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REVIEW

Study on immunity of dengue virus and dengue vaccine development

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Abstract In the study of vaccines for dengue viruses, which are multivalent immunization, genetically and antigenically distinct, we should have more sophisticated understanding of viral immune physiology. Because the immune response to dengue and its role in the pathophysiology of dengue fever and dengue hemorrhagic fever are multifaceted, several different efforts have been made to engineer a protective vaccine. Because of space limitations, this review is focused only on vaccines that have emerged from preclinical studies into clinical trial.

Keywords dengue fever, immunology, T cells, vaccines

1 Introduction

Dengue virus (DENV) infection is one of the most prevalent infectious tropical diseases, responsible for up to 100 million infections annually. Most patients infected with dengue show no symptoms or get dengue fever-a self-limiting flu-like illness. However, approximately 1% of the cases turn into dengue hemorrhagic fever (DHF), a serious condition in which blood leaks from capillaries and collects in body cavities. Nearly 1 in 20 patients with this condition dies, and most victims are children (Shekhar, 2007). Now, dengue has become impossible to eradicate and difficult to control because of massive urbanization, overpopulation, substandard living conditions, increased regional and international travel, failure to sustain mosquito control programs, and the global emergence of more virulent genotypes of DENV (Edelman, 2007). The World Health Organization has classified dengue as a major international health concern and agreed that a dengue vaccine is urgently needed.

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2 Immunity and pathogenesis in DENV infection

The DENV belongs to the family of Flaviviridae. It is a kind of single-stranded non-segmented RNA virus that has four serologically distinct serotypes (DENV-1, DENV-2, DENV-3, and DENV-4). Genetic studies of sylvatic dengue strains provide evidence that the four DENVs evolved from a common ancestor in subhuman primate populations and that, around 500 years ago, all viruses emerged separately into a human urban transmission cycle (Halstead, 2007). These four subtypes are different strains of DENV that have 60% to 80% homology with one another. The major difference for humans lies in subtle differences in the surface proteins of the different dengue subtypes (Kautner et al., 1997). All four serotypes are identical in gene structure, consisting of three structural proteins—capsid (C), premembrane (prM), and envelope (E), and seven nonstructural proteins—NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 (Holman et al., 2007).

2.1 The antigen-antibody responses

The antibody response to the envelope (E) glycoprotein of DENV is known to play a critical role in both protection from and enhancement of disease, especially after primary infection. However, the relative amounts of homologous and heterologous anti-E antibodies and their epitopes remain unclear (Holman et al., 2008).

The membrane precursor (prM) is believed to aid in the folding of the E glycoprotein. The murine monoclonal antibodies (Mabs) reactive against the prM glycoproteins of DENV were used to passively protect mice *in vivo* against lethal challenge with homologous and heterologous DENV serotypes. The results show that they protected mice from DENV challenge; however, although *in vitro* binding to virions was readily demonstrated, Mabs

had detectable neutralizing activity to only part of the prM. The neutralizing activity could not be enhanced by anti-mouse immunoglobulin or complement (Kaufman et al., 1989). The mechanism of this protection and its relevance to natural protective immunity are uncertain.

The NS1 protein is also an important target of antibodies against DENV. NS1 is expressed on the surface of infected cells and is also secreted into the circulation as a soluble multimer (Rothman, 2004). The presence of high levels of secreted NS1 in the sera of patients experiencing secondary DENV infections, and in the context of an anamnestic antibody response (Young, 2000), suggests that NS1 may contribute significantly to the formation of the circulating immune complexes that are suspected to play an important role in the pathogenesis of severe dengue disease.

DENV serotypes can be distinguished by virus-neutralizing antibodies, although non-neutralizing antibodies against the E protein and nonstructural proteins, such as NS1 and NS3, are cross-reactive (Wilder-Smith and Deen, 2008).

2.2 Specific T-cell responses

As with other viruses, the $CD4^+$ and $CD8^+$ T-cell response to DENV is directed against multiple viral proteins. Virus-specific antibody responses require help from $CD4^+$ T lymphocytes. E protein-specific $CD4^+$ cytotoxic T lymphocyte may be important mediators of virus clearance especially during reinfection with the same serotype as that in primary infection by providing help for virus-specific antibody production and lysis of virus-infected cells (Livingston et al., 1994).

It has been reported that the characterization of DENV-specific serotype cross-reactive $CD4^+$ and $CD8^+$ human T lymphocytes could predominantly recognize nonstructural proteins (Kurane et al., 1991). One of the nonstructural proteins, NS3, contains multiple dominant T-cell epitopes and seems to be particularly immunogenic (Kurane et al., 1991).

DENV-reactive T cells vary in their abilities to recognize different DENV serotypes, depending on the degree of homology at a given epitope. However, the presence of an early serotype cross-reactive immunoglobulin G (IgG) responsive to secondary DENV infection predicts the existence of serotype cross-reactive memory $CD4^+$ and $CD8^+$ T lymphocytes (Livingston et al., 1994). The extent to which these immune responses contributing to the long-term protective immunity afforded by natural primary DENV infection has not been fully defined. Symptomatic DENV infections, including cases of DHF, can occur despite the presence of antibodies capable of neutralizing *in vitro* infection of epithelial cell lines. Neutralization of infection in monocytic cells (instead of enhancement of infection) may be more strongly associated with protection from DHF (Kliks et al., 1989). There is very little published information on the association of specific T-cell responses with protection. However, broadly serotype-cross-reactive interferon γ production *in vitro* showed a weak association with mild disease (Rothman, 2004).

2.3 Antibody-dependent enhancement phenomenon

Antibody-dependent enhancement (ADE) is a phenomenon during dengue viral infection. After a person is infected with DENV, he or she develops an immune response to that dengue subtype. The immune response will produce specific antibodies to that subtype's surface proteins that prevent the virus from binding to macrophage cells (the target cell that DENVs infect) and gaining entry. However, if another subtype of DENV infects the individual, the virus will activate the immune system to attack it as if it was the first subtype. The immune system is tricked because the four subtypes have very similar surface antigens. The antibodies bind to the surface proteins but do not inactivate the virus. The immune response attracts numerous macrophages, which the virus proceeds to infect because it has not been inactivated (Kautner et al., 1997). This situation is referred to as ADE of a viral infection. This makes the viral infection much more acute. The body releases cytokines that cause the endothelial tissue to become permeable, which results in hemorrhagic fever and fluid loss from the blood vessels.

Central to the immune-enhancement mechanism in DHF is the usurping of Fc receptors on mature dendritic cells and macrophages as a means of entry for complexes of DENV and subneutralizing antibody (Monath, 2007). These complexes form in the presence of cross-reactive antibody induced by a previous infection with a heterologous dengue serotype or, in infants with maternally derived IgG, when antibody levels drop below neutralizing levels. In such instances, the viral load is increased by means of the infection of an increased proportion of Fc receptor-bearing cells and an increased level of virus in each cell. The T-cell activation and clearance of infected cells by killer cells and cross-reactive cytotoxic T cells then elicits a proinflammatory “cytokine storm” that causes endothelial damage and capillary leakage.

2.4 Animal models

Besides ADE, another central challenge facing dengue research is a lack of animal models to experimentally evaluate the molecular pathophysiology of infection. For instance, although nonhuman primates become infected and develop dengue viremia, they do not exhibit any symptoms of dengue infection. Some dengue research has been performed with genetically engineered immunodeficient mice, but clinical correlation with human disease is tenuous (Hatch et al., 2008). Thus, there is a pressing need for the creation of a valid animal model to study the

immunopathology of DENV to establish such clinical correlations.

3 DENV vaccine development

Development of a safe and effective vaccine against a disease with such strong immunologic ramifications poses considerable challenges. A dengue vaccine must provide reliable and long-term protection against all four serotypes, for two reasons: to protect the individual against diseases resulting from any serotype and to preclude the development of immune-mediated severe disease (Wilder-Smith and Deen, 2008).

3.1 Live attenuated vaccine candidates

Some universities and institutes have used conventional methods to develop DENV vaccine candidates by passage in tissue culture cells. The vaccine candidates of Mahidol University (Bangkok, Thailand) have not achieved a balanced immune response to each of the four components, and systemic symptoms have occurred in recipients of the tetravalent vaccine (Whitehead et al., 2007), so this vaccine will not be discussed further in this review.

The Walter Reed Army Institute of Research (Washington) has evaluated several live attenuated DENVs as vaccines in human volunteers. The vaccine candidates were each passaged in primary dog kidney cells with terminal passages in fetal rhesus lung cells. Four monovalent candidates were combined into a tetravalent formulation and given to flavivirus non-immune adult human volunteers (Sun et al., 2003). Neutralizing antibody seroconversion rates in the volunteers who received a single dose of tetravalent vaccine ranged from 30% to 70% among the four serotypes. Similar to the monovalent vaccines, a second dose of the tetravalent vaccine in the first month was less reactogenic and did not increase seroconversion. A third dose of the tetravalent vaccine in the fourth month resulted in three of four volunteers with trivalent or tetravalent high-titer neutralizing antibody responses (Sun et al., 2003).

3.2 Live attenuated chimeric vaccines

In addition to the classic attenuation by serial passage in tissue culture, recombinant cDNA technology is greatly facilitating the development of live attenuated vaccine candidates for DENVs. One vaccine candidate was rationally designed by Acambis through the genetic engineering of the envelope genes of dengue into a clone of the licensed yellow fever 17D vaccine (Monath, 2007). The resulting live attenuated dengue-yellow fever chimeric viruses elicit antibodies only to dengue. This vaccine has been licensed to Sanofi Pasteur and will soon enter the third-phase trials.

The vaccine candidate of Johns Hopkins Bloomberg School of Public Health, rDEN4△30, also has a promising future. The vaccine is derived from a cDNA clone of DEN-4 and contains a 30-nt deletion in the 3-untranslated region of the virus (Durbin et al., 2001). In a follow-up placebo-controlled second-phase clinical trial, rDEN4△30 was administered as a single inoculation to three separate dose cohorts. The vaccine was well tolerated at all doses studied. No vaccinee developed a dengue-like illness. The vaccine was safe and induced an antibody response that was broadly neutralizing against genotypically diverse DEN-4 viruses (Durbin et al., 2005). The △30 mutation provides a genetic backbone for the creation of chimeric viruses containing the structural genes C, PrM, and E glycoprotein of DEN-1, DEN-2, and DEN-3. Several industrial sponsors in Europe and Brazil have been awarded nonexclusive licenses for the rDEN4△30 formulations (Edelman, 2007). It is a promising vaccine candidate for inclusion in a tetravalent dengue vaccine formulation.

Both 17D and △30 vaccines contain mixtures of four dengue serotypes designed to induce long-lasting neutralizing antibodies. The co-administration of four live viruses is associated with competition among serotypes with respect to replication and the ability to stimulate neutralizing antibodies, probably owing to the activation of toll-like receptors and the induction of innate immunity (Monath, 2007). These effects have been associated with the generation of an incomplete repertoire of neutralizing antibodies after a single inoculation; therefore, complete immunization may require multiple doses.

3.3 Other dengue vaccine candidates

Preclinical development of other candidates is progressing. Examples include inactivated whole virus, infectious DNA or RNA, expression vector-based and naked DNA, and recombinant subunit dengue vaccines (Edelman, 2007).

Clearly, limited understanding of immune correlates of protection and the difficulty of distinguishing cross-reactions from the development of type-specific antibodies create further challenges. Optimistically, although the challenges facing the development of an acceptable vaccine to endemic areas are still there, the pace of vaccine development has recently been increased.

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