The Animal Models for Dengue Virus Infection

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Abstract

Currently, the mechanisms involved in the pathogenesis of DHF/DSS remain poorly understood and there is no effective vaccine available to prevent infection with DEN virus. The lack of a reliable small animal model that mimics dengue disease is a major obstacle. In this paper, the development of small animal models such as mice for dengue virus infections is reviewed.

Keywords: Dengue virus, animal model, mice.

Introduction

Dengue (DEN) viruses are mosquito-borne RNA viruses, which belong to the genus Flavivirus (family Flaviviridae), and are grouped into four antigenically distinct types (DEN-1, DEN-2, DEN-3, and DEN-4). Every year, they infect millions of people and can cause a mild-to-debilitating febrile illness (classical dengue fever, DF) or life-threatening syndrome (dengue haemorrhagic fever/dengue shock syndrome, DHF/DSS). In recent years, the geographical range of dengue in tropical and subtropical regions of the world has extended and DHF/DSS is occurring in new areas and with increased incidence[1]. Cardinal signs of DHF/DSS include haemorrhage, abrupt onset of vascular leakage and shock, accompanied by severe thrombocytopenia and massive complement activation.

However, the mechanisms involved in the pathogenesis of DHF/DSS remain poorly understood and there is no effective vaccine available to prevent infection with any of the four serotypes of DEN virus. A major technical barrier is the absence of a suitable animal model that mimics DEN disease, including DHF/DSS. So far, there are only three known hosts for DEN virus infections: mosquitoes, humans and lower primates[2]. Although these lower primates infected with wild type DEN viruses develop viremia, they generally manifest only very mild or no clinical signs of disease[3]. Since the appearance of the severe combined immunodeficiency (SCID) mice in 1983[4], efforts have been made to develop new small animal models that may be useful for the development of a future DEN virus vaccine and for studying the pathogenesis of DEN virus infections.

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Animal models based on SCID mice

The SCID mice, which do not produce functional T and B cells and lack detectable immunoglobulin (Ig), can support DEN-susceptible human cell lines xenografts, and this system has been employed to study DEN virus infection in vivo. SCID mice reconstituted with human peripheral blood lymphocytes (hu-PBL-SCID) have been used for studies on the pathogenesis of infection with the human immunodeficiency virus (HIV)\(^{[5,6]}\) and for research on treatment of HIV infection\(^{[7]}\).

The hu-PBL-SCID mice were firstly evaluated as an animal model for DEN viral infection in 1995\(^{[8]}\). SCID mice were injected intraperitoneally (ip) with hu-PBL for reconstitution and successful engraftment was demonstrated by the presence of a high serum level of human IgG. Hu-PBL-SCID mice were ip-infected with DEN-1 virus. Unfortunately, only 5 of 19 hu-PBL-SCID mice showed sensitivity to DEN-1 virus. It was suggested that the main reason for the low DEN infection rate was a scanty number of appropriate human target cells in the reconstituted mice. Thus, investigators searched for more DEN-susceptible human cell lines to improve the infection rate of the SCID mouse model. One promising candidate was K562 cell, an erythroleukemia cell line. SCID mice were engrafted ip with K562 cells (K562-SCID mice)\(^{[9]}\). After intratumor injection into the peritoneal tumor masses of DEN-2 virus, K562-SCID mice showed neurological signs of paralysis and died at approximately 2 weeks post-infection (pi). In addition to being detected in the tumour masses, high virus titers were detected in the peripheral blood and the brain tissues, indicating that DEN virus had replicated in the infected K562-SCID mice. Other serotypes of DEN viruses were also used to infect the K562-SCID mice, and the mortality rates of the infected mice varied with different challenge strains, suggesting that this animal system might potentially be utilized to define the virulence of various human DEN isolates and to characterize the molecular determinants for such viral virulence. K562-SCID mice were also challenged with DEN-2 virus and received antibody administration at the same time or one day earlier, and the results revealed that these mice exhibited a reduction in mortality and a delay of paralysis onset after DEN virus infection. These results indicated that an in vitro neutralizing antibody also defended K562-SCID mice against DEN-2 virus infection.

Target cells and organs for DEN virus replication in humans remain unclear. Unusual clinical manifestations, mostly cerebral and hepatic symptoms, have become more common in patients with DEN virus infection in recent years\(^{[10,11]}\). The involvement of liver cells in the pathogenesis of DEN virus infection has been indicated by abnormal liver function, pathological findings and detection of viral antigen in hepatocytes and Kupffer's cells at biopsies\(^{[12]}\). It was reported that DEN virus could replicate in a human heptocarcinoma cell line, HepG2, and infectious particles were released into the culture medium\(^{[13,14]}\). Therefore, HepG2 cells were transplanted into SCID mice to develop an animal model for studying the pathogenesis of DEN virus infection\(^{[15]}\). The replication of HepG2 cells in host mice was confirmed by an increase of serum human albumin and propagation of HepG2 cells in the liver. At 7-8 weeks
after transplantation, HepG2-grafted SCID mice were ip-infected with DEN-2 virus. A high titer of the virus was detected in the liver and serum but not in the brain in the early stage of the infection. When the mice showed paralysis, the highest titer of virus was detected in the serum and brain. DEN-2 antigens were also found in HepG2 cells of the liver in the early stage and some neurons of the brain in the late stage. Upon clinical examination, thrombocytopenia, prolonged partial thromboplastin time, increased haematocrit, blood urea nitrogen and tumour necrosis factor a (TNF-a) were seen in the paralyzed mice. Moreover, mild haemorrhages in the liver and tarry stool in the small intestine were observed in some mice.

All of above animal models based on SCID mice with transplanted DEN-susceptible human cells mimic some of the aspects of human disease, which may be helpful for studying DEN virus infection, especially in the areas of viral pathogenesis, virus-host interaction and vaccine development against DEN infections. However, it is generally agreed that DHF/DSS is an immune-mediated disease, and since SCID mice are unable to produce the innate immune response, this may impose some limitations on the use of these animal models to extrapolate the situation in human DHF/DSS.

**BALB/c mouse model**

Inbred four-week-old BALB/c mice were found sensitive (haplotype H-2d) to the challenge with dengue virus type 2 (strain P23085)\(^\text{(16)}\). Mice were ip-infected with a dose of 5 LD\(_{50}\) of the mouse-adapted DEN-2 virus, and the first clinical manifestations such as arching of the back, ruffling of the fur and slowing of activity appeared at end of day 4 pi. The presence of DEN-2 virus in the blood was confirmed on day 2 pi by reverse transcriptase-polymerase chain reaction (RT-PCR). The development of the experimental DEN-2 virus infection in mouse model was accompanied by the virus reproduction in all animals. Within 5 and 6 days pi, all mice showed severe sickness with anorexia and weight loss ending in limb paralysis and 100% mortality rate was noted at 7 days pi. The most impressive changes were seen with TNF-a, which abruptly and steeply increased 24 h before death. Serum levels of interleukin (IL)-1ß, IL-6, IL-10, IL-1 receptor antagonist and soluble TNF receptor I continuously increased during the time of infection. Treating animals with anti-TNF-a serum reduced the mortality rate down to 40%. This model supports the view that the activation of innate immune response is at least partially responsible for mortality in DEN-2 virus infection, and in line with this concept, anti-TNF treatment significantly reduces the mortality rates. Therefore, inbred 4-week-old BALB/c mice are useful models to research the immune activation of host in DEN-2 virus infection.

**Gene knockout mouse (AG129) model**

There is evidence that alpha and beta interferons (IFN-a/ß) and gamma IFN (IFN-?) might be involved in human DEN virus infection\(^\text{(17,18)}\). In addition, exogenously administered IFN appears to protect mice from DEN virus challenge\(^\text{(19)}\). This information suggested that mice defective in their IFN response might provide a suitable
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Intraperitoneally administered mouse-adapted DEN-2 virus was uniformly lethal in AG129 mice, which lack IFN-α/β and IFN-γ receptor genes, regardless of age. The mice showed neurological abnormalities, including hind-leg paralysis and blindness at 7 days pi, and died at 12 days after infection. The immunized mice were protected from virus challenge, and the survival time increased following passive transfer of anti-DEN polyclonal antibody. To determine which aspect of the IFN response was critical in protecting these mice from DEN virus infection, animals individually deficient in either IFN-α/β (A129) or IFN-γ (GKO) functions as well as BALB/c controls were subjected to a similar DEN virus challenge. None of these mice exhibited any overt symptoms of illness, indicating that for DEN virus infection, IFN-α, -β, and -γ abnormalities in combination were necessary for the mouse-adapted virus to be lethal when the ip challenge route was used. These results demonstrated that AG129 mice were a promising small animal model for DEN virus vaccine trials.

A/J mouse model

DEN virus infection causes DF and DHF/DSS. No animal model is available that mimics this clinical manifestation. The immunocompetent mouse (A/J strain) was reported as a mouse model for DEN virus infection that resembles the thrombocytopenia manifestation. Intravenous injection of DEN-2 virus into A/J mice induced paraplegia at 2-3 weeks, while the mock-infected controls were normal. Viremia detected by RT-PCR was found transiently at two days but at no other time after infection. Although A/J mice developed paraplegia after virus infection, they recovered after one month. However, there was transient thrombocytopenia at 10-13 days pi. When the mice were re-infected with the same DEN-2 virus two months later, thrombocytopenia was manifested again at 10 days after infection. Anti-platelet antibody was also generated after injection. And there was strain variation in DEN-2 virus infection; the A/J strain was more sensitive than BALB/c or B6 mice. These results show that this DEN-2 virus-infected mouse system accompanied by thrombocytopenia and anti-platelet antibody may be a suitable model to study the pathogenicity, especially immune activation in DEN virus infection. On the other hand, A/J mice had to be inoculated with a large quantity of DEN-2 virus; a dose of less than 1×10⁸ pfu per mouse was not effective in causing paraplegia. Furthermore, viremia was low and transient in A/J mice compared with that in SCID or IFN-deficient AG129 mice. DEN-2 virus could not be isolated from the blood of infected mice; it could only be detected by sensitive RT-PCR in A/J mice.

Conclusion

Although the above-mentioned new small animal models, which mimic some of the aspects of human DEN virus disease, may facilitate not only the study of DEN pathogenesis but also the evaluation of anti-DEN virus as well as vaccine development, there are still drawbacks in each model, especially in mimicking DHF/DSS. Presently, the molecular mechanisms underlying the pathogenesis of DHF/DSS remain unknown, such as the receptors of DEN virus which are still not clear. Even though the use of transgenic animals has been proposed in the quest for an animal model, it is apparent
that one needs to know more about the mechanisms involved in the pathogenesis of DHF/DSS at the molecular level before one can construct a transgenic animal to serve as a model for use in research on the pathogenesis, vaccine development and therapy for DHF/DSS.

Acknowledgment

This work was partially supported by grants nos. 30170848 and 30300303 from the National Science Foundation of China (NSFC).

References


