Review Article: Unresolved Problems Related to Scrub Typhus: A Seriously Neglected Life-Threatening Disease

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Ecology and epidemiology. Scrub typhus is a life-threatening infectious disease that presents as an acute undifferentiated febrile illness. Its agent, *Orientia tsutsugamushi*, is an obligately intracytosolic bacterium that is transmitted by feeding larval trombiculid mites, which are the reservoir of the agent and the only life stage that feeds on a vertebrate host.1 Nymphal and adult trombiculid mites live in the soil and feed on the eggs of insects. Mites maintain the organisms by transovarial transmission as well as transstadial transmission through the mite's lifecycle.2,3 Although mites transmit *O. tsutsugamushi* to vertebrate hosts such as rodents, only a small proportion of uninfected mites acquire *Orientia* during feeding on infected animals, and the ingested *Orientia* do not establish disseminated infection in the mites and are not transmitted transovarially to the next generation.4-7 It seems that chigger cofeeding on rodents is more relevant for effective mouse-to-mite transmission of *Orientia* than feeding on rickettsemic hosts.7,8 Thus, the true role of rodents as reservoirs requires additional investigation, because there may be marked genetic variability in chiggers with respect to the ability to acquire rickettsiae by feeding. Also, rodents may be better considered as dead-end hosts just as humans are rather than a reservoir. However, the evolutionary selection of tremendous antigenic diversity of the immunodominant major 56 kDa surface protein presents an enigma if immune variation plays no role in survival of *O. tsutsugamushi.*9 The potential immunomodulatory effects of the saliva of larval mites on the pathogenesis of and immunity to infection with *O. tsutsugamushi* remain undetermined.

One million cases of scrub typhus occur each year with an estimated 10% case fatality rate unless treated appropriately, very likely resulting in more deaths than dengue.10,11 Currently, scrub typhus has predominantly been reported from an area extending from the Russian Far East and Korea in the north to northern Australia in the south and Afghanistan in the west, and it includes islands of the western Pacific and Indian Oceans, including Japan, Taiwan, Philippines, Papua New Guinea, Indonesia, and Sri Lanka.12,13 This geographical range may be an underrepresentation, because case reports have been published from Africa and South America. The recent isolation of a novel species *O. chuto* acquired by a patient in Dubai, the detection of another divergent *Orientia* transmitted to a patient in Chile, and serologic diagnoses of scrub typhus acquired in Africa indicate that a wider geographic distribution and genetic diversity of the genus should be investigated.14-17 The burden of disease in rural areas of Asia is large, with studies showing scrub typhus causing up to 20% of febrile hospital admissions,18-20 an incidence of infections of greater than 3% of the population monthly,21 seroprevalence over 50% of the population, despite a significant annual rate of reversion to seronegativity of 50% of cases,22 and a seroconversion rate of 484 per 1,000 person-years.23

In 1999, the World Health Organization (WHO) stated, “Scrub typhus is probably one of the most underdiagnosed and underreported febrile illnesses requiring hospitalization in the region.”24 This opinion remains valid today and could justifiably be adjusted to scrub typhus is probably the single most prevalent, under-recognized, neglected, and severe but easily treatable disease in the world. This ancient disease is currently undergoing increased awareness both because of re-emergence and rising incidence in previously unrecognized areas and improved diagnostic testing. It is apparent that scrub typhus has been recognized to occur frequently now in places where the illness was nearly forgotten, including India, Sri Lanka, the Maldives, and Micronesia.25-28 However, scrub typhus has also emerged in regions north of the Yangtse River in China, where it was not known previously.29-33 The emergence of scrub typhus should also take into consideration the expansion of farmlands that have generated ideal habitats for trombiculid mites. The potential relationship with global climate change is unclear. Greater recognition in some countries, such as Thailand, Laos, Taiwan, and Japan, may reflect increased medical investigations and application of new diagnostic methods.34-37 The critical unresolved issues regarding epidemiology emphasize the need to determine the reasons for emergence and re-emergence and the true incidence of this neglected disease, for which calculated days of disability-adjusted years of life lost (DALYs) have not been determined and to which appropriate attention has not been paid (Table 1).

Clinical manifestations. Scrub typhus ranges from a mild to a fatal illness. The early clinical manifestations are an eschar, representing localized cutaneous necrosis at the site of mite feeding (which is not always present), and regional lymphadenopathy followed subsequently by fever, headache, myalgia, generalized lymphadenopathy, cough, gastrointestinal symptoms, transient hearing loss, and rash.38-39 Progression of severe scrub typhus may manifest as acute respiratory distress, meningoencephalitis, gastrointestinal bleeding, acute renal failure, hypotensive shock, and coagulopathy.40-42

Unresolved clinical issues include the reason for the wide range of occurrence of eschars (7-97%), the reason for rash and severity of illness, and the need for characterization and determination of the mechanisms of the coagulopathy, hemorrhages, interstitial pneumonia, and meningoencephalitis.43-45 There is currently no unified approach to assess and stratify...
Table 1
Unresolved issues related to ecology and epidemiology

| Determination of the true impact of scrub typhus on public health |
| Determination of whether there are other important factors for maintenance of \textit{O. tsutsugamushi} in nature other than vertical transmission in mites that explain the extent of antigenic diversity |
| Elucidation of the basis for the periodic re-emergence of scrub typhus |
| Discovery of \textit{Orientia} species other than \textit{O. tsutsugamushi} and their actual geographic distribution |

Table 2
Unresolved issues related to the pathogenesis of clinical manifestations

| Determination of the reasons for the wide variation in incidence of eschars, rash, and hemorrhages |
| Elucidation of the pathogenesis of acute respiratory failure and central nervous system complications |
| Characterization of the coagulation abnormalities in scrub typhus |
| Determination of virulence mechanisms of \textit{O. tsutsugamushi}, host-mediated pathogenic mechanisms, and mechanisms of tissue injury |
| Development of a disease severity score based on clinical features and pathophysiological markers |

Table 3
Unresolved issues related to diagnosis and prognosis

| Development of a simple, affordable point-of-care diagnostic approach (preferably a combined rapid test that detects antigen or DNA and antibody for the diagnosis of scrub typhus in the early or mid-course (analogous to the NS1/IgM algorithm in dengue) |
| Detection of a set of markers that are useful early in the disease course to assess diagnostic severity and prediction of progression |
For comparisons and evaluation of new diagnostic methods, a robust reference set of criteria covering both antibody- and bacterial DNA-based detection should be used that defines scrub typhus with a high level of confidence. Recently, a panel of scrub typhus infection criteria was proposed for diagnostic accuracy evaluations, in which one or more of the following criteria had to be fulfilled: (1) a cell culture isolate, (2) a single admission immunoglobulin M (IgM) titer ≥ 1:12,800 using the gold standard indirect IFA, (3) a fourfold increase in IgM IFA titer, and/or (4) detection of \textit{O. tsutsugamushi} DNA in at least two of three different target PCR assays.

**Mouse models.** The majority of research on animal models of scrub typhus has been conducted on various strains of mice infected through intraperitoneal inoculation. Groves and Osterman used approximately 40 cross-bred, inbred, and outbred mouse strains to study \textit{Orientia} infection. After intraperitoneal inoculation, all combinations of \textit{Orientia} strains and mouse strains resulted in illness of mice, but none reproducibly developed pathology resembling human scrub typhus. Kundin and others inoculated albino Swiss mice by intracranial, intramuscular, intraperitoneal, and subcutaneous routes, resulting in route-specific \textit{Orientia} distribution and lesions that did not represent human scrub typhus-like pathology.

Intraperitoneal inoculation of \textit{O. tsutsugamushi} into mice results in intense infection of peritoneal mesothelial cells and macrophages and \textit{Orientia} peritonitis with limited systemic spread late in the course. Subcutaneous inoculation, which differs from intradermal inoculation by a feeding chigger, results in chronic persistent infection. Intravenous inoculation of inbred C57BL/6 mice results in disseminated endothelial infection and vascular inflammatory and immune responses in lungs, brain, and other organs that represent the pathology of fatal human scrub typhus (Shelite TR, Walker DH, unpublished data). Severity of disease, including lethality, is dose-, \textit{Orientia} strain-, and mouse strain-dependent. Intradermal inoculation of C57BL/6 inbred mice does not cause eschar formation but results in disseminated endothelial infection targeting endothelium and mononuclear phagocytes and histopathologic lesions resembling human scrub typhus (Mendell N and others, unpublished data). Validated infection models using inbred mice will allow studies incorporating adoptive transfer of selected immune cells, such as CD4+ or CD8+ T cells. Valid models in C57BL/6 mice open the opportunity to study genes involved in the mechanisms of immunity and pathogenesis by the use of gene knockout mice. The ideal model would use chigger transmission of a predictably lethal or sublethal dose of \textit{O. tsutsugamushi} into C57BL/6 and other inbred strains of mice with differences in susceptibility to severe disease. The variations in levels of susceptibility reflected in bacterial loads, dissemination to specific organs, and host responses of outbred mice limit their suitability for specific immunological mechanistic investigations of the immune response.

**Non-human primates.** Clinical, pathologic, and immunologic responses to experimental scrub typhus infections have been studied in silvered leaf monkeys (\textit{Presbytis cristatus}) and cynomolagus monkeys (\textit{Macaca fascicularis}) indigenous to the endemic area for this disease; the latter was found to be the better model for the study of this disease, mainly because of the difficulty of maintaining silvered leaf monkeys in captivity.

In a detailed study, cynomolgus monkeys evaluated for cellular immune responses after intradermal infection with \textit{Karp} strain of \textit{O. tsutsugamushi} developed eschars, lymphadenopathy, rickettsemia, and elevated body temperatures. The development of IgM antibody titers was followed by IgG antibodies and cell-mediated immune responses shown by lymphocyte proliferation and interferon-\(\gamma\) (IFN-\(\gamma\)) production by \textit{O. tsutsugamushi} antigen-stimulated peripheral blood mononuclear leukocytes. When challenged 6 years after the initial infection, clinical signs and cellular responses were indistinguishable from the signs and responses of naive animals, but an anamnestic IgG antibody response was observed. However, if challenged at 1 year after primary infection, only a localized cutaneous lesion developed. The majority of animals infected previously had persisting lymphocyte responses to antigen stimulation, suggesting long-term immunologic memory that was not protective against rechallenge. In a recent study, cynomolgus macaques infected intradermally with \textit{O. tsutsugamushi} \textit{Karp} strain developed eschars and regional lymphadenopathy followed by systemic lymphadenopathy, with pathological features of the eschar closely resembling human scrub typhus. \textit{O. tsutsugamushi} was detected in mononuclear leukocytes in eschars and lymph nodes, and \textit{O. tsutsugamushi}-specific IgM and IgG antibodies and cell-mediated immune responses were observed in all infected non-human primates (Paris DH, unpublished data).

**Pathogenesis.** \textit{Orientia} inoculated in chigger saliva infects mainly dendritic cells and macrophages in the dermis underlying the eschar at the site of inoculation. The early development of lymphadenopathy in the regional drainage of the eschar suggests lymphogenous spread. Subsequently, hematogenously disseminated infection involves predominantly endothelial cells and to a lesser degree, macrophages, both of which release soluble cell-specific adhesion molecules, suggesting that these cells play key roles in systemic inflammation.

Attachment and entry of obligately intracellular \textit{O. tsutsugamushi} into host cells by a clathrin-dependent endosomal pathway involve the major 56 kDa surface protein, an auto transporter (ScaC), a 47 kDa surface protein, host fibronectin, and integrin-a5b1 and syndecan-4 host cell receptors. The cell biology of this intracytoplasmic pathogen has been reviewed recently by Ge and Rikihisa.

Key unresolved issues regarding the pathophysiology of scrub typhus include identification of the mechanisms of cell
injury, such as potentially direct Orientia-mediated intracellular events, generation of reactive radicals by host cells, pathologic levels of cytokines, and cytotoxic immune cells, and actual pathophysiologic effects, such as potentially increased vascular permeability leading to edema in vital locations, which occurs in rickettsial infections. Another unresolved topic is the prevalence and pathologic effects of persistent Orientia infection.72,73 Persistent infection by Orientia was first described in experimentally infected mice, and bacteria could be detected by inoculation of blood or homogenized tissue of previously infected mice for up to 610 days after the infection.74,75 Smedal and others75 showed that, 2 years after scrub typhus, Orientia could be isolated from the lymph node of an asymptomatic person. The agent was infectious for intraperitoneally inoculated mice with a lethal outcome.73 More recently, Chung and others73 isolated Orientia from the blood of six individuals who had scrub typhus 1–18 months earlier. The factors that allow for persistent Orientia infection are another unresolved topic in scrub typhus research.

Immunity to scrub typhus. Immunity to Orientia is complicated by the great antigenic diversity of the agent, the weak and transient cross-protection among divergent strains, and the loss of even homologous immune protection after a few years.79,76,77 Homologous immunity is provided by antibodies to the major surface protein, a 56 kDa molecule that contains strain-specific epitopes. Antibodies to Orientia increase uptake by macrophages and neutrophils and play a role in the clearance of organisms from blood.72,78,79 Antibodies to the 56 kDa surface protein inhibit entry into target cells and neutralize infectivity.80 The concentration of Orientia strain-specific antibodies wanes over a few years after infection, and heterologous strain protective immune responses last a considerably shorter length of time; therefore, there is the possibility of reinfection.72,82 Cellular immunity provides cross-protection against divergent strains that is T lymphocyte-dependent. Using an uncharacterized intravenous inoculation mouse model, Jerrells and Osterman83 showed that depletion of macrophages was detrimental to survival of the mice. Experimental studies of immunity that have used invalid animal models suggest that protective immunity is mediated by Th1 cells. Adoptive transfer of antigen-specific IFN-γ–producing T cells is protective.84,85 Thus, it is very likely that humoral immunity facilitates phagocytic removal of orientia when appropriate antibodies are present, and IFN-γ and cytotoxic T lymphocytes play key roles in controlling the infection. Boryong strain-specific immunity stimulated in experimental animals by recombinant Boryong 56 kDa protein correlates with antibodies directed against this antigen.86

Patients with scrub typhus have been reported to develop increased serum concentrations of cytokines (IFN-γ, interleukin 1β [IL-1β], IL-12p40, tumor necrosis factor-α [TNF-α], and IL-10), chemokines (CXCL-9 and CXCL-10), and granzymes A and B.87–92 However, mechanistic studies of protective or deleterious effects of these molecules have not been pursued. Indeed, the roles of CD4 and CD8 T lymphocytes, natural killer (NK) cells, NKT cells, dendritic cells, macrophages, and endothelial cells have not been evaluated in animal models with disseminated endothelial infection that appropriately represents scrub typhus (Table 4).

Attempts to develop a vaccine against Orientia. In the past 70 years, numerous approaches have failed to stimulate protective immunity against challenge with Orientia to prevent scrub typhus. Approaches have included the use of formalin-killed Orientia, inoculation with viable organisms followed by antimicrobial treatment, irradiated Orientia, and subunit vaccines.72,93–96 The results have varied from short-term protection to failure to protect. Protection was frequently defined as preventing death but not protecting from illness. As with natural infection, there was failure to stimulate cross-protection against the many strains circulating in nature. A recent review article has addressed the history of vaccine development for scrub typhus and its current status.95 Current approaches should aim to understand why the natural immune response against Orientia does not produce sterilizing, long-lasting, and cross-protective immunity. The naturally induced immune response seems inappropriate and is hampered by the immunodominance of antigens encoded by genes that are not conserved across the large number of Orientia strains.

Genome sequencing should be pursued, because data from two sequenced strains (Boryong and Ikeda) reveal other potential vaccine antigens (e.g., autotransporter surface Sca proteins) that suggest that a contemporary genomic approach to scrub typhus vaccinology has potential (Table 5).97 Orientia and scrub typhus present many unresolved problems that can be addressed by contemporary biomedical scientific approaches. Obligately intracellular bacteria have fallen outside of the scope of bacteriologists and virologists, leading to insufficient effort to undertake the required scientific projects. This situation represents an outstanding opportunity for biomedical scientists to pursue.

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