Dengue Fever in Humanized NOD/SCID Mice

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The increased transmission and geographic spread of dengue fever (DF) and its more severe presentation, dengue hemorrhagic fever (DHF), make it the most important mosquito-borne viral disease of humans (50 to 100 million infections/year) (World Health Organization, Fact sheet 117, 2002). There are no vaccines or treatment for DF or DHF because there are no animal or other models of human disease; even higher primates do not show symptoms after infection (W. F. Scherer, P. K. Russell, L. Rosen, J. Casals, and R. W. Dickerman, Am. J. Trop. Med. Hyg. 27:590–599, 1978). We demonstrate that none of these models (nonobese diabetic/severely compromised immunodeficient (NOD/SCID) mice xenografted with human CD34+ cells develop clinical signs of DF as in humans (fever, rash, and thrombocytopenia), when infected in a manner mimicking mosquito transmission (dose and mode). These results suggest this is a valuable model with which to study pathogenesis and test antidengue products.

Dengue virus infections in humans can be subclinical or can cause illnesses ranging from a mild, flu-like syndrome with rash and some hemorrhagic manifestations (dengue fever [DF]) to a severe and sometimes fatal disease, with coagulopathy, capillary leakage, and hypovolemic shock (dengue hemorrhagic fever [DHF]). The development of dengue vaccines and antivirals is complicated by the viruses’ diversity: they would have to protect against four different serotypes and numerous genotypes within each serotype. We have shown that dengue viruses differ in their rates of infection and replication in primary human cells, and these rates vary from one donor to another (3). In addition, secondary infection by a heterologous virus enhances disease severity, and multivalent vaccine preparations could prove dangerous to vaccinees if they induce subneutralizing levels of antibodies (5). Thus, it is hypothesized that viral and host (genetic and prior immune status) factors contribute to dengue pathogenesis, but there has been no in vivo system in which to measure their contributions (9).

Little is understood about the events that lead to DF or DHF after an infected mosquito (mainly Aedes aegypti) bites and injects virus into the human epidermis. The main targets for viral infection and replication seem to be dendritic cells (DCs) and macrophages, mainly Langerhans and monocytoid DCs (7, 13). A full repertoire of DCs develops in vitro (3). Six reconstituted and 15 nonreconstituted human CD34+ NOD/SCID mice (same genetic background but not receiving this virus was selected because it grows to high levels in human DCs in vitro) were inoculated subcutaneously with approximately 4.7 log10 PFU (7.7 log10 genome equivalents) of dengue serotype 2 strain K0049 (Southeast Asia genotype; C6/36 cell passage 3); this virus was selected because it grows to high levels in human DCs in vitro (3). Six reconstituted and 15 nonreconstituted NOD/SCID mice (same genetic background but not receiving human CD34+ cells) served as negative controls (inoculated with saline, C6/36 cell culture passage 3); this virus was selected because it grows to high levels in human DCs in vitro (3). Six reconstituted and 15 nonreconstituted NOD/SCID mice (same genetic background but not receiving human CD34+ cells) served as negative controls (inoculated with saline, C6/36 cell culture supernatant, or virus). The most dramatic signs of dengue infection in humanized mice were erythema and thrombocytopenia (Fig. 1a), as occurs in humans. We measured erythema using a DermaSpectrometer (Cortex Technology, Denmark) while mice were anesthetized; when compared to nonreconstituted, infected mice, erythema mean values were significantly different on days 1 through 8 (all pairwise t tests, P < 0.05; reconstituted mouse range, −1.36 to 8.44; nonreconstituted mouse range, −5.35 to −1.51). A marked decrease in platelets was measurable on day 8 postinoculation and was statistically highly significant (Mann-Whitney test, P < 0.001) when comparing six infected, reconstituted mice (range, 320,000 to 594,000/mm3) to three nonreconstituted, infected and six reconstituted, noninfected mice (normal
mouse range, 600,000 to 1,200,000/mm³; range for our negative controls, 610,000 to 1,251,000/mm³). Rash was visible on days 2 to 4 in the majority (eight of nine) of infected, reconstituted mice and continued through day 14 in some mice (Fig. 1c). Viremias were measured in sera (25- to 50-µl retro-orbital bleed on alternate days) by quantitative, real-time reverse transcription-PCR (RT-PCR) (Fig. 1b) (11); virus levels peaked on days 2 to 6 postinoculation (range, 4.2 to 5.4 log₁₀ genome equivalents/ml). A rise in body temperature followed the viremia, and temperature returned to normal levels on day 10. For reconstituted mice, changes in temperature were statistically significant by analysis of variance (P < 0.02); for nonreconstituted mice, changes in temperature were not significant by analysis of variance (P ≫ 0.05). Nonreconstituted, infected NOD/SCID mice showed consistently lower viremias (undetectable by day 10; range, 3.6 to 4.1 log₁₀ genome equivalents/ml) and, as noted above, showed no rash, rise in temperature, or decrease in platelets and therefore served as negative controls for statistical analyses throughout. Body weight decreased dramatically in some reconstituted, infected animals (20% loss in four mice); there were no gastrointestinal abnormalities on necropsy, and weight loss seemed to be due to lethargy or lack of eating at the time of viremia or fever. Several organs were tested for viral positive- and negative-strand RNA template (surrogate for replicating virus) by quantitative, real-time RT-PCR (3) at different times postinfection in reconstituted and nonreconstituted mice. Only the reconstituted, infected mice had dengue virus RNA in various tissues on day 8: spleen (six of six mice), liver (three of six mice), and skin (one of six mice) had positive-strand RNA, while negative-strand RNA was detectable in spleen (two of six mice) and skin (one of six mice) (Fig. 2). These data are consistent with viral replication in this model.

Others have used transplanted SCID or inbred AG129 or A/J mice to show variable thrombocytopenia, paralysis, or death after injection of massive amounts (8 log₁₀ PFU, intravenous) of cell-culture-passaged dengue virus or smaller amounts (4 to 6 log₁₀ PFU, intraperitoneal) of mouse-adapted dengue virus; however, these mice did not show signs of den-
gue disease as in humans (1, 2, 6, 10). The model described here is therefore highly relevant to human infection and can now be used to test antiviral preparations and vaccine attenuation. It may eventually also prove useful for understanding the immunopathogenesis (antibody-dependent enhancement of infection and cross-reactive T-cell activation) of DHF, after transplantation of other factors or tissues, to obtain a functional adaptive immune system.

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REFERENCES


12. Reference deleted.