SHORT COMMUNICATION

OT-1 mice display minimal upper genital tract pathology following primary intravaginal Chlamydia muridarum infection

Srikanth Manam1, Bruce J. Nicholson2 & Ashlesh K. Murthy1

1 Department of Pathology, Midwestern University, Downers Grove, IL, USA
2 Department of Biochemistry, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

In this straightforward study, Murthy et al. show that mice whose CD8 T cells that are ‘locked-in’ for the recognition of one antigen (OVA), do not show any deficiencies in the ability to clear chlamydia from infected oviducts. However, unlike wild type mice, these OVA-specific mice do not develop pathology, suggesting that Chlamydia-specific CD8 T cells contribute significantly to immune pathology despite a lack of a role for in vivo clearance of the pathogen.

Keywords
Chlamydia trachomatis; Chlamydia muridarum; antigen-specific CD8 T cells; upper genital tract; pathogenesis.

Correspondence
Ashlesh K. Murthy, Department of Pathology, Midwestern University, 555, 31st St, Downers Grove, IL 60515, USA.
Tel.: + 1 630 515 7458
fax: +1 630 515 6065
e-mail: amurth@midwestern.edu

Received 24 January 2013; revised 5 February 2013; accepted 5 February 2013
Final version published online 11 March 2013.
do:10.1111/2049-632X.12032

Editor: Raphael Valdivia

Abstract
Chlamydia trachomatis is the most common bacterial sexually transmitted disease worldwide and leads to serious pathological sequelae in the upper genital tract (UGT) including pelvic inflammatory disease, ectopic pregnancy, and infertility. Several components of the host immune responses have been shown to contribute to the UGT pathology following genital chlamydial infection. We have shown recently that CD8+ T cells induce the chlamydial UGT pathology via the production of TNF-α. However, those studies did not determine whether the pathology is mediated by bystander or antigen-specific CD8+ T cells. In this study, we compared chlamydial clearance and UGT pathology in OT-1 transgenic mice and the corresponding C57BL/6J wild-type mice following primary intravaginal Chlamydia muridarum infection. All CD8+ T cells in the OT-1 mice respond only to the Ova 257-264 peptide and are incapable of responding to other antigenic epitopes including those of Chlamydia. OT-1 mice displayed vaginal chlamydial clearance comparable to the wild-type animals. However, both oviduct and uterine horn pathology were minimal in the OT-1 mice compared with the high degree of pathology observed in the wild-type animals. These results strongly suggest that Chlamydia-specific, not bystander, CD8+ T cells mediate the UGT pathological sequelae following genital chlamydial infection.

Sexually transmitted infections caused by Chlamydia trachomatis affect approximately 90 million people annually worldwide (Brunham & Rey-Ladino, 2005). A significant proportion of infected females develop severe pathological sequelae in the upper genital tract (UGT) including pelvic inflammatory disease, ectopic pregnancy, and infertility (Brunham & Rey-Ladino, 2005). A mouse model using a closely related pathogen Chlamydia muridarum has been used extensively to study genital chlamydial infections, because the pathological phenotype in this model is closely comparable to those found in human C. trachomatis infections (Morrison & Caldwell, 2002). Using the mouse model, several components of the host immune response including Toll-like receptor 2 signaling (Darville et al., 2003), IL-1β (Prantner et al., 2009), and neutrophils and matrix metalloproteases (Imtiaz et al., 2007; Lee et al., 2010), have been shown to contribute to the immunopathogenesis of the disease sequelae following genital chlamydial infection. In this model, we also have shown recently that CD8+ T cells, via TNF-α production, mediate the UGT pathology following genital chlamydial infections (Murthy et al., 2011). Specifically, mice deficient in CD8 (CD8 KO mice) display similar kinetics of chlamydial clearance, but significantly reduced pathology following primary C. muridarum infection, when compared with corresponding wild-type animals. Moreover, repletion of CD8 KO mice with TNF+/+ CD8+ T cells, but not with TNF−/− CD8+ T cells, at the time of bacterial challenge, restores pathology to the levels found in wild-type animals. In these experiments, a likely but not confirmed possibility was that a very small frequency of Chlamydia-specific CD8+ T cells among the adoptively transferred naive cells proliferated clonally in the CD8 KO mouse response to the
infection and induced the pathology. However, it also is possible that the large numbers of bystander, non-
_Chlamydia_-specific, CD8⁺ T cells mediated the pathology. Bystander CD8⁺ T cells have been shown to get activated and possibly play a role in other diseases with infectious and noninfectious etiologies (Chen et al., 2005; Sobottka et al., 2009).

We sought to evaluate whether the _Chlamydia_-induced UGT pathology is mediated by antigen-specific or bystander CD8⁺ T cells. The adoptive transfer of CD8⁺ T cells derived from _Chlamydia_-infected wild-type mice into recipient CD8 KO mice may seem as a straightforward method to evaluate this possibility. However, the frequency of antigen-specific CD8⁺ T cells in _Chlamydia_-infected mouse spleens would likely be very low, and a large fraction of transferred cells would be non-_-Chlamydia_-specific. Therefore, the development of pathology in recipient CD8 KO mice could still be due to the effects of non-_Chlamydia_-specific CD8⁺ T cells. To overcome this hurdle, we made an unconventional use of the OT-1 transgenic mice (catalog number 003831, Jackson Laboratories, Bar Harbor, ME). OT-1 mice have a full CD8⁺ T-cell compartment; however, all CD8 T cells respond only to the Ova 257-264 peptide, and therefore, these mice are used typically to study antigen-specific responses against the Ova 257-264 peptide. However, as the CD8⁺ T cells in these mice cannot respond to chlamydial antigens in an antigen-specific fashion, we reasoned that a reduction in UGT pathology following chlamydial genital infection in these mice would suggest a role for _Chlamydia_-specific, not bystander, CD8⁺ T cells in chlamydial pathogenesis. On the contrary, if pathology in OT-1 mice was comparable to that in the wild types, it could be interpreted as an effect of the bystander CD8⁺ T cells.

We compared the course of chlamydial clearance from the lower genital tract and the development of UGT pathology in OT-1 mice and the corresponding C57BL/6J wild-type mice (Jackson Labs). The mice were housed in the animal facility at Midwestern University (MWU). Food and water were provided ad libitum, and all procedures were performed in accordance with an approved protocol (number 2088) from the Instrumental Animal Care and Use Committee at MWU. _Chlamydia muridarum_ Nigg was grown on confluent HeLa cell monolayers as described previously (Murthy et al., 2011). The infected cells were lysed by sonication and elementary bodies (EBs) purified on Renografin gradients. Aliquots of bacteria were stored at −70 °C in sucrose-phosphate-glutamine (SPG) buffer. Groups (n = 5 in experiment 1 and n = 6 in experiment 2) of 4- to 6-week-old female OT-1 and C57BL/6J mice were infected intravaginally with 5 × 10⁶ inclusion forming units (IFU) of _C. muridarum_ on day 0. Mice were given subcutaneous Depo-provera® (2.5 mg per mouse, Upjohn, Kalamazoo, MI) 10 and 3 days before infection to render them anestrous and more receptive to the infection (Murthy et al., 2011). Vaginal swab material was collected on the indicated days after challenge and chlamydial enumeration conducted by plating swab material on HeLa cell monolayers followed by immunofluorescent staining (Murthy et al., 2011). As shown in Fig. 1a, C57BL/6J mice displayed a high level of chlamydial shedding on day 3 followed by progressive clearance of infection and complete clearance by day 32 following the primary inoculation. The group of OT-1 mice displayed kinetics of chlamydial shedding and clearance similar to the wild-type animals. These results suggest that neither _Chlamydia_-specific nor bystander CD8⁺ T cells are required for clearance of the bacterium from the lower genital tract.

The mice were rested until day 80, euthanized, and genital tracts collected to evaluate the UGT pathology. The collected tissues were immersed in 2% paraformaldehyde for 24 h and then soaked in ethanol for 48 h. The processed tissues were photographed using a 10 megapixel Panasonic Lumix TZ3 digital camera. A representative set of three genital tracts each from C57BL/6J mice (left column) and OT-1 mice (right column) is shown in Fig. 1b. Individual mouse genital tracts were placed next to a standard metric ruler and photographed, the pictures printed out on 8 × 11.25 inch sheets of paper, the greatest cross-sectional diameter measured for each oviduct, and reported individually and as mean ± SEM in a group in Fig. 1c. As described by us previously (Murthy et al., 2011), we have found that a threshold of 0.5 mm can be used to distinguish between normal and dilated oviducts. Additionally, the greatest cross-sectional diameter for every 5-mm longitudinal section of the uterine horn was measured, and the average diameter per uterine horn was calculated. The individual averages per uterine horn and the mean ± SEM of the average uterine horn diameter in a group are reported in Fig. 1d. We also have found that a threshold of 1 mm can be used to distinguish between normal and dilated uterine horns. The enumeration of chlamydial counts, oviduct, and uterine horn measurements were conducted in a blinded fashion. In Fig. 1c and d, the ratio of oviducts or uterine horns that were normal to the total number evaluated is shown on the y-axis. The horizontal line at 0.5 mm in Fig. 1c and at 1 mm in Fig. 1d distinguishes the normal from dilated tissues. As shown in Fig. 1c, all oviducts in C57BL/6J mice displayed oviduct dilatation reflecting a significantly (P < 0.01, Fisher’s exact test) higher incidence of oviduct dilatation compared with one dilated oviduct in OT-1 mice. The degree of oviduct dilatation in C57BL/6 mice also was obviously and significantly (P < 0.01, two-tailed Student’s t-test) higher than that in the OT-1 mice. Similarly, the incidence (P < 0.01, Fisher’s exact test) and degree of uterine horn dilatation in C57BL/6J mice were significantly (P < 0.01, Fisher’s exact test) higher than that in OT-1 mice. These results suggest that non-_Chlamydia_-specific CD8⁺ T cells in the OT-1 mice were incapable of mediating UGT pathology following genital _C. muridarum_ infection.

Collectively, these results suggest that neither _Chlamydia_-specific nor bystander CD8⁺ T cells are required for clearance of primary genital chlamydial infection, whereas _Chlamydia_-specific, but not bystander, CD8⁺ T cells mediate UGT pathology. The inability of OT-1 CD8⁺ T cells to induce pathology following chlamydial infections may theoretically be due to these cells not trafficking to the infected genital tracts and/or due to the lack of clonal selection and
expansion following exposure to the infection in the genital tract. Although not evaluated specifically in this study or in the Chlamydia infection model in general, there is ample evidence in literature from other infection models to suggest the likelihood of the latter possibility. First, intravenously OT-1 CD8+ T cells have been shown to accelerate the clearance of Ova-expressing Herpes Simplex Virus-2 infection in the mouse vaginal epithelium, suggesting that these cells retain the ability to traffic to the mouse genital tract (Dobbs et al., 2005). Second, it has been shown that both antigen-specific and bystander CD8+ T cells are recruited in comparable numbers into the sites of infection within the first week of primary intracerebral infection with neurotropic corona virus in a mouse model of virus-induced encephalitis (Chen et al., 2005). Furthermore, that study demonstrated that the frequencies of bystander CD8+ T cells reduce beyond the first week, whereas the virus-specific CD8+ T cells are maintained at high frequencies in the infected tissues due to clonal selection and expansion (Chen et al., 2005). Similarly, it is likely that OT-1 CD8+ T cells traffic into the genital tract of C. muridarum-infected mice, but do not undergo clonal selection or expansion in response to the chlamydial (non-Ova 257-264) antigens presented during the infection, and therefore do not contribute to oviduct pathology.

The findings of this study have important implications. (1) Previous reports suggest that certain CD8+ T cell clones that recognize specific chlamydial epitopes may assist in bacterial clearance (Wizel et al., 2008) and thus may be suitable as vaccine candidates. Our findings do not exclude this possibility; however, they suggest the need to exercise due diligence in applying such immune responses for the
induction of protective immunity, without inducing clinically relevant pathology. (2) These findings suggest that Chlamydia-specific CD8+ T cells do not assist significantly in bacterial clearance and therefore may not recognize infected cells effectively. However, they mediate pathology suggesting the possibility that neighboring uninfected cells are targeted by the Chlamydia-specific CD8+ T cells. In essence, that begs the question as to how chlamydial antigens are acquired by uninfected cells. One straightforward possibility is that uninfected cells acquire chlamydial antigens from the extracellular matrix following lysis of Chlamydia-infected cells. Alternatively, there is ample evidence that gap junction-mediated antigen transport between neighboring cells via connexon channels may contribute to this phenomenon (Handel et al., 2007). Thus, our findings are relevant and provide new perspectives to further evaluate the mechanism of pathogenesis following genital chlamydial infections and apply them to antichlamydial vaccine development.

Acknowledgements

This work was supported by Midwestern University Faculty Start-up Fund and NIH Grant 1R03AI088342 to AKM. We thank Mr. Joshua Thomas for technical assistance. The authors declare no conflict of interest.

References

Brunham RC & Rey-Ladino J (2005) Immunology of Chlamydia infection: implications for a Chlamydia trachomatis vaccine. Nat Rev Immunol 5: 149–161.

Chen AM, Khanna N, Stohman SA & Bergmann CC (2005) Virus-specific and bystander CD8 T cells recruited during virus-induced encephalomyelitis. J Virol 79: 4700–4708.

Darville T, O’Neill JM, Andrews CW Jr, Nagarajan UM, Stahl L & Ojcius DM (2003) Toll-like receptor-2, but not Toll-like receptor-4, is essential for development of oviduct pathology in chlamydial genital tract infection. J Immunol 171: 6187–6197.

Dobbs ME, Strasser JE, Chu CF, Chalk C & Milligan GN (2005) Clearance of herpes simplex virus type 2 by CD8+ T cells requires gamma interferon and either perforin- or Fas-mediated cytolytic mechanisms. J Virol 79: 14546–14554.

Handel A, Yates A, Pilyugin SS & Antia R (2007) Gap junction-mediated antigen transport in immune responses. Trends Immunol 28: 463–466.

Imtiaz MT, Distelhorst JT, Schripsema JH, Sigs IM, Kasimos JN, Lacy SR & Ramsey KH (2007) A role for matrix metalloproteinase-9 in pathogenesis of urogenital Chlamydia muridarum infection in mice. Microbes Infect 9: 1561–1566.

Lee HY, Schripsema JH, Sigs IM, Murray CM, Lacy SR & Ramsey KH (2010) A link between neutrophils and chronic disease manifestations of Chlamydia muridarum urogenital infection of mice. FEMS Immunol Med Microbiol 59: 108–116.

Morrison RP & Caldwell HD (2002) Immunity to murine chlamydial genital infection. Infect Immun 70: 2741–2751.

Murthy AK, Li W, Chaganty BK, Kamalakaran S, Guentzel MN, Seshu J, Forsthuber TG, Zhong G & Arulananandam BP (2011) Tumor necrosis factor alpha production from CD8+ T cells mediates oviduct pathological sequelae following primary genital Chlamydia muridarum infection. Infect Immun 79: 2928–2935.

Prantner D, Darville T, Sikes JD, Andrews CW Jr, Brade H, Rank RG & Nagarajan UM (2009) Critical role for interleukin-1beta (IL-1beta) during Chlamydia muridarum genital infection and bacterial replication-independent secretion of IL-1beta in mouse macrophages. Infect Immun 77: 5334–5346.

Sobottka B, Harrer MD, Ziegler U, Fischer K, Wiendl H, Hunig T, Becher B & Goebels N (2009) Collateral bystander damage by myelin-directed CD8+ T cells causes axonal loss. Am J Pathol 175: 1160–1166.

Wizel B, Nyström-Asklin J, Cortes C & Tvinnerreim A (2008) Role of CD8+ T cells in the host response to Chlamydia. Microbes Infect 10: 1420–1430.