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CD4 T cell responses to flaviviruses

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ABSTRACT

Flaviviruses pose an increasing threat to global health with their potential to cause severe disease in millions of people. Protective and long-lived immunity is closely linked to the generation of CD4 T cells, which provide B cell help and support high affinity neutralizing antibody responses. Research performed during the last years revealed important new insights into the antigen specificities and diverse effector functions of CD4 T cell responses to flaviviruses. Moreover, the identification of mechanisms involved in the regulation of T cell specificity and function provides significant advances in our understanding of how durable protective immunity is established.

Here, we summarize what is known about human CD4 T cell responses to flaviviruses, with a special emphasis on CD4 T cells that provide direct help to B cells producing neutralizing and protective antibodies. We review recent progress in the identification of epitope sites in the context of the atomic structures of flavivirus proteins and highlight specific influences that shape the human CD4 T cell response in the context of infection or vaccination. Finally, we discuss challenges facing vaccine efforts to generate appropriate CD4 T cell responses, as well as recent strategies to enhance T cell-mediated antibody responses.

1. Introduction

Flaviviruses are typically transmitted to humans by infected mosquitoes or ticks. The most important human-pathogenic representatives are yellow fever (YF), Japanese encephalitis (JE), West Nile (WN), dengue (DEN), Zika and tick-borne encephalitis (TBE) viruses, which constitute significant health problems in many parts of the world. The expansion of flavivirus endemic areas and their ability to cause epidemics are most dramatically exemplified by the expansion of DEN hyperendemic areas [1], the expansion of WN virus in the Americas after its introduction to New York in 1999 [2] and its emergence in a number of countries of Southern Europe [3], the recent Zika epidemic in the South Pacific and the Americas [4], and the detection of new infection sites of TBE virus in certain parts of Europe (reviewed in [5,6]). It has been estimated that over half of the global population is at risk for infection with one of the four DEN virus serotypes, and YF, DEN, JE, and WN viruses collectively cause millions of infections and tens of thousands of deaths each year [7,8]. Despite this significant disease burden, vaccines exist for only a few flaviviruses (YF, TBE and JE viruses) [9]. The development of a DEN vaccine is especially challenging due to the need to simultaneously induce protection against all four serotypes and the concern that vaccination could predispose to severe disease with low levels of antibody giving rise to enhancement of subsequent infection [10]. Although significant progress has been made with the licensing of the first tetravalent DEN vaccine (Dengvaxia®) [11], the recent results of large scale efficacy trials, which revealed only 30–65% protection despite detectable neutralizing antibodies *in vitro* [12–14], emphasize the critical need to better understand the immune processes underlying protective immunity.

Flaviviruses consist of a nucleocapsid, composed of multiple copies of the capsid protein (C) and the single-stranded, positive-sense RNA genome. The nucleocapsid is surrounded by a lipid bilayer in which two transmembrane proteins are inserted, the envelope glycoprotein E and the membrane protein M (Fig. 1A). The M protein is found as a precursor protein (prM) on immature viruses, which is cleaved during exocytosis before mature virus particles are released from infected cells [15]. In mature flavivirus particles, the E proteins are arranged as ninety dimers that mediate receptor binding and membrane fusion [16]. The E proteins are composed of three domains (domains I, II and III) (Fig. 1a) and show a high level of structural homology among all flaviviruses [15,17–24], but differ by up to 60% at the amino acid level. Because of its important functions in host cell entry, the E protein is the major target of neutralizing antibodies that mediate protection and long-lived immunity following natural infection or vaccination [16]. CD4 T cells play a key role in generating effective immune responses by coordinating antibody production and the development of cellular

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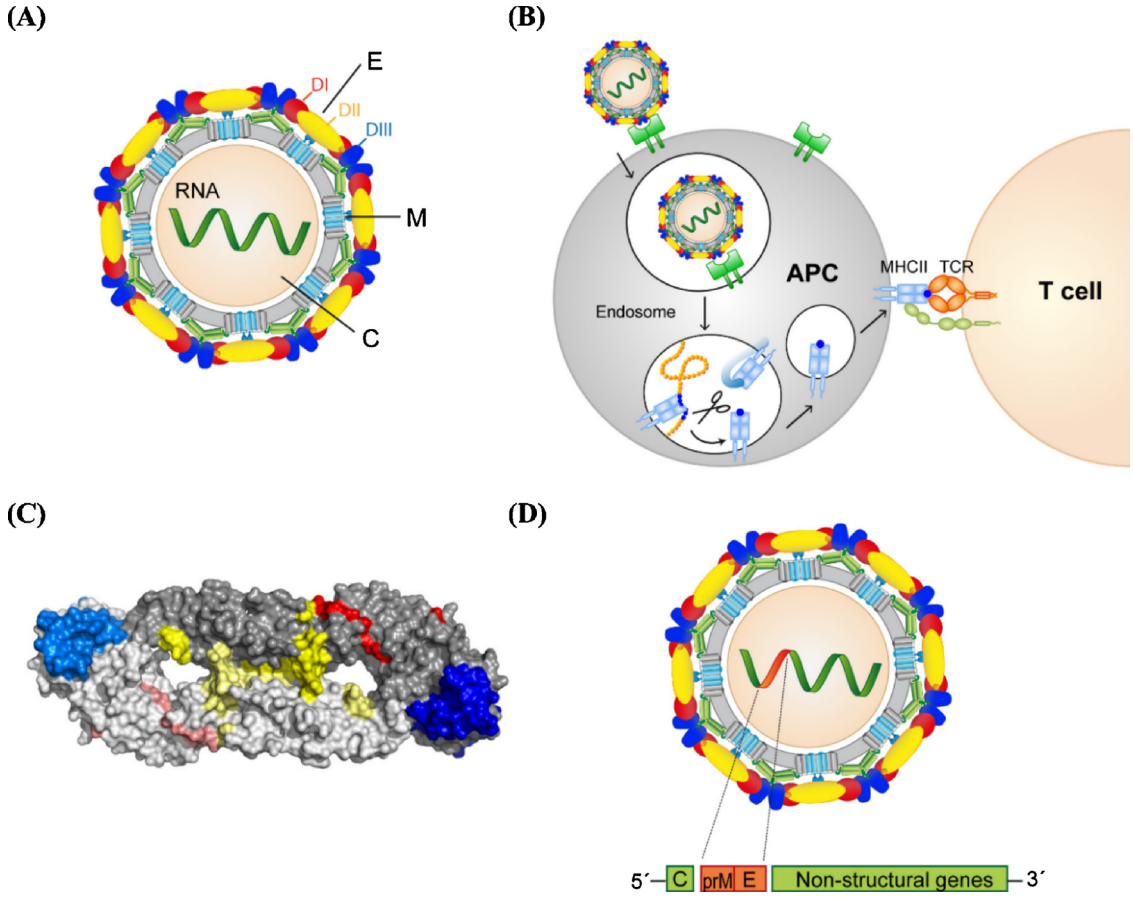

Fig. 1. Flavivirus structure and antigen presentation. (A) Schematics of a mature flavivirus particle that contains three structural proteins: C (capsid), M (membrane) and E (envelope). The three domains of the E protein are colored in red (domain I, DI), yellow (domain II, DII) and blue (domain III, DIII) (B) Schematic model of antigen processing and presentation. CD4 T cells recognize peptides that are proteolytically processed from internalized protein antigens and presented by MHC class II molecules (MHCII) on the surface of antigen-presenting cells (APC). (**C**) Surface representation of the dimeric form of TBE virus sE (PDB 1SVB) [20] in a top view; epitope hotspots of CD4 T cells at surface-exposed sites are highlighted in red (DI), yellow (DII) and blue (DIII) [25]. (D) Schematic representation of a chimeric virus particle (Chimerivax™ technology): The sequences encoding prM and E (red) of DEN or JE are engineered into the genome of the YF-17D vaccine strain (green), resulting in the expression of chimeric virus particles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

immune memory. A detailed knowledge of their distinct functions is thus imperative for resolving the mechanisms underlying protective immunity.

In order to understand the diverse protective effects of CD4 T cells, there are several important issues that need to be addressed: The first is to define the effector functions of CD4 T cells that are most critical for protective immunity, specifically those that are required for generating long-term immune protection. The second issue is the knowledge of the viral proteins/peptides recognized by CD4 T cells, and the associated viral and host genetic factors that restrict the response. Given the current epidemiological situation, in which many countries are facing outbreaks of multiple flaviviruses, prior T cell immunity to flaviviruses, either from natural infection or vaccination, is another important issue that can influence the outcome of the response. A fundamental knowledge of how prior flavivirus exposure influences the specificity and protective activity of CD4 T cell responses is necessary to fully understand individual variation of the human immune response and its consequences on protection and disease outcome. Here, we summarize results obtained from analyzing human CD4 T cell responses to flavivirus infection and vaccination and discuss specific influences on the response by different types of vaccines that may be relevant for protective immunity.

2. Effector functions of CD4 T cells following flavivirus infection

CD4 T cells are thought to control virus infection through multiple mechanisms. These include the production of cytokines, recruitment and activation of innate immune cells, help for high-affinity antibody production, enhancement of CD8 T cell responses, promotion of immune memory, as well as direct cytotoxic effects on infected cells [26–29]. A protective role of CD4 T cells against flaviviruses has been clearly shown in mouse models [30–38]. Delineating their protective roles in human infection has been more difficult because of the broad specificities in the context of highly polymorphic HLA backgrounds [39–46] and the multiple different effector functions of CD4 T cells [44,46–50]. To distinguish these functions, a combination of several parameters has to be tested. For this purpose, multiparameter flow cytometry provides simultaneous analysis of cell types, cytokines and other markers of immune function. Similarly, highly sensitive ELISPOT assays enable large-scale analysis of the complete repertoire of virus peptides recognized by CD4 T cells.

Studies examining T cell responses in human infection have shown that the quantity or magnitude of immune responses may already serve as a predictor of protection against disease [51]. However, there are also many examples indicating that the quality of these responses is equally important, and that specific effector cells, cytokine polyfunctionality (co-production of IFN-γ, TNF-α and IL-2), cytotoxic potential or epitope specificity related to certain HLA allelels correlate