CLINICAL IMPLICATIONS OF BASIC RESEARCH

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Zika Virus Vaccines — A Full Field and Looking for the Closers

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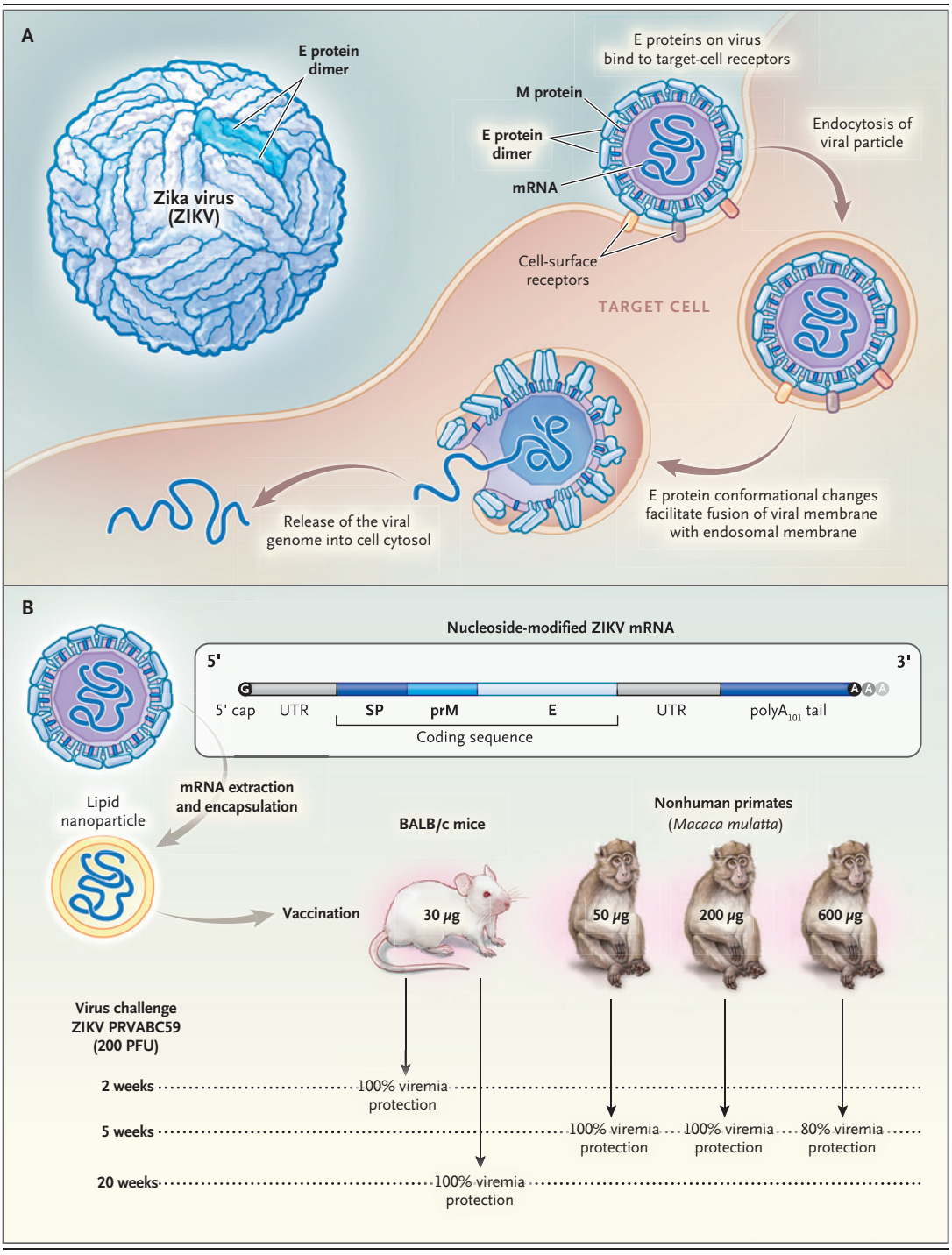
The Zika virus (ZIKV) epidemic, which started in 2015, is having a considerable effect on global public health, blood-product safety, and international travel and is further fueling the debate on elective termination of pregnancy. ZIKV infection is the latest infectious disease to reveal our limitations in preparing for and responding to biologic threats. The most profound consequence of the epidemic is the large number of congenital malformations that are known to be associated with or caused by ZIKV infection. Furthermore, as children who were exposed to ZIKV in utero grow older, new developmental abnormalities are being identified, extending the effects of the epidemic. According to a recent World Health Organization (WHO) report, 61 areas have reported ongoing ZIKV transmission since 2015, with 31 countries reporting congenital malformations that are potentially associated with infection. It is unclear whether ZIKV transmission will become endemic with seasonal peaks, like dengue, or be more episodic in nature.

There are no licensed antiviral drugs to prevent or treat ZIKV infection or disease, although groups are exploring the possibility of repurposing existing drugs and developing new compounds. There exists no licensed vaccine to prevent ZIKV infection. Once infection has occurred, diligent clinical monitoring and supportive care are the mainstays of treatment. Caring for patients with severe ZIKV disease manifestations, especially patients who were exposed in utero, is challenging for all involved and requires a substantial allocation of health care resources that are often limited in their availability. Because of these challenges, the WHO has called for development of a ZIKV vaccine, with an initial focus on protecting women of childbearing age.

Two recent reports describing the successful testing of experimental ZIKV vaccines in animal models — one by Pardi et al.[1] and another by Richner et al.[2] — are welcome news. Both groups engineered messenger RNAs (mRNAs) with sequences encoding the ZIKV precursor membrane (prM) glycoprotein and envelope (E) glycoprotein. The E protein is critical to viral attachment, entry, and replication in the infected host (Fig. 1A), which makes it a rational vaccine target. Neutralizing antibodies directed against the E protein have been identified as correlates of protection for vaccines directed against other flaviviruses, such as the Japanese encephalitis, yellow fever, and tickborne encephalitis viruses.[3]

Pardi et al. developed a nucleoside-modified mRNA vaccine candidate that was based on the prM–E sequence of a French Polynesian 2013 ZIKV strain and formulated the vaccine with lipid nanoparticles. A modified nucleoside was used to reduce indiscriminate innate immune responses after vaccination and to increase protein translation, and the lipid nanoparticles were designed to ensure prolonged protein expression. (Nucleoside molecules are the fundamental "building blocks" of nucleic acids like mRNA.) The authors vaccinated two different strains of mice (C57BL/6 and BALB/c), observed no acute safety events, and subsequently detected E-protein–specific binding IgG antibodies and neutralizing antibodies (Fig. 1B). The C57BL/6 mice were also found to have antigen-specific CD4+ T cells after vaccination. The vaccinated mice were challenged with a Puerto Rican 2015 ZIKV strain 2 or 20 weeks after vaccination. All vaccinated mice were protected from viremia (i.e., their blood tested negative for ZIKV RNA). Nonhuman primates (macaque monkeys) were then vaccinated with one of three different doses (from 50 μg to 600 μg); they had no acute safety events and had development of E-protein–specific binding IgG antibodies and neutralizing antibodies, but with-



out a dose effect. When five immunized monkeys and six control monkeys were challenged with Puerto Rican ZIKV 5 weeks after vaccination, all the control monkeys became infected, whereas four of the five vaccinated monkeys were protected from viremia; a single vaccinated monkey had transient low-level viremia 3 days after challenge.

Richner et al. used the prM–E sequence from a Micronesian 2007 ZIKV strain, the signal sequence from human IgE (IgE_{sig}), a modified nucleoside, and enzymatically synthesized mRNA

Figure 1 (facing page). The ZIKV E Protein and a Nucleoside-Modified mRNA Vaccine Candidate.

Envelope (E) glycoprotein of the Zika virus (ZIKV) interacts with receptors on the surface of target cells (Panel A), promoting viral entry, processing, and ultimately replication. The presence of a sufficient quantity of high-quality antibody directed against the E protein may neutralize the virus and reduce or prevent the replication process. Virus neutralization and decreased replication may abort infection or prevent or substantially attenuate disease. A vaccine capable of inducing robust neutralizing antibodies may also reduce the likelihood of transmission between persons and populations. Pardi and colleagues[1] based their vaccine candidate on messenger RNA (mRNA) encoding for French Polynesian 2013 ZIKV precursor membrane (prM) and E glycoproteins (Panel B). Nucleoside modification and the addition of lipid nanoparticles formed a ZIKV mRNA–lipid nanoparticle vaccine, which was tested in mice and in nonhuman primates. At varying points after vaccination, BALB/c mice and nonhuman primates were challenged with a 2015 Puerto Rican ZIKV strain. Neutralizing and binding antibodies developed after vaccination, and high levels of protection against challenge were found. PFU denotes plaque-forming units, SP signal peptide, and UTR untranslated region.

packaged in lipid nanoparticles in their experimental vaccine. They generated additional mRNA constructs by introducing mutations in or near viral DNA encoding the fusion loop of the E protein or by replacing the IgE signal sequence with one from Japanese encephalitis virus (JEV_{sig}). These modifications were made to increase the efficiency of protein production and to minimize the generation of cross-reactive — and, in theory, potentially enhancing — ZIKV antibodies directed against the highly conserved and dominant flavivirus fusion-loop epitope. ("Enhancing" antibodies do not neutralize infection but instead cross-react with and enhance infection by viruses that share an epitope with the immunizing virus or viral antigen.)

On testing of the IgE_{sig}–prM–E vaccine in mice (of strain AG129) either as a single dose or as a two-dose regimen, Richner et al. found E-protein–specific neutralizing antibodies. A higher dose (10 μg) outperformed a lower dose (2 μg) when administered as a single dose, and the two-dose regimens were superior to single-dose regimens. Six weeks after vaccination, the mice were challenged with a 1966 Malaysian ZIKV strain; all the mice that received the higher single dose or either two-dose regimen survived, and 60% of the mice that received the low single dose survived. Similarly, a two-dose, 10-μg regimen fully protected C57BL/6 mice against challenge with a 1984 Dakar ZIKV strain (none of these mice had viremia 5 days after challenge, and none died), whereas only 30% of the control mice survived.

BALB/c mice that were immunized and boosted with both wild-type and fusion-loop mutants of IgE_{sig}–prM–E or JEV_{sig}–prM–E candidates had production of similar neutralizing antibody titers. The JEV_{sig}–prM–E vaccine provided complete protection against viremia when vaccinated mice were challenged with a 1984 Dakar ZIKV strain 13 weeks after vaccination; breakthrough viremia was observed in the group of mice that were vaccinated with IgE_{sig}–prM–E. In vitro experiments revealed that the "fusion-loop" mutations reduced enhancing antibody production, and studies involving a mouse model of dengue antibody enhancement showed significantly lower morbidity and mortality in association with both the E_{sig}–prM–E fusion-loop and JEV_{sig}–prM–E fusion-loop vaccine candidates.

Data from studies in animals have now been described for numerous ZIKV vaccine candidates, which have been developed with the use of approaches that harness ZIKV DNA, protein subunit, adenovirus vectors, inactivated whole virions, and now mRNA.[4-7] The candidates produced no acute safety signals, induced ZIKV-specific humoral or cellular immune responses, and conferred at least some protection against live virus challenge. The mRNA vaccine constructs reviewed here offer numerous potential advantages, including ease and cost of manufacturing, applicability across diverse pathogens, and a favorable safety profile. Vaccinology, however, constantly warns against extrapolating conclusions from animal experiments to humans.

In the case of ZIKV vaccines, most of the available data have been generated with the use of animals that have had no previous exposure to flaviviruses; these animals are not representative of most human populations, which will probably be immunized once a vaccine is available. Will preexisting immunity to flaviviruses (such as the dengue, yellow fever, West Nile, and Japanese encephalitis viruses) affect the safety or immunogenicity of a ZIKV vaccine? Disease enhancement resulting from the immunologic interplay between ZIKV infection or vaccination

and other endemic flaviviruses has been proposed as a theoretical concern. This concern is based largely on data from in vitro studies and studies of small animals, but in vivo studies of ZIKV in nonhuman primates have not recapitulated these observations of "enhancement." Prospective studies, most likely with large sample sizes, will be required in order to most appropriately explore the concept of immune enhancement in ZIKV infection.

Past successes with other flavivirus vaccines, together with more recently obtained ZIKV data, suggest that perhaps neutralizing antibodies will be required and are sufficient to confer protection against ZIKV. What is the immune profile required to protect a pregnant woman and her fetus from disease or to prevent long-term persistence of ZIKV in fluids such as semen?

Whole-virion inactivated vaccines (which I have experience in developing), live attenuated recombinant vaccines, and DNA vaccines against ZIKV are now being tested for safety in humans. ZIKV infection may be followed by adverse neurologic outcomes, such as the Guillain-Barré syndrome or acute myelitis. The pathophysiological processes underlying these less common clinical outcomes are incompletely understood, but it has been theorized that antibodies that develop in response to ZIKV infection also recognize and target the epitopes of antigens expressed by human nervous system tissues, which may appear similar to those on ZIKV. If this is the case, vaccine developers will need to closely monitor vaccine recipients for adverse events of potential neurologic origin.

Demonstrating safety in a small number of volunteers appears feasible; demonstrating that vaccine-induced immune responses are associated with clinical efficacy will be a much more formidable task. If a vaccine is found to be safe and efficacious, producing sufficient quantities to meet the projected global need (i.e., many millions of doses) may ultimately be the most difficult undertaking.

Despite the challenges, the pace of ZIKV vaccine research and development has been impressive. If past successes with flavivirus vaccines are a guide and ZIKV behaves more like the encephalitic flaviviruses and less like dengue there would be cause for optimism. However, history has shown that the race for a vaccine typically begins with many contenders at the start, of whom very few finish the race. This observation notwithstanding, the recently published data from Pardi et al. and Richner et al. represent an important step toward the goal of protecting people from ZIKV through active immunization.

Disclosure forms provided by the author are available at NEJM.org.

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