A Dengue Fever Viremia Model in Mice Shows Reduction in Viral Replication and Suppression of the Inflammatory Response after Treatment with Antiviral Drugs

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Dengue fever is an emerging arboviral disease for which no vaccine or antiviral treatment exists and that causes thousands of fatalities each year. To develop an in vivo test system for antidengue drugs, AG129 mice, which are deficient for the interferon-α/β and -γ receptors, were injected with unadapted dengue virus, resulting in a dose-dependent transient viremia lasting several days and peaking on day 3 after infection. Additionally, nonstructural protein 1, increased levels of proinflammatory cytokines, and neutralizing IgM and IgG antibodies were found, and mice had splenomegaly. Oral administration of the antiviral compounds 7-deaza-2′-*C*-methyl-adenosine, *N*-nonyl-deoxynojirimycin, or 6-*O*-butanoyl castanospermine significantly reduced viremia in a dose-dependent manner, even after delayed treatment, leading to a reduction of splenomegaly and proinflammatory cytokine levels. The results validate this dengue viremia mouse model as a suitable system for testing antidengue drugs and indicate that antiviral treatment during the acute phase of dengue fever can reduce the severity of the disease.

Dengue fever is an infectious disease caused by the dengue virus (DENV), and it is the most prevalent mosquito-borne viral disease in humans. Dengue fever occurs in tropical and subtropical regions and threatens an estimated 2.5 billion people. Growing urbanization, failure to control the mosquito vector, and increase in long-distance travel have contributed to the continuing spread and increase of the disease [1].

DENV is a membrane-enveloped positive-strand RNA virus that belongs to the *Flavivirus* genus, a member of the flaviviridae. The virus occurs in 4 closely related but antigenically distinct viral serotypes (DENV1–4). After infection of a human subject by a mosquito, an incubation period of several days is followed by the clinical manifestation of dengue fever. The symptoms range from a mild, flulike, febrile illness to severe incapacitating fever, headache, pain behind the eyes, and pain in joints and muscles. Although in most cases fever and other symptoms abate after 3–5 days, some patients deteriorate and progress to life-threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), which can reach a fatality rate of >20%. No vaccine or antiviral treatment for dengue infection exists. [2–4].

To develop an antidengue drug, it is important to be able to test compounds with antiviral properties in an in vivo infection system, preferably a small-animal

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model of dengue fever. However, no convenient and well-characterized animal model for testing antiviral dengue drugs exists at present. From the results of many research groups, it has become clear that small rodents are not easily infectable with DENV. With the use of mouse-adapted virus strains, high-titer intracranial or intravenous injections, or severely immunocompromised mice, some progress has been made in establishing dengue infection [5–8]. Recently, grafting of human cells or tissues into severely compromised immunodeficient mice has improved infectability and shown some remarkable similarities to signs observed in patients with dengue infection [9–12]. However, what is needed for studying dengue infection and testing antiviral dengue drugs is a simple mouse infection system that, after peripheral infection, shows readily detectable increasing and sustained viremia in blood causing an innate and adaptive immune response similar to that of human patients infected with dengue.

A report by Johnson and Roehrig [13] described a mouse infection system that showed some properties of dengue fever—clear dengue viremia and dengue-neutralizing antibodies in the blood. They used AG129 mice, which have an intact immune system but are deficient for the interferon (IFN)–α/β and –γ receptors and, thus, cannot mount an antiviral response as efficiently as wild-type mice [14]. We were able to develop a straightforward, robust, and standardized dengue infection protocol for the AG129 mouse by carefully characterizing and profiling virus levels and inflammatory and immune responses. This infection protocol was then used to study how various antiviral compounds, in different doses and dosing regimens, could affect viremia and the inflammatory response during dengue infection in vivo. We established a straightforward dengue mouse model based on infection of AG129 mice, validated this model as a suitable test system for dengue antiviral drugs, and subsequently demonstrated that a reduction of viremia can ameliorate the inflammatory response to the infection.

MATERIALS AND METHODS

Virus production. DENV was produced in C6/36 mosquito cells (ATCC CRL-1660) grown in RPMI 1640 medium (Gibco) plus 5% fetal bovine serum (Hyclone) at 28°C. The DENV strains used were Hawaii (DENV1; ATCC VR-71), New Guinea C (NGC, DENV2; ATCC VR-1255), H241 (DENV4; ATCC VR-1490), and TSV01 (DENV2) [15], passaged in cell cultures 10–15 times. For virus levels $>1 \times 10^7$ pfu/mL, the cell culture supernatant was centrifuged for 1 h at 30,000 *g* and resuspended in RPMI 1640 medium.

Mouse infection and blood sampling. AG129 mice were obtained from B&K Universal. All research involving mice was performed in compliance with national laws and institutional policies and with the permission of the Institutional Animal Welfare Committee. Mice were housed in individually ventilated cages (TechniPlast) and used between 7 and 9 weeks of age. Mice were injected intraperitoneally with 0.4 mL of virus suspension. Blood samples were obtained by retro-orbital puncture under ketamine and xylazine anaesthesia. Blood samples were collected in tubes that contained sodium citrate to a final concentration of 0.4% and were immediately centrifuged for 3 min at 6000 *g* to obtain plasma. Then, 20 μL of plasma was diluted in 780 μL of RPMI 1640 medium and snap frozen in liquid nitrogen for plaque assay analysis. Remaining plasma was used for the determination of cytokine, antibody, and nonstructural protein 1 (NS1) levels.

Compound treatment. Compounds tested for antiviral treatment were ribavirin (Sigma-Aldrich), 7-deaza-2′-*C*-methyl-adenosine (7-DMA; in-house synthesis), *N*-nonyl-deoxynojirimycin (*N*N-DNJ; Toronto Research Chemicals), and 6-*O*-butanoyl castanospermine (BuCast; in-house synthesis).

Plaque assay and plaque neutralization assay. Plaque assays and plaque neutralization assays were performed as described by Morens et al. [16]. In brief, BHK-21 cells were seeded in 24-well plates (Nunc) and grown to confluency. Cell layers were incubated with serially diluted virus samples for 1 h and overlaid with 0.8% methylcellulose. Plates were incubated for 5 days at 37°C, fixed in 10% formaldehyde, and stained with 1% crystal violet in water for 20 min. Plaques were counted by eye. For plaque neutralization assays, plasma samples were heat treated for 15 min at 55°C to inactivate virus, serial diluted, and mixed with a standard amount of DENV. The mixture was incubated for 30 min on ice before addition to the cell layer to be processed for plaque assay analysis as described above.

Quantitative real-time polymerase chain reaction (PCR). Viral RNA was isolated from plasma samples or from serial diluted reference samples of known virus level. Samples were diluted 10-fold in PBS (Gibco) and processed using the QIAamp Viral RNA Mini Kit (Qiagen). Viral RNA was used in a reverse-transcriptase reaction to produce cDNA using SuperScript III (InVitrogen). This cDNA was used in a quantitative real-time PCR with the FastStart DNA Master^{PLUS} SYBR Green I system (Roche Diagnostics), using primers D1 and D2 [17], and was analyzed on a Roche Diagnostics LightCycler 2.0 instrument.

Determining NS1 levels. Microtitration plates (Maxisorp; Nunc) were coated overnight at 4°C with purified anti-NS1 monoclonal antibodies (MAbs) 17A12 and 4F7. Wells were washed with PBS that contained 0.05% Tween 20 and blocked with 3% milk. Plasma samples were diluted in PBS-Tween and incubated for 1 h at 37°C, followed by an overnight incubation at 4°C. Wells were washed again and probed for NS1 for 1 h at 37°C with peroxidase-labeled MAb 12E5. After a final wash, peroxidase activity was detected with 3,3′,5,5′-tetramethylbenzidine (TMB) solution (Kirkegaard & Perry Laboratories), and

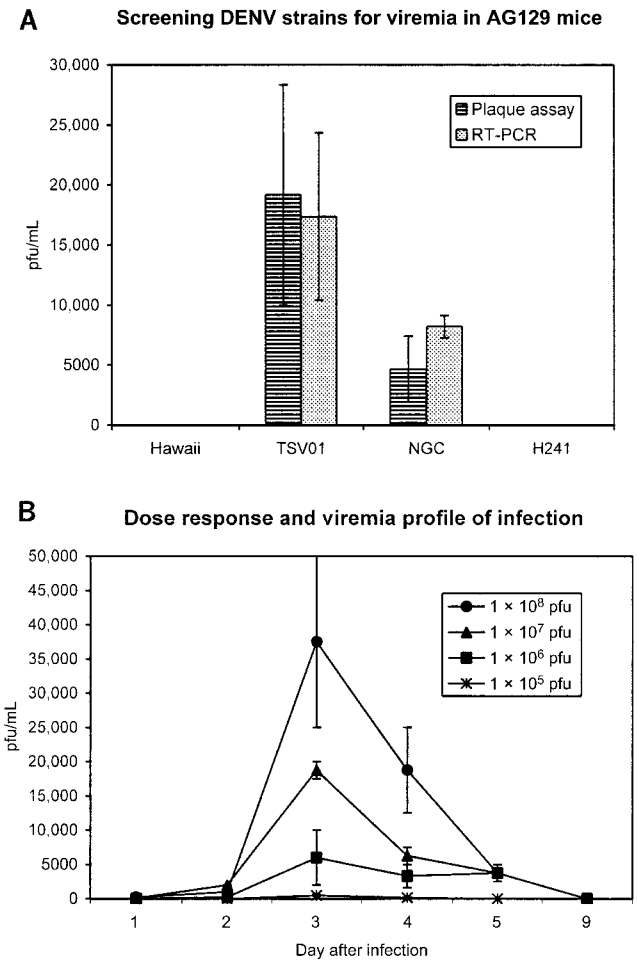

Figure 1. *A,* AG129 mice infected by intraperitoneal injection with 5×10^6 pfu of unadapted dengue virus (DENV) strains. Plasma was obtained on day 3 after infection and analyzed by plaque assay and quantitative reverse-transcription polymerase chain reaction (RT-PCR). Injection of Hawaii serotype 1 or H241 serotype 4 virus did not result in any detectable viremia. However, the TSV01 and New Guinea C (NGC) serotype 2 strains showed clear viremia in both plaque assay and RT-PCR analysis. *B,* Blood samples from AG129 mice injected with different doses of TSV01 DENV, 1×10^5–1×10^8 pfu/mouse, obtained from days 1 to 9 after infection and analyzed by plaque assay. This showed a dose-dependent viremia with very low virus levels on day 1 after infection, increasing to peak on day 3 and subsiding over the following days to be cleared completely on day 9 after infection. $n = 3$ mice per data point; error bars indicate SEs.

the color reaction was stopped with 2.5 *N* sulfuric acid. Plates were read at 450 nm.

Determining inflammatory cytokine levels. Cytokine levels in plasma samples were measured using the BD Cytometric Bead Array (CBA)–Mouse Inflammation Kit system (BD Biosciences Pharmingen). A volume of 50 μL of plasma was used undiluted in the CBA assay system. Samples were analyzed on a Becton Dickinson FACSCalibur flow cytometer using BD CellQuest Pro software (version 5.2).

Determining dengue antibody levels. UV-inactivated TSV01 virus was diluted in 0.2 mol/L sodium phosphate buffer (pH 6.5), and 50 μL was applied to 96-well microtitration plates (Maxisorp; Nunc) and incubated overnight at 4°C. Plasma samples were applied at 10-fold dilution and incubated for 3 h at room temperature. Secondary antibodies specific for mouse IgM or mouse IgG conjugated to peroxidase (Sigma) were used. Peroxidase activity was detected using TMB solution (Sigma), and the color reaction was stopped with 0.5 mol/L sulfuric acid. Plates were read at 450 nm.

Statistical analysis. Statistical analysis comparing average values of differently treated groups of mice with the control group was done using the unpaired *t* test.

RESULTS

DENV strains causing viremia in AG129 mice. The goal of the present study was to establish a dengue viremia mouse model that could be routinely and reproducibly used for testing the efficacy of antiviral compounds for treating dengue infection. We first tested 4 unadapted tissue culture–produced dengue strains—Hawaii, TSV01, NGC, and H241—by intraperitoneally injecting 5×10^6 pfu/AG129 mouse, and we found considerable differences in their infectivity (figure 1*A*). Only the NGC and TSV01 strains showed readily detectable virus levels in plasma from infected mice as tested by plaque assay and quantitative PCR. Because the TSV01 strain resulted in the highest viremia, it was selected for further studies.

To determine the dose and time dependence of the infection, mice were infected with different doses of the TSV01 virus, ranging from 1×10^5 to 1×10^8 pfu/mouse. Plasma samples were obtained daily after injection and were analyzed by plaque assay (figure 1*B*). All doses showed the same profile of infection,

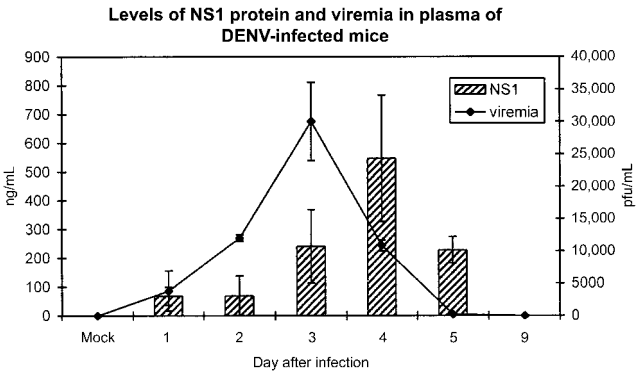

Figure 2. Mice infected with dengue virus (DENV) and analyzed for nonstructural protein 1 (NS1) plasma levels by ELISA and for viremia by plaque assay on days 1–9 after infection. NS1 could be detected from day 1 after infection and increased to peak on day 4 after infection, a day after the peak of viremia. No virus or NS1 was detected in mock-infected (mock) mice or on day 9 after infection. $n = 3$ mice per data point; error bars indicate SEs.

starting with very low virus levels on day 1 after infection, higher levels on day 2, and consistent peaks on day 3 after infection. From day 4 to day 5, virus levels decreased, and virus was no longer detectable on day 9 after infection.

Although the profile of viremia was independent of the dose, the level of viremia was found to be dose dependent (figure 1*B*). The lowest dose of 1×10^5 pfu gave very low levels of viremia, whereas higher doses of 1×10^6, 1×10^7, and 1×10^8 pfu caused increasing virus levels in the blood, consistent peaking at day 3, and clearance by day 9 after infection. Death or overt signs of illness were not observed for up to 20 days after infection. This viremia profile is very similar to that reported by Johnson and Roehrig [13] and is somewhat comparable to the viremia profile of patients with dengue fever.

To confirm productive infection, we looked for the presence of the dengue NS1, a hallmark of dengue infection in patients with dengue fever [18, 19]. NS1-specific ELISA analysis of blood samples from dengue-infected mice showed significant amounts of NS1 to be present during infection (figure 2). The NS1 protein could be detected above background level as early as day 1, increasing over the succeeding days to peak on day 4 after infection, a day after the peak of viremia. NS1 was no longer detectable on day 9 after infection, when the infection had been overcome.

Inflammatory immune response during dengue infection. To investigate the effect of dengue infection on the AG129 mice, we studied the extent of the inflammatory immune response that has been linked to the severity of symptoms in patients with dengue fever [20–23]. A clearly visible marker for the virus infection–induced inflammatory response is the enlargement of the spleen, as is also observed in patients with dengue fever [24, 25]. We weighed the spleens of infected mice after infection and found a 2-phase response (figure 3). A modest acute increase in spleen size was observed on day 1 after infection that diminished on day 2 after infection. From day 3, with increasing virus levels, spleen size increased, continuing further on the succeeding days to more than double the size of uninfected spleens on day 9 after infection. This splenomegaly pointed to a strong inflammatory response to dengue infection in this mouse infection model.

The extent of the inflammatory immune response in dengue-infected mice was further explored by analyzing the levels of a set of proinflammatory cytokines. Tumor necrosis factor (TNF)–α, interleukin (IL)–6, IL-12, IFN-γ, and monocyte che-

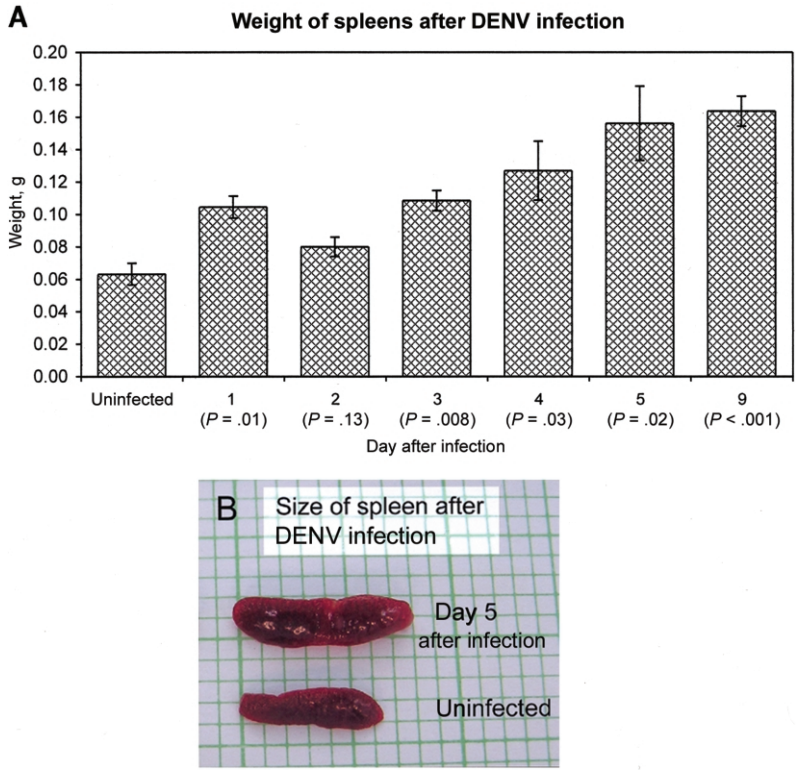

Figure 3. Spleens of mice infected with dengue virus (DENV) and of age-matched uninfected controls, taken out and weighed on subsequent days after infection. *A,* Two-phase response observed with an acute increase in spleen weight on day 1 after infection that subsided on day 2, followed by a second increase over the following days leading to a spleen size more than twice the weight of that of uninfected controls. *B,* Increased spleen weight reflecting easily observed splenomegaly. $n = 6$ mice per data point; error bars indicate SEs. *P* values reflect the probability of each infected group differing from the uninfected control group, analyzed by *t* test.

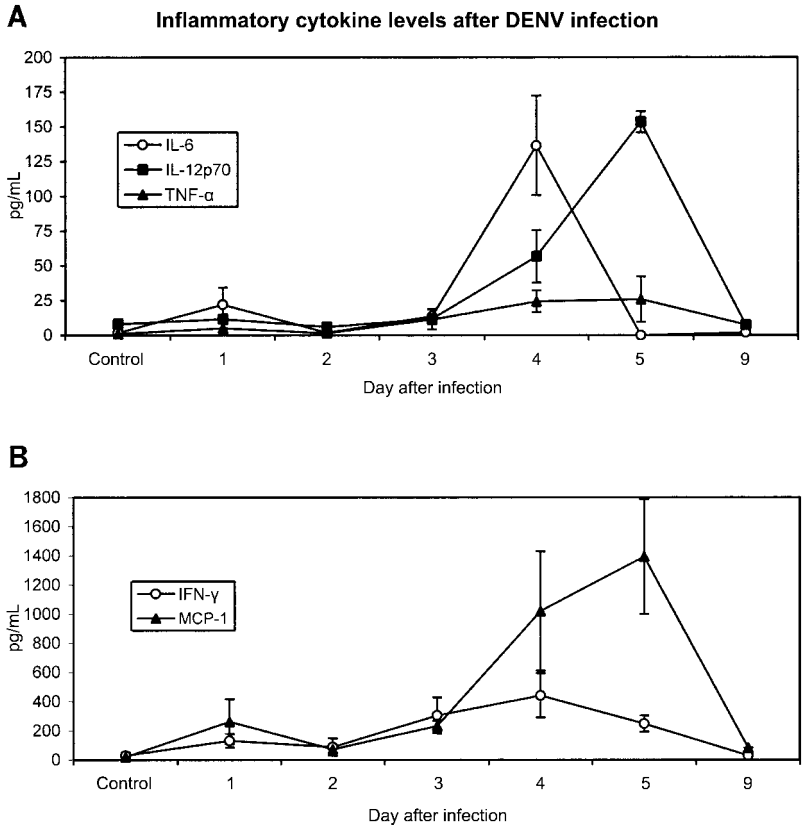

Figure 4. Plasma samples from dengue virus (DENV)–infected mice, analyzed for proinflammatory cytokine levels on subsequent days after infection. Interleukin (IL)–6, IL-12, and tumor necrosis factor (TNF)–α *(A)* and interferon (IFN)–γ and monocyte chemotactic protein (MCP)–1 *(B)* all showed a similar increase from day 3 after infection followed by a peak on days 4 and 5 after infection. MCP-1 showed the highest increase, to levels >1000 pg/mL. Cytokine levels returned to background levels (similar to those of uninfected controls) on day 9 after infection. $n = 5$ mice per data point; error bars represent SDs.

moattractant protein (MCP)–1 are inflammation markers that have been reported to be higher in patients with acute dengue infection [26–28]. Interestingly, the levels of all these proinflammatory cytokines started to increase on day 3 after infection and peaked on day 4 or 5 after infection (figure 4). Levels returned to normal on day 9 after infection, when virus infection was overcome. These results corroborate dengue infection causing a clear inflammatory response in our dengue-infected AG129 mice.

Adaptive immune response to dengue infection. Additional characterization of the adaptive immune response to dengue infection focused on the occurrence of dengue-specific neutralizing antibodies. ELISAs of plasma samples showed that dengue-specific IgM antibodies appeared on day 3 and increased on days 4 and 5 after infection (figure 5*A*). The increase in dengue-specific IgM antibody levels coincided with enlargement of the spleen, increased inflammatory cytokine levels, and reduced viremia. IgM levels subsequently dropped slowly over the next weeks, as indicated for days 10 and 20 after infection. Dengue-specific IgG antibodies became detectable on day 4 after infection and increased in level up to day 20.

To determine whether these antibodies possessed neutralizing properties, a series of plaque neutralization assays was performed with plasma samples obtained on day 10 after infection (figure 5*B*). Plaque formation of TSV01 virus was neutralized by antibodies from the infected mice, whereas plasma from uninfected mice had no effect. Plasma from infected mice could also reduce plaque formation of the homologous DENV2 strain NGC. It can be concluded that dengue infection of AG129 mice induced an adaptive immune response, giving rise to neutralizing antibodies against the infection.

Effect of antiviral treatment. After establishing our dengue viremia model, we proceeded to validate it as a test system for antidengue drugs. A standard infection protocol was developed in which mice were injected with 2×10^6 pfu of strain TSV01 and then treated with potential antiviral compounds. Mice were killed for sampling at the peak of viremia (i.e., day 3 after infection).

The well-known viral inhibitor ribavirin can inhibit DENV proliferation in cell culture at an EC_{50} of ~40 μmol/L [29]; however, after the subcutaneous injection of 100 mg/kg ribavirin once daily for 3 days, virus levels were unchanged (figure

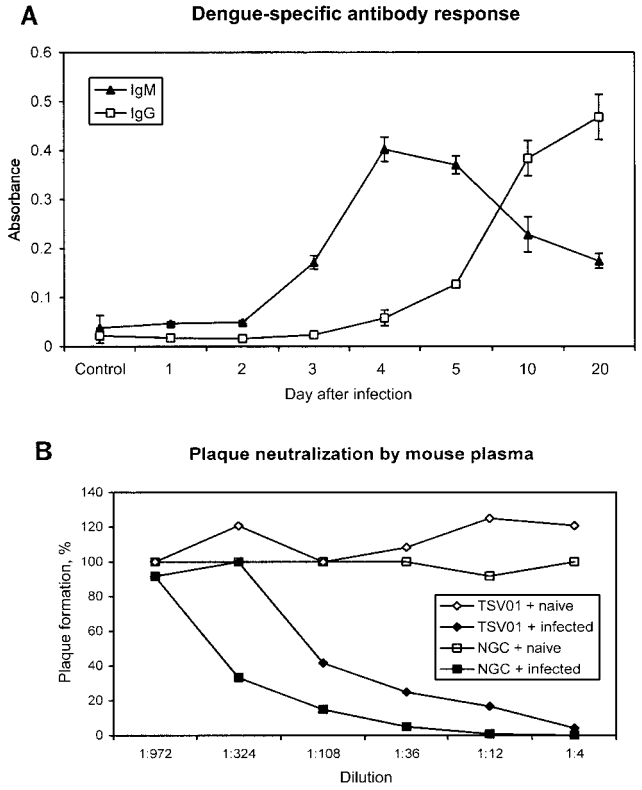

Figure 5. *A,* Plasma from uninfected naive mice and from dengue virus (DENV)–2 TSV01–infected mice tested by ELISA for the presence of IgM and IgG antibodies against DENV. Dengue-specific IgM antibodies were found from day 3 after infection, peaking on day 4, after which their level slowly decreased. Dengue-specific IgG antibodies were found from day 4 after infection and were still increasing by day 20. *B,* Antibodies neutralizing virus in a plaque neutralization assay, as shown for plasma from day 10 after infection. The antibodies could neutralize plaque formation of TSV01 virus and other DENV-2 strains, such as New Guinea C (NGC). $n = 5$ mice per data point; error bars indicate SDs.

6*A*). This is similar to findings by Leyssen et al. [30], who showed that mice infected with the Modoc flavivirus did not benefit from ribavirin treatment.

The compound 7-DMA, which inhibits DENV RNA–dependent RNA polymerase, proved to be more effective. It has an inhibitory effect on dengue growth in cell culture at an EC_{50} of 15 μmol/L [31]. Because this compound has good oral availability but a short half-life, we chose to administer doses orally twice daily at 50 mg/kg for 3 days. This caused a significant (70%) reduction of viremia in dengue-infected mice (figure 6*A*), showing, to our knowledge for the first time, that treatment with a small antiviral compound can reduce viremia in an in vivo dengue infection system.

A different class of viral inhibitors was subsequently tested, not targeting the virus directly but inhibiting the α-glucosidase enzymes involved in the processing of glycoprotein oligosaccharides in the endoplasmic reticulum. Inhibitors of α-glucosidase I are known to have inhibitory activity against flaviviridae, including dengue virus [32–34]. The compounds *N*N-DNJ and BuCast are α-glucosidase I inhibitors known to inhibit dengue proliferation in cell culture at micromolar concentrations [35] and to be active in vivo against HIV, bovine viral diarrhea virus, and herpes simplex virus [36–38]. Both have good oral availability and were administered twice daily at 75 mg/kg for 3 days. Treatment with *N*N-DNJ or BuCast resulted in a significant and potent reduction (93% and 88%, respectively) in viremia (figure 6*A*). This demonstrated that not only viral enzymes but also human host enzymes can serve as targets to reduce viremia during dengue infection.

On the basis of the above findings, we proceeded to investigate how different doses and dosing regimens would affect antiviral efficacy. BuCast was orally administered at a low dose of 7.5 mg/kg or at a high dose of 75 mg/kg twice daily for 3 days, starting on the day of dengue infection. Additionally, a shorter 2-day treatment with the 75 mg/kg dose, starting 1 day after infection, was included to determine how delayed treatment after infection affected drug efficacy. This study showed a dose-dependent response—that is, low-dose treatment caused a modest but significant lowering of viremia by 62%, whereas high-dose treatment reduced viremia more extensively, by 88% (figure 6*B*). Delayed treatment starting 1 day after infection was less effective but still caused a significant 55% reduction of viremia, compared with the dengue-infected control group (figure 6*B*). These results demonstrate that the AG129 dengue viremia model we have developed can be used to test and compare the antiviral efficacy of different antidengue drugs in different doses and dosing regimens.

Effect of antiviral treatment on inflammation. It is the aim of antiviral treatment to lower virus levels and, thereby, to ameliorate symptoms of viral infection, such as inflammation and fever, thus reducing the morbidity and, hopefully, the mortality of the disease. We therefore tested whether compounds shown to reduce dengue viremia were able to lower the inflammatory immune response in the mice.

Weighing the spleens of mice treated with 7-DMA or *N*N-DNJ showed that splenomegaly was significantly less severe than in vehicle-treated control mice: a reduction of 37% and 68%, respectively, was observed (figure 7*A*). We subsequently analyzed whether antiviral treatment reduced the proinflammatory cytokine response to dengue infection. Cytokine quantification studies revealed that TNF-α, IL-6, IL-12, IFN-γ, and MCP-1 levels were all markedly lower in mice treated with 7-DMA and *N*N-DNJ than in untreated dengue-infected controls (figure 7*B*). These data show that lowering of viremia by antidengue drug treatment could reduce the acute inflammatory response, as represented by splenomegaly and by inflammatory cytokine levels in this in vivo dengue infection model.

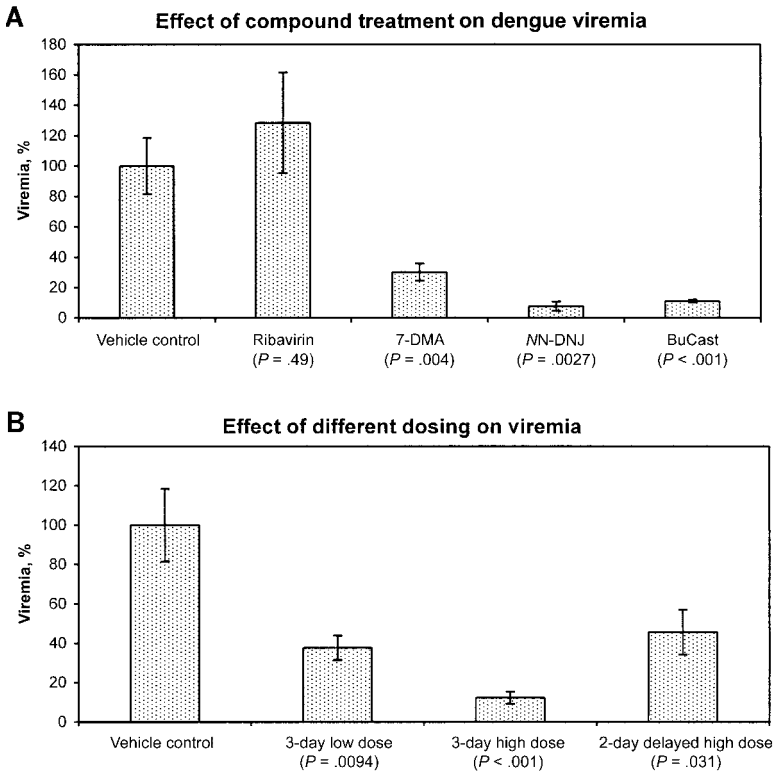

Figure 6. Dengue virus–infected mice treated daily with different antiviral compounds or with vehicle as a control, starting on the day of infection and sampled on day 3 after infection for determination of the level of viremia by plaque assay. Viremia in infected vehicle-treated control mice was consistently between 15,000 and 25,000 pfu/mL and set to 100% for comparison with compound-treated mice. *A,* Effect on viremia of subcutaneous injection of ribavirin (100 mg/kg) and oral dosing of 7-deaza-2′-*C*-methyl-adenosine (7-DMA; 50 mg/kg), *N*-nonyl-deoxynojirimycin (*N*N-DNJ; 75 mg/kg), or 6-*O*-butanoyl castanospermine (BuCast; 75 mg/kg). *B,* Reductions in viremia with different dosing regimes of BuCast: a low dose (7.5 mg/kg) and a delayed high dose (75 mg/kg), compared with the full 3-day high-dose treatment. $n = 8$ mice per data point; error bars indicate SEs. *P* values reflect the probability of each compound group differing from the vehicle control group, analyzed by *t* test.

DISCUSSION

We have established a straightforward dengue infection model using easy-to-breed AG129 mice and simple intraperitoneal injection of the unadapted tissue culture–produced DENV strain TSV01. This infection model is based on work by Johnson and Roehrig [13], who showed the same levels and profile of viremia as we report here. By using daily readings of viremia, spleen size, and levels of viral NS1, inflammatory cytokines, and IgM and IgG antibody, we have obtained a more thorough and detailed characterization of dengue infection in the AG129 mouse. Viremia was low for the first day after infection, followed by increasing virus levels over the next day to consistently peak on day 3 and decreasing levels over the next few days to clear by day 9. Although DENV2 strain TSV01 was primarily used in these experiments, our ongoing studies have identified several clinical DENV1 and DENV2 isolates that can cause similar viremia in AG129 mice (data not shown).

Dengue NS1 protein was found in the blood as early as day 1 after infection, when virus levels were still very low. NS1 levels peaked on day 4 after infection, a day after the peak of viremia, and were no longer detectable when the virus was cleared. This profile is very similar to that observed during acute dengue infection in patients [18, 19] and confirms that NS1 may be a marker for dengue infection even before the occurrence of high viremia and clinical symptoms.

From day 3 after infection, infected mice had systemic inflammation, as was evident from the splenomegaly and high proinflammatory cytokine levels, indicating activation of both the innate and the adaptive immune system. The activation of the immune system and the appearance of neutralizing antibodies are the mostly likely cause of the reduction of viremia observed from day 4 after infection.

Taken together, the serological profile of dengue viremia, NS1 protein, dengue-specific IgM and IgG antibodies, and increased levels of proinflammatory cytokines (figures 2, 4, and 5) are very similar to what is observed during acute dengue fever in humans. This leads us to propose our infection system to be a mouse model for dengue fever. Although this model does not

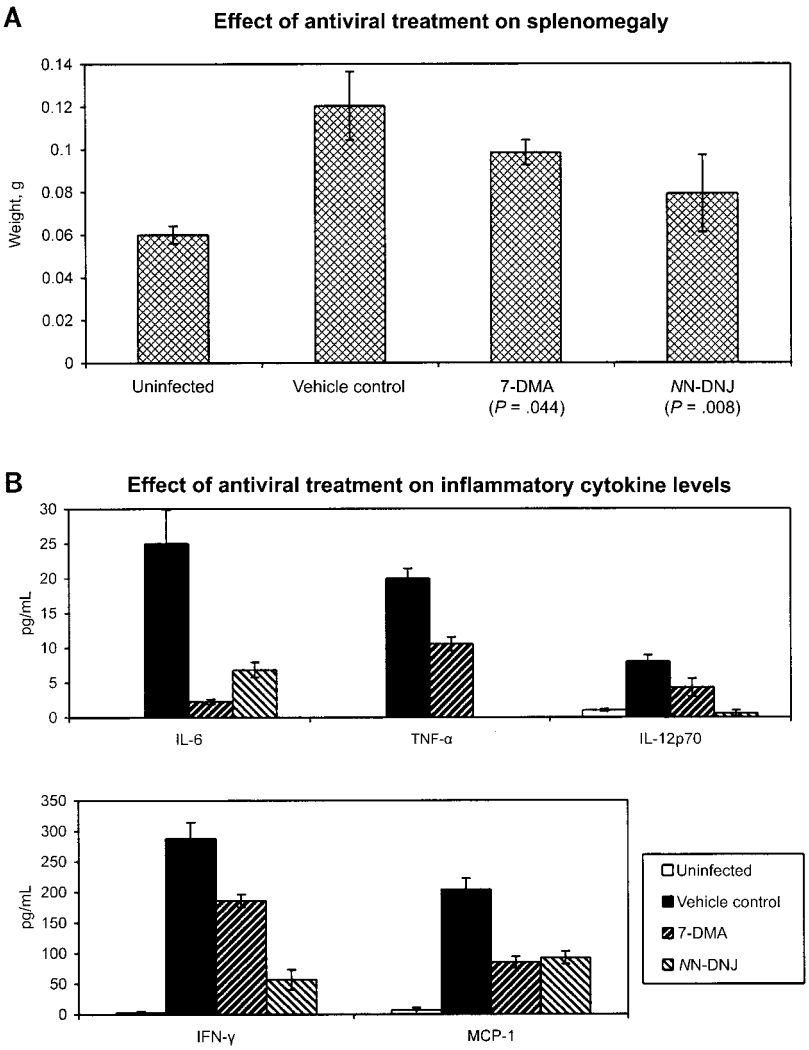

Figure 7. Dengue virus–infected mice dosed orally with antiviral compounds 7-deaza-2′-*C*-methyl-adenosine (7-DMA; 50 mg/kg), *N*-nonyl-deoxynojirimycin (*N*N-DNJ; 75 mg/kg), or vehicle alone for 3 days, starting on the day of infection. *A,* Spleens from mice, harvested on day 3 after infection and weighed. Vehicle control–treated dengue infection caused a doubling of spleen weight that was significantly reduced by 7-DMA or *N*N-DNJ compound treatment. *B,* Plasma obtained on day 3 after infection and analyzed for interleukin (IL)–6, tumor necrosis factor (TNF)–α, IL-12, interferon (IFN)–γ, and monocyte chemotactic protein (MCP)–1 cytokine levels. Vehicle control–treated dengue infection caused a strong induction of all 5 cytokines that was significantly reduced ($P < .05$) after compound treatment. $n = 8$ mice per data point; error bars indicate SDs. *P* values reflect the probability of each compound group differing from the vehicle control group, analyzed by *t* test.

represent the clinically more relevant DHF and DSS, it allows convenient testing of potential drugs against dengue fever in an in vivo system.

To validate this dengue fever mouse model for the testing of potential antidengue drugs, we administered several known antiviral compounds. Oral treatment with compounds targeting dengue RNA–dependent RNA polymerase or the human host enzyme α-glucosidase necessary for virus replication were found to significantly reduce viremia in a dose-dependent manner. Additionally, different dosing regimes were tested—notably, BuCast showed a significant reduction of viremia even when treatment was delayed. These results establish the AG129 dengue fever model as a suitable system for testing, comparing, and characterizing potential antidengue drugs for their in vivo antiviral efficacy. They also indicate that α-glucosidase inhibitors may be interesting drug candidates for dengue fever, similar to their proposed use in anti–hepatitis C treatment [36], whereas ribavirin treatment may not be effective during dengue fever.

So far, it has been unclear how the level of dengue viremia contributes to the severity of the disease, and, consequently, whether a lowering of viremia by antiviral drug treatment will reduce the symptoms of dengue fever. Vaughn et al. [39] showed that higher virus levels are associated with increased

dengue disease severity, which suggests that reducing viremia may lower morbidity and reduce the risk of progression to DHF or DSS. Although the dengue-infected AG129 mice did not show overt signs of disease, increased IL-6, IFN-γ, and TNF-α levels are known to be the main triggers for fever [40], headache [41], and other features of dengue fever. Additionally, the splenomegaly that we observed is a reported clinical feature of dengue fever [24, 25]. We have shown that lowering viremia by use of antiviral treatment significantly reduced splenomegaly and brought down proinflammatory cytokine levels, reflecting a marked reduction of inflammation in these mice. These results are the first indication that lowering viremia by an order of magnitude through timely antiviral drug treatment may ameliorate the severity of dengue fever symptoms and possibly reduce the risk of progression to DHF or DSS.

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