help lower the cost of vaccines. One is granting tax incentives for a vaccine manufacturer. Another mechanism to make vaccines more affordable in developing countries is the so-called advance market commitment (see *VAX* September 2005 *Spotlight* article, *An industrial incentive*). This is an arrangement that requires governments to pay the difference between the price of a vaccine that a developing country can pay and the price that would make it profitable for the manufacturer to develop and produce it.

Got GMP?

Finding a manufacturer that can make a vaccine under GMP conditions is not that easy, says Sayeed, who is in charge of finding companies to manufacture vaccines IAVI has developed. That's especially true for vaccines based on viral vectors. "There is a waiting list," he says. Only a handful of manufacturers worldwide can do that type of work, and some of them are booked for at least nine months. There are also vaccine manufacturers in countries like India, South Korea, Brazil, and China that can do the job, but researchers are hesitant to go there because they are concerned about protecting their intellectual property, adds Sayeed.

Meanwhile, some academic or nonprofit research organizations have started to make candidate vaccines in their own facilities. The VRC, for example, has its own facility, and the University of Oxford also may use its own facility to manufacture adenovirus-based AIDS vaccine candidates in the future. This is generally cheaper than using a commercial manufacturer, according to Pru Bird, head of research at the Oxford facility.

AERAS also manufactures vaccines in its own facility, and earlier this year, the Canadian government, in collaboration with the Bill & Melinda Gates Foundation, announced the creation of the Canadian HIV Vaccine Initiative (CHVI). The initiative has proposed building a vaccine manufacturing facility in Canada, according to Ingrid Wellmeier of the Public Health Agency of Canada, which is part of CHVI. This plan is in response to a limited global capacity to manufacture vaccine candidates for clinical trials, Wellmeier says.

There are currently no large-scale facilities in place that could immediately take over production if an AIDS vaccine was proven to work in efficacy trials, Sayeed says. Manufacturers have to strike a careful balance between building a facility—which can take several years and cost a significant amount—and the risk that it may become useless if a vaccine eventually fails in late stage clinical trials. Sayeed adds that one

strategy adopted by some of the big pharmaceutical companies, with several products in the pipeline, is to build generic facilities that can accommodate different types of vaccine technologies. This way, their construction is flexible enough to switch to the vaccine that is successful, even midway into the building process.

Sayeed, for his part, remains optimistic. "People ask if there is scarcity for large scale HIV vaccine manufacturing," he says. "The answer is yes, but when it comes to crunch time, the capacity will be identified."

Global News

New HIV/AIDS estimates released

In advance of World AIDS Day, commemorated on December 1, the Joint United Nations Programme on HIV/AIDS (UNAIDS) and the World Health Organization (WHO) released annually-updated HIV prevalence and incidence figures that help to gauge the scope of the global HIV/AIDS epidemic. According to the 2007 data, there are an estimated 33.2 million individuals worldwide who are living with HIV and of those, 2.5 million individuals were newly infected this year. Also, more than two million deaths over the past year were attributed to HIV/AIDS-related causes, raising the cumulative death toll to 20 million.

This year's prevalence figures are significantly lower than in previous years. In 2006, UNAIDS and the WHO estimated that just under 40 million individuals were HIV infected globally, which was also lower than the 2005 estimate. The difference between this year's numbers and those for 2006 is largely attributed to improved efforts to monitor the epidemic and the implementation of better modeling tools, which are used to extrapolate available data from HIV surveillance systems and generate estimates of regional HIV prevalence. Much of this year's drop was attributed to India. In July the Indian government drastically revised the estimated number of individuals in the country who were HIV infected from 5.7 million to 2.5 million. This substantial revision helped push global estimates downward, but other countries lowered their estimates as well, including Angola, Kenya, Nigeria, Mozambique, and Zimbabwe. The UNAIDS/WHO 2007 AIDS epidemic update also attributes lower HIV prevalence in these countries to the success of HIV prevention and treatment programs (http://www.unaids.org/en/HIV_data/2007EpiUpdate/default.asp).

Many of the earlier estimates on the extent of the HIV/AIDS pandemic were based on data collected primarily from pregnant women (see *VAX* September 2007 *Spotlight* article, *HIV prevalence estimates: Fact or fiction?*). This data was easiest to collect because these women were more likely to seek healthcare services. However, this method also tended to overestimate the number of people who were actually infected with HIV since the prevalence was then based mostly on wealthier, sexually active women in urban areas who weren't representative of the country as a whole. Over the years many countries have started conducting household surveys, in which healthcare workers move from house to house counseling and testing individuals for HIV infection, to collect more accurate data. In almost all countries where this method was used, it has resulted in lower prevalence estimates.

UNAIDS and the WHO now also say that the global HIV incidence peaked sometime late in the last decade, when approximately three million people were newly infected with the virus in a single year. Since then, the number of new infections each year has slowly declined, reflecting both the natural course of the global epidemic and the success of HIV prevention efforts, according to the report. Many statisticians and scientists have warned for some time that the UNAIDS/WHO estimates were overblown. But with 2.5 million new HIV infections occurring this year, the battle against AIDS is far from over.

Despite a declining HIV prevalence in some African countries, the continent is still the most severely affected. Sub-Saharan Africa is home to 68% of the world's HIV-infected individuals, and the majority of them are women. Just this year, 1.6 million people in that region died from HIV/AIDS. In other areas, including Eastern Europe and Asia, HIV infection rates continue to rise, though most of the new infections still occur within populations at increased risk of infection, including men who have sex with men, injection-drug users, and sex workers.

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IAVI is a global not-for-profit organization working to speed the search for a vaccine to prevent HIV infection and AIDS. Founded in 1996 and operational in 24 countries, IAVI and its network of partners research and develop vaccine candidates. IAVI also advocates for a vaccine to be a global priority and works to assure that a future vaccine will be accessible to all who need it. For more information, go to www.iavi.org.

What are the advantages of using replicating viral vectors in AIDS vaccine research?

Many of the already licensed vaccines are based on either a weakened or killed version of the disease-causing virus or bacteria against which the vaccine is designed to protect. The vaccine against measles, for example, is a weakened version of the measles virus. This is a common approach in vaccine development and usually stimulates strong and varied immune responses.

However, this approach is not being pursued in AIDS vaccine research because of safety concerns. HIV can mutate rapidly and extensively and researchers are concerned that an attenuated or killed version of HIV may recover or retain its ability to cause disease once inside the body. As it is not feasible to develop a preventive AIDS vaccine using this approach, researchers have explored alternative strategies. One of these is using other viruses as delivery systems or vectors (see *VAX* September 2004 *Primer* on *Understanding Viral Vectors*). The virus particles used as vectors are weakened, or attenuated, by researchers so that they can't cause disease, and are also manipulated so that instead of containing their own genes, they carry fragments of HIV. These virus vectors shuttle the HIV fragments or immunogens into human cells, where they are then presented to the immune system. This triggers an immune response against HIV. These viral vector-based AIDS vaccine candidates include only parts of the virus and therefore can not cause HIV infection.

Non-replicating vectors

Most of the current AIDS vaccine candidates in clinical trials utilize viral vectors to induce cellular immune responses against HIV. The STEP and Phambili trials both used a candidate based on adenovirus serotype 5 (Ad5; see *VAX* October-November 2007 *Spotlight* article, *A STEP back?*). The naturally-circulating form of this virus is one of many that cause the common cold, but the version used as a vector is intentionally attenuated so that it can not cause disease. The Ad5-based vector tested in these trials, as well as others being tested, was also modified by researchers to carry HIV immunogens and was further attenuated by genetic modification so that it could not replicate or multiply. All viruses cause infection and disease by infecting cells and then using the cell's machinery to churn out multiple copies of the virus. This is referred to as replication. Copies of the virus that are produced can then infect other cells—setting off an infectious cycle. Researchers prevent the Ad5 vector from replicating once it enters the body by removing a single gene from the virus.

This means that each Ad5 particle used as a vector could infect only a single cell and present the HIV immunogens it carries only once before the vector is processed and the infected cells are destroyed by the immune system. Each dose of the vaccine candidate contains a billion or more Ad5 particles, meaning an equivalent number of cells could be infected. This may sound like a large number, but using non-replicating vectors substantially limits the immune system's exposure to HIV and therefore the magnitude of HIV-specific immune responses that can be induced. The outcome of the STEP trial showed that this specific Ad5 vector was not effective at providing any level of protection against HIV. It is still unclear why this vaccine candidate failed, but even before these disappointing results, researchers had started exploring alternative strategies for developing AIDS vaccine candidates.

Replicating vectors

One of these strategies is using viral vectors that retain their ability to replicate. This type of vector could greatly increase the amount of cellular immune responses generated against HIV. With a replicating virus as a vector, many more cells would be infected, increasing the immune system's exposure to the HIV immunogens included in the vector and potentially increasing the immunogenicity of the vaccine candidate (see *VAX* August 2007 *Primer* on *Understanding Immunogenicity*).

To develop a replicating viral vector, researchers manipulate the viruses so that they are unable to replicate at their full capacity and therefore can't cause disease. For some viruses, researchers remove some of their genetic material, which in turn slows down their replication rate and minimizes their ability to cause disease. This allows the immune system to eventually catch up, typically within a few weeks, and rid the body of the viral vector. AIDS vaccine researchers are also studying several animal viruses that do not naturally infect humans and therefore do not replicate as well in human cells.

Some of the replicating viral vectors that are currently being studied include vesicular stomatitis virus or VSV, which primarily infects cattle; sendai virus, which infects rodents; and an attenuated strain of measles virus. Some research groups are also studying serotypes of adenoviruses that retain their ability to replicate.

So far no AIDS vaccine candidates based on replicating viral vectors have entered clinical trials, but many researchers are hopeful that replicating viral vectors will improve the efficacy of AIDS vaccine candidates that induce primarily cellular immune responses. Although it is unlikely that cellular immune responses alone will be sufficient to protect against HIV infection, after the recent results of the STEP trial researchers are looking for vaccine candidates that will induce more robust immune responses that can provide some level of partial protection against HIV infection (see *VAX* May 2007 *Primer* on *Understanding Partially-Effective AIDS Vaccines*).

But safety is also a concern. Although replicating viral vectors will be attenuated so that they are unable to cause disease, there is still some concern amongst regulatory agencies about the potential risks associated with this approach. Further study of these vectors will be essential to sorting out any possible safety issues.