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Perspective Piece

Ideal Criteria for Accurate Mouse Models of Vector-Borne Diseases with Emphasis on Scrub Typhus and Dengue

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Abstract. Nine criteria regarding the infectious agent, mode of transmission, portal of entry, route of spread, target organs, target cells, pathologic lesions, incubation period, and modifiable spectrum of disease and outcomes appropriate to the intended experimental purpose are described. To provide context for each criterion, mouse models of two vector-borne zoonotic infectious diseases, scrub typhus and dengue, are summarized. Application of the criteria indicates that intravenous inoculation of *Orientia tsutsugamushi* into inbred mice is the best current model for life-threatening scrub typhus, and intradermal inoculation accurately models sublethal human scrub typhus, whereas the immunocompromised mouse models of dengue provide disease outcomes most closely associated with human dengue. In addition to addressing basic questions of immune and pathogenic mechanisms, mouse models are useful for preclinical testing of experimental vaccines and therapeutics. The nine criteria serve as guidelines to evaluate and compare models of vector-borne infectious diseases.

INTRODUCTION

Mouse models provide a key platform to generate reproducible results to dissect mechanisms of disease and immunity. Perhaps the most important aspect is to know the limitations of a specific model to benefit from its advantages and mitigate is disadvantages. Nine criteria for an accurate model of vector-borne infectious diseases are presented. Specifically, the infectious organism, mode of transmission, portal of entry, route of spread, target organs, target cells, pathologic lesions, and incubation period should be similar to those of the human disease (Table 1). Furthermore, the spectrum of disease with regard to outcomes should be experimentally modifiable, and the model should be appropriate for its intended experimental purpose. Mouse models of dengue and scrub typhus provide major contributions to the study of their respective diseases. Critical evaluation of these models through the lens of the nine criteria reveals the strengths and drawbacks of their applicability (Table 1). 1) The challenge infectious agent should have the same virulence as and a close genetic relationship to the pathogen of interest. The rationale for this criterion is that the infectious agent should contain the virulence factors that cause the appropriate disease in the mouse strain used. For example, *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever, does not cause a disseminated infectious illness in mice, but *Rickettsia conorii*, a very closely related bacterium, causes dose-dependent disease in mice with all the features of the life-threatening Rocky Mountain spotted fever in C3HeN mice. Furthermore, infectious disease vaccines and therapeutics are developed to elicit a response against wild-type bacterial or viral strains, so a challenge model should use a strain that is similar to the strain causing human infections for testing in mice. 2) Ideally, the mode of transmission should be the same as in the human illness, for example, transmission of *Orientia tsutsugamushi* by a feeding chigger. This mode of inoculation contains the actual feeding dynamics of the chigger including the duration of inoculation and the effects of chigger saliva on host defenses in the skin. Similarly, mosquito transmission of dengue virus (DENV) would introduce salivary components that may affect local responses. 3) The portal of entry should be the same as that in human disease, that is, intradermally for *O. tsutsugamushi* (not subcutaneously or intraperitoneally). 4) The route of spread in the body should represent what occurs in the human disease, that is, lymphatic spread from the skin to regional lymph nodes and subsequently hematogenous systemic spread for scrub typhus and human dengue. 5) The target cells of these obligately intracellular agents should conform to those of the human infection. The portal of entry, route of spread, and target cells dictate the interactions of the infectious agent sequentially with the same components of the immune system with the same antigenic stimuli from dendritic cells, endothelial cells, and macrophages in the same temporal order as in the human infection. 6) The target organs and 7) pathologic lesions should reflect those of the human disease, for example, the lungs and brain in scrub typhus, where the presence of the pathogens and disease manifestations of disseminated vasculitis causing interstitial pneumonia/acute respiratory distress syndrome and meningoencephalitis are critical components of the disease. These criteria are important because the infectious agent should cause injury to the same target cells and organs as the human disease. Because the manifestations of the disease are determined by the pathologic lesions and host immunity, this model should represent these highly similar manifestations rather than those of other approaches such as peritonitis following the commonly used intraperitoneal inoculation. Furthermore, in individuals with dengue disease, the internal organs suffer from both infection and the host response to the virus, such as a cytokine storm, which contribute to pathology. 8) Likewise, a similar incubation period as that of the human disease favors the appropriate period of time for the dissemination and replication of the pathogen and generation of innate and adaptive cellular and humoral immune responses. 9) An important experimentally modifiable spectrum of outcomes according to the purpose of the study can be determined by dose- or agent-

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TABLE 1
Ideal criteria for accuracy of mouse models of vector-borne infectious diseases

Criteria	Scrub typhus and other rickettsioses	Dengue
Infectious organism should represent the etiologic agent	In C3H/HeN mice, *Rickettsia conorii* is available to accurately model *Rickettsia rickettsii* Rocky Mountain spotted fever, and *Rickettsia typhi* causes epidemic typhus-like disease. In C57BL/6 mice, *Orientia tsutsugamushi* Karp and Gilliam strains inoculated intravenously cause lethal and sublethal disease, respectively, representing the spectrum of illness in scrub typhus.[8,9,45,46]	AG129 mouse models with non–mouse-adapted DENV-1–4 are systemic and lethal.[38-41]
Mode of transmission should be similar to natural infection	Models are available for tick transmission for ehrlichiosis (*Ehrlichia muris eauclariensis* by *Ixodes scapularis*) and for spotted fever rickettsiosis (*R. parkeri* by *Amblyomma maculatum*). Chigger-transmitted models of scrub typhus have not been developed in inbred mice.[47,48]	AG129 mouse models use needle inoculation.[37,39,40] Salivary gland extract augments pathogenesis of DENV-2 needle inoculation in immunocompromised IFNAR$^{-/-}$ mice.[35] In asymptomatic IFN response factor-3 and -7 knockout mice, DENV-2 transmission from mouse to mosquito to mouse yielded low viremia levels.[30] Infection via mosquito bite or with saliva alone exacerbates clinical outcomes and prolongs infection in humanized mice.[26]
Portal of entry should mimic that of natural infection	Intradermal infection by needle inoculation provides the same portal of entry as chigger feeding does.[5]	Intradermal inoculation of DENV-2 into IFNAR$^{-/-}$ mice leads to cellular recruitment to the dermis and infection of dendritic cells and macrophages,[49] whereas intraperitoneal or intravascular inoculation of AG129 mice bypasses early immune and pathogenic steps.[44,50]
Route of spread should be the same as natural infection	Intravenous and intradermal inoculation of *O. tsutsugamushi* into inbred mice leads to hematogeneous spread.[5,8]	AG129 mouse models of DENV-1–4 show that infection peaks in lymphoid tissues before nonlymphoid tissues.[51,52]
Target organ(s) should be the same as natural infection	Intravenous inoculation of *O. tsutsugamushi* into inbred mice targets the lungs, brain, kidney, and liver.[8]	AG129 mouse models of DENV-1–4 using intravenous or intraperitoneal inoculation lead to systemic infection and multi-organ failure.[29,37,40]
Target cells (for intracellular organisms) should be the same as natural infection	Intravenous inoculation of *O. tsutsugamushi* into inbred mice targets endothelial cells as in human scrub typhus.[8,9]	AG129 and A129 models of primary DENV-1–4 infection result in infection of macrophages and dendritic cells, and secondary DENV-2 infection models show infected endothelial cells.[33,40,51,53]
Pathological lesions should be the same as those of the disease	Human scrub typhus and intravenous infection of mice with *O. tsutsugamushi* are characterized by disseminated vasculitis, interstitial pneumonia, meningoencephalitis, and multifocal hepatocellular cell death and associated cellular immune responses.[3,8]	AG129 mouse models of DENV-1–4 infection via the intravenous, subcutaneous, or intraperitoneal route lead to liver, spleen, and intestinal pathology, which occur during human infections.[39–42,52,50]
Incubation period should be reasonable	Intravenous inoculation of mice with a high dose of orientiae or rickettsiae results in toxic death within 1 day, compared with the onset of human scrub typhus or rickettsial infections typically one week or more after arthropod inoculation and dose-dependent onset with *O. tsutsugamushi*, *Rickettsia conorii*, or *R. typhi* 4–12 days after experimental inoculation.[45,46,54–56]	AG129 mouse models of primary, lethal DENV-1–4 infection show that mice are infected within 1 day and die approximately 4–8 days after infection.[29,37,39,41] A129 and AG129 antibody-enhanced infection models use passive strategies to mimic primary infection.[33,34] Five-week-old AG129 pups born to DENV-immune dams develop enhanced disease following infection with low-dose inoculum.[36]
Modifiable spectrum of disease and clinical outcomes appropriate to the intended experimental purpose	Inbred mouse model of intravenous *O. tsutsugamushi* inoculation is dose- and strain-dependent allowing for different outcomes and allows adoptive transfer or depletion of immune cells or genetic knockout studies to study immunity.[7,9]	AG129 mouse models of DENV-3 can be mild or lethal and used to study pathogenesis; lethal models can be used to determine efficacy of antivirals and live-attenuated vaccines (because AG129 mice mount insufficient responses to inactivated or killed vaccines).[29,40,44,57] Humanized mice have prolonged viremia and do not develop severe disease but can be used to study human immunity to dengue and for antiviral reduction of viral loads.[58,59]

DENV = dengue virus; IFNAR = IFN-α/β-receptor; *O. tsutsugamushi* = *Orientia tsutsugamushi*.

or host strain–dependent severity of illness. To determine the important role for a particular immune effector or genetic condition, it is useful to have a sublethal infection model to evaluate whether depletion or genetic absence of the factor converts the ordinarily sublethal infection to a lethal outcome. Conversely, an important role for an immune component can be determined by its adoptive transfer into a susceptible mouse strain that is subsequently challenged with an ordinarily lethal infectious inoculum and observing protection by the immune effector.

Mouse models of scrub typhus. *Orientia tsutsugamushi* is maintained transovarially and transmitted by larval *Leptotrombidium* mites during the chigger's feeding when organisms are inoculated into the dermis of the human skin. The initial targets are dermal dendritic cells, and macrophages[1,2] and spread occurs via the lymphatic vessels to the regional lymph nodes and subsequently by the bloodstream to infect endothelial cells and macrophages throughout the body. Infection of endothelial cells in the lungs and brain results in vasculitis manifesting as interstitial pneumonia and meningoencephalitis.[2,3] It was discovered more than eight decades ago that intraperitoneal inoculation of mice is an effective means to isolate *O. tsutsugamushi*, frequently resulting in the animal's death.[4] However, this approach is not representative of human scrub typhus in its mode of transmission, portal of entry, route of spread, principal target organs and cells, and pathologic lesions.

Orientiae are introduced into the dermis by the stylostome of the feeding larval trombiculid mite. Because the chigger stylostome does not extend beyond the superficial dermis to the depth of subcutaneous tissue, models using subcutaneous needle inoculation do not mimic natural infection. Intradermal needle inoculation of mice with *O. tsutsugamushi* accurately models sublethal human scrub typhus, except for the effects of the feeding chigger such as salivary secretions and the prolonged duration of inoculation.[5] Intradermally inoculated orientiae disseminate hematogenously in mice, infecting endothelial cells and macrophages in the lungs, liver, spleen, brain, and other organs, resulting in illness associated with disseminated vasculitis, interstitial pneumonia, meningoencephalitis, and hepatic lesions typical of human scrub typhus after an incubation period of 10–12 days.[2,3]

Chigger transmission of orientiae into CD-1 Swiss mice has not been adequately characterized. *Orientia tsutsugamushi*–infected chigger colonies maintained at the Armed Forces Research Institute of Medical Sciences in Bangkok used for transmission into mice yield variable results, presumably due to the spectrum of genetic host resistance in infected outbred mice studied.[6] *Leptotrombidium changraiensis*, *Leptotrombidium imphalum*, and *Leptotrombidium deliense* chiggers infected with one or more minimally characterized strains of *O. tsutsugamushi* cause illness in outbred mice with incubation periods, organ involvement, and mortality that vary from animal to animal. Additional variables to consider are the virulence of *O. tsutsugamushi* strains and host resistance of the individual outbred mice. By contrast, the use of inbred strains of mice allows for adoptive transfer of immune components such as T-cell subsets and infection of mice with selected gene knockouts.[7] Chigger-transmitted *O. tsutsugamushi* infection of inbred mice remains to be reported. Such a model detailing histopathology, detection of the organisms, kinetics of spread, replication, and host responses would offer the best opportunity to elucidate the mechanisms of immunity and pathogenesis as the foundation for vaccine development. The drawbacks of chigger feeding inoculation are the inability to quantify the dose of inoculum transmitted by a feeding chigger, issues regarding establishment and maintenance of infected chigger colonies, and effective biocontainment of the experiments.

The most useful currently available animal models of scrub typhus are intravenous and intradermal inoculation of inbred mice.[8] Dose- and *O. tsutsugamushi* strain–dependent sublethal and lethal outcome models can be used to determine the critical roles of cells or immune components in immunity or pathogenesis.[8,9] Although bypassing the early events of dermal entry and initial lymphogenous spread, the effects of the defined components of immunity in the setting of hematogenous disseminations, such as CD8+ T lymphocytes, on illness, survival, and hematogenous infection of endothelial cells in the lung, brain, liver, kidney, and other organs can be determined. The particular sites and cell types of infection assure that immune cell trafficking conforms to what occurs in hematogenously disseminated human infection with *O. tsutsugamushi* in stark contrast with the cell trafficking events after infection of the peritoneal lining following intraperitoneal inoculation.[8] Moreover, the animal's pathologic lesions that are lethal or ameliorated by specific immune components, namely, interstitial pneumonia, meningoencephalitis, interstitial nephritis, and multifocal hepatocellular death and associated cellular inflammation, represent the critical events in human scrub typhus.[3] Examples of mouse models of scrub typhus and other rickettsioses that demonstrate the same criteria for the relevant vector-borne diseases are presented in Table 1.

Mouse models of dengue. Dengue is caused by infection with any of the four DENV serotypes 1–4. The milder dengue fever is self-limiting and usually leads to long-term immunity to the infecting serotype. Severe dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) are life-threatening and associated with the development of a secondary DENV infection as a result of weak cross-neutralizing immunity.[10–12] Following virus introduction by a mosquito bite, DENV infects cutaneous dendritic cells and makes its way to regional lymph nodes where additional infection occurs and ultimately disseminates. The pathological features of severe dengue can include pleural effusion or pulmonary edema, splenomegaly, and liver involvement such as hepatomegaly and hepatic necrosis.[10,13] Also, dengue patients may develop gastrointestinal bleeding, diarrhea, and hematemesis. Clinical markers include thrombocytopenia and increased serum liver enzyme concentrations.[14]

Dengue mouse models are often categorized into those using immunocompetent, immunocompromised, or humanized mice. Although the mouse is not a natural host of DENV, passaging DENV in mice using the intracranial route is a historical method of viral propagation that leads to genotypic and phenotypic changes in the virus, resulting in disease not typically observed in humans.[15] Intracranial inoculation of neuroadapted DENV into immunocompetent C57Bl/6 mice leads to encephalitis and paralysis, whereas intraperitoneal inoculation results in lethal dengue-like disease in the absence or presence of neurotropism.[16–19] Paralysis is rare in dengue patients, but central nervous system involvement, such as impaired consciousness, is a warning sign that is increasingly reported.[20,21] Recent studies explain the inability of DENV to naturally cause infection in mice by showing that DENV

interacts with and degrades human, but not mouse, immune signaling mediators.[22,23] Contemporary dengue mouse models focus on systemic and immunological markers of disease mimicking human infection often at the cost of natural infection routes, inocula, or incubation periods.

Subcutaneous or intradermal inoculation of 10^6 plaque forming units wild-type DENV-2 into humanized mice can lead to fever, erythema, and thrombocytopenia.[24,25] In humans, viremia lasts only days, but in humanized mice, viremia may last several weeks and be prolonged by virus delivery via mosquito bites or intradermal injection including mosquito saliva.[26] The portion of mice developing a human neutralizing antibody response varies, and mosquito inoculation increases the number of mice developing anti-DENV antibodies, as does refinement of the engraftment procedure used to generate humanized mice.[26,27] Overall, humanized mouse infection is not severe, probably because of impaired interactions between the human immune cells and the mouse nonimmune components.

Interferons (IFNs) induce an antiviral response in infected and neighboring cells, and mice that are deficient in one or more components of IFN signaling are immunocompromised. Dengue virus inoculation of mice with a deficiency of the IFN-α/β-receptor ($IFNAR^{-/-}$ or A129 single knockout), IFN-α/β-receptor and IFN-γ-receptor (AG129, double knockout), or the IFN signaling mediator signal transducer and activator of transcription-1 (STAT-1) shows that the doubly deficient AG129 mice are more immunocompromised and, thus, more sensitive to DENV.[28,29] Interestingly, mice doubly deficient in IFN response factors 3 and 7 exhibit resistance to primary dengue disease but can develop a low-level viremia following either needle or mosquito inoculation of DENV-2.[28,30] Overall, $IFNAR^{-/-}$ and A129 mice require higher doses of DENV-2 inoculum to cause severe disease.[31,32] To circumvent this, DENV-reactive antibodies are administered 1 day before inoculation, leading to antibody-dependent enhancement of infection, which can then mimic signs of human illness and cause mortality.[31–34] Also, $IFNAR^{-/-}$ mice infected with DENV-2 in the presence of *Aedes aegypti* mosquito salivary gland extract exhibit increased DENV pathogenesis.[35] Enhancement of infection is also observed in a pregnancy model of immunocompromised mice in which pups are born to DENV-immune mothers.[36] Primary infection models for DENV-1–4 are available in AG129 mice via intravenous, subcutaneous, or intraperitoneal inoculation of 10^7 pfu.[37–42] The mice develop signs of human DHF and DSS, such as high systemic viral loads in the blood; thrombocytopenia; organ pathology; cytokine storm, including TNF-α–mediated disease; and vascular leakage, ultimately succumbing to infection within approximately 4–8 days.[29,42,43] Also, AG129 mice generate a neutralizing antibody response to DENV. Furthermore, AG129 mice are the only model of nonlethal symptomatic dengue wherein mice become systemically infected with DENV-3, generate cytokines, and develop acute thrombocytopenia, mild organ pathology, and a neutralizing antibody response, yet recover 8–10 days after infection.[44] A criticism of immunocompromised DENV infection mouse models is that the antiviral response is impaired and is, thus, limited in its capacity to faithfully reproduce innate immunity to DENV. However, DENV proteins target and degrade human STAT-2 and STING, which impairs IFN signaling in humans.[22,23] These DENV proteins do not target mouse STAT-2 and STING; therefore, IFN-receptor–deficient mice allow DENV to establish infection similar to that of humans. Furthermore, AG129 mice retain the ability to induce an acute elevated cytokine response and develop TNF-α–mediated disease, leading to mortality.[37,40] The route of spread, target cells and organs, modifiable clinical outcomes and spectrum of disease, infectivity by non-adapted virus strains, and appropriateness for testing vaccines and therapies indicate that the AG129 mouse models are the most accurate models currently available for human disease caused by DENV-1–4.

CONCLUSION

Animal models remain one of the most important tools in the study of infectious diseases to understand pathogenesis and immunity and to evaluate therapies and vaccines. Establishing accurate disease models for vector-borne infections comes with an additional set of challenges insomuch that variables associated with arthropod transmission require consideration. Mouse models for scrub typhus and dengue have significantly advanced the knowledge in their respective fields, yet their application relies on critical evaluation of the advantages and disadvantages of each model.

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