Flavivirus DNA vaccine with a kick

Alan D T Barrett

Immunization with single-round infectious particles enhances potency of a DNA vaccine against West Nile virus.

Dengue[1] and West Nile[2–4] viruses are major human pathogens for which there are no approved vaccines or antiviral drugs. Concerns about the safety of vaccination with inactivated or live attenuated viruses have stimulated interest in DNA vaccines, but thus far these have not proved sufficiently immunogenic. In this issue, Chang *et al.*[5] describe a strategy for boosting the efficacy of a DNA vaccine against West Nile virus (**Fig. 1**). Their vaccine, which generates immunogenic viral particles that undergo one round of replication and infection, confers better protection against challenge by West Nile virus in mice compared with a conventional DNA vaccine.

Dengue is caused by four serologically and genetically related viruses and is the most common arthropod-borne viral disease in humans, with >50 million infections annually, including ~500,000 cases of a severe form of infection called dengue hemorrhagic fever. West Nile virus was introduced into North America in 1999 and causes annual epidemics of neurologic disease in the United States[6], occasional outbreaks in Southern Europe and isolated cases in Central and South America. Although there are effective vaccines to control infection by flaviviruses such as the Japanese encephalitis, tick-borne encephalitis and yellow fever viruses[7], the most advanced candidate vaccines for dengue and West Nile viruses are in phase 2 clinical trials and still some way from licensure[8].

For dengue, the most advanced candidates are a tetravalent live attenuated vaccine and a tetravalent live chimeric virus based on the yellow fever 17D vaccine backbone. In the latter, the membrane and envelope protein genes have been replaced by those of each of the four

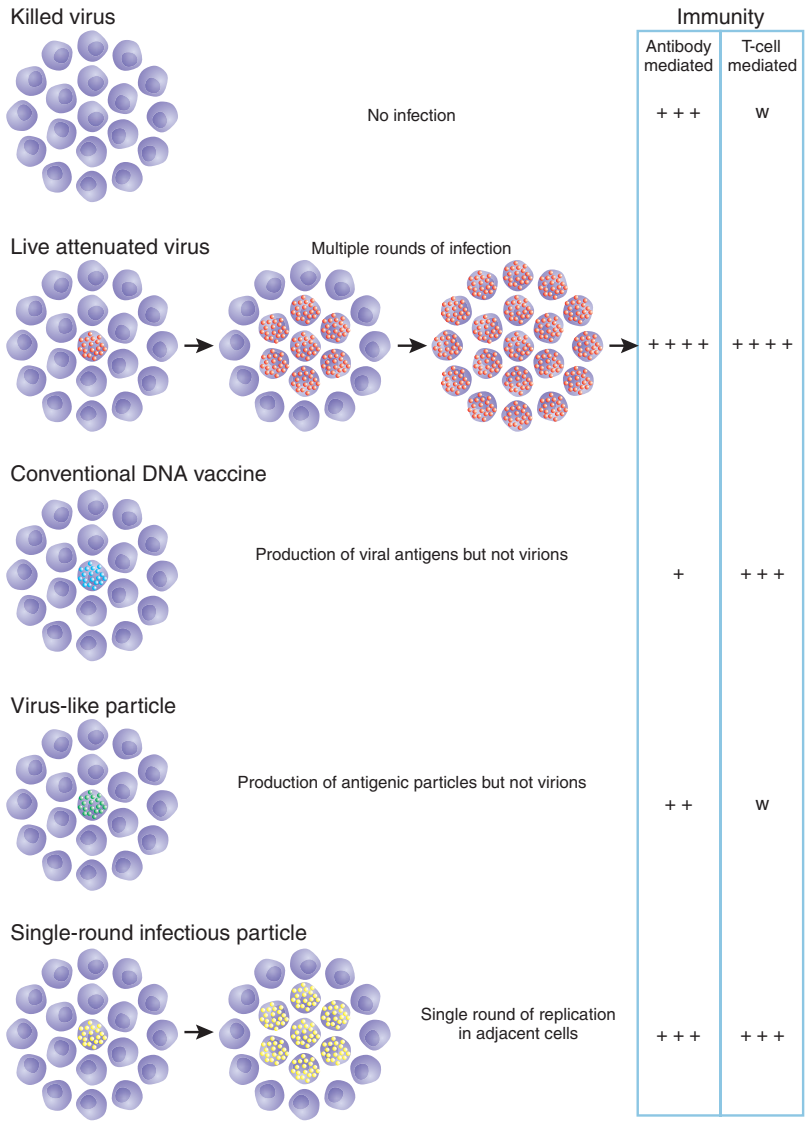

Figure 1 Comparison of the mechanisms associated with different vaccination strategies. Whereas immunization with killed virus elicits mainly antibody-mediated immunity, and delivery of conventional DNA vaccines confers primarily cell-mediated immunity, a 'split-genome' DNA construct generates single-round infectious particles that generate both humoral and cell-mediated protection almost as potent as the response to a live attenuated virus. Single-round infectious particles eliminate concerns about the safety of infection with live attenuated viruses. w, weak response.

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dengue viruses. These candidate vaccines seem promising, but studies are still underway to determine whether they can confer a balanced immune response against all four dengue viruses and avoid immunological interference. For West Nile virus, the lead candidate vaccines—a DNA vaccine and a chimeric yellow fever 17D virus—also appear promising, but there is still no consensus on what constitutes a long-term protective immune response.

Chang *et al.*[5] build on earlier work by Kofler *et al.*[9,10], who showed that the tick-borne encephalitis flavivirus still forms immunogenic virus particles even when much of the capsid gene sequence has been deleted. Using Kunjin, a subtype of West Nile virus found in Australia, Chang *et al.*[5] have developed a 'split-genome' vaccine that generates two RNA species, one encoding the entire Kunjin virus genome except the capsid gene and the other encoding only the capsid gene. As both RNAs are encoded on the same DNA plasmid under the control of two cytomegalovirus promoters configured in a back-to-back orientation, transfected cells transcribe and translate all the viral genes. The capsid protein acts as a helper to assemble virus particles containing the viral genomic RNA lacking the capsid gene. These so-called single-round infectious particles (SRIPs) then infect adjacent cells (**Fig. 1**), in contrast to DNA vaccines that produce viral antigens only in the cells initially infected. Because the viral genome transmitted to neighboring cells does not encode capsid protein, no further viral replication can occur.

Chang *et al.*[5] compare the immunogenicity of SRIPs in mice to a live virus, a traditional DNA vaccine (encoding the viral genome, with the exception of functional capsid) and a DNA vaccine that produces virus-like particles composed of the pre-membrane and envelope proteins. SRIPs confer a superior antibody-mediated immune response in mice and horses, as well as protective immunity in mice, at lower doses of DNA compared with the traditional DNA vaccine. $CD8^+$ T-cell responses elicited by SRIPs in mice were also significantly greater than those produced by the virus-like particle vaccine, although smaller than those following immunization with live virus. Neutralizing antibodies are considered critical for achieving protective immunity, but it is clear that a vaccine must elicit both antibody- and cell-mediated immunity to ensure long-term protection.

Although these results represent an important proof of concept of a technology that should in theory be applicable to any flavivirus, a couple of important points should be considered. First, comparison of DNA-based vaccine strategies is very difficult given the many variables involved (e.g., viral strain, viral gene(s) selected, different parental virus strains and codon optimization). Second, it remains to be seen whether the present findings translate to primates. Several candidate DNA vaccines have performed impressively in lower animals only to disappoint in clinical trials. Prospects for using a SRIP-based approach in veterinary vaccines, such as those against Japanese encephalitis and West Nile virus infections of horses, seem more promising in the short term, especially as killed vaccines do not induce long-term protective immunity and booster doses are required to maintain immunity.

The major issues surrounding new candidate vaccines always concern efficacy and safety. With regard to efficacy, we know that current licensed flavivirus vaccines have neutralizing antibody as the correlate of protection and that only low levels of neutralizing antibodies are required for protective immunity. We do not know whether this will be true for vaccines against dengue and West Nile viruses—and even if it is, as most investigators believe, it is unclear what level of neutralizing antibodies will be required. This question is particularly complicated for a dengue vaccine, as the disease is caused by four genetically and serologically related viruses. For a tetravalent vaccine, higher levels of neutralizing antibodies might be needed to control four viruses simultaneously. Candidate vaccines, such as those involving SRIPs, may help achieve this goal, possibly through a prime-boost regimen, although it remains to be shown that SRIP-based vaccines are effective over the long term.

In the 21st century, safety has become the paramount attribute of a vaccine, even more so then efficacy, as society will not accept any adverse events associated with a vaccine. In this study, Chang *et al.*[5] have boosted efficacy using viral particles that have clear safety advantages over live attenuated vaccines.

COMPETING INTERESTS STATEMENT
The author declares competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturebiotechnology/.

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Customized signaling with reconfigurable protein scaffolds

Patrick Guye & Ron Weiss

Engineering protein scaffolds creates signaling networks with novel properties.

The emerging field of synthetic biology aims to design sophisticated biological systems that exploit diverse mechanisms for regulating information flow. New functions have been implemented using engineered transcriptional and translational networks, but little progress has been achieved in constructing protein-protein networks with complex connectivities. A recent report in *Science* by Lim and colleagues[1] addresses this challenge with a general approach for controlling signaling by protein scaffolds, as demonstrated by engineering of the pheromone response of the mitogen-activated protein kinase (MAPK) pathway in yeast.

The ability to design and implement sophisticated information-processing circuits was fundamental to the success of the computer revolution. To obtain a desired behavior, a circuit designer connects well-characterized components and modules into particular topologies that actuate the behavior. Similarly, in biological systems, connections between regulatory components (that is, circuit topology) help determine how cells process and react to information.

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