Tumor Necrosis Factor (TNF) Receptor Superfamily Member 1b on CD8⁺ T Cells and TNF Receptor Superfamily Member 1a on Non-CD8⁺ T Cells Contribute Significantly to Upper Genital Tract Pathology Following Chlamydial Infection

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Background. We demonstrated previously that tumor necrosis factor α (TNF- α)-producing *Chlamydia*-specific CD8⁺ T cells cause oviduct pathological sequelae.

Methods. In the current study, we used wild-type C57BL/6J (WT) mice with a deficiency in genes encoding TNF receptor superfamily member 1a (TNFR1; TNFR1 knockout [KO] mice), TNF receptor superfamily member 1b (TNFR2; TNFR2 KO mice), and both TNFR1 and TNFR2 (TNFR1/2 double KO [DKO] mice) and mix-match adoptive transfers of CD8⁺ T cells to study chlamydial pathogenesis.

Results. TNFR1 KO, TNFR2 KO, and TNFR1/2 DKO mice displayed comparable clearance of primary or secondary genital *Chlamydia muridarum* infection but significantly reduced oviduct pathology, compared with WT animals. The *Chlamydia*-specific total cellular cytokine response in splenic and draining lymph nodes and the antibody response in serum were comparable between the WT and KO animals. However, CD8⁺ T cells from TNFR2 KO mice displayed significantly reduced activation (CD11a expression and cytokine production), compared with TNFR1 KO or WT animals. Repletion of TNFR2 KO mice with WT CD8⁺ T cells but not with TNFR2 KO CD8⁺ T cells and repletion of TNFR1 KO mice with either WT or TNFR1 KO CD8⁺ T cells restored oviduct pathology to WT levels in both KO groups.

Conclusions. Collectively, these results demonstrate that TNFR2-bearing CD8⁺ T cells and TNFR1-bearing non-CD8⁺ T cells contribute significantly to oviduct pathology following genital chlamydial infection.

Keywords. *Chlamydia*; genital infection; oviduct pathology; TNF receptor 1; TNF receptor 2; CD8⁺ T cells.

Chlamydia trachomatis is the leading cause of sexually transmitted bacterial disease worldwide [1, 2]. Left

untreated, chlamydial infections in women ascend to the upper reproductive tract and induce severe immunopathology in the uterus and fallopian tubes, including pelvic inflammatory disease, and complications such as ectopic pregnancy and infertility [3, 4]. Because of host tropism dictated by interferon γ (IFN- γ) evasion mechanisms, *C. trachomatis* does not productively infect and cause such pathologies in mice [5]. *Chlamydia muridarum* is a mouse pathogen that causes genital infection and reproductive pathology in mice, similar to the effects of *C. trachomatis* in humans [6, 7]. We demonstrated recently that tumor necrosis factor α (TNF- α)-producing CD8⁺ T cells cause chlamydial

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immunopathology [8], likely in an antigen-specific fashion [9]. However, the mechanisms downstream of TNF- α in chlamydial pathogenesis remained to be elaborated.

TNF- α has been shown to act primarily via 2 cognate receptors, TNF receptor superfamily member 1a (*Tnfrsf1a*, which encodes TNFR1) and TNF receptor superfamily member 1b (*Tnfrsf1b*, which encodes TNFR2), in the induction of pathogenesis. TNFR1 is present on most cells of the body and is a weak inducer of death signaling [10]. TNFR2 is present on cells of the immune system and typically induces prosurvival signals but may enhance or suppress TNFR1-mediated effects [10]. TNFR2 signaling may respond to membrane-bound TNF- α signaling and promote both the activation of CD8⁺ T cells and later contraction of the activated CD8⁺ T-cell population [11]. The complex effects of TNFR1 and TNFR2 have been studied in various disease systems, including kidney disease, hepatic injury, and, of particular relevance to this study, the pathogenesis of viral infections [10, 11]. The effects of these receptors may complement or oppose each other, based on the specific pathogen [12], and therefore the effects on chlamydial pathogenesis cannot be directly extrapolated from past studies and need to be determined separately. Zhong et al recently reported that TNFR1 contributes significantly to chlamydial oviduct pathology [13], whereas the relationship of TNFR1 to CD8⁺ T cells and the role of TNFR2 in general had not been studied in context of chlamydial infections.

In the current study, we evaluated *C. muridarum*-mediated upper genital tract pathologies in wild-type C57BL/6J (WT) mice and mice with a deficiency in genes encoding TNFR1 (TNFR1 knockout [KO] mice), TNFR2 (TNFR2 KO mice), and both TNFR1 and TNFR2 (TNFR1/2 double KO [DKO] mice), all on a C57BL/6J background, and mix-match adoptive transfers of CD8⁺ T cells to study chlamydial pathogenesis.

MATERIALS AND METHODS

C. muridarum and Mice

Chlamydia muridarum strain Nigg (hereafter, “*C. muridarum*”) was grown in HeLa 229 cells, and elementary bodies were obtained using a Renograffin gradient separation method as described previously [14]. UV irradiation-inactivated organisms were generated by subjecting purified elementary bodies to 30 minutes of UV irradiation, using a UV crosslinker. Female 4–6-week-old C57BL/6J WT mice and breeding pairs of mice with a deficiency in *Tnfrsf1a* (TNFR1 KO mice), *Tnfrsf1b* (TNFR2 KO mice), or both *Tnfrsf1a* and *Tnfrsf1b* (TNFR1/2 DKO mice) [15, 16] were purchased from the Jackson Laboratory and maintained at Midwestern University. Food and water were supplied ad libitum, and all experimental procedures described in this article were approved by the Institutional Animal Care and Use Committee at Midwestern University.

Enrichment of CD8⁺ T Cells From Splenocytes and Adoptive Transfer

Naive donor mice were euthanized, spleens were collected, and single-cell suspensions of splenocytes were prepared. CD8⁺ T cells were enriched and purified by negative selection, using magnetic beads (Easysep; Stemcell Technologies, California), according to the manufacturer's instructions. The enriched (>95%) cells (10⁷ cells/mouse) were injected intravenously into recipient mice in 100 μ L of sterile 1X phosphate-buffered saline 2 hours after intravaginal infection with *C. muridarum*. Three additional mice in each group received CFSE-labeled CD8⁺ T cells and were euthanized at day 7 after chlamydial inoculation for flow cytometry-based confirmation that transferred CD8⁺ T cells were present in the infected genital tracts.

Intravaginal Infection of Mice and Monitoring of Bacterial Shedding

Mice received 2.5 mg of medroxyprogesterone acetate subcutaneously 10 and 3 days before vaginal challenge to render them anestrus and receptive to genital infection. The mice were then challenged with 5×10^4 inclusion-forming units (IFU) of *C. muridarum* contained in 10 μ L of sucrose-phosphate-glutamate buffer placed into the cervicovaginal vault. The course of infection was followed by swabbing the cervicovaginal vault at the specified intervals following inoculation. Chlamydial counts in swabs were determined by infection of HeLa229 cells, followed by immunofluorescence staining and enumeration, as described previously [17].

Evaluation of Cellular Activation, Cytokine Production, and Antibody Responses

Four separate investigations were performed to evaluate cellular activation, cytokine production, and antibody responses. Mice (3 per group) were euthanized 9 days after challenge with *C. muridarum*, genital tracts were collected and homogenized individually in phosphate-buffered saline containing protease inhibitor, supernatants were collected. Second, mice (3 per group) were euthanized 14 days after primary infection with *C. muridarum*, medial iliac lymph nodes (ie, those draining the mouse female genital tract) and spleens were collected, and single-cell suspensions were made. Splenocytes or lymph node cells (10⁶ cells/well) were stimulated with 10⁴ UV-irradiated elementary bodies, the unrelated protein bovine serum albumin, or medium alone and incubated for 72 hours, after which supernatants were collected. Supernatants were assayed for IFN- γ and TNF- α , using enzyme-linked immunosorbent assay (ELISA) kits (Ebioscience, San Diego, California), according to the manufacturer's instructions.

Third, splenic single-cell suspensions created from samples collected on day 15 after infection were evaluated for the percentage of CD8⁺ T cells expressing OX40, and the mean fluorescence intensity of CD11a expression on these cells was evaluated by flow cytometry. Furthermore, enriched (>95%) CD8⁺ T cells

(2×10^5 cells/well) were cultured with an equal number of mouse antigen-presenting cells (prepared by treatment of WT mouse splenocytes with 20 $\mu\text{g/mL}$ mitomycin C) in the presence of chlamydial or control antigens. After incubation for 72 hours, supernatants were collected to analyze cytokine production.

Fourth, ELISA analysis of anti-chlamydia total immunoglobulin (H+L), immunoglobulin G2c (IgG2c), and IgG1 antibodies was performed on sera collected 14 and 60 days after chlamydial inoculation, as described previously [18]. The reciprocal serum dilution corresponding to the 50% maximal binding titer was used for comparisons.

Estimation of Oviduct and Uterine Horn Pathology

Upper genital tract pathology was evaluated on day 80 after challenge, as described previously [19]. Mice (8 per group) were euthanized, and genital tracts removed and examined for the presence of gross hydrosalpinx. The tissues were then aligned next to a standard metric ruler and photographed from a fixed distance, using a 20 megapixel Panasonic ZS20 camera. Images were stored at high resolution and printed on sheets of paper (size A4). Dilated oviducts measuring >0.5 mm in diameter were used as an indicator of hydrosalpinx. When multiple oviduct loops were present, the one with the greatest diameter was reported. For uterine horns, the greatest cross-sectional diameter of each 5-mm longitudinal section of individual uterine horns was measured, and the average per uterine horn was reported. The baseline normal mouse oviduct diameter was determined to be 0.5 mm and the normal uterine horn diameter to be 1 mm by prior analysis of a group of age-matched naive mice.

Statistical Analyses

Sigma Stat (Systat Software, San Jose, California) was used to perform all tests of significance. Unless otherwise stated, analysis of variance (Systat Software) was used for all comparisons between multiple groups. The differences in incidence of oviduct pathology were measured using the Fisher exact test. Differences between groups were considered statistically significant if P values were $\leq .05$. All experiments were repeated at least twice, and each experiment was analyzed independently.

RESULTS

Vaginal Bacterial Clearance Following Genital *C. muridarum* Infection

Groups ($n = 8$) of WT, TNFR1 KO, TNFR2 KO, and TNFR1/2 DKO mice were infected with 5×10^4 IFU of *C. muridarum*, and chlamydial numbers in vaginal swabs were monitored at the indicated periods for 32 days. WT mice shed high numbers of bacteria at early periods and displayed a progressive reduction in vaginal chlamydial shedding, with complete clearance by day 32 after primary infection (Figure 1). The kinetics of resolution

Figure 1. Chlamydial shedding after genital *Chlamydia muridarum* challenge. Groups ($n = 8$) of mice (C57BL/6J, TNFR1 KO, TNFR2 KO, and TNFR1/2 DKO; see text for descriptions of each group) were pretreated (10 and 3 days prior to infection) with medroxyprogesterone acetate and challenged (on day 0) with 5×10^4 inclusion-forming units (IFU) of *C. muridarum*. Chlamydial shedding was enumerated at the indicated time points for 1 month. The mean vaginal shedding of chlamydia ($\pm$ standard error of the mean) per group at each time point is shown.

of infection in TNFR1 KO, TNFR2 KO, and TNFR1/2 DKO mice was comparable to that in WT mice. These results suggest that TNFR1 and TNFR2 individually or in combination are not required and do not contribute significantly to the resolution of vaginal chlamydial infection following primary intravaginal chlamydial infection.

Immune Responses Following Genital *C. muridarum* Infection

T-helper type 1 (Th1) cytokine responses and antibody are generally thought to contribute to protective immunity against genital chlamydial infections. Serum levels of antichlamydia total antibody, IgG2c (Th1 immunity), and IgG1 (Th2 immunity) were measured 14 and 60 days after primary infection. As shown in Figure 2A, mice deficient in TNFR1 and/or TNFR2 displayed a serum antibody response comparable to that of the WT mice, suggesting that antibody responses following primary genital chlamydial infection are not significantly affected by the absence of either TNF receptor.

We also measured the production of IFN- γ and interleukin 5 (IL-5) as representative cytokines of the Th1 and Th2 immune responses, respectively. Th1 immunity is known to promote the clearance of genital chlamydial infections, whereas Th2 immunity is deleterious to the clearance of genital chlamydial infections. As shown in Figure 2B, the de novo production of IFN- γ and IL-5 in the genital tract and the antigen-specific cytokine production following in vitro chlamydial stimulation of medial iliac lymph node cells or splenocytes were largely comparable among the WT and the TNFR1 KO, TNFR2 KO, and TNFR1/2 DKO mice. The only exception was the significantly enhanced production of IFN- γ in the genital tract in TNFR1/2

Figure 2. Immune responses after genital *Chlamydia muridarum* challenge. Groups of mice (C57BL/6J, TNFR1 KO, TNFR2 KO, and TNFR1/2 DKO; see text for descriptions of each group) were pretreated (10 and 3 days prior to infection) with medroxyprogesterone acetate and challenged (on day 0) with 5×10^4 inclusion-forming units of *C. muridarum*. **A**, Anti-*C. muridarum* antibody (Ab) responses were measured 14 and 60 days following primary infection. Anti-*C. muridarum* total Ab, immunoglobulin G2c (IgG2c), and IgG1 were assayed. Reciprocal antibody titers corresponding to 50% maximal binding are shown. **B**, On day 9 after infection, 3 mice per group were euthanized, and the genital tracts were homogenized (left column). The levels of interferon γ (IFN- γ), interleukin 5 (IL-5), and tumor necrosis factor α (TNF- α) were evaluated using an enzyme-linked immunosorbent assay. Draining medial iliac lymph nodes (middle column) and spleens (right column) were isolated from euthanized mice on day 14 after inoculation. Single cells were prepared and stimulated in vitro with UV irradiation-inactivated *C. muridarum* for 72 hours. Subsequently, supernatants were analyzed for the indicated cytokines. The mean cytokine levels ($\pm$ standard error of the mean) per group at each time point is shown. * $P < .05$, by analysis of variance, between the indicated group and wild-type mice.

Figure 3. Upper genital tract pathology following genital *Chlamydia muridarum* challenge. Groups (n = 8) of mice (C57BL/6J, TNFR1 KO, TNFR2 KO, and TNFR1/2 DKO; see text for descriptions of each group) were challenged with 5×10^4 inclusion-forming units of *C. muridarum* on day 0, and the macroscopic oviduct diameter (A) and uterine horn diameter (B) were measured. Each individual marker represents 1 oviduct or uterine horn, and the mean oviduct and uterine horn diameters ($\pm$ standard error of the mean) are also shown. The number of dilated oviducts/uterine horns (numerator) and the number of oviducts and uterine horns evaluated (denominator) per respective group of mice are indicated in parentheses. The horizontal lines at 0.5 mm (A) and 1 mm (B) depict the distinction between normal and dilated oviducts and uterine horns, respectively. $P < .05$, by analysis of variance, for the difference in the severity of dilatation between the specified group and wild-type mice (Ψ); $P < .05$, by the Fisher exact test, for the difference in incidence between the specified group and C57BL/6J mice (*) or TNFR2 KO mice (**).

DKO mice in comparison to the other mouse groups, possibly indicating a compensatory increase in the production of this cytokine within this mouse group. These results suggest that TNFR1 and TNFR2 collectively, not individually, may significantly downregulate the production of the protective cytokine IFN- γ , but not to an extent to affect chlamydial clearance.

Oviduct and Uterine Horn Pathology Following Genital *C. muridarum* Infection

The development of hydrosalpinx, a characteristic marker of reproductive tract pathological sequelae, and uterine horn dilatation was evaluated at day 80 following primary genital chlamydial inoculation in the 3 different mouse models of TNF receptor deficiency. We have shown previously that oviduct and uterine horn sequelae are fully developed by day 80 after primary genital chlamydial inoculation in mice [14]. As shown in Figure 3A, the incidence of hydrosalpinx and severity of oviduct dilatation were significantly reduced in TNFR1 KO, TNFR2 KO, and TNFR1/2 DKO mice, compared with the incidences in C57BL/6J animals. Additionally, TNFR2 KO mice displayed a significantly greater incidence of hydrosalpinx and uterine horn dilatation than TNFR1/2 DKO mice. There were no differences in incidence or severity of oviduct dilatation between TNFR1 KO mice, compared with either TNFR2 KO or TNFR1/2 DKO mice. Uterine horn dilatation followed similar trends as oviduct pathology (Figure 3B). Importantly, the levels of TNF- α production in the genital tract after infection or from in vitro *Chlamydia*-stimulated splenocytes and lymphocytes from infected mice were comparable or significantly greater in

the KO mice than in WT mice (Figure 2B). This suggested that the observed reductions in pathology were not due to reduction in the ligand TNF- α but specifically were due to the deficiency of the receptors TNFR1 and/or TNFR2. Collectively, these results suggest that a deficiency of either TNFR1 or TNFR2 leads to significant reduction in upper genital tract pathological sequelae following primary chlamydial challenge.

CD8⁺ T-Cell Responses Following Genital *C. muridarum* Infection

We have shown previously that TNF- α production from CD8⁺ T cells contributes significantly to the induction of upper reproductive pathology following primary genital *C. muridarum* infection [8]. Since CD8⁺ T cells are one of the few cell types in the body that express both TNFR1 and TNFR2 [10] and because both receptors contribute to chlamydial upper genital tract pathology, we hypothesized that *Chlamydia*-specific response of CD8⁺ T cells will be reduced in TNFR1- and TNFR2-deficient mice. Since both TNFR1 and TNFR2 mediate pathology and do not oppose each other's effects, the further use of TNFR1/2 DKO mice was deemed unnecessary. At an early (day 4) and late (day 15) time point following intravaginal infection, CD8⁺ T cells were enriched from splenocytes and analyzed for activation by measuring OX40 expression [20] and the mean fluorescent intensity of CD11a expression [21] on CD8⁺ T cells. As shown in Figure 4A, the frequency of OX40-positive CD8⁺ T cells was comparable between the different mouse groups. However, CD11a expression was significantly reduced in TNFR2 KO mice, compared with WT and TNFR1 KO

Figure 4. CD8⁺ T-cell activation profiles during infection in the absence of TNFR1 or TNFR2 (see text for descriptions of these and other groups). Groups of (n = 3) mice (C57BL/6J, TNFR1 KO, and TNFR2 KO) were challenged with 5×10^4 inclusion-forming units/mouse of *Chlamydia muridarum* on day 0, with spleens removed on days 4 or 15. **A**, Expression of cell surface markers was determined by fluorescence-activated cell-sorter analysis. The mean number ($\pm$ standard error of the mean [SEM]) of OX40-positive CD8⁺ T cells per 10³ CD8⁺ T cells is shown at left. The mean $\pm$ SEM of the mean fluorescence intensity (MFI) of CD11a expression on CD8⁺ T cells is shown at right. **B**, Purified CD8⁺ T cells were cocultured with APC and UV irradiation-inactivated *C. muridarum*, and supernatants were collected 72 hours later for analysis of tumor necrosis factor α (TNF- α) and interferon γ (IFN- γ) production. * $P < .05$, by analysis of variance, for the difference between TNFR2 KO mice and wild-type and TNFR1 KO mice.

mice. Furthermore, we evaluated the production of TNF- α and IFN- γ following in vitro stimulation of CD8⁺ T cells with *C. muridarum*. As shown in Figure 4B, TNF- α levels were comparable between all mouse groups. However, IFN- γ production was significantly reduced in TNFR2 KO mice, compared with WT and TNFR1 KO mice. Collectively, these results suggest that TNFR2, not TNFR1, primarily promotes the activation of CD8⁺ T cells following primary genital chlamydial infection.

Induction of Pathology by CD8⁺ T Cells Following Genital *C. muridarum* Infection

Because both TNFR1 and TNFR2 contribute to pathogenesis and TNFR2 KO, not TNFR1 KO, CD8⁺ T cells display reduced activation following chlamydial infection, we hypothesized that TNFR2, not TNFR1, on CD8⁺ T cells is important in chlamydial pathogenesis. Recipient TNFR1 KO or TNFR2 KO mice were repleted with CD8⁺ T cells from WT mice or from TNFR1 KO or TNFR2 KO mice, respectively, and infected intravaginally with *C. muridarum*. Vaginal chlamydial shedding was monitored and found to be comparable among all mouse groups (data not shown). Furthermore, upper reproductive tract

pathology was evaluated. As shown in Figure 5A, TNFR1 KO mice expectedly displayed a significant reduction in incidence and severity of hydrosalpinx, compared with WT animals. However, repletion of TNFR1 KO mice with WT or an equal number of TNFR1 KO CD8⁺ T cells at the time of challenge did not enhance the incidence or severity of oviduct pathology significantly. It is to be noted that repletion of TNFR1 KO mice with either WT or TNFR1 KO CD8⁺ T cells enhanced the incidence and severity of oviduct pathology to an extent that was intermediate between but not significantly different from that in WT and TNFR1 KO mice. This suggests that increased WT or TNFR1 KO CD8⁺ T cell numbers have a similar effect in partially enhancing oviduct pathology within TNFR1 KO mice. Furthermore, as shown in Figure 5B, TNFR2 KO mice expectedly displayed significant reduction in the incidence and severity of hydrosalpinx, compared with WT animals. Importantly, repletion of TNFR2 KO mice with WT CD8⁺ T cells resulted in a significant enhancement of the incidence and severity of oviduct pathology to a level comparable to that of WT mice. However, repletion of TNFR2 KO mice with an equal number of TNFR2KO CD8⁺ T cells only produced partial

Figure 5. Upper genital tract pathology induction by repletion of wild-type (WT) CD8⁺ T cells in TNFR1 KO or TNFR2 KO mice (see text for descriptions of these and other groups). Groups (n=7) of mice (C57BL/6J, TNFR1 KO, and TNFR2 KO) were pretreated (10 and 3 days prior to infection) with medroxyprogesterone acetate and challenged (on day 0) with 5×10^4 inclusion-forming units of *Chlamydia muridarum*. **A**, Groups (n=7) of TNFR1 KO mice received purified CD8⁺ T cells (1×10^7 cells/mouse) from C57BL/6J or TNFR1 KO mice at the time of bacterial inoculation. **B**, Groups (n=7) of TNFR2 KO mice received purified CD8⁺ T cells (1×10^7 cells/mouse) from C57BL/6J or TNFR2 KO mice. On day 80 after bacterial inoculation, oviduct dilatation was measured. $P < .05$, by analysis of variance (ANOVA), for the difference in severity of dilatation between the indicated group and WT mice (Ψ), TNFR2 KO mice (ΨΨ), or TNFR2 KO mice repleted with TNFR2 KO CD8⁺ T cells (ΨΨΨ); $P \leq .05$, by ANOVA, for the difference in the severity of dilatation between the indicated group and WT mice (*) or TNFR2 KO mice (**).

enhancement in pathology, which was not significantly increased, compared with that for TNFR2 KO mice, but was significantly lower in severity than that produced by repletion of TNFR2 KO mice with an equal number of TNFR2KO CD8⁺ T cells. The uterine horn pathology followed the same trend as oviduct pathology in TNFR1 and TNFR2 KO mice (data not shown). These results indicate that TNFR2, not TNFR1, on CD8⁺ T cells contributes significantly to upper genital tract pathology following primary genital *C. muridarum* infection.

DISCUSSION

We have demonstrated previously that TNF- α -producing CD8⁺ T cells mediate *Chlamydia*-induced upper genital tract pathology [8]. While a recent report described the contribution of TNFR1 [13], the relative contributions of TNFR1 and TNFR2 to chlamydial pathology and their relationship to CD8⁺ T cells had not been elaborated. In this study, we have shown that both TNFR1 and TNFR2 contribute significantly to *Chlamydia*-induced upper genital tract pathology, not to clearance of vaginal chlamydial infection. In addition, it appears that TNFR1 can mediate partial pathology in the absence of TNFR2, whereas TNFR2 cannot mediate pathology in the absence of TNFR1. Furthermore, we demonstrated that activation of and induction of pathology by CD8⁺ T cells following genital chlamydial infection requires the expression of TNFR2 on these cells.

We found that TNFR1 and TNFR2 contribute significantly to upper genital pathology but not to clearance of vaginal *C. muridarum* infection. These results confirm and extend our

previous observations that TNF- α is primarily a mediator of reproductive pathology, not pathogen clearance, during genital chlamydial infections [8, 22], and confirm the demonstration by Zhong et al that TNFR1 contributes significantly to chlamydial pathology [13]. In this study, by comparing TNFR1 KO, TNFR2 KO, and TNFR1/2 DKO, we have shown that absence of TNFR1 alone leads to near-total reduction of chlamydial pathology, which is comparable to that in TNFR1/2 DKO mice. Additionally, TNFR2 KO mice displayed significant reduction in pathology, compared with WT mice, but had a significantly greater incidence of pathology than TNFR1/2 DKO mice. However, pathology in TNFR1 KO mice was comparable to that in TNFR1/2 DKO mice. This suggests that deficiency of TNFR1 completely abrogates TNF-mediated pathology, whereas deficiency of TNFR2 partially abrogates TNF-mediated pathology. Conversely, TNFR1 can mediate partial pathology in the absence of TNFR2, whereas TNFR2 cannot mediate pathology in the absence of TNFR1. This interpretation suggests that TNFR1 may act downstream of TNFR2 in the induction of chlamydial reproductive tract pathologies.

TNFR1 is expressed on all nucleated cells of the body, whereas TNFR2 has a limited distribution, including T cells in the immune system. Because we demonstrated previously the contribution of CD8⁺ T cells [8, 9] and we found that both TNFR1 and TNFR2 contribute to chlamydial pathology, in this study we evaluated the role of TNFR1 and TNFR2 on CD8⁺ T cells in this process. The deficiency of TNFR1 on CD8⁺ T cells did not significantly affect activation of this cell type during chlamydial infection. Additionally, TNFR1-bearing CD8⁺ T cells could not induce chlamydial pathologies when transferred

into TNFR1 KO recipient mice infected intravaginally with *Chlamydia*. This suggested to us that, although expression of TNFR1 on CD8⁺ T cells may play a role in chlamydial pathology, the contribution of TNFR1 is mediated primarily by non-CD8⁺ T cells. On the other hand, deficiency of TNFR2 on CD8⁺ T cells led to significantly reduced activation of this cell type during chlamydial infection. A similar role of TNFR2 in activating CD8⁺ T cells and enhancing their survival has been demonstrated previously [23, 24]. Conversely, a role of TNFR2 in the contraction phase of the CD8⁺ T-cell response also has been reported [25]. In our experiments, only TNFR2-bearing CD8⁺ T cells could restore genital tract pathology to WT levels following repletion into TNFR2 KO recipient mice infected with *Chlamydia*. When taken with our previous finding that TNF- α production by CD8⁺ T cells mediated chlamydial pathology [8], the results from this study indicate that TNF- α production by CD8⁺ T cells acts in an autocrine fashion to activate this cell type via TNFR2 during chlamydial infection.

Chlamydia muridarum-induced oviduct pathology has been shown to involve components of the bacterium, including plasmid-coding sequence 5 [26, 27], and various host immunological mediators, including but not limited to neutrophils and matrix metalloproteases [28–32]; CD8⁺ T cells, TNF- α , and perforin [8, 33]; interleukin 1 β [34]; and interleukin 17 [35]. The results from this study provide new insights into the involvement of TNFR1 and TNFR2 in the contribution of CD8⁺ T cells to chlamydial pathogenesis. Further studies will be required to elaborate the interface between the bacterial pathogenic components and CD8⁺ T cells and the interactions between CD8⁺ T cells and other immunological mediators of chlamydial pathogenesis.

In summary, we have demonstrated that (1) TNFR1 and TNFR2 both contribute to *Chlamydia*-induced upper genital tract pathology, with a seemingly larger role for TNFR1; (B) TNFR2 expression on CD8⁺ T cells and TNFR1 expression on non-CD8⁺ T cells play an important role; and (C) TNFR1-mediated chlamydial pathogenesis appears to be potentiated by TNFR2-expressing CD8⁺ T cells. These insights into *C. muridarum* infection in mice remain to be confirmed by those from *C. trachomatis* infections of human, but they have important therapeutic implications, including targeted interception of individual TNF receptors. There are several studies aimed at using TNF or TNF receptor blockade as a strategy to reduce chronic inflammatory pathologies. In this regard, TNFR2 is expressed on limited cell types and may be an attractive target for safe and effective intervention in various diseases [10]. The results from our study support this line of thought in the context of reducing the morbidity of chlamydial reproductive pathologies.

Notes

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