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Immune Response to Dengue and Zika

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Abstract

Flaviviruses such as dengue (DENV), yellow fever (YFV), West Nile (WNV), and Zika (ZIKV) are human pathogens of global significance. In particular, DENV causes the most prevalent mosquito-borne viral diseases in humans, and ZIKV emerged from obscurity into the spotlight in 2016 as the etiologic agent of congenital Zika syndrome. Owing to the recent emergence of ZIKV as a global pandemic threat, the roles of the immune system during ZIKV infections are as yet unclear. In contrast, decades of DENV research implicate a dual role for the immune system in protection against and pathogenesis of DENV infection. As DENV and ZIKV are closely related, knowledge based on DENV studies has been used to prioritize investigation of ZIKV immunity and pathogenesis, and to accelerate ZIKV diagnostic, therapeutic, and vaccine design. This review discusses the following topics related to innate and adaptive immune responses to DENV and ZIKV: the interferon system as the key mechanism of host defense and viral target for immune evasion, antibody-mediated protection versus antibody-dependent enhancement, and T cell–mediated protection versus original T cell antigenic sin. Understanding the mechanisms that regulate the balance between immune-mediated protection and pathogenesis during DENV and ZIKV infections is critical toward development of safe and effective DENV and ZIKV therapeutics and vaccines.

INTRODUCTION

The genus *Flavivirus* of the family *Flaviviridae* consists of more than 70 members and includes several medically important viruses, such as dengue (DENV) and Zika (ZIKV). The four serotypes of DENV cause dengue fever and dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS), the most important viral illnesses transmitted by mosquitoes, with a global estimate of 3.6 billion people at risk for infection, 400 million new infections, and 100 million new symptomatic cases annually (1). Over 2 million DHF/DSS cases and 20,000 deaths are estimated to occur each year (2). Dengue fever is characterized by fever, arthralgia, myalgia, abdominal pain, rash, and a viremia that begins 3–4 days following infection by mosquito bite. DHF/DSS manifests, as the fever subsides, with a sudden onset of plasma leakage that can result in hemoconcentration, pleural effusion, ascites, and shock. A small subset of DHF/DSS patients may die because of hypotensive shock. Although coagulation abnormalities and thrombocytopenia are prominent features, plasma leakage is the hallmark of DHF/DSS. In rare cases of DHF/DSS, neurologic abnormalities, including encephalitis, may also occur (3). DENV is transmitted to humans by the mosquitoes *Aedes aegypti* and *Aedes albopictus*. Owing to uncontrolled urbanization, globalization, and the spread of DENV-transmitting mosquitoes, multiple DENV serotypes cocirculate, resulting in increased frequency of epidemics and the continued emergence and spread of DHF/DSS. As a result, DENV is a major public health threat throughout the world. Despite the significance of this pathogen, no DENV-specific therapies are available, and a DENV vaccine that elicits protection in people with prior DENV exposure but not in naive individuals and that is not equally protective against all four serotypes has recently begun to be licensed on a country-by-country basis. This is in large part due to an incomplete understanding of the interplay between viral and host factors that contribute to DENV pathogenesis. On the virus side, some DENV lineages are more virologically and epidemiologically fit than others and are thus associated with DHF/DSS manifestations (4–6). On the host side, DENV infection history is the primary determinant associated with development of more severe dengue disease, with potential contributions from genetic variation, age, and sex (7).

Similar to DENV, ZIKV is transmitted by *Aedes* mosquitoes, and most infected people are asymptomatic or develop a dengue fever–like, self-limiting febrile disease. ZIKV, discovered in 1947, was thus not considered to be a significant human pathogen for 60 years, until large-scale outbreaks started in 2007 in the Pacific Islands, and multiple modes of transmission and new syndromes became recognized during the 2015–2016 outbreak in Latin America. ZIKV is now known to cause fetal infection and congenital Zika syndrome, which includes microcephaly, cerebral malformations, ophthalmological and hearing defects, and arthrogryposis (8). In adults, ZIKV infection may induce Guillain-Barré syndrome, an autoimmune peripheral neuropathy characterized by acute, symmetric limb weakness with decreased or absent deep-tendon reflexes (9–12). Additionally, case reports of ZIKV sexual transmission (13, 14) and ZIKV persistence in semen (15, 16) and vaginal secretions (17) are also mounting. Thus unlike DENV, ZIKV is characterized by transplacental and sexual transmission, life-threatening neurological complications, and viral persistence. Likely owing to its multiple modes of transmission and sharing of DENV's mosquito vector, ZIKV has now spread to many DENV-endemic regions beyond Latin America, including Southeast Asia and India.

ZIKV-specific therapeutic and in particular vaccine candidates have begun to be developed rapidly (18, 19). However, ZIKV's unusual transmission modes and features may present many challenges for drug and vaccine development. Moreover, cocirculation of DENV and ZIKV, which share a close antigenic relationship at antibody and T cell levels, may complicate vaccine development efforts for both viruses owing to potential dual roles for antibodies and T cells in protection and pathogenesis. The amino acid sequences of DENV strains within a serotype are approximately 98.1% to 99.0% identical, and the four DENV serotypes share 68.7% to 78.1%

identity (20). ZIKV strains (representing both the Asian and African lineages) exist as a single serotype (21) and are 99.2% identical, comparable to sequence identity among different DENV strains, which also exist as multiple lineages. Among pathogenic human flaviviruses, DENV and ZIKV are most closely related to each other, with 55.1% to 56.3% amino acid sequence identity. Accordingly, emerging literature indicates many similarities between these two viruses in terms of interactions between the virus and host immune system. For both viruses, the interferon system is the central mediator of host defense and target of viral counterattack, whereas complex interplays between antibody and T cell responses likely determine the outcome of infection in flavivirus-immune settings. Thus, virus-host interactions related to interferon, antibody, and T cell responses to DENV and ZIKV are the major topics of discussion in this review. A thorough understanding of the immune response to both viruses is paramount for developing urgently needed DENV and ZIKV therapeutics and vaccines that are both safe and effective.

DENV AND ZIKV VIROLOGY

DENV and ZIKV are enveloped, positive-sense, single-stranded RNA viruses. The flaviviral genome is approximately 10.7 kb in length and encodes a polyprotein with three structural proteins [capsid (C)-premembrane (prM)-envelope (E)] at the N terminus and seven nonstructural proteins (NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5) at the C terminus flanked by 5′ and 3′ untranslated regions (UTRs) (22) (**Figure 1**). The single polyprotein is cleaved into these ten viral proteins

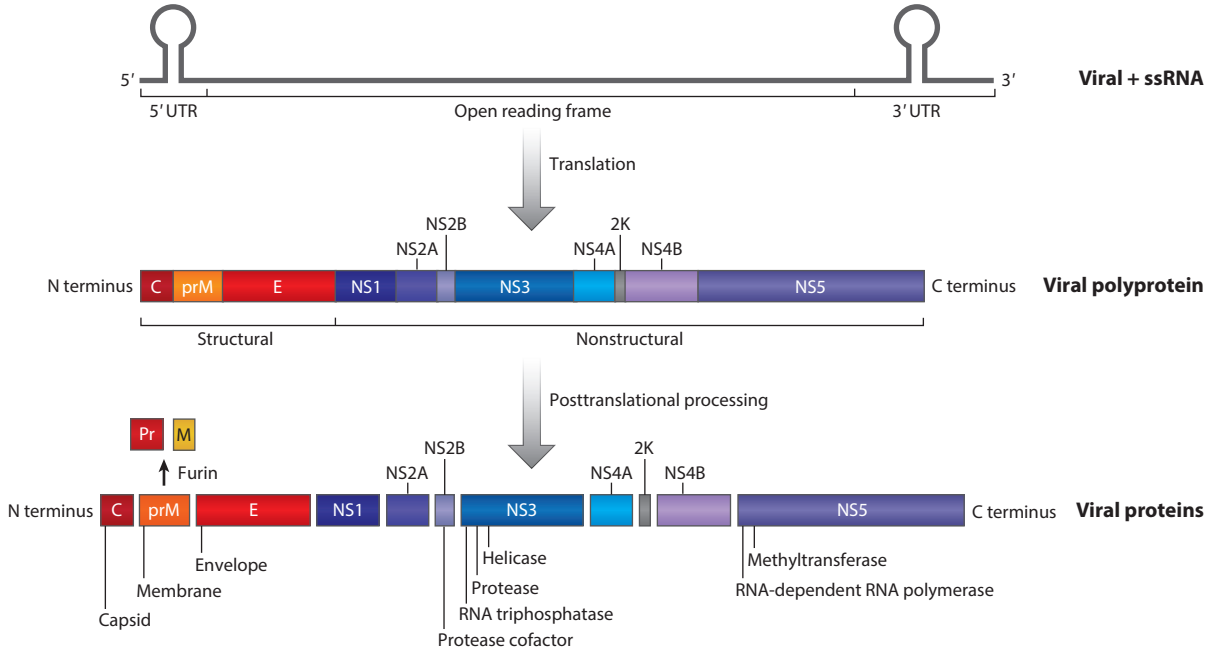


Figure 1

The flaviviral genome is approximately 10.7 kb and is a positive-sense, single strand of RNA. Translation results in a polyprotein composed of three structural proteins at the N terminus and seven nonstructural proteins at the C terminus. Posttranslational cleavage and processing produce viral proteins: capsid, premembrane, envelope, NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. The 2K signal peptide is a sequence of 17 amino acids linking NS4A and NS4B. Abbreviations: C, capsid; E, envelope; NS, nonstructural; prM, premembrane; ssRNA, single-stranded RNA.

by both viral and host proteases. The structural proteins form the virion particle. The C protein surrounds the viral genome, forming a nucleocapsid. The prM protein acts as a chaperone for E during virion assembly and is cleaved by furin to M in the *trans*-Golgi, resulting in the formation of mature virions that contain E and M (23). However, this cleavage is incomplete, resulting in the release of many immature virions containing prM (24, 25). The M protein regulates fusion of the viral envelope with the host membrane. The E protein is involved in receptor binding, membrane fusion, and viral assembly. E protein is also the major target of neutralizing antibodies and contains each of the four DENV serotype-specific antigenic epitopes. Based on the crystal structure of the DENV E protein ectodomain (26, 27), E is composed of three domains: Domain I (EDI) participates in E protein structural rearrangements required for fusion, domain II (EDII) contains the fusion loop involved in pH-dependent fusion of virus and host cell membranes, and domain III (EDIII) includes the receptor-binding region. The nonstructural proteins are involved in viral polyprotein processing, replication, and innate immune antagonism (28). In particular, NS5, approximately 900 amino acids long, is the largest and most conserved of the flavivirus proteins. It encodes an RNA-dependent RNA polymerase for genome replication, methyltransferase for 5′ RNA cap formation and methylation, and a type I interferon antagonist. NS3 encodes a protease and associates with NS2B (which acts as a cofactor) to form the NS2B3 viral protease complex, and it antagonizes the type I interferon pathway using both proteolytic-dependent and proteolytic-independent activities. In addition to nonstructural proteins, 5′ and 3′ UTRs are part of the viral replication complex, and subgenomic flavivirus RNA (sfRNA) encoded by the 3′ UTR is also involved in viral evasion of the innate immune response (5, 29, 30). During productive infection, DENV binds its cellular receptor, enters cells via receptor-mediated endocytosis, uncoats the viral genome in acidified endosomal vesicles, and releases its RNA into the cytoplasm to initiate viral translation, which is required to generate individual viral proteins, including the viral replicase necessary for subsequent viral RNA replication. Viral particles assemble by budding into the endoplasmic reticulum, pass through the Golgi, where surface glycoprotein modification occurs, and are exocytosed via secretory vesicles.

Viral Entry Receptors and Cellular Tropism

Central to understanding mechanisms of viral immunity and pathogenesis is the knowledge of viral entry receptors and cellular tropism. Although the E protein has been known to mediate receptor binding and fusion, the precise identity of entry receptors for DENV and ZIKV in humans remains uncertain. DENV appears to use multiple cell surface molecules for binding to and infecting target cells, depending on the cell type. Several candidate molecules—including glycosaminoglycans (31, 32); C-type lectins; dendritic cell–specific ICAM3-grabbing nonintegrin (DC-SIGN) and liver/lymph node–specific ICAM3-grabbing integrin (L-SIGN) (33–35); mannose receptor (36); the phosphatidylserine receptors T cell immunoglobulin and mucin domain (TIM) and Tyro3, Axl, and Mertk (TAM) (37); and the phospholipid receptor CD300a (38)—have been proposed to serve as receptors for DENV based primarily on in vitro studies with cell lines and primary human cells. One TAM family member, Axl, has also been implicated as a ZIKV entry receptor in studies with cell lines and primary human cells. However, recent studies using CRISPR-Cas9-based gene editing in primary human neural progenitor cells and brain organoid cultures (39) and gene-deficient mice lacking Axl or both Axl and Mertk showed no effect of either Axl or Mertk on ZIKV infection (40–42). Therefore, bona fide entry receptors required for DENV and ZIKV infection in humans are as yet unknown, or they may not exist. The exact functions of the various candidate receptors identified to date are unclear. These candidates, individually or in various combinations in a cell type–dependent manner, may serve as true entry receptors that mediate fusion, or they

may serve as attachment factors that promote virus adsorption and internalization, leading to either productive or abortive infection, or perhaps influencing the level of productive infection.

Viral entry may also occur via the process known as antibody-dependent enhancement (ADE) of infection, which is discussed in detail in the section titled The Debate Continues: The Role of Adaptive Immunity in Protection Versus Pathogenesis. Owing to incomplete cleavage of the pr domain from the prM protein during viral maturation, DENV and ZIKV populations exist as a mixture of mature, partially mature, and immature virions (43). This structural heterogeneity allows the virion population not only to limit the uniform availability of neutralizing antibody epitopes but also to enter into cells. In particular, immature virions that are not infectious can enter Fcγ receptor (FcγR)-bearing cells through antibody-dependent entry and become infectious (44). Thus, DENV and ZIKV not only counteract but also usurp the host antibody response via FcγR-mediated entry into cells as immune complexes.

In large part owing to incomplete knowledge about DENV entry receptors and the highly heterogeneous and plastic nature of FcγR-expressing cells such as macrophages, the precise cellular tropism of DENV in humans is still not fully defined. Human autopsy studies have reported that cells belonging to the monocyte/macrophage/dendritic cell lineage in the spleen, lymph nodes, lungs, liver, kidney, and stomach are the primary cellular hosts of DENV (45–48). Additionally, endothelial cells, hepatocytes, and neurons in the brain may also support DENV infection (45–51). Likely owing to the use of reagents that might not have distinguished virus-infected cells from those that might have endocytosed or phagocytosed viral antigen, some of these immunohistochemistry- and autopsy-based publications have differed over whether particular cell types are infected by DENV. In peripheral blood samples from DENV-infected patients, monocytes have been identified as the principal DENV hosts (52). In skin biopsy samples from uninfected human donors, Langerhans cells, dendritic cells, and macrophages can serve as DENV cellular hosts (53, 54). Similarly, in spleens from uninfected human donors, macrophages are the target cells for DENV infection in vitro (55). In agreement with human studies, detailed analyses of cellular tropism in highly susceptible mice lacking type I interferon receptor signaling have demonstrated that both dermal dendritic cells and macrophages in the skin (56), but primarily macrophages in other lymphoid (spleen, lymph nodes, thymus, bone marrow, Peyer patches) and nonlymphoid (kidney, heart, lung, and gastrointestinal tract) tissues are the principal cellular hosts of DENV (57, 58). Thus, human and mouse model studies to date indicate cells of the monocyte/macrophage/dendritic cell lineage as the major DENV hosts in the skin and many lymphoid and nonlymphoid organs.

Studies on human clinical samples and cell culture and nonhuman primate and mouse models suggest that ZIKV is pantropic. In clinical samples in situ hybridization has identified neural progenitor cells, glial cells, amniotic epithelial cells, fetal mesenchymal cells (59), and Hofbauer cells (macrophages of the placenta) (60–63) as permissive. Ex vivo and in vitro studies on human cells also suggest that ZIKV replicates in astrocytes (64), trophoblasts (65, 66), fibroblasts (placental, uterine, pulmonary) (67), endothelial cells, various epithelial cells, and peripheral blood mononuclear cells (PBMCs) (68). Numerous other immunohistochemistry-based studies using immune serum or anti-E antibody clone 4G2 on clinical samples have also demonstrated immunoreactivity in neurons, pneumocytes, hepatocytes, and renal epithelial cells (69–71). In vitro studies using 4G2 (39, 72–75) have also shown ZIKV E in glomerular podocytes, mesangial cells (76), endometrial stromal cells (77), retinal pericytes and pigmented epithelial cells (78), dermal fibroblasts and dendritic cells, epidermal keratinocytes (79), and endothelial cells (glomerular, retinal) (80). In rhesus macaques, in situ hybridization and NS2B immunohistochemistry have demonstrated that neurons, macrophages, neutrophils, and dendritic cells are permissive (81–83). Results in macaques have differed in regards to whether ZIKV replicates in B cells (82, 83). Further, independent studies have shown that ZIKV can persist in the macaque central nervous system and lymphoid tissue

for over 4 weeks after infection (83, 84). Similar findings in immunocompetent and interferon axis–compromised mouse models suggest pantropism (85, 86). Notably in C57BL/6 mice treated with a monoclonal type I interferon receptor (IFNAR)-blocking antibody, neurons in the retina and optic nerve were permissive to infection (42, 87). In a separate study also using IFNAR blockade, ZIKV was detected in spermatogonia, primary spermatocytes, and Sertoli cells (40). In *Ifnar1*$^{-/-}$ mice, Leydig cell infection was associated with decreased testosterone production and testicular atrophy (88). Following intravaginal infection in mice, trafficking of permissive round cells (likely macrophages) through the subcapsular sinus of iliac lymph nodes has also been demonstrated (89). Reflecting ZIKV's broad tropism, viral RNA has been isolated from several human body fluids, including saliva, urine, tears, aqueous humor, breast milk, semen, and vaginal washes (14, 17, 90–93).

Approached from the clinical side, susceptibilities of various placental, blood-brain barrier, and fetal brain cells are most directly relevant to understanding the pathogenesis of microcephaly and fetal Zika syndrome. Permissiveness of cells of the blood-testis barrier and male and female urogenital tracts is most relevant to investigating sexual transmission. The strong association between ZIKV infection and Guillain-Barré syndrome may be related to already-documented susceptibility of neurons to ZIKV replication.

Most pantropic viruses employ a similar dissemination strategy. At the inoculation site virions replicate in tissue macrophages and dendritic cells, which traffic virus to the draining lymph nodes and other lymphoid tissues. In lymphoid tissues, large numbers of macrophages are recruited and amplify the virus. Following a period of mostly cell-associated viremia, the virus spreads to monocytes, macrophages, or dendritic cells in multiple tissues. Assembled virions then spread to infect the remaining local tissues. Although ZIKV tropism studies to date are consistent with this model, many additional questions remain, including how the virus crosses intact epithelia, enters specific body fluids, enters sites of immune privilege, and persists in specific sites and the possible influence of ADE on tropism. Due to the rarity of ZIKV autopsy samples, inability to conduct experiments in human hosts, and the large numbers of immunologic tools available for mouse modeling, further studies in mice and nonhuman primates will accelerate investigations of ZIKV tropism by allowing for phenotyping and genotyping of permissive and restrictive cell populations in stringently controlled experiments.

TYPE I INTERFERONS: A CRITICAL HOST DEFENSE SYSTEM AGAINST DENV AND ZIKV

A short course of illness and self-limiting febrile symptoms in most DENV and ZIKV cases implicate a key role for the innate immune system in controlling DENV and ZIKV infections. The interferon system, comprising type I interferons (IFN-α, β), type II interferon (IFN-γ), and type III interferons (IFN-λ1–4), is the primary mechanism by which the innate immune system defends against viruses (94). Type I interferons are encoded by a family of closely related IFN-α genes and one IFN-β gene; type II interferon is encoded by one IFN-γ gene; and type III interferons, IFN-λ1–4, are also known as IL-29, 28A–C. Nearly all nucleated cells can respond to secreted type I interferons, and any nucleated cell can produce type I interferons; thus type I interferons are the primary interferons that are generated in most cell types during viral infections. The type I interferon system is triggered within hours of viral infection, upon recognition of viruses by cellular pattern recognition receptors (PRRs) such as the retinoic acid–inducible gene I (RIG-I)-like receptors (RLRs) and Toll-like receptors (TLRs). Engagement of these PRRs by viral nucleic acids results in the activation of multiple transcription factors, including interferon regulatory factors (IRFs) and NF-κB, that cooperate in inducing interferons and various other inflammatory cytokines and chemokines that orchestrate the innate and adaptive immune responses. RNA viruses

that replicate in the cytosol are recognized mainly via the RLRs RIG-I and melanoma-associated differentiation antigen 5 (MDA5). RNA virus replication generates 5′-triphosphorylated single-stranded RNA and double-stranded RNA that are detected by RIG-I and MDA5. Upon RNA binding, RIG-I/MDA5 recruits the adaptor mitochondrial antiviral-signaling protein (MAVS) to trigger activation of the noncanonical IKK-related kinases IKKε and TBK1, which then activate IRF3 and NF-κB to induce type I interferon production (95) (**Figure 2**). The biological activity of type I interferons is mediated by triggering of their own transcriptional program, which results in

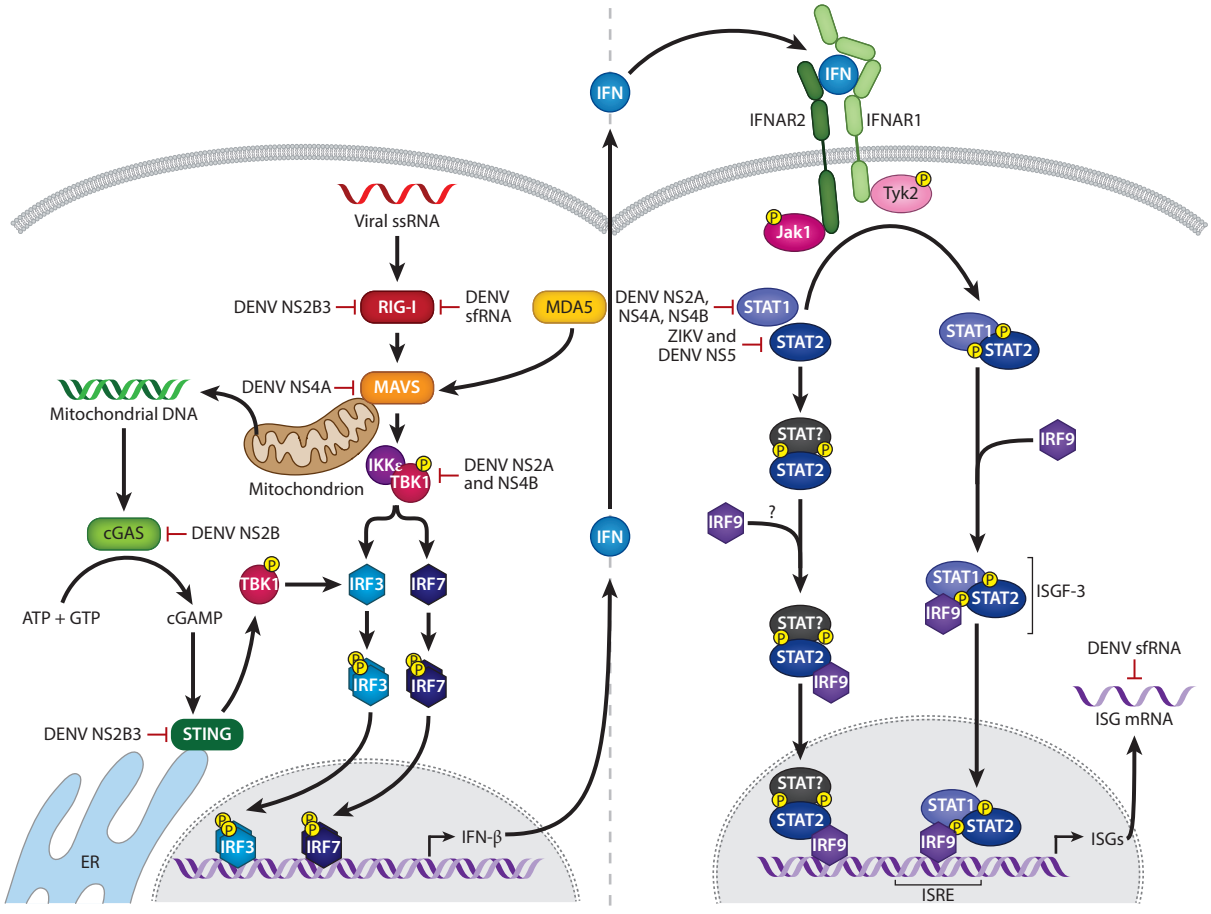


Figure 2

The interferon system. Following flaviviral infection, viral RNA is sensed by cellular pattern recognition receptors, including RLRs, which induce multiple transcription factors including the IRFs and NF-κB, leading to transcription of interferon. Interferon is secreted and binds the type I interferon receptor to activate the JAK-STAT signal transduction pathway. STAT1 and STAT2 are phosphorylated and form a heterodimer that joins IRF9 to form ISGF-3. The ISGF-3 complex then binds to ISREs in the genome to promote expression of ISGs. DENV may evade the interferon system by multiple mechanisms, including degradation of STAT2, blocking of STAT1 and STAT2 phosphorylation, suppression of STAT1 signaling, blocking of TBK1 phosphorylation, blocking of MAVS binding to RIG-I, induction of mitochondrial elongation linked to viral replication complexes, cleavage of mitochondrial fusion proteins, prevention of RIG-I translocation to mitochondria, binding of TRIM25, cleavage of STING, degradation of cGAS, and inactivation of host RNA-binding proteins. Abbreviations: ER, endoplasmic reticulum; IRF, interferon regulatory factor; ISGF, interferon-stimulated gene factor; ISRE, interferon-stimulated response element; MAVS, mitochondrial antiviral-signaling protein; RLR, RIG-I-like receptor; sfRNA, subgenomic flavivirus RNA; ssRNA, single-stranded RNA.

expression of hundreds of interferon-stimulated genes (ISGs) (96). Specifically, type I interferons bind to IFNAR, a heterodimer consisting of IFNAR1 and IFNAR2 chains, and signals through the Janus kinase–signal transducer and activation of transcription (JAK-STAT) pathway. The JAK kinases JAK1 and TYK2 phosphorylate and activate STAT1 and STAT2, followed by heterodimerization of the activated STAT1 and STAT2 and association with another DNA-binding protein, interferon regulatory factor 9 (IRF-9), to form ISG factor 3 (ISGF-3). The ISGF-3 complex promotes gene expression by binding to interferon-stimulated response elements (ISREs) of type I interferon–dependent genes. These ISGs influence many cellular processes, including RNA processing, protein stability, and cell viability, thereby directly affecting specific steps of the viral life cycle and thus virus replication. ISGs in antigen-presenting cells such as macrophages and dendritic cells are also important for T and B cell activation, affecting the magnitude and quality of the adaptive immune response and eventual virus clearance.

Several lines of evidence indicate that the type I interferon system is the central mediator of protection against DENV and ZIKV. First, studies with DENV-infected patient samples have shown that, during the early febrile period, patients contain high levels of type I interferons in the serum (97, 98), and the type I interferon signatures are prominent in transcriptional profiling of PBMCs (99–101). Second, cell culture studies have demonstrated that DENV is recognized by both RIG-I and MDA5 (102), and the phosphatases PP1α and PP1γ are required for RIG-I- and MDA5-mediated type I interferon response to DENV (103); type I interferons inhibit DENV infection in a variety of cells (104, 105); autocrine type I interferon action in infected cells, rather than paracrine type I interferon signaling in naive cells, is important for controlling DENV infection in cell cultures (106); and expression of type I interferon–inducible ISGs such as IFITM2/3, viperin, ISG15, ISG20, OAS, BST2, and RyDEN (also known as IRAV) blocks DENV infection (107–113). IFITMs, in particular IFITM3, also inhibit ZIKV infection (114), and IFITM3 interacts with ZMPSTE24 to mediate its antiviral activity against ZIKV (115). Third, mouse models of experimental DENV and ZIKV infection have shown that the interferon system is essential and more important than T and B cell–dependent immunity in controlling DENV infection in mice (40, 116–118); these mouse model data are further described below. Finally, all flaviviruses studied to date must evade the type I interferon system–mediated antiviral defense in order to replicate and cause disease in vertebrate hosts; thus, DENV and ZIKV employ multiple viral mechanisms to antagonize both type I interferon induction and type I interferon signaling (28, 119), underscoring the importance of the type I interferon system in anti-DENV/ZIKV immunity as discussed below.

Murine Studies: Identification of the Type I Interferon Components That Are Required for Protection Against DENV and ZIKV Infection In Vivo

Studies with various gene-deficient mice have revealed the key type I interferon components that are required for mediating protection against DENV in vivo. In particular, MAVS and the combined activities of IRF-3 and IRF-7 are essential for the initial type I interferon induction and restriction of DENV in mice (120, 121), revealing the presence of MAVS-independent and IRF-3- and IRF-7-independent pathways that control DENV infection in vivo in a delayed manner. These late-acting pathways that restrict DENV infection in vivo are as yet to be identified. Based on studies showing that TLR3 and TLR7 can sense DENV infection in cells (122, 123) and that IRF-5 (85, 124, 125) and IRF-1 (126) contribute to host defense against ZIKV and the closely related WNV, MyD88/TRIF and IRF-5/IRF-1 signaling, respectively, are likely candidates for mediating MAVS-independent and IRF-3- and IRF-7-independent antiviral defenses against DENV. At the level of type I interferon signaling, mice lacking IFNAR1 are highly sensitive to DENV infection

and thus can succumb to DENV infection depending on the viral challenge strain and dose (127, 128). Dissection of the IFNAR signaling mechanism has revealed that the IFNAR signaling in myeloid cells is essential for protection against DENV (129, 130), and the early STAT1-dependent and the late STAT1-independent pathways can each control DENV infection to a degree but are most effective in concert (131). The later STAT1-independent mechanism of protection against DENV involves the noncanonical IFNAR-STAT2 pathway (132), indicating that the IFNAR-STAT2 pathway, in the absence of STAT1, modulates type I interferon production and ISG response in a delayed manner during DENV infection in vivo. Similarly, studies using mouse models of systemic ZIKV infection have demonstrated that wild-type mice treated with blocking anti-IFNAR monoclonal antibody, *Ifnar1*$^{-/-}$ mice, *Irf3*$^{-/-}$*Irf5*$^{-/-}$*Irf7*$^{-/-}$ triple knockout mice, and *Stat2*$^{-/-}$ mice, but not wild-type, *Irf3*$^{-/-}$, *Irf5*$^{-/-}$, *Mavs*$^{-/-}$, *Tmem173*(STING)$^{-/-}$, and *Mb21d1* (cGAS)$^{-/-}$ mice, can succumb to ZIKV infection (85, 125, 133–135). As *Stat2*$^{-/-}$ mice can succumb to infection with ZIKV (133) but not DENV (132, 136), STAT2-directed signaling may play a more important role against ZIKV than DENV. Additionally, studies using mouse models of intravaginal ZIKV infection have shown a requirement for IFNAR signaling in myeloid cells (89) and combined activities of IRF-3 and -7 or TLR7 and MAVS to restrict local ZIKV infection in the female reproductive tract (118). Another study using direct inoculation of ZIKV into mouse eyes reported a role for ISG15 in controlling ZIKV infection of eyes (87). Collectively, these findings indicate that multiple, redundant pathways at both type I interferon induction and signaling levels contribute to host defense against DENV and ZIKV. They have also set the foundation to determine the precise virus-specific (DENV versus ZIKV), time-specific (early versus late stage of DENV/ZIKV infection, or acute versus persisting ZIKV infection), route-specific (systemic versus mucosal ZIKV infection), tissue-specific (e.g., lymphoid versus nonlymphoid organs/genital organs/placenta), and cell type–specific (e.g., immune versus nonimmune cells, or macrophages versus dendritic cells) mechanisms by which the type I interferon system protects against DENV and ZIKV.

However, some of the signaling molecules involved in the type I interferon system may also provide protective immunity in a type I interferon–independent manner. Based on the following results, PRR-induced, type I interferon–independent, type III interferon–dependent ISGs may be important in protecting against DENV and ZIKV infection depending on the context: (*a*) The combined activities of IRF-3, -5, and -7 are more important than that of IFNAR in restricting systemic ZIKV infection in mice (125), and IRF-3 and -7 are more important than IFNAR in limiting ZIKV infection in the murine female reproductive tract (118); (*b*) RIG-I agonists have a part in inhibiting DENV replication in human cells via the RIG-I/MAVS/TBK1/IRF3 signaling axis that is largely type I interferon independent (137); (*c*) type III interferons have a role in protection against ZIKV infection in human syncytiotrophoblasts from term placenta (138); and (*d*) the peroxisomal MAVS-based type III interferon–mediated ISG response is protective against DENV infection in various human cells (139). Additionally, based on results revealing the type II interferon receptor–mediated control of DENV infection in the *Ifnar1*$^{-/-}$ mouse brain (127) and a higher susceptibility of mice lacking both type I and II interferon receptors to DENV and ZIKV infection than mice lacking type I interferon receptors alone (116, 117, 134), type II interferon signaling may be synergistic or nonredundant with the IFNAR pathway in protecting against DENV and ZIKV. Similarly, in terms of virus sensing, TLRs may contribute to host defense against DENV and ZIKV in a context-dependent manner. DENV employs multiple mechanisms to evade the type I interferon system, particularly at the RLR pathway level, as discussed below. The TLR- and plasmacytoid dendritic cell (PDC)-based mechanisms may be key in fighting against DENV and ZIKV infection in settings where the virus inhibits the RLR pathway and in *Mavs*$^{-/-}$ mice (120), as cell culture studies have suggested roles for TLR3, TLR7, and PDCs in detecting

DENV and producing type I interferons (122, 123, 140). However, under certain circumstances, activation of the TLR pathway may contribute to pathogenesis rather than protection. For instance, TLR3 activation following ZIKV infection in human embryonic stem cell–derived cerebral organoids has been associated with attenuated neurogenesis (141), and TLR2, 4, and 6 activation upon binding of DENV NS1 protein to endothelial cells results in proinflammatory cytokine production and loss of endothelial cell integrity (142–144). Deciphering how signaling via type I, II, and III interferons and other inflammatory cytokines intersects and regulates the balance between protection and pathogenesis will be crucial for understanding the precise mechanisms by which the type I interferon system mediates host defense against DENV and ZIKV.

Antagonism of the Type I Interferon System by DENV and ZIKV

To facilitate viral replication/dissemination and cause disease in humans, each pathogenic flavivirus has evolved multiple yet distinct mechanisms for inhibiting the signal transduction pathways that induce type I interferon production and ISG expression. Given the recent emergence of ZIKV, only one mechanism of viral antagonism of the type I interferon signaling has been identified thus far (145). In contrast, the past decade of research has revealed numerous mechanisms by which DENV antagonizes the signal transduction pathways involved in the type I interferon system (28, 119). Multiple DENV proteins as well as sfRNA are dedicated to inhibition of both the type I interferon induction and signaling axes, further emphasizing a critical role for type I interferons in host defense against DENV.

In particular, DENV NS2A, NS4A, NS4B, NS2B3, and the sfRNA have been observed to target distinct steps of the RIG-I/MDA5 signaling to inhibit type I interferon production. DENV NS2A and NS4B overexpression can inhibit RIG-I/MDA5-dependent type I interferon production by blocking TBK1 phosphorylation (146). DENV NS4A can associate with MAVS, preventing MAVS binding to RIG-I and subsequent IRF3 activation and type I interferon induction (147). DENV NS4B has also been demonstrated to induce the formation of elongated mitochondria that are physically linked with DENV-induced replication factories and favor viral replication by limiting RIG-I-dependent induction of type I and III interferons (148). However, in another study DENV protease NS2B3 was shown to cleave mitochondrial fusion proteins, thereby disrupting mitochondrial fusion and membrane potential and attenuating MAVS activation (149). These two conflicting reports on mitochondrial elongation versus fusion during DENV infection may be specific to the cell line and experimental approach, and both are as yet to be confirmed using relevant primary human cells. The protease-independent function of DENV NS2B3 can also antagonize the RIG-I pathway by binding of NS3 to the adaptor protein 14-3-3ε and thus preventing RIG-I translocation to mitochondria (150). Finally, sfRNA of DENV strains that are associated with greater epidemic potential binds more strongly to the ubiquitin ligase tripartite motif protein 25 (TRIM25), thereby preventing the ubiquitination-dependent activation of RIG-I (5). The existence of so many different mechanisms involving multiple viral proteins and sfRNA to subvert the RIG-I/MDA5 pathway suggests a dominant role for the RLR sensors in inducing a type I interferon response against DENV, consistent with *Mavs*$^{-/-}$ mouse data demonstrating an essential role for MAVS in mounting the initial antiviral innate immune response against DENV (120). RIG-I and MDA5 function redundantly in detecting DENV in cells (102) and are thus likely to play a similar role in vivo. Based on a study suggesting that overexpression of ZIKV sfRNA limits both RIG-I- and MDA5-directed type I interferon induction, whereas DENV sfRNA inhibited only RIG-I-mediated type I interferon induction (30), ZIKV sfRNA may have a broader antagonistic activity than DENV sfRNA at the level of RLR signaling.

DENV also blocks type I interferon production in human cells via manipulation of the cytosolic DNA receptor cGAS and its adapter STING, an endoplasmic reticulum–resident protein (151–153). cGAS binds to double-stranded DNA and catalyzes the formation of cyclic dinucleotides, which then form cGAMP that activates STING to trigger type I interferon production. cGAS can recognize DNA in various contexts, including microbial and cellular DNA. In DENV-infected cells, cGAS senses mitochondrial DNA (153). DENV NS2B3 protease mediates cleavage of human but not murine STING (151, 152), revealing STING as a species-specific restriction factor against DENV in mice. To counteract NS2B3-mediated cleavage of STING, the endoplasmic reticulum protein SCAP prevents association of NS2B with STING and inhibits the formation of NS2B3 (154), suggesting that STING and SCAP levels may dictate differential susceptibility of cells to DENV infection. The DENV protease also targets cGAS for degradation, but independently of its proteolytic activity and via NS2B rather than the NS2B3 complex (153). DENV targeting of both cGAS and STING (i.e., members of the same pathway) suggests an important role for this pathway in sensing DENV. Thus, redundant targeting of the RLR and cGAS/STING pathways and multiple members of a common pathway may ensure efficient blockade of type I interferon production. In the future, in vivo studies using mice deficient in particular components of the RLR and cGAS pathways and in vitro studies using primary human cells should elucidate the relative importance of the RLR versus cGAS pathways in the host defense against DENV and ZIKV.

DENV also uses multiple viral proteins and sfRNA to antagonize type I interferon signaling. DENV NS2A, NS4A, and NS4B can suppress STAT1 activation and downstream ISG expression (155). To prevent expression of ISGs, DENV sfRNA binds to and inactivates RNA-binding proteins G3BP1, G3BP2, and CAPRIN1, which are required for translation of ISG mRNAs (29). Studies to date indicate that DENV NS5 may be the most potent antagonist of type I interferon signaling. Specifically, DENV NS5 targets human but not murine STAT2 for degradation via interaction with UBR4, a cellular protein with E3 ligase activity responsible for degrading proteins (136, 156). Thus, murine STAT2 is another species-specific restriction factor against DENV besides STING. Recently, ZIKV NS5 was also identified as an antagonist of type I interferon signaling (145). Similarly to DENV NS5, ZIKV NS5 binds and degrades human but not mouse STAT2 and acts as a species-specific restriction factor against ZIKV in mice; however, unlike DENV, ZIKV does not use UBR4, suggesting that ZIKV uses an as yet to be identified mechanism to degrade STAT2. Based on another study showing that ZIKV blocks phosphorylation of both STAT1 and STAT2 in primary human dendritic cells to antagonize type I interferon signaling and limit cytokine production and activation marker expression (157), ZIKV is also likely to use multiple viral proteins and mechanisms to evade type I interferon signaling.

The 5′ methylated cap of the DENV and ZIKV genomes may also be involved in counteracting the interferon system. In general, 2′-*O*-methylation of the 5′ cap on the virus genomic RNA mimics cellular mRNAs and conceals the viral genome from detection via RLRs (158, 159) and the type I interferon–dependent IFIT family of proteins (160). For DENV, 2′-*O*-methylation of the 5′ cap is also important for evading an early type I interferon–independent response (161). MAVS localizes to peroxisomes in addition to mitochondria, and peroxisomal MAVS can rapidly induce type III interferon–dependent expression of ISGs that restrict DENV (139). Additionally, DENV capsid, the first viral protein made in flavivirus-infected cells, may be involved in reducing peroxisome numbers and type III interferon production (162). Thus, consistent with murine studies implicating roles for PRR-induced, type I interferon–independent, type III interferon–dependent responses in host defense against DENV and ZIKV infection in vivo, DENV and ZIKV may have evolved strategies to counteract the antiviral effects of early ISGs. These ISGs may be rapidly induced in a type I interferon–independent manner to provide short-term protection, while mitochondrial MAVS activates type I interferon signaling with delayed kinetics to amplify and stabilize the

antiviral response. At present, cross talk among various viral proteins and interferon antagonism strategies, and the importance of peroxisome- versus mitochondrion-mediated responses and early type I interferon–independent versus –dependent responses against DENV and ZIKV infection in vivo are as yet to be explored.

Collectively, studies have provided a solid foundation to explore the interactions between the interferon system and virus during DENV/ZIKV infections in animal models, relevant human cell culture models, and humans using state-of-the-art technologies. Despite decades of research, the mechanisms by which the type I interferon system mediates antiviral defense are as yet to be fully understood. Considering their similarity in genome structures and replication strategies, DENV and ZIKV likely share many aspects of their interactions with the type I interferon system. However, due to differences in the biology of DENV and ZIKV and the context-dependent nature of the type I interferon system, many interactions between the type I interferon system and DENV/ZIKV should also vary depending on the virus. Answers to questions related to how ZIKV interactions with the interferon system influence the outcome of infection in barrier tissues such as the placenta, testes, eyes, and brain and during pregnancy in the mother versus the fetus may also provide novel insights into the mechanism of action of the interferon system. Importantly, identifying the specific mechanisms by which DENV and ZIKV activate and evade the interferon system and potentially other components of the innate immune system, including the complement system, γδ T cells, and various types of innate lymphoid cells, is essential for understanding viral pathogenesis and developing effective therapeutics and vaccines against DENV and ZIKV. Particularly in the context of adaptive immunity and vaccine development (discussed below), studies investigating the roles of infected versus bystander cells belonging to the monocyte/macrophage/dendritic cell lineage in regulation of the adaptive immunity are urgently needed.

THE DEBATE CONTINUES: THE ROLE OF ADAPTIVE IMMUNITY IN PROTECTION VERSUS PATHOGENESIS

While primary infection by one DENV serotype confers life-long immunity to that serotype (163), multiple epidemiological studies indicate that DHF/DSS most often occurs in individuals experiencing a second DENV infection by a different (heterotypic) serotype (7, 164). For decades, the two non–mutually exclusive hypotheses of ADE and T cell original antigenic sin have competed to explain why a subset of secondary infections results in more severe dengue disease. Evaluation of these hypotheses is now crucial in the context of infection with not only the four DENV serotypes but also ZIKV, given that DENV and ZIKV share genetic similarity and structural homology with each other, and ZIKV continues to merge into DENV's established ranges. Emerging evidence indicates that the amino acid sequence conservation and structural similarities among the four DENV serotypes and ZIKV are substantial enough for these viruses to be cross-reactive at both the humoral and the cellular levels (165, 166). In this section, we focus on the potential dual roles of antibodies and T cells in DENV and ZIKV protection and pathogenesis.

Antibody-Mediated Protection

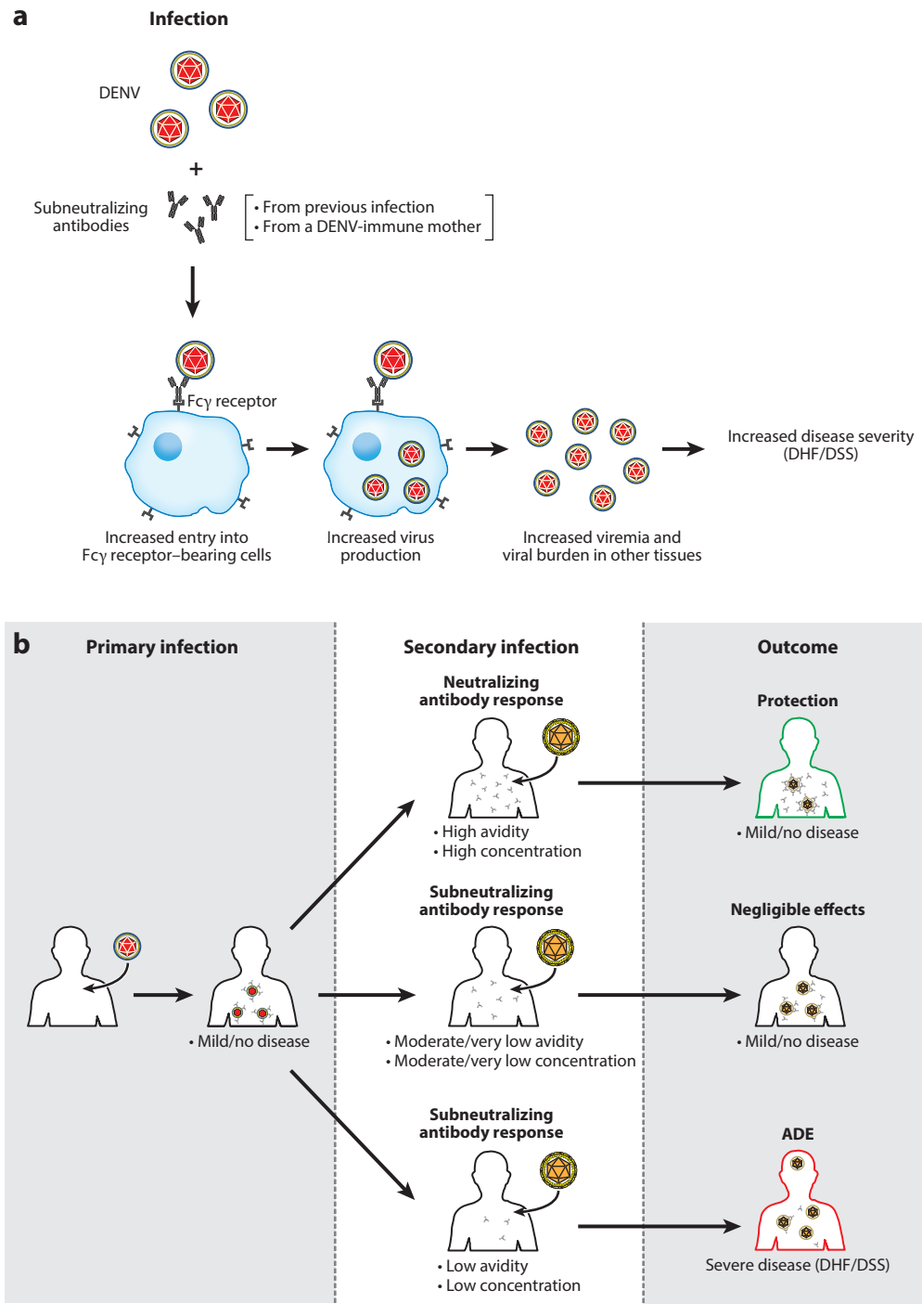
Many years of research have shown that both DENV serotype–specific and DENV serotype–cross-reactive antibodies can inhibit DENV infection (58, 117, 167–169). In natural DENV infections, DENV-specific IgM appears first, followed by IgG (mainly IgG1 and IgG3), which persists despite a steady decline in titer (170). For decades, infected patients have had detectable DENV antibodies that are partially cross-reactive for all four DENV serotypes and for other flaviviruses as well.

Primary infection induces long-term immunity to homotypic DENV. However, the cross-reactive antibody response induced by a primary DENV infection tends to be low avidity and weakly neutralizing, whereas the cross-reactive antibody response after a secondary DENV infection can be high avidity and highly neutralizing in vitro (171, 172), and subsequent reinfections are rare. Since the recent emergence of ZIKV, multiple groups have reported cross-reactivity between DENV and ZIKV sera and vice versa, in terms of both binding and neutralization (173–176). Thus, how prior DENV immunity affects the subsequent antibody response to ZIKV and vice versa, in terms of specificity/avidity and binding versus neutralizing titers, is now an important question in the field.

The E protein is the main target of neutralizing antibodies in flavivirus infections, and many recent reviews have discussed epitopes recognized by DENV and ZIKV antibodies (165, 177). In particular, EDIII contains epitopes that are recognized by serotype-specific potently neutralizing antibodies in mice (178, 179). However, these EDIII-specific antibodies represent a small proportion of antibodies in DENV-immune human sera (180); instead, the human antibody response to DENV appears to be dominated by weakly neutralizing and highly cross-reactive antibodies that bind the prM protein or fusion loop epitope (FLE) located in EDII (170). In addition, the anti-DENV antibody response in humans consists of antibodies that recognize complex, quaternary epitopes that are present on intact virions but not recombinant E proteins (181, 182). The most strongly neutralizing DENV monoclonal antibodies identified to date are directed against the conformational quaternary epitopes that span across multiple E proteins (183, 184). Recently, E dimer epitope–specific monoclonal antibodies derived from DENV patients were demonstrated to cross-neutralize ZIKV with high potency (175, 176). Other recent studies showed that EDIII, FLE, and complex quaternary epitopes on E are also targets of monoclonal antibodies isolated from ZIKV-infected patients (174, 185–188). One of these studies also found that preexisting DENV immunity is associated with increased ZIKV neutralizing antibody titers against EDIII (188), setting the stage for investigating the influence of prior DENV exposure on the development of an antibody response to ZIKV and vice versa.

Antibody-Dependent Enhancement

In addition to protection, the antibody response to DENV may contribute to viral replication and disease severity during secondary infection with heterotypic DENV via ADE. According to the ADE hypothesis, DENV-antibody complexes are formed and bind to FcγRs on cells such as macrophages, facilitating viral entry and replication; increased viral loads resulting from ADE then drive the production of inflammatory mediators that increase vascular permeability (**Figure 3*a***). Multiple lines of evidence now support this ADE model. First, mothers of infants with DHF/DSS due to primary infection have DENV-specific antibodies (189), suggesting that antibodies produced during primary infections or passively acquired contribute to DHF/DSS. DHF/DSS has not yet been observed in an infant born to a DENV-naive mother, providing the most compelling argument for the role of anti-DENV antibodies in DENV pathogenesis. Second, studies with nonhuman primates have demonstrated ADE of DENV replication in vivo. Specifically, monkeys develop higher levels and longer duration of viremia in heterologous infections with DENV than in primary infection with the same virus (190), and monkeys passively transferred with DENV-immune human sera (191) or a humanized IgG1 monoclonal antibody have higher viral loads than control monkeys (192). Third, studies with models of DENV infection in mice lacking type I interferon receptor signaling have shown ADE of both DENV replication in vivo and disease severity (58, 193, 194). Recently, a similar passive transfer study using DENV-immune human sera and a model of ZIKV infection in mice lacking STAT2 also demonstrated ADE of ZIKV



(*Caption appears on following page*)

Figure 3 (*Figure appears on preceding page*)

Simplified scheme of outcomes resulting from antibody dynamics: ADE versus protection versus negligible effects. (*a*) According to the ADE hypothesis, antibodies that are subneutralizing in quality or quantity bind a cross-reactive virion, and this immune complex gains access to permissive cells bearing the Fcγ receptor. Increased viral replication and viremia result in severe disease (DHF/DSS). (*b*) Antibodies are generated in response to primary infection. At the time of future infection by a cross-reactive virus, four states of antibody CAC result in three categories of clinical outcomes. If CAC is high, then the second infection is neutralized without causing symptoms. If CAC is moderate or minimal, then virus infection is controlled through other mechanisms and Abs have negligible effects. If CAC is low, then antibodies enhance infection and result in severe disease. Abbreviations: Abs, antibodies; ADE, antibody-dependent enhancement; CAC, combined avidity and concentration; DHF/DSS, dengue hemorrhagic fever/dengue shock syndrome.

infection and pathogenesis (195). Collectively, these findings implicate an important role for ADE in both DENV and ZIKV pathogenesis in humans.

Studies using a variety of monoclonal antibodies have shown that all neutralizing antibodies can promote ADE in vitro when used at subneutralizing concentrations (196–198). This relates to the concept that a neutralizing antibody can induce ADE if the viral occupancy threshold required for neutralization is not reached (196). Neutralization versus enhancement is simply a function of the stoichiometry of antibody binding to the viral particles (199). For neutralization, an antibody must be of high enough affinity to block correct epitopes on the surface of the virus, and antibody must be present in sufficient concentration. Failure to fulfill either condition will prevent neutralization. Therefore, even neutralizing antibodies will neutralize only if present at sufficiently high concentrations, and they may either mediate ADE if the antibody amount is insufficient for neutralization but sufficient for ADE or exert negligible effect if the antibody amount/condition is insufficient/inappropriate for both ADE and neutralization (**Figure 3*b***). The precise features and molecular mechanisms of ADE are currently unknown. Studies suggest that ADE versus negligible effects of antibodies may depend on engagement of different signaling cascades. For example, signaling-competent and -incompetent forms of FcγR1 and FcγRII induce differential enhancement of DENV immune complex infectivity (200), and FcγRIIB coligation, which requires high antibody concentrations, inhibits ADE (201, 202). ADE may also downregulate activities of key antiviral molecules such as IRF-1 and STAT-1 (203) and increase production of certain cytokines such as TNF, IL-6, and IL-10 (203, 204), and this ADE-mediated evasion of the antiviral response appears to be mediated via coligation of LILRB1, which inhibits expression of ISGs triggered via the activating FcγR pathway (205). Consistent with an important role for FcγR signaling in mediating ADE, a recent study reported a potential role for unfucosylated Fc glycans and particular IgG subclasses in promoting ADE (206). Overall, these results suggest that complex interactions among multiple signaling pathways modulate ADE, providing a framework toward further investigation of ADE mechanisms using relevant human cell culture and animal models.

T Cell Original Antigenic Sin

With or without ADE, T cell original antigenic sin postulates that T cells primed against a primary DENV serotype can inappropriately dominate the response to secondary heterotypic infection, resulting in an immune response that can worsen the active secondary infection (**Figure 4*a***). Clinical studies have shown a higher frequency of DENV serotype cross-reactive CD8 T cells with low affinity (207) in the blood of patients experiencing DHF/DSS compared to those with dengue fever, as well as altered cytokine profiles in DENV-specific CD8 T cells from DHF/DSS patients (208, 209). DENV-specific CD4 T cells also produce higher proportions of TNF, a key feature of the cytokine storm in DHF/DSS patients, in response to heterotypic antigen compared to homotypic antigen (210, 211). However, to date, direct evidence for the role of T cells in the pathogenesis of DHF/DSS is lacking.

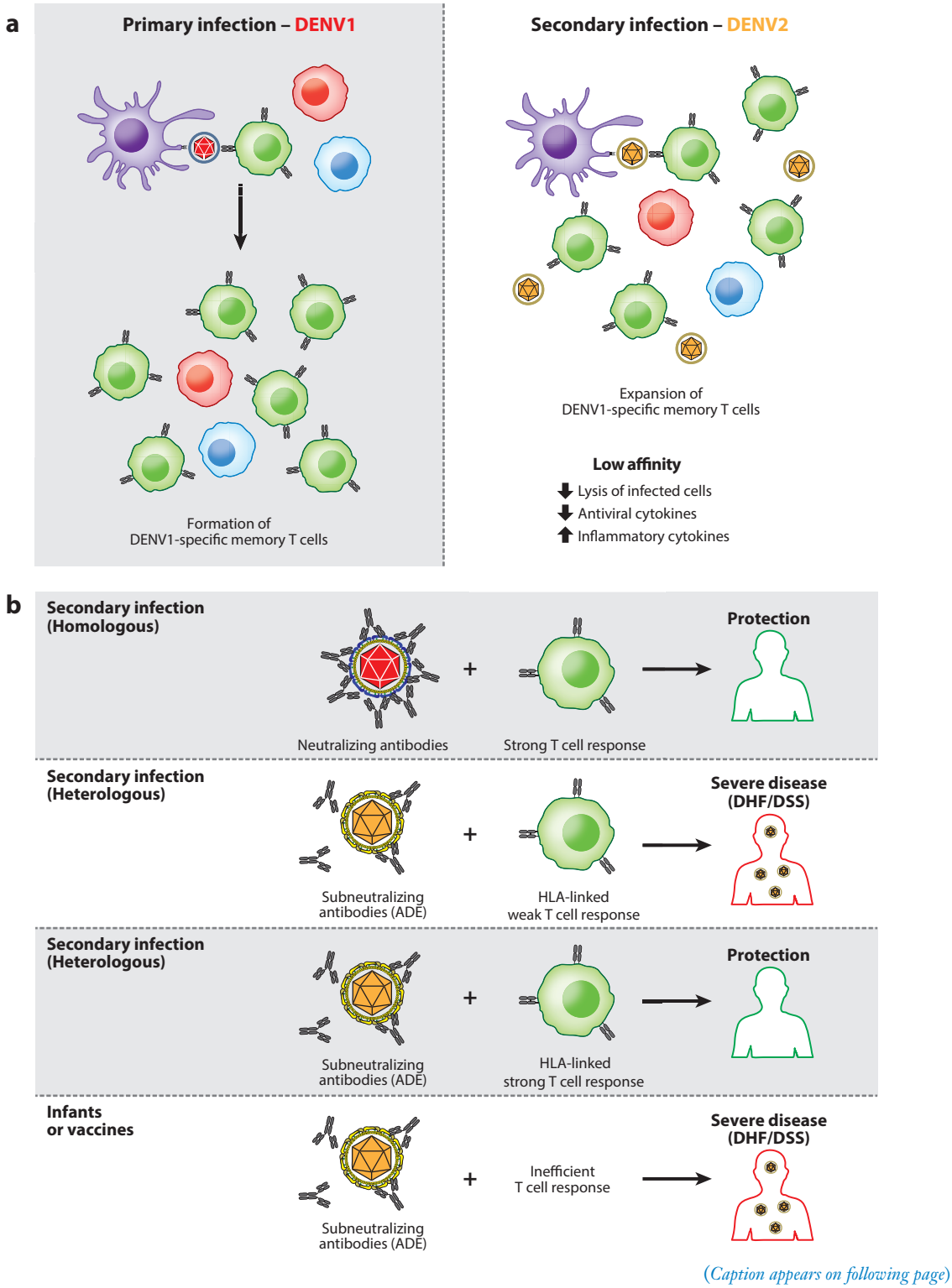


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Figure 4 (*Figure appears on preceding page*)

T cell original antigenic sin and scheme of outcomes resulting from combined humoral and T cell immunity. (*a*) T cell original antigenic sin postulates that T cells exposed to a first virus undergo appropriate clonal expansion. However, upon new infection and presentation of cross-reactive viral antigen, the previously expanded T cell populations that have low affinity to the currently infecting virus are activated. This results in a failure to adequately kill infected cells and leads to aberrant, pathogenic immune responses. (*b*) Evidence better supports a model in which T cells are generally protective against primary infection and secondary infection even under antibody-dependent enhancement (ADE) conditions. However, T cell responses may be attenuated by host factors such as HLA.

Is It Time to Forgive T Cells?

While the evidence suggesting a pathogenic role for T cells is sparse, recent data abound to indicate protective roles for serotype-specific and cross-reactive T cells against DENV infection. The CD8 T cell response to DENV mainly targets the nonstructural proteins NS3, NS4B, and NS5 (212). Studies analyzing epitope-specific CD8 T cell responses in blood samples from populations with prior DENV exposure and individuals that have been vaccinated with a live-attenuated tetravalent DENV formulation have revealed that although T cell specificity is skewed toward conserved epitopes, this skew is not associated with impairment of the actual immune response (212–214). In fact, in these populations with prior DENV exposure, the magnitude and breadth of the CD8 T cell response was found to be associated with HLA alleles that correlate with disease susceptibility, suggesting that a high-magnitude/breadth CD8 T cell response is a correlate of protection (212, 215). DENV-specific CD8 T cell responses that were associated with protective HLA alleles were polyfunctional, producing both IFN-γ and TNF-α, and the majority of these cells were effector memory T cells (both $CD45RA^{-}CCR7^{-}$ T effector memory and $CD45RA^{+}CCR7^{-}$ T effector memory RA subsets) that expressed high levels of the programmed death 1 (PD-1) receptor (212, 215). DENV-specific CD8 T cells in the blood may also express CXCR3, CCR5, and the skin-homing marker cutaneous lymphocyte-associated antigen (CLA); these cells can home to the skin during acute DENV infection (216). Finally, higher frequencies of DENV-specific IFN-γ-producing CD8 T cells were also seen in children who developed subclinical infection, compared to those who developed symptomatic secondary DENV infection (217).

Unlike the CD8 T cell response, the anti-DENV CD4 T cell response targets structural proteins (C and E) over nonstructural proteins (218). However, similar to CD8 T cells, CD4 T cells may contribute to protection against DENV. Phase I trials in humans using a live-attenuated vaccine suggested that multifunctional CD4 T cells that produce proinflammatory cytokines might facilitate control of DENV replication or mediate viral clearance (219). In addition, the $CD45RA^{+}CCR7^{-}$ subset of CD4 T cells was expanded in humans with secondary (multiple) DENV infections, particularly in individuals carrying an HLA allele associated with protection from severe disease (220). These CD4 T cells that expand depending on DENV infection history and HLA alleles are likely cytotoxic effectors, based on their expression of granzyme B, perforin, CD107a, and ex vivo DENV-specific cytolytic activity. Thus, both CD4 and CD8 T cells may protect against DENV infection in large part via their cytotoxic effector functions, and HLA alleles seem to dictate the magnitude and breadth of both CD4 and CD8 T cell responses.

In parallel to the evidence in humans, murine studies have demonstrated a critical role for T cells in protection against DENV infection. Specifically, studies using mice lacking type I and/or type II interferon receptors—which support robust DENV replication, unlike their wild-type counterparts, as discussed above—have revealed that CD8 T cells are more important than CD4 T cells in protection against both primary and secondary DENV infections (127, 221–226). In particular, these studies have demonstrated that (*a*) primary DENV infection elicits polyfunctional effector CD8 T cells that express IFN-γ, TNF, and CD107a and contribute to

viral clearance (222), (*b*) CD8 T cells are required for DENV clearance from the central nervous system (127), (*c*) CD8 T cells prevent ADE (227), (*d*) a DENV E protein–based vaccine candidate confers protection via CD8 T cells (226), (*e*) CD8 T cells are essential for protection against heterotypic reinfection (224), and (*f*) cross-reactive CD8 T cells that exhibit lower avidity and magnitude of response to heterotypic infection contribute to viral clearance (225). Although CD4 T cells appear not to be essential in controlling DENV infection, they can provide protection in a peptide vaccination setting, also likely through their cytotoxic activities (223). These results for T cell–mediated protection in mice are likely relevant to human infection, as the following observations based on studies with HLA transgenic mice have been validated: (*a*) The epitopes identified in mice were also recognized by T cells from DENV-exposed humans (225, 228, 229); (*b*) a dominance of HLA B*0702–restricted responses was detected in both mice and humans (212, 228); (*c*) CD8 T cell responses target both structural and nonstructural proteins in DENV3 but predominantly nonstructural proteins in the other three DENV serotypes in both mice and humans (214, 229, 230); and (*d*) CD8 T cell responses were broad following primary and homotypic secondary DENV infection in both mice and humans, whereas CD8 T cell responses following heterotypic secondary infection in mice (229) and vaccination with tetravalent live-attenuated DENV (214, 231) and natural reinfections in humans (208, 212, 218) were focused toward the conserved nonstructural proteins.

Researchers are also beginning to explore the role of T cells in ZIKV immunity using animal models. T cell responses have been detected in ZIKV-infected nonhuman primates and the peak of activation correlates with ZIKV viral RNA reduction, suggesting protective roles for both CD4 and CD8 T cells in controlling ZIKV replication (232). In all cases, ZIKV rechallenge resulted in complete protection, indicating that primary ZIKV infection induces protective immunity (82, 232). In mice with interferon receptor–competent T cells, CD8 T cells expand and play a critical role in protection against ZIKV infection (233–235). These CD8 T cells express the cytotoxic markers CD107a and granzyme B and exhibit in vivo cytotoxicity. During pregnancy, ZIKV-infected mice have decreased CD8 T cell activation, which could facilitate vertical transmission (235). CD8 T cell responses have also been examined in HLA transgenic mice (166). This study showed that both ZIKV-specific and DENV/ZIKV-cross-reactive CD8 T cells could protect against ZIKV infection. It also revealed that the CD8 T response to ZIKV, similar to the anti-DENV CD8 T cell response, elicits a weak T cell response in HLA-A*0101 transgenic mice compared to HLA-B*0702 transgenic mice; and immunodominance patterns in DENV/ZIKV cross-reactive T cell responses are modulated in a similar manner as heterotypic DENV responses. Collectively, these studies implicate important roles for both ZIKV-specific and DENV/ZIKV-cross-reactive CD8 T cells in protection against ZIKV in humans. Future investigation of both primary and sequential DENV-ZIKV and ZIKV-DENV infections in mice, nonhuman primates, and humans should provide insights into the precise quality and quantity of protective CD8 T cell responses to both viruses, and they may also provide evidence in support of a protective role for CD4 T cells against ZIKV in certain settings.

CONCLUSION

While vaccines have successfully enlisted the antibody response to prevent large-scale morbidity and mortality caused by other viruses, the studies reviewed herein indicate that the four serotypes of DENV present a cross-reactive challenge requiring a different approach. Early studies of DENV-ZIKV cross-immunity further suggest that a more complex, potentially high-risk immunological puzzle is emerging. Two principles to guide vaccine development for these flaviviruses emerge

from our investigative experience and assessment of clinical and research studies: Embrace complexity. Do no harm.

Viral species and strain/serotype, host age, prior infection/vaccination status, time since seroconversion, host genetic factors (in particular HLA), and individual immune status all must be considered when interpreting clinical and research data. While some data would implicate innate factors, antibodies, or T cells in protection or pathogenesis, clinical outcomes reflect the sum of all these factors. The model we present is one of innate immunity backstopped by humoral immunity backstopped by cellular immunity. In most primary and secondary cases of DENV and ZIKV infection, innate immunity is sufficiently backstopped by adaptive immunity to neutralize the virus and result in asymptomatic or negligible infection. However, in a minority of primary infections a weakened innate immune response can result in more severe clinical signs. Secondary infection by a cross-reactive serotype or virus challenges the host to produce an appropriate adaptive response based on memory of a different virus. A strong antibody response will neutralize the second virus and result in negligible disease. A minimal antibody response is insufficient in combined antibody avidity and concentration (CAC) to generate ADE conditions, and a strong T cell response stands behind humoral immunity to neutralize the infection. But if at the time of secondary infection CAC is in a low range conducive to ADE, then a strong T cell response is required to neutralize the infection and abrogate the effects of ADE. If the T cell response is attenuated by host factors such as HLA or inadequate antigen presentation, then ADE will progress unchecked (**Figure 4*b***).

Finally, beginning with epidemiologic observations made nearly 50 years ago of dengue patients in Thailand, enough scientific evidence has been established to indicate that modulation of host flavivirus immunity carries the risk of producing harmful effects. Given this documented risk, flaviviral vaccines should be designed to elicit strong responses from all branches of immunity, and vaccines and therapies should be diligently tested prior to release. Today no animal model matches the mouse for combined capacity to isolate and manipulate single variables, measure in vivo response, repeat and refine experiments, and generate statistical power. Further investigations of flaviviral infection in mouse models, in combination with studies using nonhuman primates and human samples, will prove invaluable to generating safe and effective vaccines and therapeutics.

DISCLOSURE STATEMENT

S.S. is listed as an inventor on US patent application 14/800468, "Dengue Virus (DV) Polypeptide Sequences, T Cell Epitopes, and Methods and Uses Thereof."

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