A Mouse Model for Studying Dengue Virus Pathogenesis and Immune Response

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A small animal model for studying dengue disease is of critical importance to furthering many areas of dengue research, including host immunity, disease pathogenesis, and drug and vaccine development. Recent characterization of the AG129 mouse model has demonstrated it to be one of the only models at this time that permits infection by all four serotypes of dengue virus (DENV), supports replication in relevant cell and tissue types comparable to human infection, and allows antibody-mediated protection and enhancement of DENV infection. Thus, this model enables testing hypotheses arising from epidemiological observations and *in vitro* experiments in an *in vivo* system with a functional adaptive immune response. This review provides a brief overview of the development of a mouse model of DENV infection, describes the work completed to date characterizing the AG129 model, and examines several of the unanswered questions remaining in the field.

Key words: **dengue; mouse model; antibody-dependent enhancement; AG129 mice**

Introduction

Dengue virus (DENV) is a mosquito-borne virus of the *Flaviviridae* family and is related to yellow fever, West Nile (WNV), and Japanese Encephalitis viruses.[1] Endemic to tropical and subtropical regions of the world, DENV is the most medically important arthropod-borne virus worldwide and a major public health challenge. Three billion people are at risk for DENV infection, with an estimated 50 million cases of dengue fever (DF) annually and 250,000–500,000 cases of the potentially fatal dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS), characterized by vascular leak leading to hypotensive shock.[2] The four DENV serotypes (DENV1-4) are transmitted to humans primarily by the mosquitoes *Aedes aegypti* and *Ae. albopictus*. The clinical course of DHF/DSS is initially quite similar to DF; however, at defervescence, DHF/DSS patients rapidly deteriorate into life-threatening conditions characterized by vascular leak, hemorrhagic manifestations, and thrombocytopenia with or without shock.[3] The inability to differentiate between DF and DHF/DSS at early stages of illness contributes to the difficulty in treating the disease. Further, individuals previously infected with DENV are at increased risk of severe disease upon secondary infection with a different (heterologous) serotype.[4] At present, no effective antiviral therapy or vaccine exists, and treatment is largely supportive in nature.

Role of the Adaptive Immune Response in Modulating Secondary DENV Infection

Host factors, including human leukocyte antigen (HLA) polymorphisms and prior

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T and B cell immunity, are key determinants of susceptibility to severe dengue disease.[5] Interferon (IFN) α/β, and γ are necessary for resistance to DENV-induced disease,[6] and both arms of the adaptive immune response have been demonstrated to play an important role in both protection and enhancement of dengue disease. In general, most individuals infected with DENV for the first time (primary infection) experience inapparent infection, undifferentiated fever, or DF. In contrast, epidemiological data and experimental human studies suggest that the greatest risk factor for development of DHF/DSS is prior infection with a different DENV serotype[4]; Halstead[7] estimated the rate of DHF/DSS to be 40 times more frequent during secondary than primary infections.

Two nonmutually exclusive hypotheses have been proposed to explain the role of the adaptive immune response in the development of DHF/DSS. The first implicates antibodies in mediating enhanced disease. In hallmark studies, Halstead and O'Rourke[8] demonstrated that DENV did not replicate in peripheral blood leukocytes (PBL) of nonimmune primates but did replicate in PBL isolated from immune primates; however, nonimmune PBL could be infected by adding anti-DENV antisera. This illustrates a phenomenon termed "antibody-dependent enhancement" (ADE),[7] in which non-neutralizing anti-DENV antibodies facilitate entry of the virus into Fcγ receptor (FcγR)-bearing cells.[7,9] This increase in infected cells directly contributes to the higher viremia levels associated with DHF/DSS,[7] and the infection of target monocyte/macrophage cells leads to activation, ultimately promoting the "cytokine storm" that characterizes DHF/DSS.[10] Additional evidence for ADE *in vivo* is derived from the observation that most severe cases in primary DENV infections occur in infants in endemic areas, where DENV-specific antibodies are transferred transplacentally to infants from their dengue-immune mothers.[11] These antibodies wane over time until they can enhance DENV infection,[11,12] suggesting that pre-existing antibodies alone are sufficient to promote severe DENV illness.[13] The second hypothesis explaining the immunopathology underlying severe secondary DENV infection implicates T cells. Specifically, low-affinity, cross-reactive $CD4^+$ and $CD8^+$ memory T cells resulting from a primary infection are over-activated during secondary DENV infection with a different serotype, and these serotype cross-reactive T cells produce high levels of cytokines, such as tumor necrosis factor-α (TNF)-α, which contribute to increased vascular permeability.[5,14,15] While *in vitro* and *ex vivo* experiments provide insight into individual contributions of the humoral and cell-mediated immune response, an *in vivo* model is required to mechanistically define their combined contribution to protection and enhancement of DENV infection and disease.

Development of a Mouse Model for DENV Infection and Disease

A small animal model for studying dengue disease is of critical importance to furthering many areas of dengue research, including host immunity, disease pathogenesis, and drug and vaccine development. Although many attempts were made over the past several decades to develop an animal model for studying DENV, most animal species proved to be resistant to DENV infection.[16] Over the past 7 years, our group and others[17,18] have developed and characterized the AG129 mouse (IFN-α/β, and -γ receptor-deficient) as a tool for studying dengue pathogenesis and immunology. This review will briefly outline the development of a small animal model of DENV infection and disease, discuss the contributions of the AG129 model to date, and elaborate upon its utility in studying aspects of DENV pathogenesis, immunity, and therapeutic drug development. For more extensive recent reviews of dengue mouse models, see Bente and Rico-Hesse,[19] Balsitis and Harris,[16] and Yauch and Shresta.[20]

Initial attempts to develop a mouse model for dengue in immunocompetent mice required

high doses of virus and were hindered by the inability to recapitulate several important aspects of human DENV infection, including replication in peripheral tissues and development of the hallmark symptoms of DENV disease.[16] As another approach, humanized mice developed by engrafting severe combined immunodeficient (SCID) mice with either human peripheral blood lymphocytes or a variety of DENV-susceptible tumor cells, including human K562 erythroleukemic cells and human liver HepG2 and Huh-7 cells, were explored as potential DENV mouse models.[16] Improvement of the humanized mouse model has included the development and characterization of nonobese diabetic (NOD)/SCID mice[21] and RAG2$^{-/-}\gamma^{-/-}$ mice[22] engrafted with CD34^{+} human cord blood hematopoietic stem cells. Interestingly, the reconstituted NOD/SCID mice developed viremia as well as clinical signs of dengue, including fever, rash, and thrombocytopenia following a subcutaneous (sc) infection with DENV2. However, the interaction between human cells and the mouse immune system may not completely reproduce a functional immune system, limiting the study of the adaptive immune response to DENV infection. Additionally, these mice are difficult and expensive to generate, thus hindering large-scale studies.

Severely immunocompromised strains, BALB/c athymic nu/nu mice[23] and RAG1$^{-/-}$ mice,[6] both succumb to infection with DENV, but death results from paralysis instead of a defined vascular leak phenotype. Given the importance of IFNs in controlling viral infections, studies were conducted in IFN-α/β, and -γ receptor-[6,24] and STAT1-deficient[25,26] mice. Johnson and Roehrig[24] initially established the utility of AG129 mice to study primary DENV infection and vaccine challenge. Comparison between mice lacking either the IFN-α/β or IFN-γ receptor or both suggested that both knockouts are required for early viral replication in peripheral tissues and subsequent disease.[6] Despite the immunodeficiency, the AG129 mouse model for dengue allows for investigation of tropism and pathogenesis in context of a functional adaptive immune system.

Characterization of the AG129 Mouse Model

Over the past several years, multiple studies have characterized the tissue and cellular tropism of DENV in the AG129 model,[27,28] demonstrating significant parallels with human infection.[27,29,30] Specifically, initial tropism studies using the AG129 model demonstrated that clinical isolates from all four DENV serotypes replicate efficiently in spleen, lymph node, bone marrow, and muscle. Negative-strand viral RNA was detected in dendritic cells and macrophages of the lymph node and spleen.[28] Similarly, antibodies directed against the nonstructural NS3 DENV protein indicated active viral replication in macrophages, dendritic cells, hepatocytes, and bone marrow-derived myeloid cells in infected AG129 mice.[27] Both of these studies coincide with tropism data from human autopsy studies[27,30] and flow cytometry analysis of infected human peripheral blood mononuclear cells,[29] where the infected cells were predominantly of the myeloid lineage. Importantly, the sc route of infection and 10^2–10^5 pfu inoculum used in these murine tropism studies are compatible with the natural route and viral inoculum found in a mosquito bite.[31] In addition, AG129 mice exhibit thrombocytopenia inversely related to viral load and develop high levels of soluble NS1 during DENV infection comparable to levels in humans[18] (data not shown).

Characterization of the AG129 immune response revealed a functional adaptive immune response to DENV infection. Specifically, antibodies elicited by infection are a mixture of serotype-specific and serotype–cross-reactive antibodies, including long-lasting neutralizing antibodies,[32] and the distribution of IgG isotypes among DENV-specific antibodies include IgG1, IgG2a, and IgG2b in ratios similar to the ratios of isotypes elicited by viral infection of

wild-type 129 mice[33] (data not shown). Sequential infections of one DENV serotype followed 4–52 weeks later by another serotype demonstrated reduction in viral load in the second infection as compared to naive mice experiencing a primary infection.[32] Passive transfer studies demonstrated that transfer of high doses of monoclonal antibodies (mAbs) directed against different epitopes on the envelope [E] protein (domain II fusion loop or domain III lateral ridge; see below) or anti-DENV polyclonal sera 24 h prior to DENV infection protect against viral challenge with either the same or a different serotype as measured by a reduction in viral load in spleen, lymph node, and serum[32] (data not shown). Additionally, studies examining the T cell response to DENV infection in AG129 mice determined that $CD8^+$ T cells release IFN-γ and TNF-α and have cytotoxic effects *in vivo*.[17] Mapping studies identified 12 immunodominant epitopes that mapped to six DENV proteins, and vaccination with four of these epitopes supported clearance of viral proteins.[17] Taken together, these results support the role of the mouse model in studying both serotype-specific and cross-reactive antibody-mediated protection.

To further investigate the role of dengue pathogenesis *in vivo,* a virulent, mouse peripherally adapted strain of DENV2, D2S10, was generated by alternately passaging the virus through mice and mosquito cells 10 times, harvesting mouse serum in each cycle.[34] A high inoculum of D2S10 administered to AG129 mice results in a lethal "vascular-leak" syndrome within 4–5 days. D2S10 has only two mutations, N124D and K128E, in the E protein that differentiate it from the parental clinical isolate PL046. These mutations decrease heparan sulfate binding and consequently reduce clearance of the virus, thus increasing viremia and contributing to the lethal disease phenotype.[35] A triple plaque-purified clone of D2S10, S221, has an additional mutation in NS1 and causes the same phenotype as D2S10.[17]

To study the role of antibodies in mediating enhancement of DENV disease, Balsitis *et al.* (manuscript submitted) tested whether mAbs or polyclonal sera raised in AG129 mice could enhance a sublethal dose of DENV2 strain D2S10. Passive transfer of both cross-reactive mAbs and heterotypic sera was found to be capable of lethal enhancement. Interestingly, although a higher dose of homotypic serum was protective, enhancement was observed following transfer of lower doses of homotypic sera, implying that once the dilution of serum falls below the threshold required for neutralization, the serum can become enhancing regardless of serotype specificity. This is consistent with *in vitro* observations of neutralization and enhancement.[36] Additionally, mice that succumbed to lethal disease had significantly increased levels of TNF-α and IL-10 and reduction in platelets, characteristics of human DHF/DSS. Viral tropism data indicated a significant increase in viral load in bone marrow, serum, white blood cells, lymph node, liver, and small and large intestine between mice infected with a sublethal DENV2 dose and mice experiencing lethal ADE of DENV2 infection. Interestingly, this increased viral load was not significantly different than that caused by a lethal dose of D2S10. Cellular tropism data supported an increase in infection in macrophages and dendritic cells in numerous organs as well as sinusoidal endothelial cells in the liver. Taken together, the viral and cellular tropism data imply that the pathogenesis of an enhanced infection is fundamentally comparable to that of a lethal, non-enhanced infection, where the main outcome is increased infection of targeted cell types resulting in elevated viral load. Again, the cell types targeted are similar to those infected by DENV in humans. In this model, infection with human DENV1 and DENV2 clinical isolates was similarly enhanced with pretransfer of either heterotypic anti-DENV sera or mAbs. In summary, the AG129 mouse model is currently the only model of DENV infection that supports replication of all four serotypes, demonstrates infection in relevant cell types and tissues, succumbs to a fatal vascular leak syndrome from a lethal dose of a mouse-adapted

strain of DENV, and can reproduce both protection and ADE. Together these data indicate that the AG129 mouse model can be used to study DENV pathogenesis and answer pertinent questions regarding the roles of both humoral and cell-mediated immune responses in contributing to protection and enhancement of DENV disease.

Unanswered Questions about Dengue Pathogenesis and Immune Response

To date, a majority of observations regarding factors contributing to the severity of DENV disease have resulted from epidemiological observations and *in vitro* analysis. As most aspects of the immune system are difficult to duplicate *in vitro*, many questions regarding the contribution of the adaptive immune response to modulating secondary DENV infection still remain. With the development of a small animal model to study DENV disease, we can now test hypotheses generated from epidemiological and *in vitro* data in an *in vivo* system. Specifically, what components of the immune response contribute to protection and enhancement? Are both the humoral and cell-mediated components necessary for protection? Dissecting the humoral immune response to DENV requires consideration of multiple contributing factors, including serotype-, domain-, and epitope-specificity of antibodies, stoichiometry and mechanism of neutralization, interaction between specific isotypes, complement and FcγR subtypes, and maturation state of the virus[37] (Fig. 1). Which antibody characteristics are associated with protection versus enhancement *in vivo*? How does the ratio of mature to immature virus particles contribute to antibody-mediated neutralization or enhancement? What candidate antivirals are protective against DENV infection and disease *in vivo*? Last, identification of a functional *in vitro* correlate of *in vivo* protection is critical for vaccine development. These are some of the issues that can now be addressed using the AG129 dengue mouse model.

Understanding the Role of Serotype-, Domain-, and Epitope-Specificity in Mediating Protection or Enhancement during a Secondary DENV Infection

One important question that remains unanswered is which characteristics of the antibody response contribute to modulation of infection outcome. For instance, to what degree are cross-reactive antibodies protective? To answer this question in an *in vivo* system, we are directly comparing the ability of serotype-specific versus cross-reactive antibodies to protect/neutralize or enhance DENV infection by diluting sera derived against each of the four serotypes to the same neutralizing titer (NT_{50}) against DENV2, inoculating mice with this sera followed by infection with DENV2 D2S10, and then measuring viral load and morbidity/mortality. This should address the contribution of quantity (titer) versus quality (serotype-specificity). The role of antibodies directed against different domains of the DENV E protein is also unclear. DENV E consists of three domains (EDI, II, III),[38] and antibodies targeted to EDI/II are often cross-reactive among the four DENV serotypes and other flaviviruses,[39,40] while antibodies against EDIII are more likely to be neutralizing and DENV serotype-specific.[39,41] Further, in mice, the immunodominant and most neutralizing antibodies are directed against EDIII, while in humans, the immunodominant response to WNV[42] and DENV[43] (A. deSilva, personal communication) appears to be directed against EDI/II, though strongly neutralizing anti-EDIII antibodies exist[43] (F. Sallusto, personal communication). By depleting mouse and human sera of EDIII-specific antibodies, measuring neutralization capacity *in vitro*, and inoculating mice with depleted and nondepleted sera followed by DENV2 challenge, we can directly determine how domain-specific antibodies contribute to neutralization *in vitro* and protection *in vivo*.

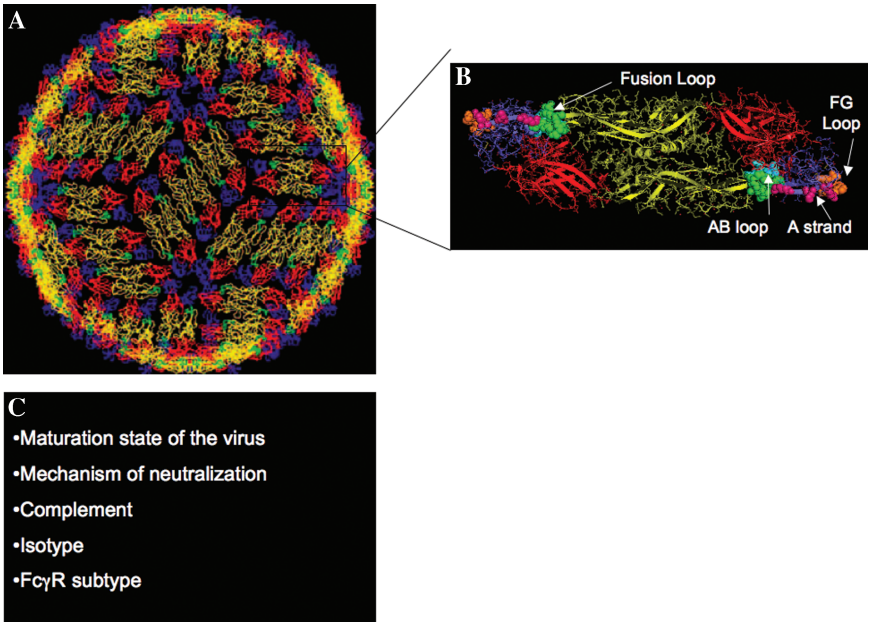

Figure 1. Factors influencing function of anti-dengue virus (DENV) antibodies. **(A)** Cryo-electron microscopy reconstruction of a mature DENV virion, with individual domains EDI, EDII, and EDIII indicated by red, yellow, and blue, respectively. Reprinted with permission from Kuhn *et al.*[56] **(B)** Ribbon diagram of the antiparallel DENV E protein homodimer viewed from the top. The fusion peptide and individual domains (EDI, red; EDII, yellow; EDIII blue) are indicated. Arrows indicated well-characterized epitopes. Modified from Modis *et al.*[57] **(C)** Additional viral and host factors that may modulate protection and enhancement of secondary DENV infection *in vivo*.

Throughout these experiments, the correlation between *in vitro* assays and *in vivo* outcomes will be addressed.

Likewise, the role of specific epitopes in protection/enhancement is not well-understood. At least eight epitopes on the flavivirus E protein with distinct biologic activities have been defined by antibody mapping,[44] including two strongly neutralizing epitopes (the FG loop of DENV E, analogous to the lateral ridge in WNV[45] and the A strand of EDIII[46]) and a cross-reactive epitope in the fusion loop in EDII.[44] Using Reporter Viral Particles (RVPs)[47] and infectious DENV2 D2S10 clones ablated for particular epitopes, the role of these epitopes in neutralization *in vitro* and protection *in vivo* can be assessed. Conversely, alphavirus/DENV virus replicon particles expressing particular B cell epitopes can be used to immunize mice and then test the contribution of these epitopes to protection upon subsequent viral challenge. Little is known about prM/M antibodies; this can be addressed directly by comparing the ability of anti-prM/M mAbs and anti-E mAbs to protect or enhance in the mice. Since anti-prM/M antibodies interact with immature/partially immature virions, these experiments can be combined with questions of virion maturation, which can be experimentally manipulated by preparing virions that are fully mature, partially immature, or fully immature.[48,49] By ablating the site of FcγR or C1q interaction in recombinant mAbs (e.g., mAbs containing the N297Q mutation in the heavy chain constrant region), the role of antibody effector functions in enhancement can be determined (Balsitis *et al.*, manuscript submitted). We have shown that N297Q-containing mAbs added after infection can protect against ADE elicited by priming with the same mAb

prior to infection (see below). This then allows investigation of whether ADE triggered by priming mice with mAbs directed to one epitope can be prevented by treatment with mAbs directed against another epitope or domain. Thus, many aspects of the humoral response to DENV infection can be examined mechanistically using this mouse model.

Establishing the Role of the B Cell Response in Modulating Immune Response to Secondary DENV Infection

While the memory immune response is clearly involved in both protection and enhancement of secondary DENV infection in humans,[5] little mechanistic data dissecting the roles of individual subsets of the memory response currently exists. Specifically, what components of the memory response—long-lived plasma cells (LPCs) or memory B and T cells—are important in controlling disease of a secondary DENV infection? Additionally, there is little data identifying and characterizing the epitope-specific memory B cells that contribute to protection or enhancement of DENV infection.

We have previously reported protection against secondary heterologous DENV infection between 4 and 52 weeks after primary infection, using sequential DENV1-DENV2 and DENV2-DENV4 infections in AG129 mice.[32] This *in vivo* protection correlated with detectable titers of heterologous neutralizing antibodies. Moreover, prior infection with DENV1 protects mice against both a sublethal (10^5 pfu) and lethal (10^7 pfu) secondary infection with DENV2 D2S10 8 weeks later (data not shown). Passive transfer of high-titer polyclonal sera or mAbs against E provided protection against secondary infection, whereas adoptive transfer of DENV1-immune splenocytes to naïve recipients provided only partial protection against secondary DENV2 infection.[32] These data underscore the importance of preformed antibodies for protection against DENV *in vivo* during sequential infections and imply a role for DENV-immune cells in the spleen.

The importance of the cellular immune response has been further illustrated by recent experiments using cyclophosphamide (CP). CP is an alkylating agent and immunosuppressive drug that primarily affects proliferating lymphocytes. CP-treated mice show a marked decrease in $CD4^+$ and $CD8^+$ T cell subsets, antibody-forming cells, and antibody levels.[50,51] CP has previously been shown to decrease survival of mice infected intracerebrally with DENV, and passive transfer of immune sera after CP treatment protected mice against fatal DENV infection.[52] Using the AG129 model, we showed that immune mice treated with CP prior to secondary DENV infection died on day 7.5–8.5, demonstrating a role for the cellular memory response in protection (Fig. 2A). Furthermore, CP-treated naïve mice experiencing a primary DENV infection died on day 4 (with no antibodies or cellular immune response), in contrast to the delayed death of CP-treated immune mice with a secondary DENV infection (containing LPC and preformed antibodies), supporting the contribution of preformed antibodies to protection (Fig. 2B). Antibody levels did not change significantly between days -1 and day 6 p.i., but viremia increased on average 2.5 logs between days 4 and 6.5 p.i. only in CP-treated mice (Fig. 2C). At day 6.5, or 24–48 h prior to death, viral load in CP-treated mice was ~1 log lower than that of mice with lethal ADE 10 h prior to death (data not shown). This indicates an initial role for antibodies in early protection and a later role for the cellular immune response. Together, these data imply the importance of a functional B cell response and LPCs in protection against secondary DENV infection.

The partial protection provided by the cellular immune response may have been induced either by memory $CD4^+$ cells, memory $CD8^+$ cells, or by memory B cells that rapidly differentiated into antibody-producing cells upon secondary DENV infection, thus producing high titers of neutralizing antibody. Depletion of the

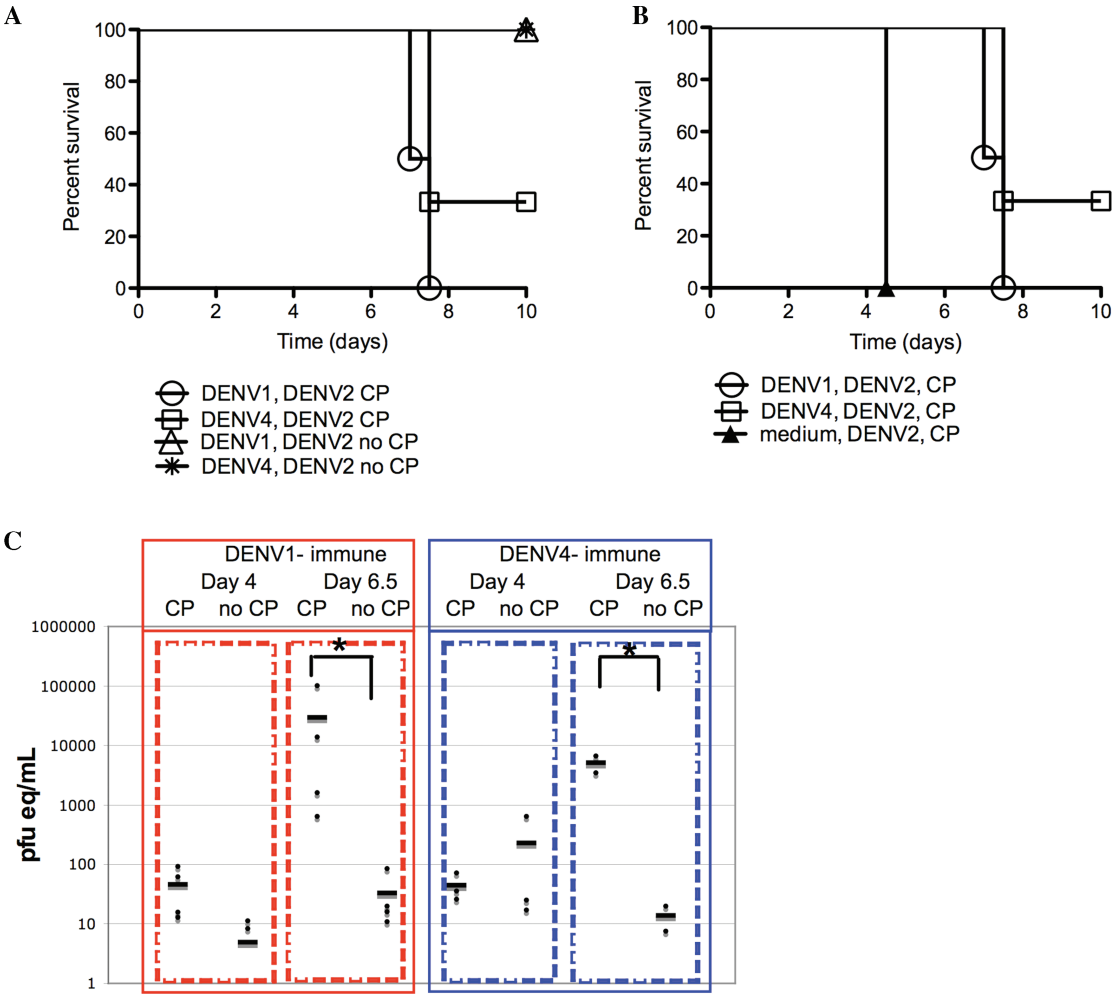

Figure 2. Role of memory immune response in dengue virus (DENV)-infected AG129 mice. (**A**) Mice (n = 3–4/group) were infected with 10^5 pfu sc of DENV1 or DENV4 and 6 weeks later were infected with 10^5 pfu of DENV2 D2S10 intravenously (iv). On days −1, 0, 1, 2, 3, and 4, mice were treated with 50 mg/kg of cyclophosphamide (CP) or PBS (no CP). Survival was monitored for 10 days. (**B**) As in **A**, mice (n = 3–4/group) were infected with 10^5 pfu sc of DENV1 or DENV4 or C6/36 cell medium and 6 weeks later infected with 10^5 pfu iv of DENV2 D2S10. On days −1, 0, 1, 2, 3, and 4, mice were treated with 50 mg/kg of CP. (**C**) DENV1- and DENV4-immune mice treated or not treated with CP were infected with 10^5 DENV2 D2S10 iv 6 weeks after 1° infection. Retro-orbital eye bleeds were performed on days 4 and 6.5, and DENV viremia was quantified by qRT-PCR. Significant differences were measured by Wilcoxon rank sum analysis; * $P < 0.05$.

different cellular components using mABS and adoptive transfer of presorted T and B cells will allow us to differentiate which component(s) are necessary and/or sufficient to induce protection during secondary DENV infection, as well as to investigate memory B cells and LPCs and the specific contributions of individual B cell epitopes.

Development of Therapeutics for DENV Infection

While no antivirals for dengue currently exist, there has recently been an upsurge in interest in development of anti-dengue therapeutic agents.[18,53] While *in vitro* analysis of different compounds is necessary for screening

purposes, preclinical testing using a small animal model is crucial to further development of candidate therapeutic agents. Several classes of potential anti-dengue drugs currently exist: antivirals that reduce viral load by targeting either the virus or host processes that are critical for the virus, compounds that interfere with the antibody-FcγR interaction and prevent ADE, and drugs that prevent severe systemic inflammatory disease (e.g., vascular leak). The rationale for the first class of drugs that target a reduction in viral load arises from clinical studies showing that DHF/DSS patients have 1- to 2-log higher viremia than DF cases.[54,55] This data suggests that compounds that target virus replication early in disease may lower viral load and prevent progress to severe disease. One such class of reagents are iminosugars designed to inhibit host cell proteins required for E glycoprotein folding and maturation. One iminosugar, *N*-nonyl-deoxynojirimycin (*N*N-DNJ) tested in AG129 mice reduced viremia and splenomegaly.[18] Consistent with these results, we have obtained preliminary data demonstrating efficacy for additional iminosugars in our AG129 mouse model of ADE.

The second class of drugs involves a novel therapeutic approach that derives from the critical role of the antibody–FcγR interaction for ADE *in vitro*,[9] which we have recently demonstrated is essential for ADE *in vivo* (Balsitis *et al.*, manuscript submitted). Interestingly, recombinant mAbs lacking the binding site for FcγR (N297Q) transferred either concurrently with heterotypic sera or enhancing mAbs in the AG129 ADE model, or 24 h after antibody-enhanced lethal DENV infection completely prevented mortality. Treatment with this mAb variant significantly decreased viremia, tissue viral burden, and serum TNF-α levels as compared to lethal infection. Moreover, when transferred 48 h post infection, 80% survival was observed with higher concentrations of mAb and 40% survival with lower doses. In addition to therapeutic use, mAbs lacking the FcγR binding site can be used to ask fundamental questions regarding the mechanism of ADE (see above).

The third class of drugs are anti-inflammatory compounds designed to reduce the pathogenic response to DENV infection. We tested an anti-inflammatory peptide and found it to modestly but significantly delay mortality in our mouse model of ADE (data not shown). For all three classes of drugs, *in vivo* analysis in a small animal model is essential for further consideration of these candidate therapeutic agents in treating the clinical manifestations of DENV disease. Wide-scale use of dengue antivirals is much more feasible now, after development and increasingly widespread distribution of an acute-phase diagnostic based on detection of NS1, a viral protein that is secreted from DENV-infected cells whose antigenemia correlates with DENV viremia.[54] As dengue continues to spread worldwide and increase in both incidence and severity, there is a heightened sense of urgency to move animal testing of lead antiviral compounds forward.

In conclusion, the AG129 mouse model is now positioned as the first robust small animal model to study the role of the adaptive immune system in modulating protection and enhancement of DENV infection. Epidemiological data has implicated the adaptive immune response in mediating severe secondary infections; however, crucial questions regarding the specific role of the humoral and cell-mediated immune response currently remain unanswered. With the development of the AG129 mouse model, necessary experimental tools now exist to further examine the role of antibody repertoire and function in mediating DENV protection and enhancement as well as to unravel the contribution of LPCs and B and T memory cells in modulating secondary DENV infection. Finally, this model can be used to evaluate therapeutic drug candidates to treat DENV disease.

Conflicts of Interest

The authors declare no conflicts of interest.

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