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Absence of TLR11 in mice does not confer susceptibility to *Salmonella* Typhi

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Salmonella enterica serovar Typhi (*S. Typhi*) causes typhoid fever, a systemic disease of humans that is estimated to cause more than 200,000 annual deaths (Butler, 2011; Crump and Mintz, 2010; Parry et al., 2002). Unlike other *Salmonella enterica* serovars, which can infect a broad range of animals, *S. Typhi* can only infect humans, which has hampered the development of a convenient animal model for the study of typhoid fever. Recently, it was reported in *Cell* (Mathur et al., 2012) that mice lacking Toll-like receptor 11 (TLR11) could be lethally infected with *S. Typhi* after oral or systemic inoculation. It was also postulated that TLR11-mediated recognition of *Salmonella* flagellin prevents *S. Typhi* infection in wild-type (C57BL/6) mice and that the lack of functional TLR11 renders humans susceptible to the bacterial infection (Mathur et al., 2012). It was therefore proposed that TLR11-deficient mice could serve as a convenient animal model for typhoid fever (Mathur

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et al., 2012). However, we report here that infection studies conducted in 4 different laboratories have found that TLR11-deficient mice do not show enhanced susceptibility to *S. Typhi* regardless the route of inoculation. We also observed no binding of flagellin to TLR11 and found no differences in the response of wild type and TLR11-deficient mice to the administration of bacterial flagellin, which are inconsistent with the proposed role of this Toll receptor in the detection of this bacterial protein (Mathur et al., 2012).

We infected TLR11^{-/-} and wild-type isogenic control mice either orally or intraperitoneally with different strains of *S. Typhi*. In addition to the strain used in the original study (Ty2) (Mathur et al., 2012), we used other virulent *S. Typhi* strains including ISP2825 (Galán and Curtiss III, 1991), and A8/14353363984-6 and 3290A481, two clinical isolates obtained from the Yale Clinical Microbiology laboratory. Mice were obtained from Dr. Sankar Ghosh and their genotype verified by standard genotyping techniques (see supplementary experimental procedures). None of the mice showed any signs of disease even when inoculated with doses up to 100-fold higher than those used by Mathur et al (Mathur et al., 2012) (Fig. 1 and Supplementary Materials). Consistent with this finding, no *S. Typhi* colony forming units (c. f. u.) were recovered from the spleens of animals (WT or TLR11^{-/-}) orally or intraperitoneally inoculated with *S. Typhi* (Fig. 1 A–G). Mice orally or intravenously inoculated with *S. Typhi* Ty2 showed no differences in CFU detected in fresh fecal pellets (Fig. 1H), spleens (Fig. 1I), or livers (Fig. 1J). Survival studies independently conducted at 4 different geographical sites demonstrated that mice defective in TLR11 receptor do not show enhanced susceptibility to *S. Typhi*. Taken together these results indicate that TLR11^{-/-} mice cannot serve as a model for the study of typhoid fever and *S. Typhi* pathogenesis as originally proposed (Mathur et al., 2012). Unlike the previous report (Mathur et al., 2012), we found no evidence of flagellin binding to TLR11. We injected WT and TLR11^{-/-} mice with recombinant *Salmonella* flagellin and measured serum levels of IL-12 and IL-6 two hours post-injection. We found no differences in the levels of these cytokines in WT and TLR11^{-/-} mice (Fig. S1). Similarly, we found no differences in the levels of IL-12, IL-6, and TNF produced by sort-purified WT and TLR11^{-/-} lamina propria mouse macrophages or splenic dendritic cells in response to flagellin (Fig. S1). These results are inconsistent with the proposed recognitions of flagellin by TLR11 (Mathur et al., 2012).

We cannot offer an explanation for the variance between our collective studies and those reported by Mathur et al. The differences in the results obtained are drastic in that TLR11^{-/-} mice not only did not succumb to *S. Typhi* after oral or systemic infection, but the animals cleared the infection. The strain of *S. Typhi* used in this study was the same as that used in the study of Mathur et al (Mathur et al., 2012) and same observations were made with several other *S. Typhi* clinical isolates. Furthermore the infection protocols were similar (see Supplement Experimental Procedures).

All laboratories used the TLR11^{-/-} mice that were originally generated at Yale University (Zhang et al., 2004). Differences in animal facilities are unlikely to account for the vastly different results obtained since animal husbandry is largely consistent across different animal facilities and in one case, experiments were conducted in the same animal facility (Yale University) as that used to conduct the previous study (Mathur et al., 2012).

We are also uncertain why we did not find evidence of TLR11-mediated flagellin-sensing, and note that the Ghosh lab had previously reported that flagellin was not able to stimulate TLR11 (Zhang et al., 2004), a variance that was not discussed by Mathur et al.

In conclusion, 5 independent laboratories at 4 different institutions were unable to reproduce the reported increased susceptibility of TLR11-deficient mice to *S. Typhi*, and therefore these animals cannot serve as a model to study typhoid fever. The conclusion that species-specific expression of TLR11 and its binding to flagellin determines *S. Typhi* susceptibility is also questioned by our studies. Urgent efforts to develop a convenient animal model for typhoid fever must therefore continue.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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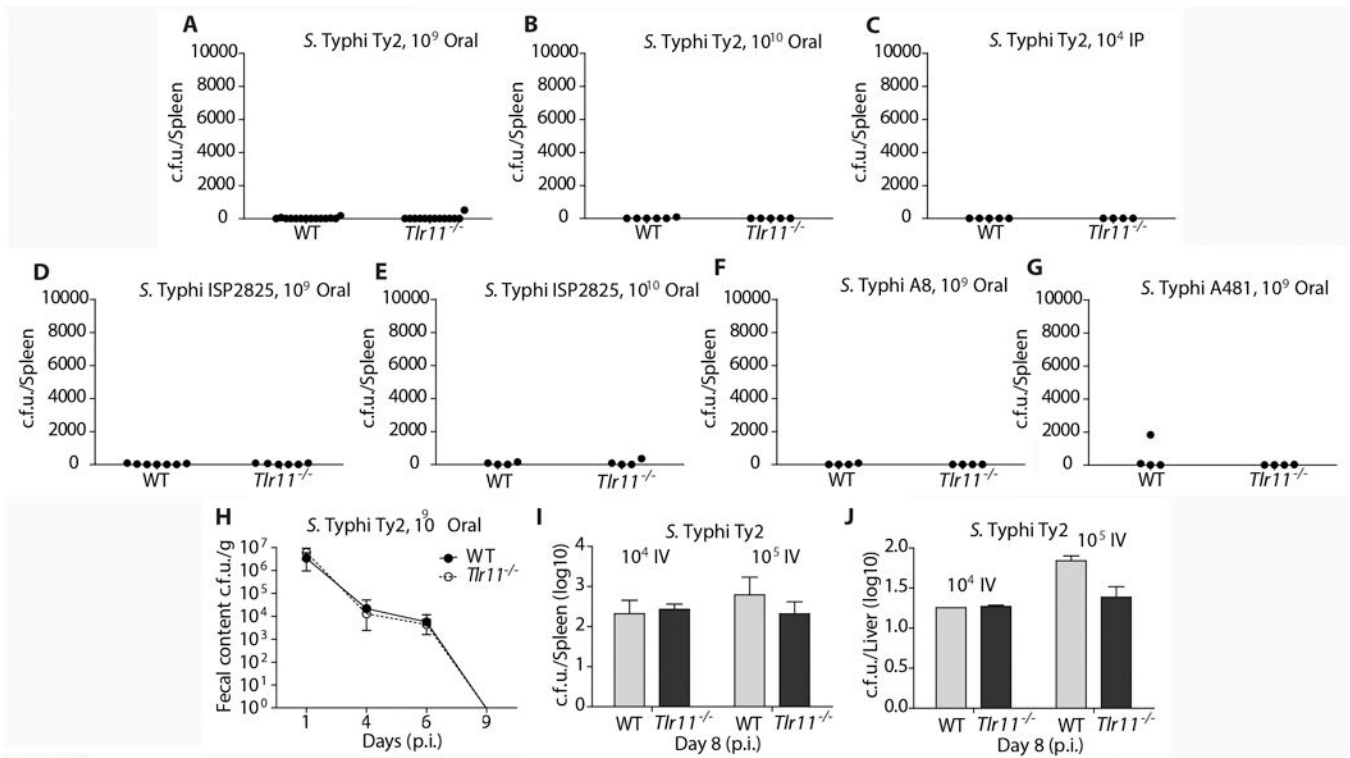


Figure 1. TLR11^{-/-} mice do not support *S. Typhi* replication

(A–G) Groups of sex- and age (6 to 10 weeks)-matched control and TLR11^{-/-} mice were infected with the indicated *S. Typhi* strains. Infection routes and doses are indicated in the figure panels. Shown are c. f. u. 24 days after infection obtained from spleens of animals infected with *S. Typhi* Ty2 (A–C), ISP2825 (D and E), A8/14353363984-6 (F) and 3290A481 (G). (H) Groups (n = 5) of male and female mice aged 9 to 14 weeks were inoculated via oral gavage with approximately 1×10¹⁰ CFU of *S. Typhi* Ty2 in a volume of 0.2 ml. Fresh fecal pellets were collected at indicated time points. (I and J) Mice infected intravenously infected with the indicated doses of *S. Typhi* Ty2 and the c. f. u. in spleens (I) and livers (J) enumerated 8 days after infection. See supplemental experimental procedures for information on each laboratory’s experimental contributions.