



# Suspected Mycoplasma Contamination in the Study “Toll-Like Receptor 2 Recognizes *Orientia tsutsugamushi* and Increases Susceptibility to Murine Experimental Scrub Typhus”

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**KEYWORDS** *Orientia tsutsugamushi*, *Mycoplasma*, Toll-like receptor 2

In a recent publication (1), Gharaibeh et al. demonstrated that human Toll-like receptor 2 (TLR2) recognizes *Orientia tsutsugamushi* by using TLR2-transfected HEK293 cells. They demonstrated that murine TLR2 is required for inflammatory cytokine production by bone marrow-derived dendritic cells. Additionally, heat-killed *O. tsutsugamushi* induced higher levels of cytokine production than live organisms. Next, they performed *in vivo* experiments with wild-type and TLR2-deficient mice but the results were inconclusive. They revealed that TLR2 is dispensable for intradermal infection with *O. tsutsugamushi* but might be partially required for bacterial clearance after intraperitoneal infection. Despite its protective role in bacterial restriction, TLR2 seems to cause tissue injury, as well as increase disease severity and the mortality rate.

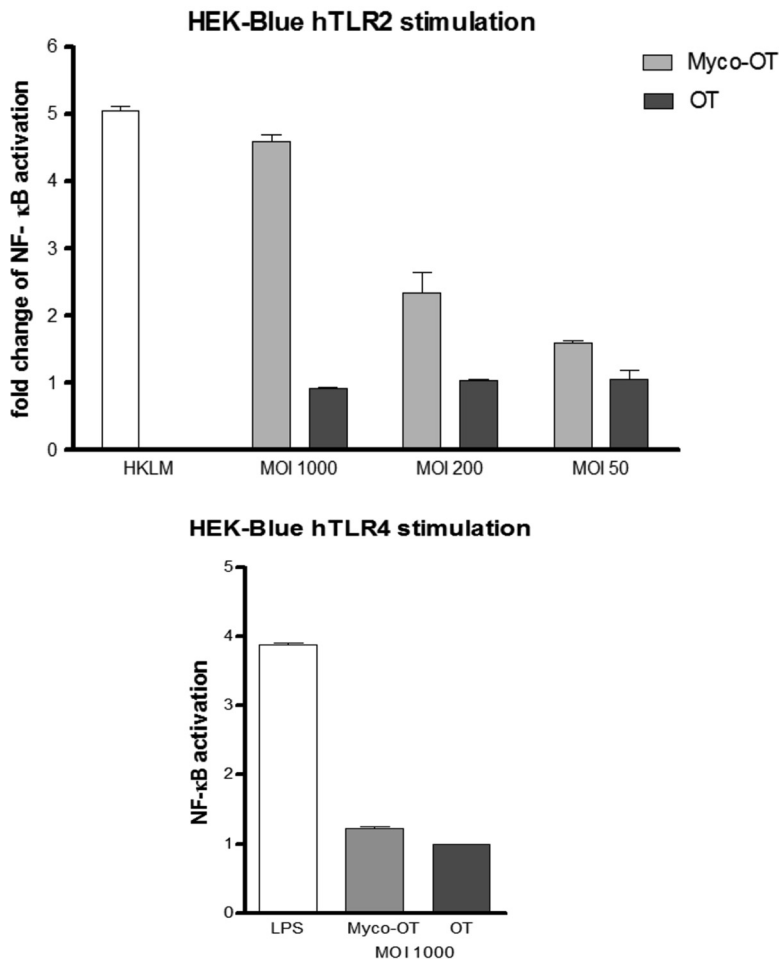
In contrast to that study, our unpublished data have demonstrated that neither TLR2 nor TLR4 recognizes *O. tsutsugamushi*. However, if we use *O. tsutsugamushi* with mycoplasma contamination to stimulate TLR2-transfected HEK293 cells, we obtain false-positive results (Fig. 1). Mycoplasma organisms are well known to possess several lipoproteins, including macrophage-activating lipopeptide 2 (2), which contains diacylated lipopeptides (Pam2Cys) as a TLR2 ligand (3). Heat killing of mycoplasma organisms can cause bacterial lysis and the subsequent release of more lipopeptides into the extracellular milieu. This hypothesis may explain why heat-killed organisms induce a stronger inflammatory response in mouse dendritic cells (1). In contrast, our unpublished data and previous studies have demonstrated that live *O. tsutsugamushi* without mycoplasma contamination induces higher cytokine response levels in human monocytes and macrophages (4, 5). Moreover, it is noteworthy that Gharaibeh et al. collected *O. tsutsugamushi* Karp from infected L929 cells at 14 days postinfection. However, on the basis of our experience, infected cells usually show cytopathic effects within 6 to 9 days, whereas mycoplasma-contaminated cells exhibit growth retardation of *O. tsutsugamushi* Karp and then show cytopathic effects at about 2 weeks postinfection. Therefore, our observations suggest that the results presented by Gharaibeh et al. (1) may be confounded by mycoplasma contamination, which can cause false-positive *in vitro* and *in vivo* results.

**Citation** Tantibhedhyangkul W, Inthasin N, Wongprompitak P, Ekpo P. 2017. Suspected mycoplasma contamination in the study “Toll-like receptor 2 recognizes *Orientia tsutsugamushi* and increases susceptibility to murine experimental scrub typhus.” *Infect Immun* 85:e00269-17. <https://doi.org/10.1128/IAI.00269-17>.

**Editor** Craig R. Roy, Yale University School of Medicine

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For the author reply, see <https://doi.org/10.1128/IAI.00326-17>.



**FIG 1** *O. tsutsugamushi* (OT) Karp organisms with or without *Mycoplasma orale* (Myco) contamination were used to infect HEK-Blue cells (InvivoGen, San Diego, CA), which stably coexpress a human TLR (hTLR) gene and an NF- $\kappa$ B-inducible secreted embryonic alkaline phosphatase reporter gene. Alkaline phosphatase levels in supernatants were measured by spectrophotometry, normalized to those of unstimulated cells, and correlated with fold changes in NF- $\kappa$ B activation. Data represent the mean  $\pm$  the standard error of the mean of triplicate values and are representative of three independent experiments. Heat-killed *Listeria monocytogenes* (HKLM) is a positive control for TLR2 activation. To calculate multiplicities of infection (MOIs), *O. tsutsugamushi* was quantified by real-time PCR. LPS, lipopolysaccharide.

## REFERENCES

- Gharaibeh M, Hagedorn M, Lilla S, Hauptmann M, Heine H, Fleischer B, Keller C. 2016. Toll-like receptor 2 recognizes *Orientia tsutsugamushi* and increases susceptibility to murine experimental scrub typhus. *Infect Immun* 84:3379–3387. <https://doi.org/10.1128/IAI.00185-16>.
- Takeuchi O, Kaufmann A, Grote K, Kawai T, Hoshino K, Morr M, Muhradat PF, Akira S. 2000. Cutting edge: preferentially the R-stereoisomer of the mycoplasmal lipopeptide macrophage-activating lipopeptide-2 activates immune cells through a Toll-like receptor 2- and MyD88-dependent signaling pathway. *J Immunol* 164:554–557. <https://doi.org/10.4049/jimmunol.164.2.554>.
- Okusawa T, Fujita M, Nakamura J, Into T, Yasuda M, Yoshimura A, Hara Y, Hasebe A, Golenbock DT, Morita M, Kuroki Y, Ogawa T, Shibata K. 2004. Relationship between structures and biological activities of mycoplasmal diacylated lipopeptides and their recognition by Toll-like receptors 2 and 6. *Infect Immun* 72:1657–1665. <https://doi.org/10.1128/IAI.72.3.1657-1665.2004>.
- Tantibhedhyangkul W, Prachason T, Waywa D, El Filali A, Ghigo E, Thongnoppakhun W, Raoult D, Suputtamongkol Y, Capo C, Limwongse C, Mege JL. 2011. *Orientia tsutsugamushi* stimulates an original gene expression program in monocytes: relationship with gene expression in patients with scrub typhus. *PLoS Negl Trop Dis* 5:e1028. <https://doi.org/10.1371/journal.pntd.0001028>.
- Tantibhedhyangkul W, Ben Amara A, Textoris J, Gorvel L, Ghigo E, Capo C, Mege JL. 2013. *Orientia tsutsugamushi*, the causative agent of scrub typhus, induces an inflammatory program in human macrophages. *Microb Pathog* 55:55–63. <https://doi.org/10.1016/j.micpath.2012.10.001>.