



COMMENTARY

Humoral Immune Response to *Orientia tsutsugamushi* Infection: Recent Research Findings and Future Studies

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Abstract

Orientia tsutsugamushi (*Ot*), an intracellular bacterium in the family Rickettsiaceae, is the etiological agent of scrub typhus. Understanding of the immunologic responses to *Ot* infection, particularly immunity after infection, has remained a major knowledge gap. Although anti-*Ot* IgG appears to be short-lived in infected humans, recent studies have reported autoreactive IgM antibodies in people with scrub typhus, including antibodies reactive to nuclear components, DNA, and platelets. However, mechanistic studies of B cell responses in the secondary lymphoid organs of infected humans are severely lacking. This article highlights feasible methods to investigate important aspects of humoral immunity, and avenues to obtain samples from post-mortem and ante-mortem *Ot*-infected people. A pioneer study identified that serum passive transfer could protect against homologous *Ot* challenge. Studies comparing humoral immune responses to *Ot* Karp versus Gilliam strains have revealed impaired B cell and germinal center (GC) responses during severe, but not self-limiting, infection in C57BL/6 mice. Further studies are needed, particularly in people with scrub typhus, to help understand and define deficiencies in the acute B cell response and long-lived humoral immunity.

Key words: Scrub typhus, humoral immunity, B cell, germinal center, splenic architecture, *Orientia tsutsugamushi*

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INTRODUCTION

Scrub typhus is an emerging but neglected tropical disease. Human studies have revealed that anti-*Ot* IgM responses are transient; while, anti-*Ot* IgG titers can persist for several years, yet, their functional roles in disease outcomes remain unclear. Notably, recent findings indicating the presence of autoreactive antibodies in people with scrub typhus have prompted new questions regarding immune dysregulation. Our research using mouse models of *Ot* infection has demonstrated strain-specific differences

in disease severity. Severe scrub typhus was found to lead to disruption of (GCs), loss of splenic architecture, diminished B cell activation, and proinflammatory immune environments. In contrast, mice with self-limiting infection were found to induce adequate humoral responses and preserve lymphoid structures. These results highlight the effects of excessive inflammation and altered splenic microenvironments on humoral immunity, and have implications regarding long-term immune competence, vaccine development, and cross-protection. Future animal model studies using

different *Ot* strains and transcriptomic imaging technologies would aid in the exploration of GC function, memory B cell responses, and autoreactive antibody generation at different disease stages.

HUMORAL IMMUNE RESPONSES IN PEOPLE WITH SCRUB TYPHUS

Humoral immunity after *Ot* infection varies but is generally considered short-lived: previous reports have demonstrated seronegativity in people with previous scrub typhus at 49 weeks post-infection [1] or 1–3 years post-infection [2,3]. For *Ot*-specific IgM responses, one study (in a cohort of 197 people with scrub typhus) has reported that 50% of people were IgM seronegative at 82 days post-infection [3], whereas another study has found that only 3.5% of a hospitalized surveyed population was IgM seropositive [4]. For *Ot*-specific IgG responses, the titers wane to baseline levels approximately 2–3 years post-infection [2,5]. IgG seropositivity appears common: 20–40% IgG seropositivity has been reported in surveyed populations [3,6]. In those studies, ELISAs were based on recombinant proteins derived from a major *Ot* surface antigen (56-kDa type-specific antigen) and/or ScaA (an autotransporter protein). Although the initial IgM titers between people with severe or non-severe scrub typhus did not significantly differ [7], intermediate or high initial IgG titers appeared to be associated with elevated risk of severe scrub typhus [7]. Although higher initial IgG titers correlated with greater risk of scrub typhus with complications, IgG waned more slowly in people with higher initial IgG levels [3]. In a recent study aimed at evaluating whether antibodies generated in people with scrub typhus might be autoreactive, people with scrub typhus, compared with healthy controls, displayed higher antinuclear antibodies and anti-double-stranded deoxyribonucleic acid IgM titers [8]. Reports have also described anti-platelet antibodies, which might contribute to thrombocytopenia, in people with scrub typhus [9,10]. These self-reactive IgM titers increase throughout infection, particularly after the onset of symptoms and signs of scrub typhus [8]. Whether scrub typhus-induced autoreactive antibodies might be linked to disease severity remains to be explored, and the functional roles of antibodies in people with scrub typhus remain poorly defined.

No study has reported the development or alterations in humoral immune responses in people with scrub typhus, partially because examination of secondary lymphoid organs has been limited. Although spleen sampling is unlikely to be safe in living people with scrub typhus, several human studies examining other pathogens that target endothelial cells, macrophages, and dendritic cells might offer valuable tools and methods for sampling other secondary lymphoid organs from people [11–14]. For the evaluation of GCs in human lymph nodes, much progress has been made through COVID-19 studies post-infection or vaccination [11–14]. The possibility of

lymph node biopsies and ultrasound-guided fine-needle lymph node aspiration may provide new routes to study the GC response in people with scrub typhus without a need for postmortem sampling. A future study using these valuable human samples might potentially inform scrub typhus vaccine development and provide critical information regarding adaptive immune responses in relevant immune sites such as draining lymph nodes. Even postmortem spleen and lymph node samples from people with scrub typhus would have substantial future study value. For example, postmortem samples of spleens and thoracic lymph nodes from people with COVID-19 have revealed a lack of GCs, including BCL-6⁺ GC B cells and BCL-6⁺ Tfh cells, corresponding to Th1 cell infiltration and excessive TNF α [15]. Spleen and lymph node postmortem samples from humans with scrub typhus will allow for further examination of GCs and other cell types to findings of in severe scrub typhus mouse models [16].

HUMORAL IMMUNE RESPONSES IN MOUSE MODELS OF SCRUB TYPHUS

Inbred and outbred mouse models of scrub typhus are valuable tools for examining the scrub typhus disease (development, severity, and pathogenesis) and immune responses (development, magnitude, and alterations) [17–19]. Nearly half a century ago, Shirai et al. reported that passive transfer of anti-Gilliam and anti-Karp sera protected three of five tested mice against lethal Karp challenge [20]. Three decades earlier, Topping described the protective ability of anti-*Ot* hyperimmune sera in rabbits [21]. Limited studies have demonstrated that, during infection, antibodies inhibit bacterial entry into target cells, and increase bacterial uptake in neutrophils and macrophages [22–24]. In 2023, our team examined B cell responses in a C57BL/6 mouse model (intravenous, Karp strain) of severe scrub typhus [25]. Immunofluorescence staining revealed disruption of GC structures in the spleens during severe infection stages. Furthermore, RNAseq analysis indicated that B cells from Karp-infected spleens showed downregulation of adhesion molecule and costimulatory genes, and impaired B cell activation and BCR signaling in late stages of infection.

Our team further explored the extent of humoral immune impairment and spleen-related pathological changes during acute stages of severe scrub typhus (Karp strain) versus self-limited infection (Gilliam strain) (Fig 1) [16]. In contrast to Karp-infected mice, Gilliam-infected mice had a significantly lower bacterial burden in the spleen during acute infection stages, but exhibited higher levels of IgM and IgG antibodies at day 12 post-infection, and greater absolute numbers of splenic B cells, follicular B cells, and marginal zone (MZ) B cells. Only Karp infection induced GC collapse and abrogation of splenic MZs, according to immunohistology analysis. Karp infection induced an inflammatory transcriptomic environment, including upregulation of inflammation genes (*Cd2*, *Il6*,

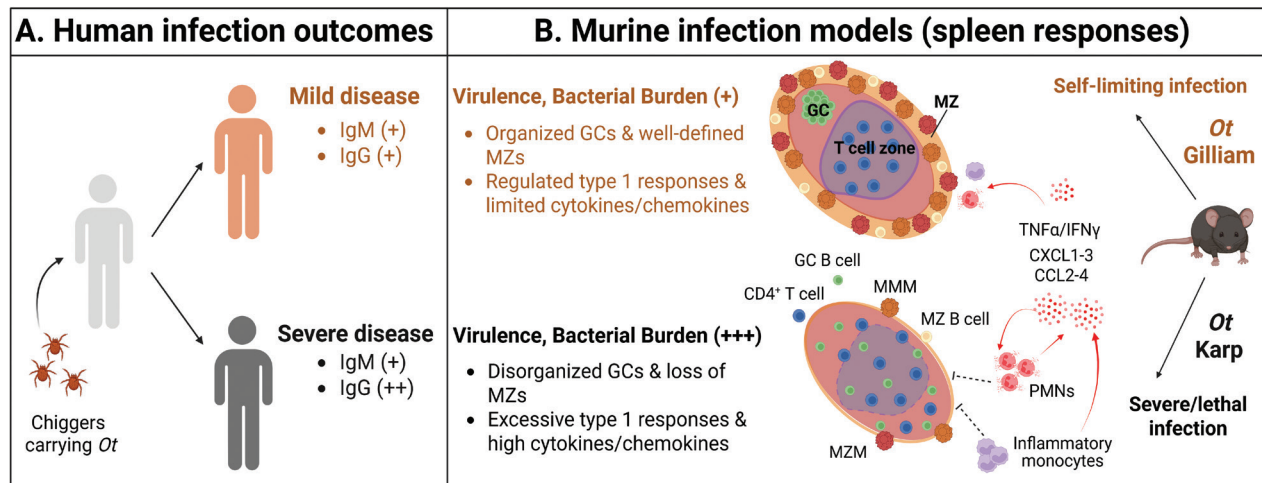


FIGURE 1 | Overview of human scrub typhus and mouse models for spleen-focused studies.

A) Disease development, severity, and antibody responses in people with scrub typhus after exposure to bacterium-carrying chiggers. B) C57BL/6 mouse models after infection with the *Ot* Karp or Gilliam strain (at the same viable dose via an intravenous route), which resulted in 0% lethality (Gilliam) or ~50% lethality (Karp), respectively. Spleen-focused studies have revealed differential bacterial burdens, germinal center (GC) responses, marginal zone (MZ) organization, and recruitment of innate and adaptive immune cell subsets in acute stages of Gilliam versus Karp infection. Possible mechanisms of splenic microarchitecture disruption, GC collapse, and MZ abrogation are graphically illustrated, and include inflammatory monocytes, degranulating neutrophils (PMNs), and excessive inflammation. Red arrows represent stimulation, and dashed lines represent inhibition.

Il10, and *Ifng*) and related pathways (TNF, IFN γ , IL-1, and IL-17). Additionally, flow cytometry confirmed that Karp-infected spleens showed significant infiltration of activated neutrophils, macrophages, M1 macrophages, monocytes, and inflammatory monocytes. Gilliam-infected spleens, in contrast, demonstrated upregulation of adaptive immune response pathways, such as T cell receptor signaling, T cell differentiation, and B cell activation, at day 12 post-infection. Importantly, the study was limited in its lack of assessment of antigen-specific immune responses, T cell dependent or independent immune responses, affinity maturation, GC responses in peripheral or other secondary lymphoid organs, or underlying mechanisms of GC collapse. Nevertheless, this study was the first, to our knowledge, to identify and define differential immune responses in mouse models with unique disease outcomes. Through these analyses within the splenic immunological niche, we revealed critical insights into the impairment of B cell responses and potential mechanisms of GC collapse during severe scrub typhus.

MOUSE MODELS FOR MECHANISTIC INVESTIGATION

Our recent studies have provided the first lines of evidence, to our knowledge, that humoral immune responses are impaired during severe *Ot* infection, particularly GCs and splenic microarchitecture [16,25]. We identified that altered splenic architecture, exemplified by a lack of MZs and the presence of shrunken white pulp regions, persisted in early convalescence (day 26) after sublethal Karp infection (unpublished data). Therefore, future studies directed at assessing (1) the durability of altered splenic

microarchitecture, (2) the development of GC responses in late convalescence timepoints, and (3) underlying mechanisms of GC collapse (inflammation-driven GC disruption, inflammatory monocyte-mediated asphyxiation of GCs, and/or neutrophil-mediated destabilization and disorganization of GCs) are necessary. In particular, studies assessing immunity as inflammatory responses resolve and recovery begins will be important, to understand which factors might drive dysfunctional humoral immune responses. Additional studies should examine draining lymph nodes to determine whether similar impairments in microarchitecture and organization occur in organs other than the spleen. Such studies will be important in the context of the intradermal inoculation model [17,26], which mimics natural infection routes and early events in the skin and draining lymph nodes. Additional analyses might assess the differentiation of memory B cells by flow cytometry with antibodies to IgD, CD80, CD73, and PDL2, and bone marrow or tissue resident plasma cells, to enhance understanding of the development of long-lived humoral immunity. The roles of GCs during acute infection remain undefined, and whether GCs might lead to protective antibody responses in Gilliam infection remains to be determined. Given that autoreactive IgM has been found in people with scrub typhus [8], future studies should examine levels of autoreactive IgM and even IgG antibodies in mouse scrub typhus models. Furthermore, systems serology analysis in mice with severe and self-limited infection might enhance understanding of the roles of antibodies during *Ot* infection. This approach would enable exploration of concepts such as antibody dependent enhancement or Fc γ R-mediated inflammation.

Although *Ot* has been established to spread to multiple organs, probably via the blood, the initial infection site in the spleen remains undefined. Given the known tropism of *Ot* and the structure–function relationship in the spleen, *Ot* might take advantage of the splenic architecture by infecting antigen-presenting, phagocytic cells residing within the MZ, such as dendritic cells or macrophage subtypes. The infection of antigen-presenting cells might enable *Ot* to subvert or evade immune responses through several mechanisms, such as altering antigen presentation and suppressing cytokine production. An understanding of the key target cells that allow *Ot* to establish infection in all organs would provide key insights into the infection dynamics and any differing patterns among *Ot* strains with differing virulence.

The identified changes in splenic lymphoid architecture and GC dysfunction [16,25] have broad implications beyond *Ot* infection. Although they are likely to be multifactorial, the mechanisms underlying GC collapse and disorganization of splenic architecture might not only affect immune responses to *Ot* infection but also weaken responses after subsequent encounters with different pathogens or vaccination. Furthermore, we have identified that the splenic lymphoid architecture does not show complete recovery by early convalescence (unpublished data), and the lack of MZs might alter the initiation of adaptive immune responses to other infections, as shown in humans with altered splenic MZs. Biram et al. have demonstrated that GC-collapsing inflammatory monocytes derived from LPS hinder subsequent vaccine-induced GC responses [27].

Although current understanding of humoral immune responses in mouse scrub typhus models remains limited, the effectiveness of antibody-mediated protection against homologous challenge is substantial [21]. Experimental models of severe versus mild *Ot* infection during acute disease stages warrant further investigation [16,25], particularly in the context of innate responses and cellular immunity to clear acute infection. The development of a scrub typhus vaccine eliciting both humoral and cellular immunity should be feasible, to provide protection against homologous and heterologous challenge [28]. More comprehensive understanding of the *Ot*-specific T cell- and B cell-mediated immune responses, correlates of protection, and critical vaccine components is necessary to help achieve protection against homologous and heterologous challenge.

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CONFLICT OF INTEREST

Lynn Soong is Co-Editor-in-Chief of Zoonoses. She was not involved in the peer review or handling of the manuscript. Casey Gonzales has no competing interests to disclose.

REFERENCES

1. Saunders JP, Brown GW, Shirai A, Huxsoll DL. The longevity of antibody to *Rickettsia tsutsugamushi* in patients with confirmed scrub typhus. *Trans R Soc Trop Med Hyg.* 1980;74(2):253-257.
2. Ha NY, Kim Y, Min CK, Kim HI, Yen NTH, Choi MS, et al. Longevity of antibody and T-cell responses against outer membrane antigens of *Orientia tsutsugamushi* in scrub typhus patients. *Emerg Microbes Infect.* 2017;6(12):e116.
3. Schmidt WP, Devamani CS, Rose W, Alexander N, Prakash JAJ. Antibody response following scrub typhus infection: clinical cohort study. *Trop Med Int Health.* 2019;24(12):1455-1464.
4. Devamani CS, Prakash JAJ, Alexander N, Suzuki M, Schmidt WP. Hospitalisations and outpatient visits for undifferentiated fever attributable to scrub typhus in rural South India: retrospective cohort and nested case-control study. *PLoS Negl Trop Dis.* 2019;13(2):e0007160.
5. Trowbridge P, Divya P, Premkumar PS, Varghese GM. Prevalence and risk factors for scrub typhus in South India. *Trop Med Int Health.* 2017;22(5):576-582.
6. Bonell A, Lubell Y, Newton PN, Crump JA, Paris DH. Estimating the burden of scrub typhus: a systematic review. *PLoS Negl Trop Dis.* 2017;11(9):e0005838.
7. Devamani CS, Prakash JAJ, Alexander N, Stone W, Gunasekaran K, Rose W, et al. High initial IgG antibody levels against *Orientia tsutsugamushi* are associated with an increased risk of severe scrub typhus infection. *PLoS Negl Trop Dis.* 2021;15(3):e0009283.
8. You H, Song J, Kim S, Oh SM, Hwang JH, Hwang JH, et al. Scrub typhus association with autoimmune biomarkers and clinical implications. *PLoS Negl Trop Dis.* 2025;19(1):e0012766.
9. Chowdhary PK, Agrawal RK, Kumar S, Kale SA, Kumar V. Rare and unusual presentation as immune thrombocytopenic purpura in scrub typhus complicated by meningitis and acute kidney injury. *Indian J Crit Care Med.* 2022;26(6):748-751.
10. Auxilia J, Daniel NJ, Rajan N. Secondary immune thrombocytopenic purpura: an unusual complication of scrub typhus. *J Current Res Sci Med.* 2025;11(1):76-78.
11. Kim W, Zhou JQ, Horvath SC, Schmitz AJ, Sturtz AJ, Lei T, et al. Germinal centre-driven maturation of B cell response to mRNA vaccination. *Nature.* 2022;604(7904):141-145.
12. Mudd PA, Minervina AA, Pogorelyy MV, Turner JS, Kim W, Kalaidina E, et al. SARS-CoV-2 mRNA vaccination elicits a robust and persistent T follicular helper cell response in humans. *Cell.* 2022;185(4):603-613.e15.
13. Sakharkar M, Rappazzo CG, Wieland-Alter WF, Hsieh CL, Wrapp D, Esterman ES, et al. Prolonged evolution of the human B cell response to SARS-CoV-2 infection. *Sci Immunol.* 2021;6(56):eabg6916.
14. Turner JS, O'Halloran JA, Kalaidina E, Kim W, Schmitz AJ, Zhou JQ, et al. SARS-CoV-2 mRNA vaccines induce persistent human germinal centre responses. *Nature.* 2021;596(7870):109-113.
15. Kaneko N, Kuo HH, Boucay J, Farmer JR, Allard-Chamard H, Mahajan VS, et al. Loss of Bcl-6-expressing T follicular helper cells and germinal centers in COVID-19. *Cell.* 2020;183(1):143-157.e13.
16. Gonzales C, Liang Y, Thiriot J, Wang H, Villacreses D, Sun J, et al. Unique B cell and germinal center responses in mice with severe versus mild *Orientia tsutsugamushi* infection. *bioRxiv.* 2025. <https://doi.org/10.1101/2025.03.27.645674>
17. Soong L, Mendell NL, Olano JP, Rockx-Brouwer D, Xu G, Goez-Rivillas Y, et al. An intradermal inoculation mouse model

- for immunological investigations of acute scrub typhus and persistent infection. *PLoS Negl Trop Dis*. 2016;10(8):e0004884.
18. Thiriot JD, Liang Y, Gonzales C, Sun J, Yu X, Soong L. Differential cellular immune responses against *Orientia tsutsugamushi* Karp and Gilliam strains following acute infection in mice. *PLoS Negl Trop Dis*. 2023;17(12):e0011445.
 19. Shelite TR, Saito TB, Mendell NL, Gong B, Xu G, Soong L, et al. Hematogenously disseminated *Orientia tsutsugamushi*-infected murine model of scrub typhus [corrected]. *PLoS Negl Trop Dis*. 2014;8(7):e2966.
 20. Shirai A, Catanzaro PJ, Phillips SM, Osterman JV. Host defenses in experimental scrub typhus: role of cellular immunity in heterologous protection. *Infect Immun*. 1976;14(1):39-46.
 21. Topping NH. Tsutsugamushi disease (scrub typhus); the effects of an immune rabbit serum in experimentally infected mice. *Public Health Rep*. 1945;60:1215-1220.
 22. Seong SY, Huh MS, Jang WJ, Park SG, Kim JG, Woo SG, et al. Induction of homologous immune response to *Rickettsia tsutsugamushi* Boryong with a partial 56-kilodalton recombinant antigen fused with the maltose-binding protein MBP-Bor56. *Infect Immun*. 1997;65(4):1541-1545.
 23. Rikihisa Y, Ito S. Effect of antibody on entry of *Rickettsia tsutsugamushi* into polymorphonuclear leukocyte cytoplasm. *Infect Immun*. 1983;39(2):928-938.
 24. Nacy CA, Osterman JV. Host defenses in experimental scrub typhus: role of normal and activated macrophages. *Infect Immun*. 1979;26(2):744-750.
 25. Gonzales C, Liang Y, Fisher J, Card G, Sun J, Soong L. Alterations in germinal center formation and B cell activation during severe *Orientia tsutsugamushi* infection in mice. *PLoS Negl Trop Dis*. 2023;17(5):e0011090.
 26. Liang Y, Wang H, Sun K, Sun J, Soong L. Lack of the IFN- γ signal leads to lethal *Orientia tsutsugamushi* infection in mice with skin eschar lesions. *PLoS Pathog*. 2024;20(5):e1012020.
 27. Biram A, Liu J, Hezroni H, Davidzohn N, Schmiedel D, Khatib-Massalha E, et al. Bacterial infection disrupts established germinal center reactions through monocyte recruitment and impaired metabolic adaptation. *Immunity*. 2022;55(3):442-458.e8.
 28. Walker DH, Mendell NL. A scrub typhus vaccine presents a challenging unmet need. *NPJ Vaccines*. 2023;8(1):11.



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