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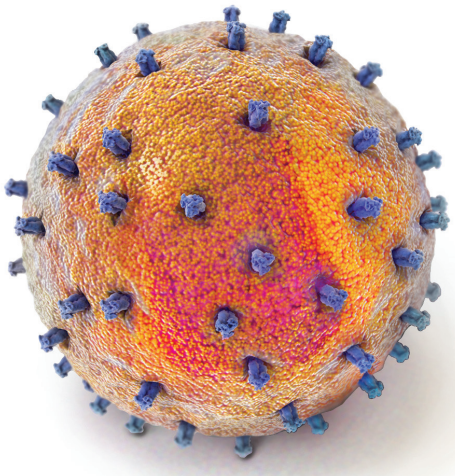

[SPOTLIGHT]

Vaccine Science Goes Holistic

As a growing number of researchers are studying biological systems in their entirety to better understand—and even predict—immune responses to vaccines, there is hope that such "systems biology" approaches might one day help accelerate clinical trials.

By Andreas von Bubnoff

A system, according to Merriam Webster's online dictionary, is a "group of related parts that move or work together." The term also evokes thoughts of looking at something in its entirety.

That's certainly true for systems biology, an emerging branch of biology that involves studying all parts of biological systems such as the immune system at once. One common approach systems biologists use to study the immune system is to measure changes in the activity of not just some, but all or most of the genes of an organism in response to certain stimuli that are known to cause an immune response, such as vaccination. This "systems" approach has grown a lot in popularity in recent years, and is already starting to yield novel biological insights.

Researchers are even starting to use it to predict the future: A few years ago, they measured changes in the activity of genes a few days after vaccination with the yellow fever vaccine. They found that a certain "signature" of such gene activity changes could be used to predict the level of the later adaptive antibody and cellular immune responses to that vaccine (see *VAX* Feb. and Mar. 2004 *Primers* on *Understanding the Immune System, Part I* and *II*). That study was the first that used a systems approach to identify signatures that could predict immune responses to a vaccine.

The yellow fever vaccine is just one of many different types of vaccines: As a so-called live viral vaccine, it contains a weakened version of the actual pathogen that causes the disease, while certain other vaccines only contain parts of the pathogen they are protecting against. Interestingly, systems biologists have found that the combination of gene activity changes that predict adaptive immune responses to different vaccine types differ from each other: For example, the gene activity signatures that predict later immune responses to two meningococcal vaccines (which contain certain sugars) are similar to each other, but differ from signatures that predict immune responses to yellow fever and other live-viral vaccines.

One day, it might even be possible to use this approach to predict immune responses to candidate HIV vaccines. Research teams have already shown that this might be possible for simian immunodeficiency virus (SIV), the monkey version of HIV that infects rhesus macaques. They measured gene activity changes within two weeks of the animals receiving a vaccine that contained SIV proteins. One year later, they infected the animals with SIV and checked the impact of the vaccine on virus levels.

They found that the gene activity measurements from two weeks after vaccination could predict—with about 85% accuracy—whether the vaccine reduced the viral load after the SIV challenge one year later. To efficiently process the complex data, the researchers used techniques from machine learning, where computers identify hidden patterns and relationships in enormous datasets to focus on the most relevant information.

Systems biologists are also trying to predict potential adverse effects of vaccines. For example, almost half of the almost 400 children who were vaccinated in a Japanese

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clinical trial that tested an inactivated whole H5N1 flu virus vaccine in the 2007/2008 flu season developed fever after the first of two vaccinations. As a result, the vaccine was not approved by the Japanese health authorities.

But the trial was useful in another way: Japanese researchers studied the vaccine recipients to identify molecular markers that could predict whether they would develop fever. They measured the levels of most of the 2,000 known microRNAs—small RNA molecules that regulate gene activity—in serum samples that had been taken from 85 of the children before the vaccination. They found that the levels of 73 microRNAs differed between the children who developed fever and the ones who didn't. They hope that these microRNAs can be used to predict whether vaccinees will develop fever in other vaccine trials.

The systems approach could also be helpful in predicting whether people with latent tuberculosis (TB) infection are likely to develop active TB disease. Being able to make such a prediction is very important: One third of the world's population, or about two billion people, are estimated to have latent TB infection; of those, about 10% will develop active TB at some point in their lives. Every year, nine million people develop the active disease, and 1.5 million die.

Just why some people come out of latency while others don't is not understood. But researchers are trying to at least identify markers that can help predict whether latently infected people will develop active infection, which would make prevention and treatment of TB much easier. To see if this is possible, they measured gene activity changes in blood cells taken from more than 6,000 adolescents with latent TB in South Africa five times over a period of two years.

They found more than 1,200 genes that showed different activity in 35 people who developed TB disease during that time, compared with 70 people who didn't. The researchers could use this information to predict the development of active TB six months in advance with up to 80% accuracy. The accuracy was lower for earlier time points, but still had excellent predictive value up to 18 months before active TB developed. They hope to use the analysis to identify people at high risk of developing TB disease for prophylactic treatment or to enroll them into efficacy trials of TB vaccines or treatments.

> If we could use [this] technology [to test] ten people [with] 40,000 data points per person to predict what's going to happen, probably vaccine development would be much faster. –Rino Rappuoli

But it's not all about predicting the future. Systems biologists are also trying to better understand why some people respond to vaccines better than others. They have found, for example, that higher activity of inflammation-related genes in elderly people before vaccination corresponds with lower immune responses to flu vaccination. Age-related systemic inflammation could therefore be one reason why vaccines have less of an effect in elderly people. The researchers now want to see if reducing inflammation before vaccination can improve immune responses to yellow fever and hepatitis B vaccines in elderly people.

Systems biology can also help to better understand results from clinical trials of HIV candidate vaccines. In a trial called Step, researchers tested an HIV vaccine candidate called MRKAd5, which uses a common cold virus (adenovirus serotype 5, or Ad5) as a vector to deliver fragments of HIV to the immune system. MRKAd5 didn't protect from infection; in fact, people with preexisting immune responses to Ad5 showed increased HIV infection risk.

Recent measurements of gene expression changes in people who received the vaccine suggest a possible explanation for this: One day after vaccination with MRKAd5, people with preexisting immunity to Ad5 activated fewer inflammation-related genes. This suggests that insufficient activation of appropriate "danger signals" by the vaccine may have something to do with the increased HIV infection risk in this population.

Because the "systems" approach involves measuring everything, researchers are not constrained by their preconceptions of what to expect. That's why systems biology can also lead to unexpected insights. When researchers measured global gene expression changes in response to flu vaccination, they found that the upregulation of a gene called *TLR5* one week after vaccination correlated with the level of the subsequent antibody response to the vaccine.

That surprised the researchers, because TLR5 is a receptor that senses bacterial flagellin, which is not present in viruses. At first, they thought the flu vaccine they were studying might be contaminated with bacterial products. But there was no evidence

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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, IAVI works with partners in 25 countries to research, design and develop AIDS vaccine candidates. In addition, IAVI conducts policy analyses and serves as an advocate for the AIDS vaccine field. For more information, see www.iavi.org.

for any such contaminants, and further investigation revealed that mice without *TLR5*, or without bacteria in their gut, had fewer cells that produce antibodies. This suggests that the sensing of our own gut bacteria by TLR5 might help induce the antibody response to vaccines, and that things that disturb bacteria, like antibiotics, might be harmful to some vaccine responses.

If recent trends are any indication, systems biology has a lot of promise in helping researchers to develop better vaccines. Some researchers believe that one day, the approach might even be able to help accelerate the process of testing vaccine candidates in clinical trials, which can take as many as 15 years.

While clinical trials today test, say, 10 parameters in 10,000 people, systems biologists might be able to find ways to do the same thing with much less effort, says Rino Rappuoli, a vaccinologist at the company Novartis. "If we could use [this] technology [to test] ten people [with] 40,000 data points per person to predict what's going to happen, probably vaccine development would be much faster." ■

GLOBAL NEWS *by Andreas von Bubnoff*

Tiny change in Envelope can make SIV resistant to antibodies

HIV uses structures that are called Envelope spikes on its surface to enter target cells, a process that's intercepted by antibodies, molecules the immune system makes to defend against pathogens. This interception of infection by antibodies is called neutralization. Researchers have in recent years found dozens of so-called broadly neutralizing antibodies (bNAbs) in chronically HIV-infected people that can neutralize many, if not most, HIV strains at very low concentrations. Their goal now is to develop a vaccine that can bring the immune system to make such bNAbs.

One challenge is that many HIV variants are much more resistant to antibodies than other, sensitive ones. The reason for this has been unclear—but researchers have now found a tiny change in the Envelope protein that can make simian immunodeficiency virus (SIV), the monkey version of HIV, more resistant to neutralization by some antibodies (*Nature* **505**, 502, 2014).

If this is also true for HIV—and preliminary results suggest it is—it could explain why candidate HIV vaccines have so far shown no or only low efficacy in human trials: they failed to induce antibodies to this resistant form of Envelope. "It probably can explain why RV144 worked a little bit but not a lot," says Oregon Health & Science University researcher Louis Picker, who was not involved in the study, referring to RV144, the only human trial to date that showed some, albeit low, efficacy of an HIV candidate vaccine of 31.2%.

The new findings come from experiments where the researchers, led by Mario Roederer of the Vaccine Research Center in Bethesda, Md., gave rhesus macaques different types of experimental vaccines that contained certain parts of SIV. Only one of them, a vaccine that contained only the SIV Envelope protein, protected the animals: it reduced the probability of infection after each SIV exposure threefold.

But some of the animals still got infected—even though they had received the vaccine. When the researchers took a closer look at what might have enabled viruses to break through the vaccine protection, they found a tiny change in their Envelope protein: two amino acids (the building blocks of proteins) called alanine (A) and lysine (K) were in certain positions close to one end of the Env protein that are usually occupied by two different amino acids called threonine and arginine.

That's a small difference, given that the entire Envelope protein is more then 850 amino acids long. But later experiments showed that the difference was enough to make half of these "A/K" viruses resistant to the antibodies in the blood of the vaccinated animals. The researchers calculated that for this to happen, only a tiny fraction—about 2%—of the A/K Envelope proteins must have become resistant to the antibodies, because each virus carries many Env proteins on its surface, and just one of them needs to be antibody resistant to infect a target cell.

Why the different amino acids made only a tiny fraction of Envelope proteins resistant to antibody binding is unclear. But the researchers speculate that they somehow allowed the Envelope to sometimes fold into a different shape, which made it more difficult for antibodies to bind to most parts of it.

To better understand how this works, the researchers now plan to isolate the resistant A/K form of the SIV Envelope protein and study its structure. But the fact that the resistant A/K Envelope form is so rare could make this quite difficult—and is probably the reason why the resistant form has so far escaped the attention of researchers.

Isolating the resistant form would also enable researchers to create structures that mimic it to develop a vaccine that can induce antibodies to resistant virus variants. "If we could purify that form, or figure out how to stabilize it in some way and make it in large quantities, we could immunize with it," Roederer says.

To be sure, the A/K change doesn't seem to make all parts of Envelope resistant to antibody binding. One part of Envelope that the virus uses to bind to a target cell protein called CD4 when it enters target cells seems to be unaffected, because the researchers found that antibody-like molecules that resemble CD4 could still bind the resistant A/K form of Envelope.

Therefore, another way to make a vaccine that protects from the resistant A/K viruses is to make sure that the vaccine induces CD4-specific bNAbs, such as one called VRC01. "If we figure out an immunogen that elicits VRC01 in everyone, then we are done," Roederer says.

That approach, of course, has its own challenges (see *VAX* May 2013 *Primer* on *Understanding How a Vaccine May be Designed to Induce Broadly Neutralizing Antibodies)*. Says Roederer: "If it was easy, it would have been done." ■

[PRIMER]

Understanding VLP Vaccines

What are virus-like particles and how are they being used in the design of AIDS vaccine candidates?

Many vaccines teach the body how to fend off a chosen bacterium, virus or parasite by presenting it with a killed or weakened form of that pathogen.

These approaches are not, however, viable for HIV due to concerns that any such virus preparation may not be completely inactivated, or that its weakened form might mutate and regain its ability to cause disease. So scientists have relied instead on delivering purified proteins derived from recombinant HIV genes, or the genes themselves, to trigger cellular (T-cell) and antibody (B-cell) responses against HIV (see *VAX* July 2008 Special Issue, *Understanding the Immune System and AIDS Vaccine Strategies*).

AIDS researchers have tended to favor recombinant vaccines, in which parts of the pathogen are synthesized from scratch and used as immunogens (the active ingredient in the vaccine candidate). In some cases, the vaccine candidates have consisted of soluble proteins, which, as the phrase suggests, dissolve easily in water. This is the approach that was used in one vaccine candidate used in the RV144 trial (see *VAX* Sep. 2009 *Spotlight* article, *First Evidence of Efficacy from Large-Scale HIV Vaccine Trial*), which demonstrated modest efficacy, and the candidate used in the Step trial (see *VAX* Oct.-Nov. 2007 *Spotlight* article, *A STEP Back?),* which did not.

A viral imitator

Another type of recombinant vaccine that has captured the attention of scientists in recent years relies on virus-like particles (VLPs) to deliver HIV's payload. While VLP vaccine candidates present their own challenges, these multi-protein structures have yielded impressive results in studies and represent a safe and potentially more effective alternative for HIV vaccines.

Studies suggest that VLP vaccines against the influenza virus might be able to provide more potent and longer-lasting protection than do the current seasonal vaccines. AIDS researchers are developing VLP-based vaccines as well. A variety of VLPs are currently in various stages of preclinical and clinical development.

So how do these candidates work? As you may know, viruses need a human host to multiply. A virus particle—or virion—is essentially a combination of DNA or RNA material packaged in a protein capsule that's made by infected cells and spreads by budding. A number of years ago, researchers described during their study of the hepatitis B virus that it's possible to assemble particles that lack a viral genome and some of its proteins, but can still be recognized by the immune system.

VLPs present parts of the proteins specific to the targeted pathogen, such as the Envelope (see *VAX* March 2011 *Primer* on *Understanding HIV's Envelope Protein*) that sits on HIV's surface and is used by the virus to invade cells.

VLPs are similar in size and conformation to intact virions. Because they lack crucial genetic material, they are non-infectious and so provide a safer alternative to weakened viruses. Many VLP vaccine candidates are also built from viruses that infect bacteria, or those that infect plants, animals, or even humans. Studies have found that VLP vaccine candidates can be highly immunogenic, in part because they can display multiple antigens on their surface, improving interaction with components of the immune system and thus increasing the odds of inducing a potent antibody response.

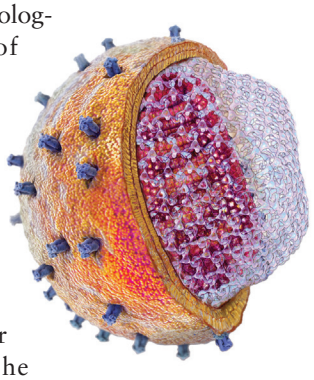
VLPs in HIV science

AIDS vaccine researchers are employing VLPs in different ways. One group of researchers is using them to induce antibodies to a part of the protein spike that protrudes from HIV's Envelope called the membrane proximal external region. This part of the spike is important for fusion of the viral membrane with the target cell membrane. Researchers are using a baculovirus—which infects cultured insect cells—to express the recombinant HIV genes. The VLPs are then purified from infected cells.

Researchers have also created a VLP vaccine candidate that swapped one segment of HIV's spike—another name for the Envelope, or trimer—with a smaller protein from the influenza virus. They devised this method to try and make portions of the HIV spike more accessible to the human immune system.

Finally, researchers have developed a test that uses VLPs to screen for proteins that bind to the earliest ancestors of broadly neutralizing antibodies (bNAbs), which prevent a broad variety of HIV variants from invading cells in laboratory studies. Scientists have identified dozens of these bNAbs in people with chronic HIV, but the antibodies take years to develop. So engaging these early cells, known as germ-line precursors, represents a potential road to success in developing a vaccine candidate that might induce these coveted bNAbs (see *VAX* May 2013 *Primer* on *Understanding How a Vaccine May be Designed to Induce Broadly Neutralizing Antibodies*).

While VLP vaccine candidates are an attractive alternative, they present manufacturing challenges that developers will need to overcome. Some VLP candidates are too costly to produce in significant quantities and the biological structures of some VLPs are, in some cases, too complicated for large-scale production.



Still, there are now two recombinant vaccines on the market—one for hepatitis B and the other for human papilloma virus—that employ VLP platforms. Another VLP-based vaccine candidate, GlaxoSmithKline's malaria vaccine candidate RTS,S, is in late-stage clinical testing (see *VAX* Nov. 2012 *Global News*).

The hope is that VLPs may help AIDS vaccine scientists achieve similar success. ■