Innate immunity is sufficient for the clearance of *Chlamydia trachomatis* from the female mouse genital tract

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This short communication addresses a well-defined and focused question that is particularly relevant to the pursuit of a chlamydial vaccine. The finding that innate immunity is sufficient on its own to clear a chlamydial infection caused by a human isolate in one of the most widely used mouse models should cause a re-evaluation of previous results obtained using this model. In the authors’ own words, “studies designed to ascertain vaccine mediated protective immunity should be interpreted with cautionary implications.”

Keywords

*Chlamydiae*, human and mouse strains; Rag1−/− mice; female genital tract; innate immunity; adaptive immunity.

**Abstract**

*Chlamydia muridarum* and *Chlamydia trachomatis*, mouse and human strains, respectively, have been used to study immunity in a murine model of female genital tract infection. Despite evidence that unique genes of these otherwise genomically similar strains could play a role in innate immune evasion in their respective mouse and human hosts, there have been no animal model findings to directly support this conclusion. Here, we infected C57BL/6 and adaptive immune-deficient Rag1−/− female mice with these strains and evaluated their ability to spontaneously resolve genital infection. Predictably, C57BL/6 mice spontaneously cleared *C. muridarum* infection but spontaneously cleared *C. trachomatis* infection. In contrast, Rag1−/− mice which lack mature T and B cell immunity but maintain functional innate immune effectors were incapable of resolving *C. muridarum* infection but spontaneously cleared *C. trachomatis* infection. This distinct dichotomy in adaptive and innate immune-mediated clearance between mouse and human strains has important cautionary implications for the study of natural immunity and vaccine development in the mouse model.

**Chlamydia trachomatis** is an obligate intracellular bacterial pathogen that infects mucosal surfaces of the eye and urogenital tract. In the United States, infections of the urogenital tract represent the most common cause of bacterial sexually transmitted infection (STI) (CDC Grand Rounds, 2011) with an estimated 92 million STI occurring annually worldwide (WHO, 2001). Complications of chlamydial STI in women can be severe resulting in pelvic inflammatory disease, ectopic pregnancy and tubal factor infertility (Brunham & Rey-Ladino, 2005). Control of chlamydial STI in women can be severe resulting in pelvic inflammatory disease, ectopic pregnancy and tubal factor infertility (Brunham & Rey-Ladino, 2005). Control of chlamydial STI is currently focused on national screening programs and antibiotic therapy (Johnson et al., 2002); however, the effectiveness of this approach in interrupting chlamydial transmission has been questioned (Rekart & Brunham, 2008). Consequently, there has been a focus on vaccine development as the next step for controlling chlamydial STI (Brunham & Rappuoli, 2013).

Towards this end, investigators have employed a female mouse urogenital infection model where they have interchangeably used a naturally occurring *Chlamydia muridarum* strain (Barron et al., 1981; Swenson et al., 1983) or human *C. trachomatis* urogenital isolates (Tuffrey et al., 1986) to study infection mediated immunity and vaccinology. The general paradigm that has collectively emerged from this work is that immunity against both mouse and human strains is largely the result of the adaptive immune response; specifically, Th1 cells producing IFN-γ (Johanson et al., 1997; Morrison & Caldwell, 2002). However, there have been no reports that have directly examined the antichlamydial effects of the adaptive vs. the innate arms of the host’s immune response against mouse and human chlamydial organisms in a head on comparison. Defining the roles of adaptive and innate immunity in this model is important as it directly affects conclusions about the relative
roles of Th1-mediated immunity which have important consequences in the design and development of chlamydial vaccines.

*Chlamydia trachomatis* and *C. muridarum* are remarkably similar genetically sharing a high conservation in gene content and order (Read et al., 2003). Only a few open-reading frames differ between the species, and they are primarily located within the organism’s plasticity zone (PZ) (Read et al., 2003). It has been hypothesized that these pathogen-specific PZ genes play an important role in avoiding host-specific IFN-γ-induced immunity in mice and humans (Nelson et al., 2005). Thus, this host-pathogen interaction might influence both the susceptibility and infection-dependent immunity observed by these strains in their natural hosts.

Here, we addressed the relative roles of murine innate and adaptive immunity in the spontaneous clearance of female urogenital tract infections caused by *C. muridarum* and *C. trachomatis* in recombination activation gene 1-deficient (Rag1−/−) mice. Rag-deficient mice lack mature T and B cell adaptive immunity (Mombaerts et al., 1992), but retain normal innate immune functions including IFN-γ-secreting NK cells (Shinkai et al., 1992). An advantage of using Rag1−/− mice instead of nude or severe combined immunodeficiency (SCID) mice for these experiments is that Rag1−/− mice are not ‘leaky’ (Mombaerts et al., 1992) thereby providing an unambiguous interpretation for the roles of innate and adaptive immunity to chlamydial infection. We show that resolution of *C. muridarum* infection is dependent on adaptive immunity. Conversely, resolution of *C. trachomatis* infection is largely independent of adaptive immunity and is controlled by innate immunity.

Progesterone treated female eight-week-old C57BL/6 wild-type and C57BL/6-derived Rag1−/− mice (Jackson Laboratory) were each infected cervico-vaginally with 1 × 10⁵ inclusion-forming units (IFU) of either *C. muridarum* (Weiss strain) or *C. trachomatis* serovar D, strain D-LC (Sturdevant et al., 2010). Five to ten mice were infected with each chlamydial strain. All animal procedures used throughout this study were conducted in accordance with Animal Care and Use Guidelines and were reviewed and approved by the Animal Care and Use Committee at RML. Chlamydial burdens and infection duration were monitored at weekly intervals by swabbing the vaginal vault and culturing recoverable organisms on monolayers of McCoy cells. Two-way ANOVA statistical analyses were calculated comparing strain infection course curves. The results are shown in Fig. 1. *Chlamydia muridarum* genital tract infection of C57BL/6 female mice produced a self-limiting infection that cleared spontaneously by day 34 postinfection (PI) (Fig. 1a). Infectious burdens were high (10⁶ IFU) during the early time periods PI (days 3–7) and then rapidly decreased until infections resolved. In contrast, *C. muridarum*-infected Rag1−/− mice yielded similarly high numbers of recoverable IFUs that were sustained over the first 28 days PI (P ≤ 0.02 at days 7, 21; Fig. 1b). *Chlamydia muridarum*-infected Rag1−/− mice developed a rampant lethal systemic infection by 28 days postvaginal infection as determined by the isolation of *C. muridarum* from the spleen, lung, and liver of infected animals; findings similar to those reported by Cotter et al. (1997) using IFN-γ knockout (KO) mice. These results demonstrate that spontaneous clearance of *C. muridarum* infection from the female genital tract and prevention of disseminating genital tract infection are dependent on an adaptive immune response.

In contrast, C57BL/6 and Rag1−/− female mice infected with *C. trachomatis* spontaneously cleared infection (Fig. 1). Rag1−/− mice (Fig. 1d) produced higher infectious burdens (P ≤ 0.05 at days 7, 10, 21) than C57BL/6 animals (Fig. 1c) over the entire culture positive period with a similar time required to completely resolve infection. These results show that the mouse innate immune...
response alone is capable of eradicating C. trachomatis genital tract infection. The reduction in infectious burdens between C57BL/6 and Rag1−/− over the entire infection period implicates a dual role for adaptive and innate immunity in spontaneous clearance; nevertheless, it is patently clear that the primary immune component that controls C. trachomatis infection in the mouse genital tract is innate, not adaptive, immunity. These results are the first to definitively show in a side-by-side study that adaptive and innate immunity play distinct roles in control of C. muridarum and C. trachomatis infection of the female mouse genital tract.

Tuffrey et al. (1982) and Rank et al. (1985) previously reported on C. trachomatis and C. muridarum infection of the female genital tract in nude mice, respectively. Their findings were similar to those described herein; C. trachomatis infection of the female mouse genital tract resolved spontaneously in the absence of T cells (Tuffrey et al., 1982), whereas T cells were required for the resolution of C. muridarum infection (Rank et al., 1985). An important difference between those studies and ours is that these investigators used nude mice which have a greatly reduced, but not complete deficiency, in T cell immunity (Belizario, 2009). Consequently, a definitive role, or lack of a role, for T cells in immunity cannot be concluded from their work. In contrast, Rag1−/− mice are entirely deficient for both T and B cell immunity. Therefore, our findings provide a conclusive answer with respect to the roles of innate and adaptive immunity against the human and mouse strains. We do not know what innate immune mechanism(s) are responsible for the resolution of C. trachomatis infection. We speculate, because of the strong inhibitory function of IFN-γ in both in vivo (Johansson et al., 1997; Perry et al., 1999) and in vitro (Nelson et al., 2005; Roshick et al., 2006) murine infection models, that IFN-γ-secreting local NK cells could be important. However, additional studies using NK KO and double NK-IFN-γ KO mice will be required to answer this question. Lastly, our findings and those of Williams et al. (1988) and Rank et al. (1992) are in complete agreement. They showed that C. muridarum infection of both the lung and genital tract can be protracted by IFN-γ treatment. In contrast, we (Perry et al., 1999) and others ( Cotter et al., 1997) have shown that IFN-γ is not essential for the clearance of C. muridarum from the genital tract but does prevent disseminating infection and death. A possible explanation for these discrepancies is that different C. muridarum strains were used in these reports. The Perry, Cotter and Williams studies used the C. muridarum Weiss strain, whereas Rank used the Nigg strain. Interestingly, Ramsey et al. (2009) recently showed that the Weiss strain is more virulent than the Nigg strain in the mouse model. We believe that the use of strains differing in virulence could at least in part explain these conflicting findings. Nevertheless, collectively, the results warrant further studies in this model using additional strains and isolates.

In summary, because the mouse innate immune system is capable of independently controlling urogenital infections of the female genital tract caused by human C. trachomatis strains, studies designed to ascertain natural adaptive immune control or vaccine-mediated protective immunity using human isolates should be interpreted with cautionary implications.

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References

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