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Influence of antibodies and T cells on dengue disease outcome: insights from interferon receptor-deficient mouse models

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Dengue virus (DENV) is a globally important mosquito-borne virus that causes a spectrum of diseases ranging from dengue fever (DF) to dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS), affecting 3.6 billion people in 128 countries [1,2•]. There is an urgent need for a drug or vaccine against DENV, yet none are presently available. In fact, results from recent Phase IIb and III trials of an attenuated tetrameric vaccine revealed that the vaccine provided limited protection against DENV serotype 2 in DENV-immune people, and no protection against any serotype in naïve individuals [3–5], highlighting the difficulties associated with dengue vaccine development. A challenge in the development of a DENV vaccine is that a vaccine must protect against all four DENV serotypes, which co-circulate in endemic areas. Further complicating DENV vaccine development is that the correlates of protection are not fully defined, mechanisms regulating the generation of protective antibody and T cell responses against all four DENV serotypes are as yet to be deciphered, and the adaptive immune response may actually contribute to severe disease. Recent studies using the only available animal model of DHF/DSS in mice lacking one or more components of the interferon (IFN) system have begun to provide crucial insights into the protective versus pathogenic nature of both antibody and T cell responses to DENV. Herein, we highlight key studies using the IFN receptor-deficient mouse models toward understanding the contribution of antibodies and T cells in impacting the outcome of DENV infection.

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DENV as a human pathogen

The four serotypes of dengue viruses (DENV1–4) together infect an estimated 390 million people every year, and 96 million of these manifest with overt disease [2•]. DF is an acute illness with symptoms that include fever, headache, myalgia, and retro-orbital pain. DHF and DSS are characterized by thrombocytopenia, elevated hematocrit and cytokine levels, vascular leakage, abnormal hemostasis, and in DSS, shock, which can be fatal if untreated. DENV threatens half the world's population, generally in tropical and subtropical climates including Latin America and Southeast Asia [6]. While many pathogens occur in a spectrum of more or less closely related species, the existence of four closely related yet distinct serotypes is unique to DENV [7]. The four serotypes circulate concomitantly and are transmitted by both *Aedes aegypti* and *Aedes albopictus* mosquitoes. Symptoms of DENV vary widely, ranging from asymptomatic to more severe, potentially lethal disease manifestations [8]. In contrast to other pathogens, second DENV infections with a different virus serotype are associated with more severe disease than primary infections. Because multiple serotypes of DENV now circulate in many countries, large outbreaks can result in numerous severe secondary infections that may overwhelm medical facilities. Due in part to increasing disease severity, DENV has emerged as an important disease burden. There are currently no approved vaccines or antivirals for DENV [9]. Available treatments are generally supportive, including rehydration and in-patient monitoring. Despite extensive experience and training of physicians in DENV-endemic countries, complex physiological changes in DHF/DSS patients can result in major complications, and mortality remains around 4% [10].

DENV pathogenesis

Neutralizing antibodies raised against one DENV serotype are thought to confer life-long protection against reinfection with that serotype, but only transient protection against the other three serotypes. In fact, severe dengue disease is most often observed in patients experiencing a secondary infection with a heterologous DENV serotype, and during primary infections in infants born to DENV-immune mothers. Therefore, two central questions in the DENV field are: Why do secondary infections with DENV result in more severe illness than primary infections? Why do infants born to DENV-immune but not DENV-naïve mothers develop severe disease? To explain these epidemiological observations, two dominant hypotheses have been postulated: first, antibody-dependent enhancement of infection (ADE)

and second, original T cell antigenic sin. According to the ADE hypothesis, DENV-antibody complexes are formed and bind to Fcγ receptors on cells, facilitating viral entry and increased replication; increased viral burden resulting from ADE then drives the production of inflammatory mediators that increase vascular permeability [1,11]. The 'original T cell antigenic sin' hypothesis involves inappropriate T cell responses. According to this hypothesis, a secondary infection with a heterotypic DENV serotype can stimulate the expansion of serotype cross-reactive, low affinity T cells that contribute to severe disease manifestations instead of protective anti-viral immunity [12,13]. This hypothesis could explain the severe disease resulting from secondary infection, but not in infants born to DENV-immune mothers. Maternal dengue antibodies are present in infants for several months after birth, while T cells are not transferred to the infant. Therefore, this hypothesis may not explain what is occurring in infants born to DENV-immune mothers. In contrast, ADE could explain DHF/DSS pathogenesis in the context of both secondary infections and infants born to DENV-immune mothers. Another hypothesis states that some dengue viruses exhibit increased 'fitness,' that is, they produce more severe disease during a secondary dengue infection [14–18]. Increases in rates of occurrence of DHF/DSS have been associated with single amino acid substitutions in NS1 or more complex clade changes in circulating DENV 2 viruses.

The IFN receptor-deficient mouse models of DENV infection

An animal model offering the ability to manipulate individual pathogenesis components is required to perform mechanistic studies in which the exact infecting DENV strain, as well as the order and times of infection, are known. Several mouse models of DENV infection, including immunodeficient and humanized mice, are available (reviewed in [19]). Wild-type (WT) mice are resistant to parenteral infection with DENV, as the virus is able to block type I and type II interferon (IFN) receptor signaling in human but not murine cells [20–22]. The antiviral IFN response must be disrupted in mice to make them susceptible to DENV infection and manifest signs of severe disease. Therefore, a model of DHF/DSS-like disease has been developed in mice lacking the type I IFN receptor (IFNAR) and the IFN-γR (IFNAR$^{-/-}$; IFN-γR$^{-/-}$, also known as AG129) [23–25]. This mouse model reproduces key pathophysiological features of DHF/DSS, including similar cellular and tissue tropism and lethal vascular leakage, cytokine storm, low platelet count, elevated hematocrit, and hemorrhage. To increase pathogenesis relevance of results obtained using AG129 mice, a similar dengue disease model has been created in single-deficient IFNAR$^{-/-}$ mice [25–28]. A further increase in relevance of the IFNAR$^{-/-}$ mouse model was achieved by crossing them with HLA transgenic mice expressing human MHC class I or class II molecules [29•,30]. Most recently, *LysM-Cre*$^{+}$*Ifnar*$^{f/f}$ and *Itgax-Cre*$^{+}$*Ifnar*$^{f/f}$ mice have been used to develop the most immunocompetent animal model of DHF/DSS developed to date [31] (unpublished data from the laboratories of Michael Diamond at Washington University in St. Louis, and Sujan Shresta at the La Jolla Institute). These mice are made by crossing C57BL/6 *LysM-Cre*$^{+}$ or *Itgax-Cre*$^{+}$ mice to C57BL/6 *Ifnar*$^{f/f}$ mice. *LysM-Cre*$^{+}$*Ifnar*$^{f/f}$ mice lack the IFNAR on macrophages and neutrophils, while *Itgax-Cre*$^{+}$*Ifnar*$^{f/f}$ mice lack the IFNAR on dendritic cells [32]. As macrophages and dendritic cells are targets of DENV in mice [33,34], the lack of IFNAR on these cells allows for DENV replication. Thus, the lack of the type I IFN receptor on only a subset of cells renders the *LysM-Cre*$^{+}$*Ifnar*$^{f/f}$ and *Itgax-Cre*$^{+}$*Ifnar*$^{f/f}$ mice susceptible to DENV-induced DHF/DSS-like disease; the rest of their immune system, including T and B cells, is WT. As macrophages are the main site of DENV replication in most tissues of infected mice [33], and dendritic cells are important for priming T and B cell responses, *LysM-Cre*$^{+}$*Ifnar*$^{f/f}$ mice (with WT dendritic cells and T and B cells) may be a more relevant model for investigating cellular and humoral responses to DENV than *Itgax-Cre*$^{+}$*Ifnar*$^{f/f}$ mice (with WT T and B cells but not dendritic cells).

Testing the ADE hypothesis

IFN receptor-deficient mice were the key to modeling ADE *in vivo*. The relevance of ADE to DHF/DSS pathogenesis had been controversial for over 40 years until 2010, when two laboratories independently demonstrated ADE in AG129 and IFNAR$^{-/-}$ mice [23,25]. The ADE hypothesis was tested by simply asking the following question: How would administration of DENV-specific antibodies change the course of infection using a sublethal dose of DENV? Upon administration of enhancing concentrations of DENV-specific antibody before infection, mice rapidly developed a DHF/DSS-like disease with cytokine storm and increased vascular permeability and ultimately TNF-mediated lethality (Figure 1). As predicted by the ADE hypothesis for infants, mice that were injected with enhancing concentrations of anti-DENV antibodies (i.e., ADE mice) exhibited an increased viral load in tissues relative to non-ADE control animals inoculated with virus alone. The effect of ADE can first be seen at 48 h post infection, when viral load is significantly higher in livers of ADE mice compared with non-ADE controls. By 72 h post infection, a significant increase in viral load can be seen in other tissues of ADE mice, including spleen and small intestine. Besides increased viral load, the other key requirement for ADE, interaction between Fc in the DENV-antibody complex and Fcγ receptors, was also confirmed in this model. Specifically, the increased viral titer in the liver of ADE mice at 48 h post-infection can be decreased to non-ADE levels by using anti-DENV antibody containing mutant Fc region that cannot bind Fcγ receptors,