

CLINICAL IMPLICATIONS OF BASIC RESEARCH

Response to Dengue Fever — The Good, the Bad, and the Ugly?

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Dengue may be the most widespread arboviral illness worldwide. Most patients with dengue infection have only mild disease or classic dengue fever, with influenza-like symptoms, severe headache, and aching joints and muscles. However, in a small percentage of patients — maybe half a million people every year — potentially lethal forms of dengue called dengue hemorrhagic fever and dengue shock syndrome develop. It has been known for many years that antibodies directed against either of the two surface proteins of the dengue virus (the precursor membrane protein and the envelope protein) can neutralize infectivity and confer protection, although envelope protein is known to be the more important protective antigen. Paradoxically, antibodies against either protein can also enhance infectivity, depending on their specificity, avidity, and concentration. Dejnirattisai et al.¹ recently reported that after a secondary dengue infection, a large proportion of the human antibody repertoire is directed against the precursor membrane protein on immature virus particles. This observation might be pertinent to the development of inactivated dengue vaccines.

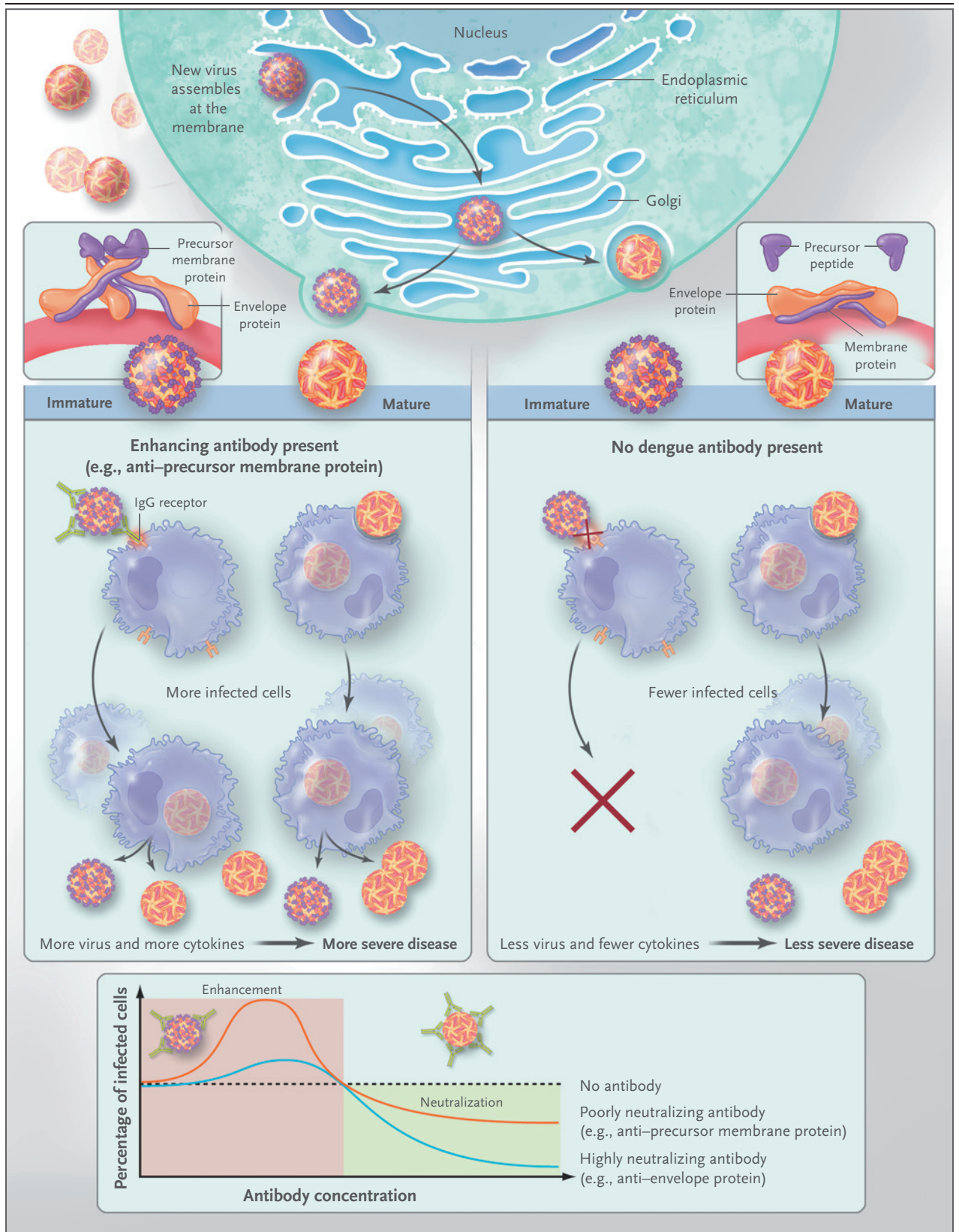
Two to three billion people in tropical and subtropical climates are at risk for dengue infection, and an estimated 50 million infections occur each year, mostly in urban areas. The four dengue serotypes are transmitted by mosquitoes, and the ongoing spread of serotypes and of the vectors (mosquitoes) has now reached more than 100 countries, including the United States. The clinical picture of dengue fever is highly variable, particularly with age. Young children might simply have fever and a rash, whereas classic dengue fever is more likely to develop in older children and adults. If dengue fever progresses to dengue hemorrhagic fever at the time of defervescence, it generally manifests as a capillary leak syndrome accompanied by thrombo-

cytopenia and abnormalities in coagulation and in liver function, potentially resulting in shock, bleeding, and organ failure.

Lifelong immunity against the infecting serotype (so-called homotypic immunity) is conferred by virus-neutralizing antibodies. Antibodies induced by one serotype can cross-react with the other serotypes (heterotypic immunity), but heterotypic neutralizing titers wane over time. Below a certain threshold and depending on antibody specificity, antibodies fail to neutralize and instead opsonize; this helps the virus to infect cells bearing IgG receptors. This phenomenon, termed “antibody-dependent enhancement,” increases the number of infected cells and is thought to lead to higher virus titers and a more pronounced inflammatory response. Antibody-dependent enhancement has been thought to

Figure 1 (facing page). Antibody-Dependent Enhancement of Dengue Infection.

Dejnirattisai et al.¹ analyzed a panel of monoclonal antibodies from human B cells isolated from patients after secondary dengue infection. Although the majority of monoclonal antibodies against anti-envelope protein neutralized dengue virus effectively, the majority of monoclonal antibodies against anti-precursor membrane protein failed to do so. The precursor membrane antigen is abundant on immature and noninfectious virions, but the precursor membrane protein density decreases as the virion matures and becomes infectious. Mature virus can always — unless neutralized — infect mononuclear cells through clathrin-mediated endocytosis. However, in the presence of non-neutralizing anti-precursor membrane protein antibodies, even immature and otherwise noninfectious virus can enter IgG receptor-bearing cells and replicate efficiently, leading to more infected cells that then produce more virus and more cytokines, potentially contributing to more severe disease. Although monoclonal antibodies against both envelope protein and precursor membrane protein, and even polyclonal convalescent serum, can enhance infection at low concentrations, the anti-precursor membrane protein response might be more likely to enhance disease because it neutralizes poorly.



contribute to dengue hemorrhagic fever² — which, in children and adults, is typically associated with secondary infection with a heterotypic serotype. Also at risk for enhanced disease are infants who are born to dengue-immune mothers and who therefore have maternally derived dengue-neutralizing IgGs. These IgGs protect for several months, but titers wane and infants enter a period of increased risk of dengue hemorrhagic fever after primary infection.

Dejnirattisai et al. generated a large panel of human monoclonal antibodies to dengue viruses from B cells obtained from persons who had recently had secondary dengue infection. More than half the monoclonal antibodies directed against structural proteins reacted with the precursor membrane protein, and less than half reacted with the envelope protein. Most of these monoclonal antibodies were cross-reactive among serotypes. Monoclonal antibodies against anti-precursor membrane protein in general were less likely than anti-envelope protein monoclonal antibodies to completely neutralize infectious virus, even at high concentrations and regardless of the dengue serotype examined. Several monoclonal antibodies against anti-precursor membrane protein neutralized no more than half of the infectious virus, suggesting that at least two populations of virions may coexist: one that is susceptible to precursor membrane protein-mediated neutralization and another that is not. As has been described for certain anti-envelope protein antibodies, all the anti-precursor membrane monoclonal antibodies were able to mediate antibody-dependent enhancement.

Knowledge of the structural biology and immunogenicity of flaviviruses has led to an understanding of the interplay among virus maturation, infectivity, and antibody binding.³ Viral morphogenesis initiates at the endoplasmic reticulum, where immature virions bud into its lumen. Three pairs of precursor membrane proteins and envelope proteins form spikes that stick out of the virus envelope at this early stage (Fig. 1). Precursor membrane protein associates with envelope protein and covers a domain on the envelope protein (the fusion loop) that would otherwise mediate fusion of the virus envelope with cellular membranes at a low pH. The precursor membrane protein thus prevents premature activation of envelope protein. As the virion matures

in the acidic trans-Golgi network, the precursor membrane and envelope protein trimeric spikes rearrange into dimers, abolishing the spikes and yielding a smooth virus surface. A cellular protease cleaves the precursor peptide from the precursor membrane protein, and once the virion buds from the surface of the cell, the precursor peptide is released, the fusion domain of the envelope protein is unmasked, and the virion is ready to infect. However, processing of the precursor membrane protein to the mature membrane protein is not always efficient, and precursor membrane protein-containing immature or partially mature viruses with decreased infectivity are produced as well (Fig. 1).

Inhibition of the cleavage of the precursor membrane protein by Dejnirattisai et al.¹ and others⁴ resulted in dengue virus preparations containing mostly immature and noninfectious virus particles. However, anti-precursor membrane monoclonal antibodies were able to enhance the infectivity of these immature virions as well by helping them to enter IgG receptor-bearing cells, where they could be activated once inside. The results reported by Dejnirattisai et al.¹ and others³ suggest that functionally different virus populations coexist: a population with a high density of precursor membrane protein that is immature and noninfectious unless opsonized by anti-precursor membrane protein antibodies, a population with an intermediate density of precursor membrane protein that is infectious but can be neutralized by anti-precursor membrane (or anti-envelope protein antibodies), and finally a population of mature virions that cannot be neutralized by anti-precursor membrane protein antibodies yet can be neutralized by anti-envelope protein antibodies.

Much progress has been made in understanding dengue; a small-animal model of dengue hemorrhagic fever is available, and promising vaccines are in development.⁵ Although there is still a great deal of work ahead to understand dengue immunity and pathogenesis, there is also cause for optimism. The studies by Dejnirattisai et al.¹ and others⁴ suggest a role for immature dengue virus particles in secondary infection in which they become infectious in the presence of non-neutralizing antibodies and thus contribute to the development of dengue hemorrhagic fever. The authors suggest that it may therefore be ad-

visible to design dengue vaccines that minimize the anti-precursor membrane protein response — by generating viruses with dengue envelope protein and non-dengue precursor membrane protein, for example. However, it is unknown whether such chimeras would be viable. More importantly, vaccine developers need to ensure that the tetravalent dengue vaccines currently in development induce a strong, long-lasting, and neutralizing antibody response against all four serotypes, probably relying heavily on envelope protein as the protective antigen. It is too early to tell whether the induction of anti-precursor membrane immunity can be prevented (for example, by reducing the precursor membrane protein content of inactivated vaccines) or whether blocking antibody binding to precursor membrane protein can be used to treat dengue hemorrhagic fever. Clinical studies that characterize the immune and inflammatory response at the

time of secondary infection are needed to better understand the correlates and causes of severe disease.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org

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