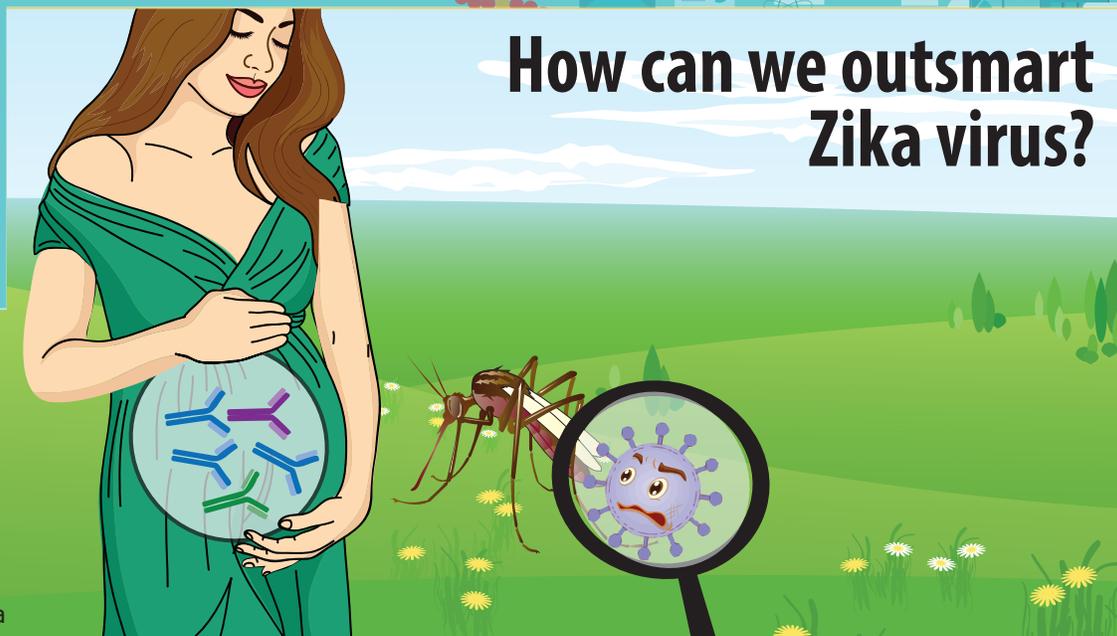


How can we outsmart Zika virus?



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Abstract

The mosquito transmitted Zika virus has been in the news a lot lately. Do you know what scientists are doing to protect us from it? When the virus infects pregnant women, it can cause neurological defects in their unborn children. *Microcephaly* (abnormally small heads) is the worst defect caused by Zika.

Currently there is no cure or *vaccine* preventing Zika infection. It is hard to develop a vaccine because of a special feature of this virus and its close relatives called *antibody-dependent enhancement* (more on that later). That's why we decided to try a new approach against this virus. Instead of

a vaccine, we wanted to introduce "ready-to-go" *antibodies* that could neutralize the infection.

We first found a patient with a natural, strong immune response against Zika virus. We then identified the antibodies that were responsible for this antiviral effect and produced them in the lab. Then, we gave the purified antibodies to four macaques monkeys and injected the animals with Zika virus to see if this treatment can prevent virus replication. Our experiment showed promising results - we found no trace of the virus in the antibodies-treated macaques.

Introduction

Until recently, we thought Zika was a rare and somewhat harmless virus. Similarly to its famous cousins, the West Nile and dengue viruses, Zika gets in your body through a mosquito bite (Fig. 1), but you rarely develop any symptoms. When you do, they are usually mild – headaches, a rash, pain in the joints and muscles and a mild fever.

Why should we worry about Zika then?

1. There is evidence that Zika can lead to birth defects. During the big Zika outbreak in Brazil in 2015, thousands of children were born with microcephaly (Fig. 2).
2. It is spreading really fast due to climate change and globalization.

Figure 1:

An *Aedes* mosquito. It can transmit Zika virus, yellow fever, West Nile virus, dengue virus, chikungunya and others. (Photo credit: Muhammad Mahdi Karim)



There is currently no medicine or *vaccine* against Zika. In fact, vaccine development for this virus and its cousins is facing a serious problem, called antibody-dependent enhancement. In short, the vaccine produces antibodies that are specific to one virus, but do not block infection of closely related viruses. Instead, the cousin viruses hitchhike on these antibodies to enter the host cells. This often means that the first time a person is infected with a virus, the illness is mild, but when infected with a similar virus later, it

is severe and sometimes deadly.

That is why we wanted to think of a better way to prevent Zika virus disease without making their cousin virus infections worse. Instead of injecting inactivated Zika viruses that prompt the body to develop antibodies (obtain 'active immunity'), we wanted to try injecting "ready-to-go" anti-Zika antibodies ('passive' immunity) that were modified to prevent disease enhancement.

Methods

First, we had to obtain some Zika antibodies. We isolated them from blood cells of a Zika virus infected patient from Colombia. We then incorporated a minor mutation into these antibodies so that antibody-dependent enhancement could not happen and multiplied the mutated antibodies by *transfection* of mammalian cells. We had to test if they can prevent Zika virus infection. To do that we infected *cell cultures* with Zika virus and treated them with different *dilutions* of the antibodies. Many showed a positive effect, but for our experiment, we chose the three most potent ones. These *in vitro* (in the test tube) tests are a good way to assess the antibody potency, but it doesn't tell us if these antibodies can actually prevent infections of people or animals. Thus, the only way to test if the antibodies will work in people is to actually test them, first in animals (preclinical trials), then in humans (clinical trials).

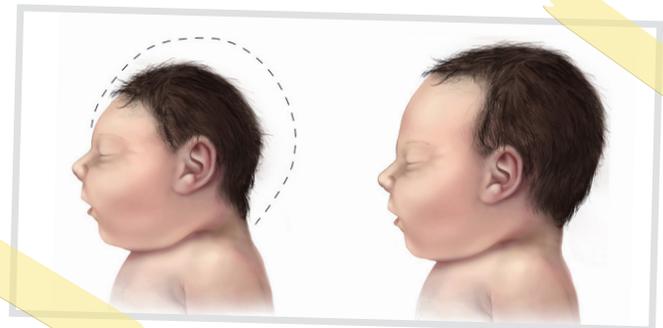


Figure 2:
Comparison between a normally born child and a child with microcephaly

For our preclinical trials, we picked eight Indian rhesus macaques (*Macaca mulatta*). To four of these macaques we gave a mix of the three Zika antibodies (test group) and to the other four we gave control antibodies which could not neutralize Zika infection (control group). On the next day we injected all of the macaques with the virus.

The only thing left was to monitor for several days:

- the antibody levels in the animals, using *ELISA* method
- their ability to prevent Zika infection, using a *neutralization test*
- the presence of the virus in the macaques' blood, using *RT-qPCR*

Results

Our mix of antibodies showed promising results. The levels of the antibodies remained high enough (for viral neutralization) even 14 days after we injected them. Using a neutralization test, we also tested the macaques' blood serum ability to neutralize Zika virus and it turned out that even when highly diluted, it still does the trick (Fig. 3).

Do higher or lower serum dilutions give better neutralization? What about higher or lower serum concentrations?

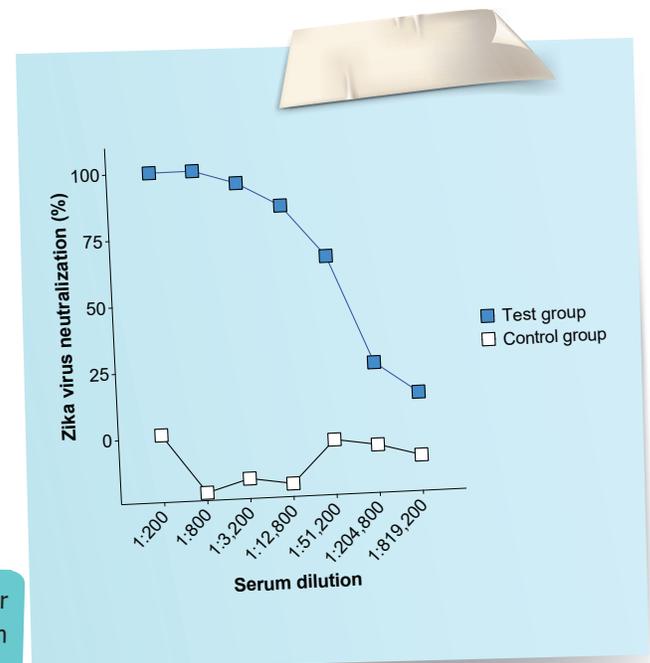
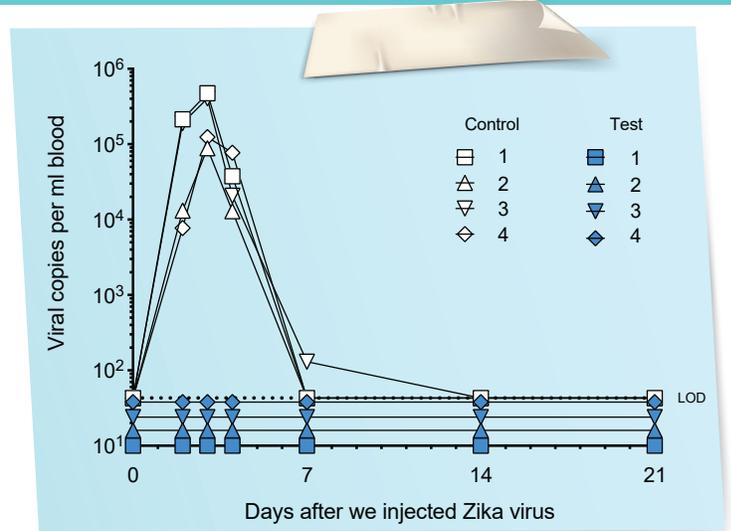


Figure 3:
Ability of macaques' blood serum to neutralize Zika virus

The antibodies prevented Zika virus replication in all macaques in our test group (Fig. 4). In contrast, we detected a large amount of virus in the animals of the control group.

Are there any viruses in the blood of the test group?

Figure 4:
Detection of Zika virus in the blood of both the test group and the control group of macaque monkeys



Discussion

Vaccines for Zika virus, like all vaccines, will probably take years to develop. A vaccine that's safe for pregnant women may take even longer (testing vaccines for pregnant women is a very tricky issue). Moreover, Zika virus and its close relatives (like dengue virus and West Nile virus) make it even harder, due to possible antibody-dependent enhancement.

For the curious:

For example, if you've had a mild dengue virus infection, you've developed antibodies against that virus. But if you then get infected with another type of dengue virus, these antibodies work

like a Trojan horse against you and the infection might become really severe, even life-threatening. The same can occur with Zika virus as scientists have described how dengue and West Nile virus antibodies enhance Zika virus infection in laboratory conditions.

That is why we think our solution to the problem may be of great help. A single injection with this mix of antibodies could effectively prevent defects in unborn children caused by the Zika virus. Of course, a lot more testing lies ahead of us as we still haven't tried the antibodies in pregnant macaques.

Conclusion

Zika virus disease is a mosquito-borne infection, so you can get it through a mosquito bite in areas where Zika virus is present. **The best way to prevent the infection is to avoid mosquito bites.** Wear long sleeved-shirts and long pants, drain standing water where mosquitoes breed, and use

insect repellents. It is important to note that the mosquitoes which spread Zika virus bite during the day as well as during the night. And they spread other infections as well so don't underestimate them!

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AMCA: Mosquito-borne diseases

<http://www.mosquito.org/page/diseases>

EPA: Find the repellent that is right for you

<https://www.epa.gov/insect-repellents/find-repellent-right-you>

Glossary of Key Terms

Antibodies – proteins which can recognize a part of a foreign target (virus, bacteria, etc). Antibodies can neutralize this target directly or they can tag it for further attack by other parts of the immune system. Example: If you had Hepatitis A as a kid, you developed antibodies against the virus and you can't get this disease again.

Antibody-dependent enhancement – antibodies against one virus allow the development of a severe disease when infection with another, closely related virus occurs. Example: Antibodies against dengue virus serotype 1 facilitates a severe dengue virus serotype 2 disease.

Cell cultures – removed cells (from humans, animals, plants) which grow in an artificial media. For example, virologists use culture cells which they infect with viruses to study viral properties.

Dilution – a liquid with a specific concentration of a biological material. E.g. Scientists prepared 10 different dilutions of antibodies for their experiment.

ELISA (Enzyme-Linked Immunosorbent Assay) – a test in which scientists use antibodies as indirect indicators for a present or past infection. For example, if you had measles as a kid, your body developed antibodies against the virus. Virologists can detect these antibodies even after many years.

In vitro – refers to the technique of performing a given procedure in a controlled environment outside of a living organism (usually in a test tube. "In vitro" is latin for "in glass").

Microcephaly – abnormally small head in babies with an often underdeveloped brain. Zika virus and Rubella virus can cause microcephaly in newborn babies.

Neutralization test – scientists put viruses and different dilution of antibodies together in cell cultures to test the ability of the antibodies to prevent infection.

Repellents - a chemical that is used to stop insects (and sometimes ticks) from biting you. Most commonly the chemical is DEET.

RT-qPCR (quantitative reverse transcriptase polymerase chain reaction) – PCR is a method for creating multiple copies of a specific part of the genetic material. By making a lot of copies, we can visualize them and eventually identify them. RT-qPCR is a type of PCR test that uses RNA as a template.

Transfection - scientists introduce nucleic acids (DNA or RNA) into eukaryotic cells. For example, scientists can insert specific DNA (which codes a specific product of interest) into a cell which will serve as a factory for this product.

Vaccine - a person receives parts of a virus/bacteria or weakened versions of pathogens and develops antibodies against them: the immune system now knows how to fight this type of infection. For instance, most children receive Measles, Mumps and Rubella vaccine (MMR) to prevent getting these diseases in the future.

Check your understanding

- 1 Why is ELISA an indirect method for detection of viral (and other) infections?
- 2 You shared popcorn with someone who turned out to have Hepatitis A, a disease you never had. Your doctor suspects you have an infection, so he gives you an injection of Immunoglobulin, containing specific hepatitis A virus antibodies. What kind of immunity will this injection induce (active or passive)?
- 3 When a pregnant woman receives a tetanus-diphtheria-pertussis (Tdap) vaccine, she passes on her antibodies she produces to the unborn child through the placenta. The antibodies protect babies who are too young to get the vaccine. What kind of immunity is this?
- 4 What is the most efficient way to prevent Zika virus disease?