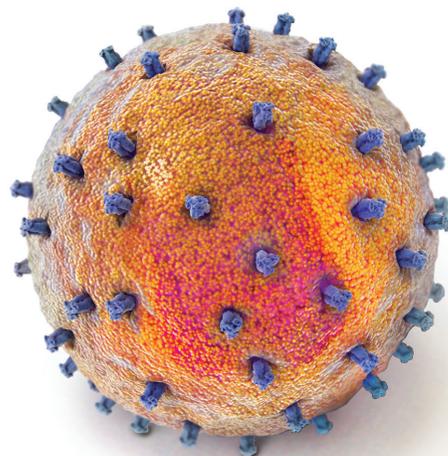


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The Bulletin on AIDS Vaccine Research



[SPOTLIGHT]

Game of Clones

Researchers are using small RNAs that inhibit the expression of specific genes to find antiviral factors, improve vaccine production, and even develop better cancer treatments *By Andreas von Bubnoff*

Scientists tend to keep quiet about the results of their experiments until they are published in a scientific journal.

Not so Benjamin tenOever: You can check in on one of his studies in real time by following his Twitter feed (@virusninja). Granted, his tweets are more technical than your typical Twitter fare: “Kinases also doing well...Cmpk2, Cdk14, TBK1, Mlk1, IKKB, IKKe, and Mapk8,” he tweeted on Sep. 3.

But it’s less complicated than it seems. The virologist at the Icahn School of Medicine at Mount Sinai in New York City is just tweeting the results of an experimental tournament he calls “Game of Clones.” The competitors are flu viruses that infect mice. In each round, tenOever infects mice with two flu viruses, waits for two days, and then checks the lungs of the animals (the preferred organ the viruses infect) to choose the winner: the virus that has multiplied better. In the next round, he infects another mouse with two winners of the previous round.

The only way the viruses differ from each other is that each one carries the genetic information for a tiny molecule called siRNA that can specifically inhibit a certain gene in the cell the virus infects. (Normally, cells use so-called messenger RNAs to copy the genetic information that then serves as the blue print to make proteins, and siRNAs are a special

kind of RNA that specifically destroys such messenger RNAs.) tenOever has made viruses that can inhibit 128 different genes that are known to be involved in the immediate (or “innate”) immune response to viruses in the first few hours after infection.

The virus that wins the tournament is therefore likely to carry an siRNA that inhibits a gene that’s very important to the host’s innate immune response to viruses. This could help researchers develop drugs that enhance the effects of anti-flu drugs or vaccines by stimulating such host genes.

Recently, tenOever did a similar experiment on a much larger scale: He made viruses carrying siRNAs that inhibit 10,000 different mouse genes. In this case, tenOever used Sindbis viruses, which are similar to West Nile virus and infect cells in the blood of mice, but are harmless to humans. tenOever infected mice with all 10,000 viruses, waited two days for the virus levels to peak, and isolated the spleen—which filters the blood—to extract all viruses. He then counted the different viruses in the spleen, and infected another mouse with the mix. After repeating this four or five times, he found that two viruses eventually came to dominate the virus population. “You are looking at evolution in real time,” he says. “We can tell that this [winning] gene is the most important gene in the context of a

real infection in the actual mouse.”

As in the Game of Clones, this means that the siRNAs that are carried by these two viruses inhibit a host gene that is very important for the host defense against these viruses. Additional experiments revealed that these host genes are so important for the host defense because they are central players in the way different parts of the cell communicate with each other. Without them, the cell is a complete mess. “When they are eliminated,” tenOever says, “the cell loses all of its organizational structure, and that is the perfect environment for a virus. The virus has a much easier time taking over a cell where the organizational ability of that cell has been thrown into complete disarray.”

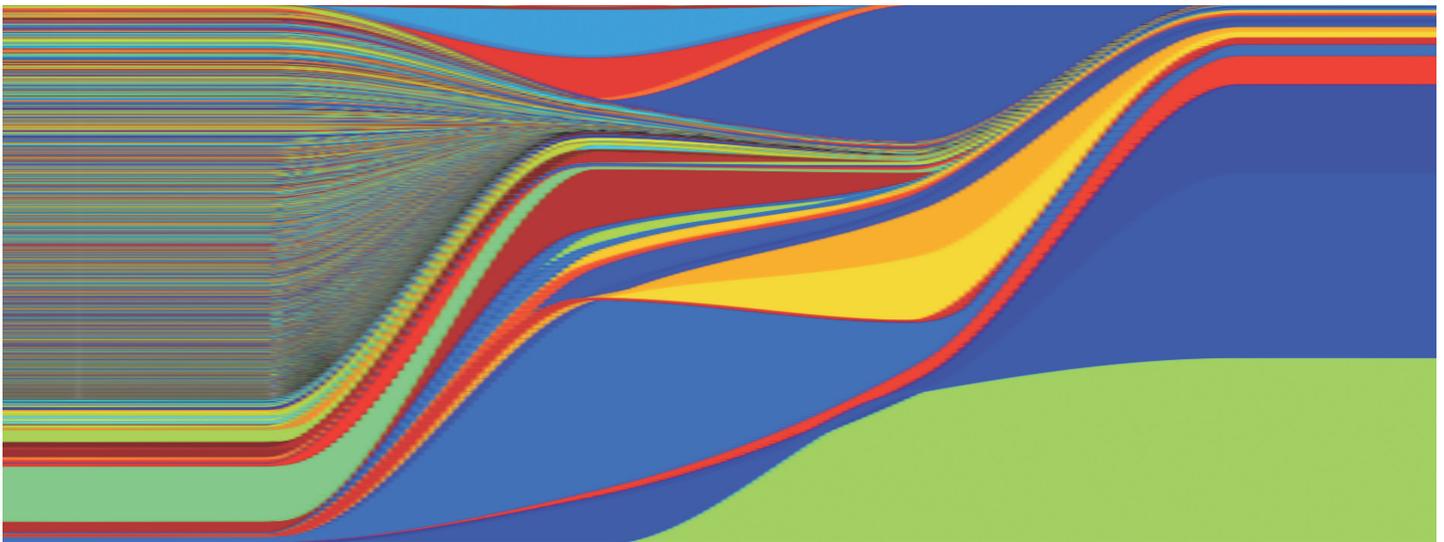
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The colors of evolution. Benjamin tenOever and colleagues infected mice with Sindbis viruses that carry siRNAs that inhibit 10,000 different host genes (left; each color shows a virus with a different siRNA). After passaging the virus mix several times to new mice, they found that very few viruses eventually dominated the virus population (right). Image courtesy of Benjamin tenOever, Icahn School of Medicine at Mount Sinai, New York City.

Interestingly, this insight could be used to fight cancer because this sort of disarray is similar to the kind that makes cancer cells a preferred target for viruses. That, in fact, is why researchers have been trying to use viruses to treat cancer. So far, the approach hasn't been all that successful, tenOever says, because the viruses aren't aggressive enough to kill off the cancer cells once they infect them.

But tenOever's siRNA-carrying viruses could be used to find siRNAs that enable these viruses to multiply so avidly in cancer cells that they destroy tumors. To do so, tenOever plans to infect tumor tissue with viruses carrying a variety of siRNAs; the viruses that grow best could then be used to fight the tumor.

tenOever's isn't the only siRNA screen around. Abe Brass of the University of Massachusetts Medical School adds siRNAs that inhibit each of the about 20,000 genes in the human genome to cultured cells. He then infects each of them with a

virus to see if the virus can still infect its target cells and multiply normally.

The screens identified cellular factors that could be inhibited to reactivate HIV production in latently infected cells. This has already led to the identification of a molecule called JQ1 that could in some instances reactivate latent HIV-1 in cells from patients who had been on long term antiretroviral therapy, Brass says.

Brass also uses his screens to find host cell factors important for dengue virus. There is still no good vaccine or treatment for dengue, which has recently been spreading into the northern hemisphere aided by climate change and global trade and travel. Each year, some 50-100 million people contract symptomatic dengue infections—a number that is increasing. Dengue cases have even appeared in Texas, Florida, and Europe.

Screens with the influenza virus enabled Brass and colleagues to identify a family of host cell factors called IFITMs that normally

block infection with many viruses, including influenza, dengue and West Nile virus. The work led Brass, in collaboration with other researchers, to a mutation in one of these factors, IFITM3, that makes people six times more susceptible to severe influenza infection, and is especially common in Chinese and Japanese populations. This knowledge could help health care providers better assess patient risk and perhaps help guide therapy.

The tenOever lab and others are also working with another type of small RNA that can specifically inhibit gene expression: so-called microRNAs. Different cells have different types of micro RNAs, and scientists now use this insight to modify viruses in such a way that they can only grow in certain cells but not in others. The trick is to add a genetic sequence to a virus that serves as the target sequence for microRNAs that are only found in certain cells. As a result, the virus cannot grow in the cells expressing microRNAs that target the sequence because the microRNA

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inhibits the viral genes; in contrast, the virus can grow in other cells that lack the microRNA in question.

tenOever has used this trick to improve the production of flu vaccines. The kind of seasonal flu vaccine that's applied as a nasal spray is live attenuated, which means that it contains a weakened version of the influenza virus that's expected to be most widespread in any given year. That way, it doesn't cause any disease but is still able to induce a protective immune response. However, the drawback is that the weakened virus also grows more slowly in chicken eggs, which have traditionally been used to manufacture seasonal flu vaccines.

tenOever modified the seasonal virus in a different way. He added a genetic sequence that serves as the target for a

It won't get really interesting until we get closer to the final elimination. The deeper you get in the tournament, the better the gene is and the more interesting it becomes.

— Benjamin tenOever

microRNA that is present in mice and humans, but not in chicken eggs. As a result, the modified virus grows completely normally in eggs, but much more slowly in humans. More recently, tenOever used the same approach to make an H5N1 bird flu

virus that only replicates well in ferrets but not in humans, and can be used to do experiments in ferrets—the main animal model system to study flu infection—without presenting much of a danger to humans.

The micro RNA technology can even be used in gene therapy, where it allows researchers to introduce genes into humans that are only active in certain cell types. “It's a very powerful technology,” says tenOever. “There are all kinds of labs doing it, all kinds of applications. It's very popular now.”

As for the Game of Clones, he says, “it won't get really interesting until we get closer to the final elimination. The deeper you get in the tournament, the better the gene is and the more interesting it becomes.”

So stay tuned—it's easy enough on Twitter. ■

GLOBAL NEWS *by Regina McEnergy*

Annual AIDS vaccine conference to stress progress, partnership, and perseverance

The scientific terrain of HIV vaccine research has changed a lot in recent years, as other new prevention strategies have come into their own. So, as the four-day conference looks beyond 2013, its agenda will entertain a number of discussions on the possible synergies between vaccines, microbicides, and ARV-based prevention. Jointly sponsored by the Global HIV Vaccine Enterprise and HIVACAT, the Catalan Program for HIV Vaccine Development, the conference is to be held Oct. 7-10 at the International Convention Center in Barcelona.

The fate of adenovirus vector vaccine candidates is likely to dominate discussion as well, beginning with an opening night talk by Magda Sobieszcyk. An assistant professor of clinical medicine at Columbia University Medical Center, Sobieszcyk will be providing highlights and lessons learned from the HVTN 505 trial, which was recently terminated for futility (see *IAVI Report* blog, *Large AIDS Vaccine Trial Shudders to a Halt*, April 26, 2013). Duke University scientist Georgia Tomaras will provide antibody profiles from the HVTN 505 study and compare them to those from RV144, the only AIDS vaccine trial to demonstrate any measure of efficacy. In that trial, which had 16,000 participants, a combination of two vaccine candidates administered sequentially in what is called a prime-boost regimen lowered the rate of HIV infection by about 31%.

There will also be a roundtable discussion on the importance of retaining and following clinical trial participants long after the conclusion of a study, a point that became clear following the Phambili study, which was halted in 2007, and drew fresh scrutiny this year (see *VAX* May 2013 *Global News*). Finally, the HIV Vaccine Trials Network (HVTN), which sponsored the HVTN 505 trial, will provide a meta-analysis of results from vaccine trials in which adenoviruses have been used as vectors.

Another hot topic at the vaccine conference will be the burgeoning field of structure-based immunogen design. That includes a four-hour satellite session organized by The Scripps Research Institute about the structure of the HIV glycoprotein trimer—or “spike”—which the virus uses to invade its target cell. This extraordinarily complex protein, which protrudes from the surface of HIV, has long resisted structural analysis. But images of this protein complex are becoming clearer, thanks to new imaging technologies and strategies. The satellite session in Barcelona will cover the promise of this structural work—and some related controversies.

And scientists from the Military HIV Research Program are expected to present preliminary data from the RV305 trial, an immunogenicity study that began in 2012 and evaluated the impact of an additional protein boost in volunteers who participated in the RV144 trial (see *VAX* Sep. 2009 *Spotlight* article, *First Evidence of Efficacy from Large-Scale AIDS Vaccine Trial*).

The vaccine conference has broken some new records this year even before it has begun. More than 325 young and early career investigators applied for scholarships, and more than 600 abstracts were submitted for consideration, about 100 more than usual. About 1,000 people are expected to attend the meeting.

Barcelona also brings to a close the single-themed focus of the meeting. Starting in 2014, attendees who converge Oct. 28-31 in Cape Town, South Africa, will get a full course of microbicide and ARV-based prevention research, along with the usual dose of vaccine science the meeting has served up every year. ■



Understanding the Role of T Follicular Helper Cells in Responding to HIV

How does this subset of cells differ from other T cells, where do you find them, and how are they important to AIDS vaccine and cure research? *By Regina McEnerly*

When a new pathogen enters the body, the immune system uses a variety of defenses to get rid of it. One of the most important responses comes from B cells, which produce antibodies—roughly Y-shaped proteins—to coat the surface of the pathogen and stop the invasion. Antibodies also label the pathogen so that other immune defenses can “see” and attack it.

Other immune cells that are among the first to respond are dendritic cells and macrophages. These cells patrol the body and pick up the pathogen or its fragments. They then migrate to the lymph nodes, which are like the hubs of the immune system. Lymph nodes are a key component of the immune system, found all over the body, most prominently under the jaw, under the arms, in the gut, chest, and groin.

In the lymph nodes, patrolling cells show or “present” the pathogen to CD4⁺ T cells. These “helper” CD4⁺ T cells coordinate the activities of antibody-producing B cells and a set of “killer” cells called CD8⁺ T cells or cytotoxic T lymphocytes. CD4⁺ and CD8⁺ T cells work together to eliminate cells that have been infected by pathogens (See *Vax* Feb. 2004 *Primer on Understanding the Immune System, Part I*).

Scientists have known for quite some time now that HIV preferentially infects and kills CD4⁺ T cells. While the immune system tries to fight off HIV by sending CD8⁺ T cells to kill HIV-infected CD4⁺ T cells, the rapidly replicating virus eventually exhausts and overwhelms the immune system.

CD4⁺ T cells play a vital role in the antibody response. One of those roles is the delivery of signals that drive the maturation and selection of B cells that generate increasingly potent antibodies. This process, referred to as affinity maturation (see *VAX* Jan. 2011 *Primer on Understanding How Broadly Neutralizing Antibodies Evolve*), is critical to the evolution of potent broadly neutralizing antibodies (bNAbs),

which have been shown in laboratory studies to neutralize many circulating variants of HIV. Many researchers believe a vaccine would have to elicit such antibodies to provide broad coverage against the virus.

A specialized subset of CD4⁺ T cells help B cells generate increasingly effective antibodies against their target pathogens. This subset is known as the T follicular helper (Tfh) cell, so named for the B-cell follicles or cavities where most Tfh cells reside. The follicles are found in germinal centers, where mature B cells proliferate and differentiate in order to make antibodies.

One reason Tfh cells eluded identification for some time is that they are primarily found in these B-cell follicles, unlike most helper T cells. Nonetheless, scientists have learned quite a lot about how these elusive cells function, and how they are affected by HIV infection.

For instance, they know that Tfh cells express high levels of certain proteins that HIV uses to enter target cells, making them a major site of HIV replication. At the same time, Tfh cells also express proteins that in some instances are helpful for B-cell survival and the production of antibodies, but in others prove to be detrimental.

Additionally, scientists have identified two types of interactions between Tfh cells and B cells—one brief and on the fringes of the germinal center, the other a much longer interaction within the germinal center. In different ways, these interactions support the development of bNAbs, many of which have recently been isolated from the serum of chronically infected HIV-positive individuals.

Unfortunately, the development of bNAbs is a relatively rare event, arising in only about 15-20% of all people infected with HIV. Scientists believe one reason this may be the case is that HIV drives an abnormal expansion of Tfh cells, thus impairing their function.

Based on this accumulating data, scientists believe a better understanding of Tfh cells

and their interaction with B cells could be essential to the design of an effective vaccine.

Curing HIV

Tfh cells are not just important in vaccine science, however. They are also important factors in the hunt for a cure for HIV. A relatively young subfield of HIV science, cure research is an area with lots of missing pieces. But it has lately shown great promise, thanks, in part, to what scientists have learned from 17 years of studying patients on highly active antiretroviral therapy (HAART) (see *VAX* March 2013 *Primer on Understanding Therapeutic Vaccination*).

HAART regimens potently suppress viral replication in the blood, allowing the body to rebuild its immune system. But such regimens cannot by themselves cure HIV infection, since the virus weaves itself into the chromosomes of resting CD4⁺ T cells, creating a population of latently infected cells known as the viral reservoir.

Scientists believe one possible way to cure HIV would be to locate and drain reservoirs of latent HIV-carrying cells. Such reservoirs persist even in people whose circulating HIV has been effectively suppressed by drugs. Scientists have been studying the blood and tissue of HIV-infected individuals—some who received HAART and others who have not—and compared them to HIV-uninfected individuals to measure Tfh populations.

Scientists believe that when the virus infects Tfh cells, it probably contributes to viral persistence because the germinal centers that house most of the Tfh cells are inaccessible to CD8⁺ T cells (killer T-cells), whose job it is to destroy HIV-hosting cells. These germinal centers also seem to serve as a kind of trap for HIV virus particles, which can then infiltrate the Tfh cells housed there.

Scientists are hoping to use what they have learned from these Tfh studies to design drugs that can counter HIV persistence and thus eradicate the virus. ■