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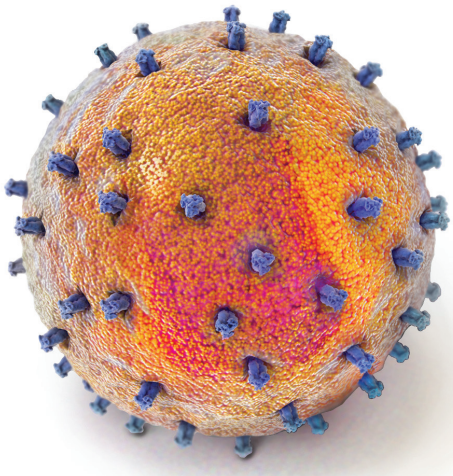
The Bulletin on AIDS Vaccine Research



[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.

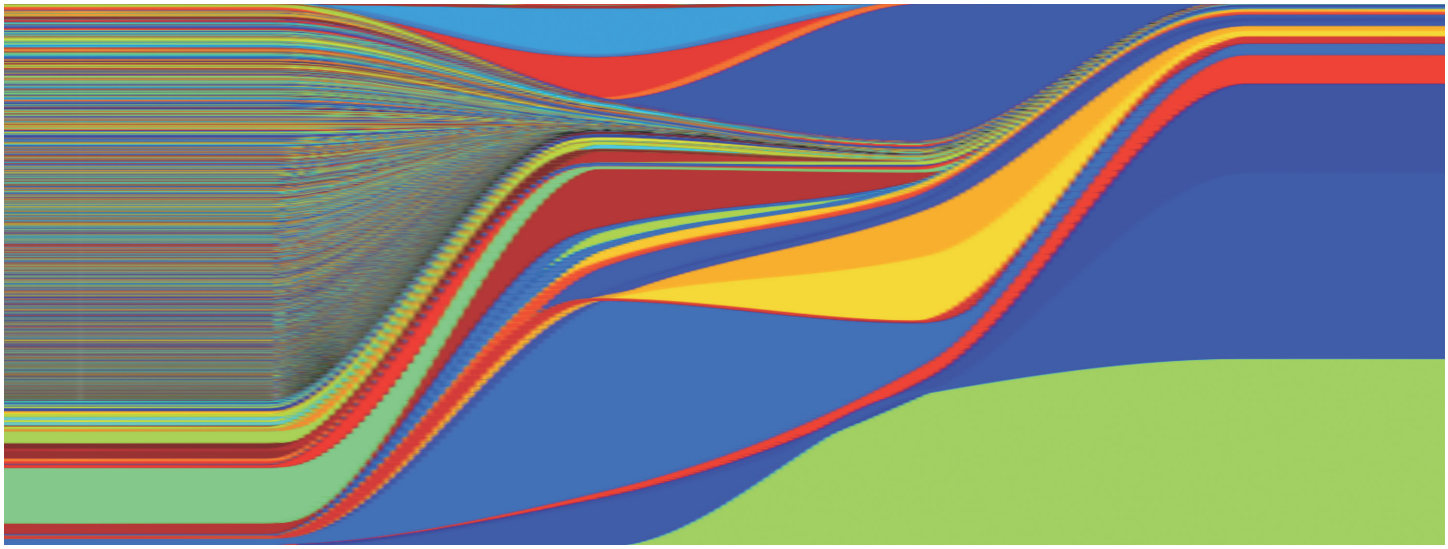
The only way the viruses differ from each other is that each one carries the genetic information for a tiny molecule called siRNA that can specifically inhibit a certain gene in the cell the virus infects. (Normally, cells use so-called messenger RNAs to copy the genetic information that then serves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

ALSO IN THIS ISSUE



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

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 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.

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>
> – Benjamin tenOever

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. ■