

Vaccine Development: Promise and Peril

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Vaccines have suppressed many scourges over the last two centuries and new vaccines could aid the fight against numerous current health threats. Yet, counterbalancing successful vaccine development are expectations and disappointments. In 2006, for instance, the United States Food and Drug Administration approved a vaccine against human papilloma virus, the virus that triggers cervical cancer; the vaccine promises to dramatically reduce the numbers of women who develop this cancer. Yet, in October 2007, a test of a promising new type of HIV vaccine was halted because the treatment did not prevent HIV infection—and in fact may have made it more likely. These contrasting events point to the public health importance of developing new vaccines and the difficulties of doing so.

A Diverse Arsenal

Vaccines stimulate the immune system to provide a defense against a specific type of invader. In many diseases, contracting an illness once provides some protection against future occurrences because the body generates a memory of the infectious agent. Vaccines accomplish this same result without producing an initial illness by using incapacitated infectious agents or molecular parts of those agents to encourage the immune system to produce antibodies. Then, if a bona fide infection hits, the body is already primed and mobilizes its antibody producing cells to fit the bacteria or virus.

A vaccine combines two components. The first component is an antigen, a foreign molecule that triggers production of antibodies. And an adjuvant is an additive to the vaccine that ensures that the immune system responds robustly to the antigen.

An antigen can be a whole virus or bacterium, deactivated to prevent disease. Whole virus or bacteria vaccines fall into two categories. Live, attenuated vaccines are based on wild viruses or bacteria that have been deactivated by growth in the laboratory until they no longer cause disease. These vaccines trigger a strong immune response and typically offer the best protection, because they contain viruses or bacteria very similar to those that cause disease. However, the chance of side effects is of concern, because the viruses or bacteria could mutate back into a disease-causing form. Live vaccines include those that protect against the viruses that cause mea-



Promise and peril standing back to back in vaccine development © stock-xchange / Brian Hoskins

sles, mumps, and rubella. Creating a live attenuated vaccine against bacteria is more difficult, and bacille Calmette Guerin, the most common vaccine against tuberculosis, and oral typhoid vaccine are the only current live attenuated vaccines available for bacterial infections.

Inactivated vaccines contain bacteria or viruses that have been killed chemically or through other means. Inactivated vaccines include vaccines against polio, hepatitis A, rabies and pertussis. They illicit a weaker immune response than live attenuated vaccines do, and typically require multiple doses, but the vaccines can't cause disease themselves.

Rather than whole cells or viruses, vaccines can also employ individual proteins from a bacteria or virus to prod a person to develop immunity. Vaccines made up of isolated molecules rather than whole particles are known as subunit vaccines. The proteins used in subunit vaccines are typically found on the surface of viruses or bacteria, so that antibodies can recognize the whole particles. In conjugate vaccines, those proteins are attached to certain types of sugars to ensure a robust immune response. Instead of carrying the antigens themselves, vaccines might contain the genes that produce those molecules—so called DNA vaccines or recombinant vaccines. DNA for the protein can be injected directly into the body, or harmless viruses or other DNA molecules called plasmids can carry these genes into cells and allow them to duplicate and activate. Once the genes generate their proteins, the body produces antibodies to those proteins. In other types of recombinant vaccines, the virus or bacteria itself is engineered genetically to be benign, forming a type of live, attenuated vaccine. And in another twist on recombinant vaccines, the currently available hepatitis B vaccine includes line of yeast engineered to produce a hepatitis B antigen. The recently approved human papilloma virus vaccine is also a recombinant vaccine. Developing DNA and recombinant vaccines is a key area for future research in the vaccine field.

Hamstrung against HIV

Almost immediately after scientists identified the HI viruses as the cause of AIDS in the early 1980s, researchers delved into creating a vaccine to prevent HIV infection. But despite two decades of work, several dozen clinical trials, and countless promising leads generated in the laboratory, no successful vaccine has emerged.

Most attempts to create an HIV vaccine have focused on the so-called envelope proteins, proteins that stick out from the outer coating of the virus particle. Early chimp studies using vaccines made up of envelope proteins appeared promising, but the chimp immune system can battle HIV without a vaccine, and the studies used a less-hardy lab strain of HIV. Envelope protein-based vaccines are the only ones to have reached Phase III testing, but have not born fruit. In 2003, VaxGen announced that two Phase III trials of its AIDSVAX—one of men in North America, and another of IV drug users in Thailand--did not show an overall reduction in HIV infection. The company still painted a bright picture, arguing that within certain ethnic groups in the North American study, HIV infection dropped. A third trial of AIDSVAX in combination with a second vaccine, called ALVAC, is ongoing in Thailand.

Envelope protein-based vaccines have proved refractory. And live attenuated vaccines wouldn't be safe—patients with HIV have weakened immune systems and HIV mutates rapidly, meaning

a live vaccine could likely cause disease. So researchers are exploring new types of vaccines. For instance, Merck developed a vaccine based on the common cold virus that contains the genes for three HIV proteins. Rather than provoke an antibody response, this combination of molecules was designed to stimulate certain immune cells called T cells to recognize and attack the body's own cells that had been overtaken by HIV. Although less precise than an antibody defense, the cell-based defense might more nimbly cope with accruing mutations in HIV that can circumvent antibodies, researchers reasoned.

A phase II trial of this vaccine, called STEP, had begun recently, in 3000 subjects in North America, South America, the Caribbean, and Australia. But after a preliminary analysis suggested that the vaccine did not prevent infection, the Data Safety Monitoring Board for the trial recommended that no new vaccinations occur. In October 2007, Merck halted the study, as well as a second phase II trial in South Africa, and two Phase I trials. Further analysis of the STEP study suggests that in some individuals—those with robust antibodies to the common cold virus—may have even been more likely to contract HIV. Despite the blow, researchers carry on down many avenues. The impact on the 2 million people who get infected with HIV each year is too great not to continue.

Malaria Mayhem

Malaria is perhaps just as deadly, killing millions each year, mostly in sub-Saharan Africa, where it is the leading killer for children under 5. Yet funding for malaria research is disproportionately less than support for AIDS research: perhaps 50 times less, when scaled for number of deaths. Malaria predominately affects poor populations who might not be able to afford a malaria vaccine, hampering interest in vaccine development.

No malaria vaccine exists, but non-profits such as the Bill and Melinda Gates Foundation have rekindled interest in the importance and potential of developing a malaria vaccine, and progress is occurring on several fronts. Advances have been catalyzed by the sequencing of the genome of *Plasmodium falciparum*, the parasite responsible for the most deadly cases of malaria in humans. Sequencing of *Plasmodium vivax*, the organism responsible for the highest number of malaria cases, is nearly complete. And this year, researchers delineated genome-wide variation in *Plasmodium*, opening the door to identifying the key molecular features responsible for causing malaria and pointing the way towards new vaccine targets. These studies might expand the number of molecules undergoing scrutiny as targets for new malaria vaccines.

The life cycle of malaria complicates the pursuit of vaccines, and so far researchers have tackled multiple life stages. After a bite by an infected mosquito, so-called sporozoites of *Plasmodium* enter the human blood stream and set up camp in liver. Liver cells disgorge merozoites that enter the blood stream and attack red blood cells, causing flu-like symptoms and, in more severe cases, seizures or coma, anemia, and kidney and liver problems. Some merozoites morph into gametophytes, which can be injected by a biting mosquito. Once in the mosquito, gametophytes fertilize each other to form an egg, which develops into sporozoites.

Researchers are teasing apart the molecular signatures of each of these life cycle stages. For instance, Hall and colleagues reported in *Science* in 2005 an analysis of the genes that are acti-

vated and the proteins that are expressed during the different segments of the parasite's life. Those findings could help define which molecules to target in order to hone in on a particular stage of life.

Several dozen malaria vaccines are currently being pursued, and at least a dozen variations target sporozoites—the liver stage. Irradiated sporozoites form an inactivated whole cell vaccine that protects animals and humans against malaria. However, protection fades over time. Moreover, safety concerns arise, especially how such a vaccine affects people with HIV, who have weakened immune systems and are especially at risk for malaria. Additional challenges remain about how to produce, store, and distribute such a vaccine. But the protective effect of inactivated sporozoites provides optimism that generating long-term immunity is possible.

Recent study results have been promising. In October 2007, researchers reported in *Lancet* that a GlaxoSmithKline vaccine called RTS,S/AS02 reduced malaria in African infants by 35%. The principal purpose of the study was to ensure the vaccine was safe in infants, and a Phase III trial is necessary to provide compelling evidence that the vaccine prevents malaria. The vaccine targets the sporozoite stage, but rather than using an inactivated sporozoite, the vaccine consists of yeast cells engineered to produce a protein found on the sporozoite surface. This vaccine is the most advanced of any currently under investigation, and Phase III trials should start in 2008.

Cancer Killers, Fever Fighters

Positive results from test studies are encouraging, but new, approved vaccines are the real payoff. In June 2006, the FDA approved the first vaccine against human papilloma virus (HPV), which causes genital warts and cervical cancer. The vaccine, called Gardasil, consists of a particular surface protein from each of the four varieties of HPV that are responsible for the vast majority of cases of genital warts and cervical tumors. The four recombinant proteins are combined to form a so-called virus-like particle, which resembles HPV but does not cause infection.

The vaccine nearly completely prevents cervical cancer, and estimates suggest that the vaccine could reduce the incidence of the cancer by 50%. The vaccine is approved for young girls (age 11-12 is the ideal time for vaccination); further studies are planned to determine if it also prevents genital warts in boys. Some detractors have expressed concern that the vaccine, by stemming the transmission of sexually transmitted virus, might promote early sexual contact. But despite bumps in the road, the vaccine promises to be a major success story in the vaccine field.

In other promising findings, researchers are making headway in developing a vaccine against dengue fever. Four different variants of *Flavivirus* cause Dengue fever. If a person is infected by one variety, they are protected against further infection by that type. But if they are infected by one of the other three, the more serious Dengue hemorrhagic fever often sets in, causing bleeding and shock. Efforts to create a Dengue vaccine are hampered by the fact that the vaccine must protect against the four *Flavivirus* varieties equally well.

Researchers have tackled the problem by separately creating live attenuated vaccines for each of the virus varieties and then mixing them together to formulate the final vaccine. Vaccines for three of the four varieties have worked well, but developing the fourth has proved difficult.

But scientists have broken through that barrier, and trials to test whether the vaccine prevents the disease should begin in 2008. Other approaches include using a modified yellow fever virus to carry proteins from each of the four varieties; Sanofi Pasteur is developing that vaccine, and plans to start Phase III studies in 2008.

The battles against numerous other diseases, including pandemic flu, Lyme disease, and staphylococcal infections would also benefit from leaps in vaccine development. Researchers will undoubtedly fine tune existing approaches and develop new strategies in the coming years, hopefully bringing success against many infectious diseases.