T Cell Responses in the Absence of IFN-γ Exacerbate Uterine Infection with *Chlamydia trachomatis*

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Infection with the obligate intracellular bacterium *Chlamydia trachomatis* is controlled primarily by IFN-γ and Th1 immunity. In this study, we used cells from a *Chlamydia*-specific CD4$^+$ TCR-transgenic mouse to assess the role of IFN-γ in development of Th1 immunity. We show that secretion of host IFN-γ or the ability of host cells to respond to secreted IFN-γ is not required to initiate a Th1 immune response. Additionally, we found that Ag-specific CD4$^+$ cells that were preskewed toward Th1 confer protection, whereas cells preskewed toward Th2 cause a previously unreported exacerbation of disease leading to higher bacterial load. *Chlamydia*-specific Th1 cells transferred into an IFN-γ$^{-/-}$ recipient mouse demonstrate protective effects, but the same cells exacerbate bacterial burden when transferred into IFN-γR$^{-/-}$ mice. Thus, we demonstrate that the secretion of IFN-γ is necessary for protection against *C. trachomatis* and that in the absence of host cell IFN-γR expression, both Th1 and Th2 cells lead to increased burden of *C. trachomatis*. *The Journal of Immunology,* 2009, 183: 1313–1319.

Chlamydiae are obligate intracellular bacteria and the etiological agents of several major human diseases. Worldwide, *Chlamydia trachomatis* is the most common cause of bacterial sexually transmitted disease (1). In many cases, *Chlamydia* infection goes undetected by the host and, if left untreated, infection of women can lead to inflammatory sequelae such as pelvic inflammatory disease, ectopic pregnancy, or sterility. In addition, *C. trachomatis* infection of the conjunctiva induces trachoma, the leading cause of preventable blindness (1). The pathology associated with infection is caused primarily by the inflammatory response; thus, a better understanding of protective vs deleterious inflammatory mediators is necessary. A thorough mechanistic understanding of *Chlamydia* immunity is also critical for development of an effective vaccine against this organism.

Adaptive immune protection against *C. trachomatis* can be demonstrated by transfer of either CD4$^+$ or CD8$^+$ T cells into infected lymphopenic mice (reviewed in Ref. 2). This protection has been shown to be dependent on secretion of IFN-γ by these populations. Although the role that IFN-γ plays in conferring protection against *C. trachomatis* has been described, the role of IFN-γ in priming Ag-specific immunity has not been explored. Similar to lymphopenic hosts, both IFN-γ$^{-/-}$ and IFN-γR$^{-/-}$ mice are unable to control *C. trachomatis* infection (3, 4), and Th1 cells, but not Th2 cells, are protective upon transfer into recipient mice (5, 6). Th1 cell-mediated protection is thought to occur through IFN-γ secretion. IFN-γ can act either indirectly to augment host immunity or directly on epithelial cells, restricting *Chlamydia* growth through a variety of mechanisms including IDO induction, NO production, and p47 GTPase-driven effects (7).

IFN-γ is key to initiating, maintaining, and permanently establishing Th1 cell identity, and both IFN-γ production and IFN-γR expression are required for development of Th1-mediated immunity to *C. trachomatis* (3, 8, 9). Moreover, the reorientation and ligation of the IFN-γ receptor on naive T cells has been thought to be an early step required for Th1 polarization (10, 11). However, this is in direct opposition to findings by Haring et al. (12), which demonstrated the development of *Listeria*-specific Th1 cells in IFN-γR-deficient mice. In this report, we elucidate how IFN-γ responses by host cells as well as IFN-γ production by immune cells impact Th1 polarization and protection against *C. trachomatis*.

Contrary to previous reports, we found that the development of *Chlamydia*-specific Th1 immunity can occur whether or not host cells express or respond to IFN-γ. However, for preskewed cells to confer protection, the recipient host must be capable of responding to IFN-γ. Th2-skewed cells induced an exacerbation of disease which was also seen when Th1 cells were transferred into a IFN-γR$^{-/-}$ mouse. These results indicate that IFN-γ is the key protective cytokine produced by Th1 cells and that other accessory Th1-immune mediators are detrimental to host immunity.

Materials and Methods

Mice

C57BL/6, B6.PL-*Thy1^a* (CD90.1 congenic), B6.129S7-*IFNγ^{tm1Agt}* (IFN-γ$^{-/-}$), and B6.129S7-*IFNγR1^{tm1Agt}* (IFN-γ receptor 1$^{-/-}$) were purchased from The Jackson Laboratory. NR1 mice were described previously (13) and are maintained and cared for within the Harvard Medical School Center for Animal Resources and Comparative Medicine (Boston, MA). All experiments were approved by Harvard's Institutional Animal Care and Use Committee.

Growth isolation and detection of bacteria

C. trachomatis serovar L2 (434/Bu) was propagated within McCoy cell monolayers grown in Eagle's MEM (Invitrogen) supplemented with 10% FCS, 1.5 g/L sodium bicarbonate, 0.1 M nonessential amino acids, and 1 mM sodium pyruvate. Infected monolayers were lifted and sonicated to disrupt the inclusion. EBs were purified by density gradient centrifugation as described (14). Aliquots were stored at −70°C in a medium containing 250 mM sucrose, 10 mM sodium phosphate, and 5 mM L-glutamic acid (SPG)[2] and thawed immediately before use.

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[2] Abbreviations used in this paper: SPG, medium containing 250 mM sucrose, 10 mM sodium phosphate, and 5 mM L-glutamic acid; IFU, inclusion-forming unit.

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Flow cytometry

Tissues were mechanically disaggregated and immediately stained for activation markers or stimulated for 5 h with 50 ng/ml PMA (Alexis Biochemical) and 500 ng/ml ionomycin (Calbiochem) in the presence of brefeldin A (GolgiStop; BD Biosciences) to determine intracellular cytokine staining. Cells were preincubated with anti-FcRγ (Bio X-Cell) before staining with anti-CD4 Pacific Blue (Biolegend) and anti-CD90.1 peridinin chlorophyll-a protein (BD Bioscience). For activation marker analysis, we examined anti-CD44 PE-CyChrome 7 (Biolegend), anti-CD62L allophycocyanin-Alexa 750 (Ebioscience), and anti-CD25 allophycocyanin (BD Bioscience). For intracellular staining, the following Abs were used: anti-IFN-γ PE or Alexa 700; anti-IL2-PE or allophycocyanin; anti-IL-4 PE or allophycocyanin; anti-IL-10 PE; anti-IL17 PE or Alexa 647; and anti-TNF-α PE or PE-CyChrome 7 (BD Biosciences). Cells were permeabilized with the Cytofix/Cytoperm Plus kit according to the manufacturer's instructions (BD Bioscience). Data were collected on a modified FACSCalibur (Cytek Development) or an LSRII (BD Bioscience) and analyzed using Flow Jo (Tree Star).

Transfer of NR1 cells, infection of mice, and preparation of tissue

Before transfer, NR1 cells were isolated from peripheral lymphoid tissues and labeled with 5 μM CFSE (carboxyfluorescein diacetate, succinimidyl ester; Invitrogen) in serum-free medium. Recipient mice were injected i.v. with either 10^6 or 10^7 *C. trachomatis* ($Cta1_{133–152}$-specific $CD4^+$ T cells (13). To infect the genital tract, mice were treated with 2.5 mg of medroxyprogestrone acetate s.c. and then infected 1 wk later in the uterine horns with 10^6 inclusion-forming units (IFU) of *C. trachomatis* L2. Five days postinfection, lymph nodes, spleen, and uterus were collected. The uterus was digested with 1 mg/ml type XI collagenase (Sigma-Aldrich) and 50 Kunitz/ml DNase (Sigma-Aldrich) for 45 min at 37°C. Single-cell suspensions were prepared for staining via mechanical disaggregation.

Skewing of NR1 cells

$CD4^+$ T cells were purified from NR1 mice using a mouse $CD4^-$ isolation kit (Dynal; Invitrogen) per the manufacturer's directions. The T cells were cultured in RPMI 1640 (Invitrogen) supplemented with 10% FCS, L-glutamine, HEPES, 50 μM 2-ME, 50 U/ml penicillin, and 50 μg/ml streptomycin. To stimulate the T cells, irradiated feeder splenocytes were pulsed with 5 μM $Cta1_{133–152}$ peptide and cocultured with the CD4-enriched NR1 cells at a stimulator to a T cell ratio of 4:1. The following conditions were used for polarization: for Th1 polarization, the T cells were incubated with 10 ng/ml IL-12 (Peprotech) and 10 μg/ml anti-IL-4 (Biolegend); for Th2 polarization, the T cells were incubated with 10 ng/ml IL-4 (Peprotech), 10 μg/ml anti-IL-12/23 (Biolegend), and 10 μg/ml anti-IFN-γ (Biolegend); for Th17 polarization the T cells were incubated with 30 ng/ml IL-6 (Peprotech), 10 ng/ml TGF-β (Peprotech), and 10 μg/ml anti-IFN-γ (Biolegend); for nonspecific polarization, Th0 T cells were incubated with peptide alone. Cells were stimulated for 5–7 days before CFSE labeling and transfer into naive $CD90.2^+$ host mice.

In vivo activation and protection assay

NR1 cells were transferred into mice, and 24 h later mice were either challenged with 10^7 IFU of *C. trachomatis* L2 i.v. or 10^6 IFU instilled in the uterus. For activation experiments, tissues were harvested 5 days after infection whereas for protection experiments spleens were harvested 3 days after infection. To assess the protective capacity of the skewed cells, spleens from infected mice were homogenized, sonicated, diluted, and applied to McCoy cell monolayers. Inclusions were counted by immunofluorescence microscopy 30 h after infection.

In vitro protection assay

NR1 cells were skewed as detailed above for 5 days and centrifuged. The supernatants were applied to semiconfluent monolayers of SV40 large T Ag-immortalized C57BL/6 MEF cells (MEF-Tag, a gift from Dr. Richard Flavell, Yale University School of Medicine, New Haven, CT) for 18 h. The supernatants were then removed, and the tissue culture wells were washed with SPG. *C. trachomatis* was applied at a multiplicity of infection of 0.5:1, or 1000 IFU/well, and centrifuged at 1928 × *g* for 1 h at 37°C. After centrifugation, the SPG was replaced with DMEM medium. After 30 h, cells were fixed and stained to enumerate inclusions.

Statistical analysis

All groups were evaluated for statistical significance through the use of unpaired two-tailed *t* tests. Where it appeared necessary to highlight significant differences between data points, the level of significance is depicted as: *, $p < 0.05$; **, $p < 0.01$; and ***, $p < 0.005$.

Results

Chlamydia-*specific Th1 immunity does not require host IFN-γR expression or the ability of host cells to produce IFN-γ*

IFN-γ is the hallmark cytokine of the Th1-type immune response. Clearance of *C. trachomatis* pivotally rests on the ability of the host to mount a Th1 response. Additionally, IFN-γ signaling in the host APCs is required to facilitate T cell skewing toward Th1 (15). Therefore, we tested both the impact of host-derived IFN-γ production and the requirement for host responsiveness to IFN-γ in the generation of a *Chlamydia*-specific T cell response. To follow $CD4^+$ T cell development, naive CFSE-labeled $CD90.1^+$ *Chlamydia*-specific NR1 TCR-transgenic cells were transferred into $CD90.2^+$ mice that were wild type, IFN-$\gamma^{-/-}$, or IFN-$\gamma R^{-/-}$. Mice were then challenged in the uterus with 10^6 IFU of *C. trachomatis* L2; 5 days later, the spleen, draining (iliac) lymph nodes, and uterus were examined for NR1 cell activation and cytokine secretion. As shown in Fig. 1*A*, regardless of whether the recipient animal could produce or respond to IFN-γ, the Ag-specific NR1 cells were able to proliferate, down-regulate CD62L, and up-regulate CD25 and CD44. Moreover, these cells were able to produce robust amounts of IFN-γ, IL-2, and TNF-α. However, the NR1 cells did not produce a significant amount of IL-17, IL-4, or IL-10 (Fig. 1*A* and data not shown). NR1 cells accumulated to a greater extent in the draining lymph nodes of mice deficient in IFN-γ and IFN-γR (Fig. 1*B*). However, cells accumulated to an equivalent extent in the spleen and uterus (Fig. 1, *C* and *D*). These data demonstrate that to mount a Th1 immune response, the host need not produce or respond to IFN-γ.

Only Th1 cells accumulate in mice infected with C. trachomatis

To explore the impact of preskewing of NR1-transgenic cells, we preactivated NR1 cells under conditions designed to skew the cells toward a Th1, Th2, Th17, or Th0 phenotype. After 7 days of stimulation, the cells were assessed for cytokine production to confirm their skewed phenotype (Fig. 2, *A* and *B*). For each phenotype, a total of 10^6 skewed cells were transferred into recipient mice. The following day, the recipient mice were challenged in the uterus with 10^6 IFU of *C. trachomatis*. Five days after challenge, we found that only Th1 cells were able to accumulate in the uterus, draining lymph node, and spleen (Fig. 2, *C–E*). Despite differences in cellular accumulation, all cells showed a similar level of CFSE dilution and activation marker expression (data not shown). However, after transfer and subsequent infection with *C. trachomatis*, IFN-γ was being produced by all skewed groups to a similar extent, despite the cytokine secretion pattern observed before transfer (Fig. 2*F*). Thus, only NR1 cells that are polarized toward the IFN-γ-secreting Th1 phenotype are able to persist after infection with *C. trachomatis*.

Th1 cells confer protection against C. trachomatis

At early time points after infection, the clearance of *C. trachomatis* is primarily mediated by the innate immune response. However, if preactivated Ag-specific cells that are secreting IFN-γ are transferred into naive mice, early protection can also be observed. For example, others have reported that the transfer of Th1 clones into recipient mice confers protection against *Chlamydia muridarum* (6, 16). To compare the protective effect of unskewed cells with cells skewed toward Th1, Th2, and Th17, we adoptively transferred 10^7 NR1 cells of each phenotype into naive B6 mice. Mice were then challenged i.v. with 10^7 IFU of *C. trachomatis* L2 and assessed 3 days later for the number of IFUs in the spleen.

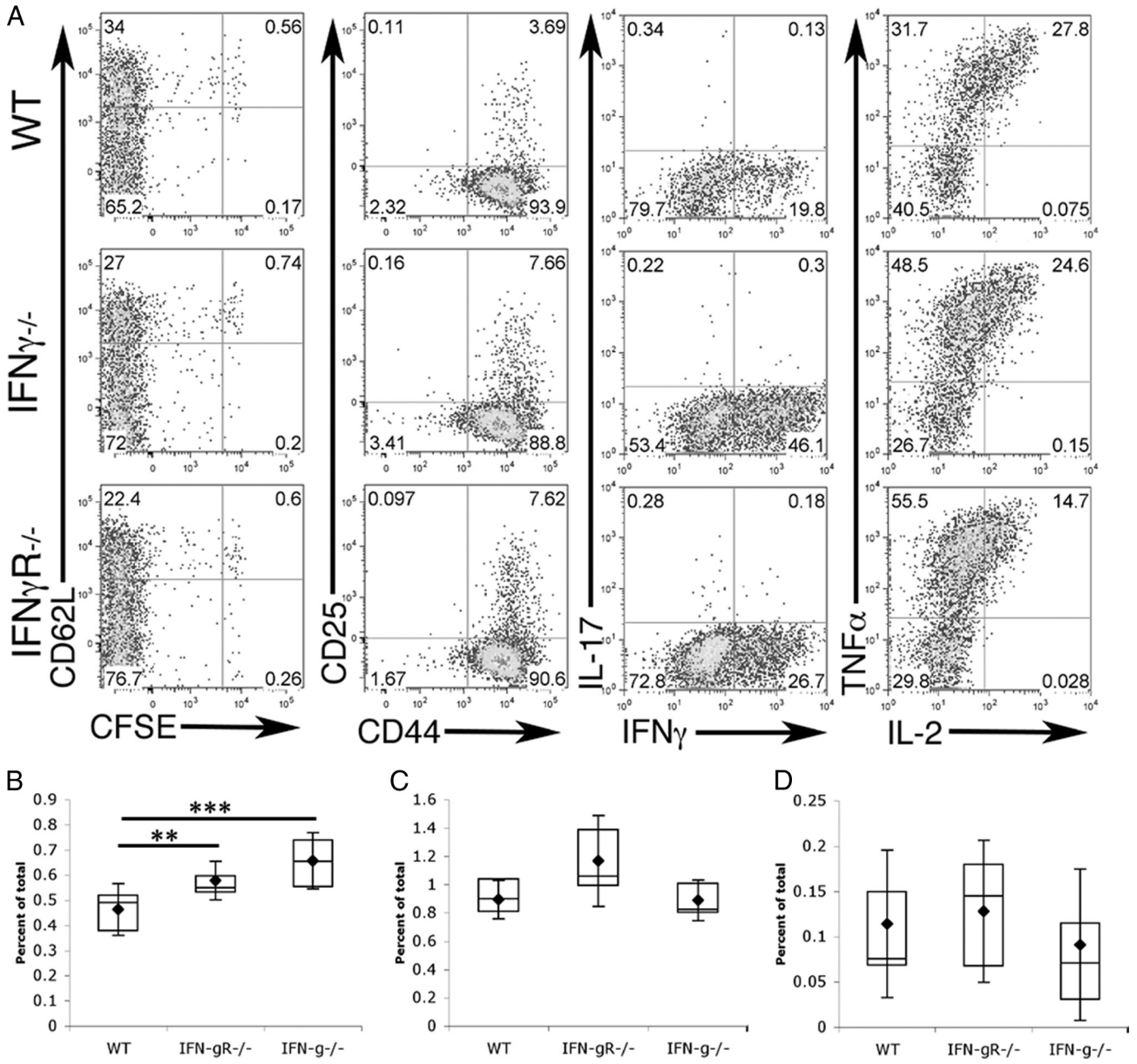


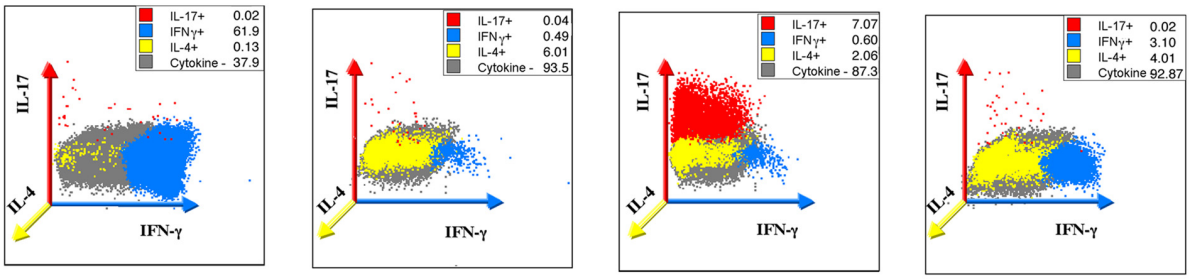
FIGURE 1. CD4$^+$ T cells specific for *C. trachomatis* can be stimulated in IFN-γ (IFN-g)$^{-/-}$ or IFN-γR$^{-/-}$ mice. IFN-γ$^{-/-}$, IFN-γR$^{-/-}$, or wild-type (WT) C57BL/6 mice were injected with naive NR1 cells and challenged the next day with *C. trachomatis*. On day 5 postinfection, flow cytometry was used to analyze cells from the uterus, draining lymph node, and spleen (*A*). Cells were assessed for activation markers (left two panels) or restimulated for 5 h with PMA-ionomycin and assessed for intracellular cytokine staining (right two panels). Flow cytometry data were first gated on live, CD4$^+$, CD90.1$^+$ cells and are representative of three independent experiments. The percentage of total live cells was calculated for draining lymph node (*B*), spleen (*C*), and uterus (*D*). Statistical analysis was performed via Student's *t* test. ∗, $p < 0.05$; ∗∗, $p < 0.01$.

Consistent with published results (16), Th1-skewed cells are capable of conferring protection; $p < 0.001$ (Fig. 3). However, in contrast to the findings of Igietseme et al. (16), we identified an exacerbation of infection following transfer of Th2 *Chlamydia*-specific cells. Mice receiving the Th2 cells had 2-fold higher IFU in the spleen than naive recipients did and 10-fold more IFUs than did recipients that had received Th1 cells (Fig. 3). Mice receiving the Th17 cells had IFUs in the spleen comparable with those of mice that did not receive a transfer, whereas mice receiving the TH0 cells had significantly higher recoverable IFUs then did naive mice. Transfer of 10^7 naive *Chlamydia*-specific cells also did not impart protection (data not shown). From these data, we concluded that the presence of Th2-skewed *Chlamydia* Ag-specific cells is deleterious to the host's clearance of *C. trachomatis*.

Response to IFN-γ, but not production of endogenous IFN-γ, is required for protective immunity

One possible mechanism by which Th1 cells may protect against *C. trachomatis* infection is by inducing endogenous IFN-γ production. To test the requirement for host-derived IFN-γ in Th1-mediated protective immunity, IFN-γ$^{-/-}$ mice were injected with 10^7 preskewed cells and challenged the next day with 10^7 IFU of *C. trachomatis*. As shown in Fig. 4, despite higher bacterial burden in the IFN-γ$^{-/-}$ mice, Th1-skewed NR1 cells were able to confer protection. Thus, the protective

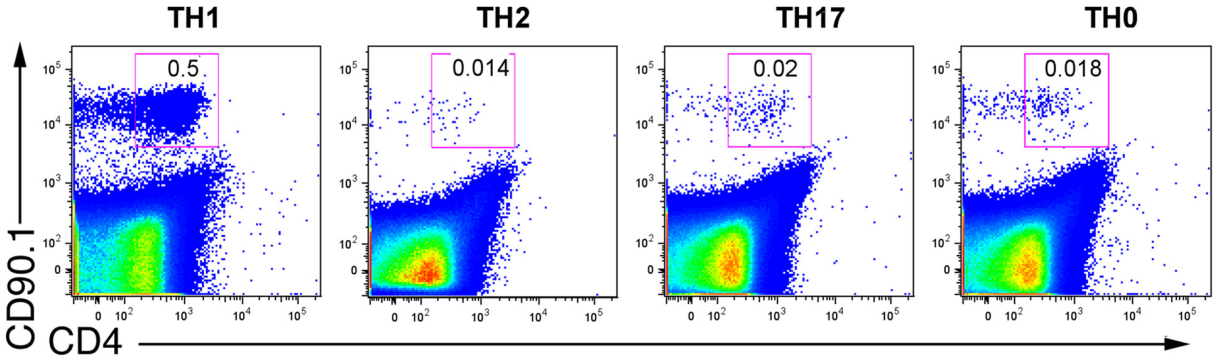
A



B

	TH1				TH2				TH17				TH0			
	% NR1		MFI		% NR1		MFI		% NR1		MFI		% NR1		MFI	
IFN-γ	**83.00**	*1.55*	**1174.62**	*55.84*	**3.56**	*0.50*	**335.23**	*45.02*	**5.41**	*0.15*	**311.64**	*31.35*	**10.96**	*0.31*	**480.28**	*43.09*
IL-10	**6.82**	*1.58*	**54.58**	*3.00*	**1.95**	*0.11*	**132.15**	*6.25*	**6.88**	*0.62*	**65.41**	*2.06*	**2.38**	*0.12*	**62.14**	*6.17*
IL-17	**1.25**	*0.23*	**143.13**	*51.47*	**6.61**	*1.23*	**70.19**	*0.32*	**12.57**	*0.54*	**687.24**	*10.22*	**1.32**	*0.09*	**141.10**	*35.52*
IL-2	**7.01**	*1.03*	**103.09**	*20.55*	**2.05**	*0.32*	**163.21**	*21.33*	**12.48**	*0.55*	**252.99**	*13.68*	**10.98**	*0.15*	**182.36**	*7.15*
IL-4	**0.62**	*0.21*	**88.60**	*19.55*	**13.73**	*0.56*	**225.18**	*10.49*	**0.59**	*0.11*	**157.28**	*91.80*	**1.74**	*0.11*	**87.00**	*14.83*
TNF-α	**75.35**	*0.42*	**291.17**	*0.42*	**48.89**	*0.96*	**171.29**	*5.26*	**40.15**	*0.24*	**302.73**	*4.63*	**51.89**	*0.77*	**195.46**	*3.26*

C



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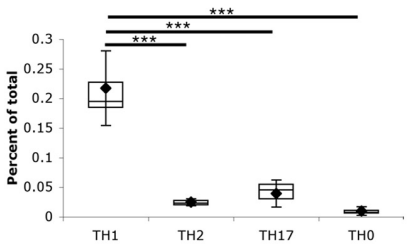

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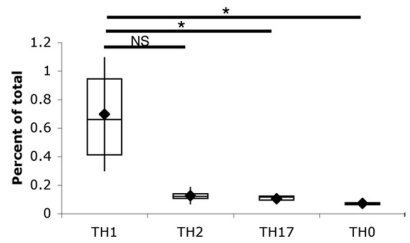

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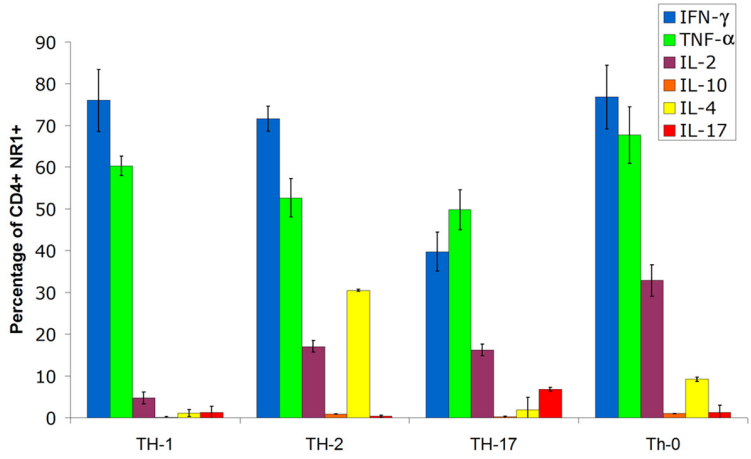

FIGURE 2. Th1 cells preferentially survive after *C. trachomatis* infection. $CD4^+$ cells purified from naive NR1 mice were stimulated for 5–7 days under conditions that left the cells unskewed or that skewed the cells toward Th1, Th2, or Th17. Cells were assessed for their cytokine profile via simultaneous stain (*A*, polyvariate plot) or via singular stains with PE-conjugated Abs (*B*). Shown is the mean percentage of NR1 cells or mean fluorescence intensity (MFI) in bold with SD in italics. Skewed cells were injected into naive mice before infection with *C. trachomatis*. Five days postinfection, the percentage of NR1 cells that were skewed was assessed in the uterus (*C*), lymph node (*D*), and spleen (*E*). Cells were restimulated for 5 h with PMA-ionomycin and stained for intracellular cytokines (*F*). The gates shown were preset on $CD4^+CD90.1^+$ cells, and data are representative of four independent experiments. Statistical analysis of the box-and-whisker plots was conducted using Student's *t* test, ∗, $p < 0.05$; ∗∗∗, $p < 0.005$.

capacity of Th1 cells does not require endogenous production of IFN-γ.

To determine whether it was IFN-γ alone that was responsible for bacterial clearance or whether other Th1 mediators were also contributing to the clearance of *C. trachomatis*, we tested the ability of IFN-$\gamma R^{-/-}$ mice to clear infection after receiving Th1-skewed NR1 cells. After transfer of the cells and challenge of the mice with *C. trachomatis*, we found that not only were

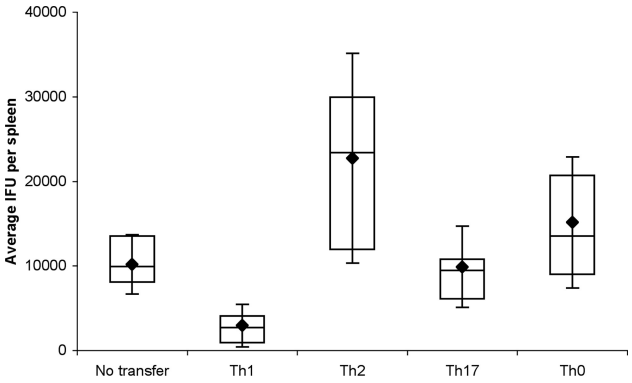

FIGURE 3. Th1 cells protect whereas Th2 cells exacerbate infection with *C. trachomatis*. Skewed NR1$^+$ cells were adoptively transferred into mice followed by infection with 10^7 IFU *C. trachomatis* i.v. Three days postinfection, spleens were assessed for recoverable IFUs. Data are representative of three independent experiments. Statistical analysis of box-and-whisker plots was done via Student's *t* test. By pairwise analysis, no transfer vs Th17 was not significant, no transfer vs TH0 = $p < 0.05$, all other groups were significant greater then $p < 0.01$.

IFN-γR$^{-/-}$ mice unprotected, but also the presence of the NR1 Th1 cells significantly exacerbated bacterial burden when they were unable to produce IFN-γ. The lack of IFN-γ production by the Th1 cells resulted in nearly 2-fold higher numbers of recoverable IFUs (Fig. 4). Bacterial burden increase was not due to a deficit of Th1 cell infiltration, given that NR1 cells were readily recruited to the site of infection in an IFN-γR$^{-/-}$ mouse (data not shown). Thus, the protective effect of NR1 Th1 cells requires that the host be able to respond to IFN-γ being produced by the NR1 cells; moreover, non-IFN-γ cytokines produced by the Th1 NR1 appear to be deleterious to host immunity.

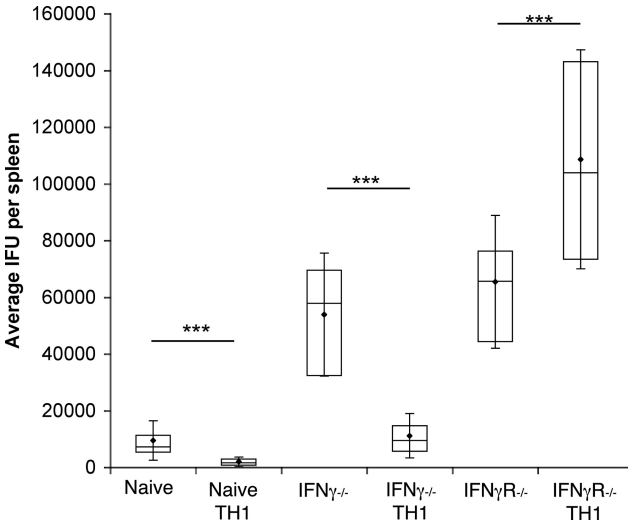

FIGURE 4. IFN-γ receptor expression but not production of endogenous IFN-γ is required for host protection. NR1$^+$ cells skewed toward the Th1 phenotype were transferred into naive IFN-$\gamma^{-/-}$, IFN-γR$^{-/-}$, or wild-type mice. Mice were then challenged with 10^7 IFUs of *C. trachomatis*. Three days after infection, the spleens were assessed for *C. trachomatis* IFUs. Data are representative of two independent experiments. Statistical analysis of box-and-whisker plots was conducted using Student's *t* test; ***, $p < 0.005$.

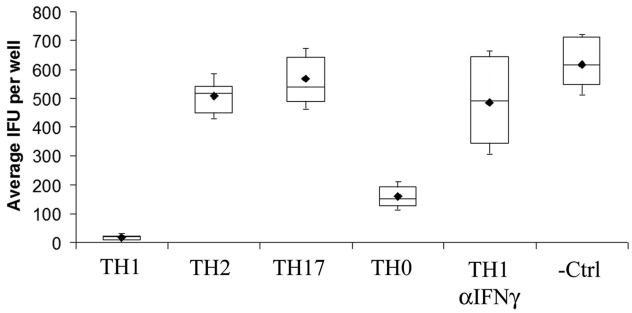

FIGURE 5. Of the cytokines secreted by Th1 cells, IFN-γ alone is able to limit *C. trachomatis* infection in epithelial cells. NR1$^+$ cells were skewed for 7 days. Supernatants were then collected and applied to B6-Mef-TAg cells overnight. Wells were infected the next day with 1000 IFUs of *C. trachomatis* and incubated for an additional 30 h before enumeration of inclusions. α-IFN-γ, anti-IFN-γ; Ctrl, control.

Production of IFN-γ is directly protective whereas Th2 and non-γ Th1 cytokines do not impact innate epithelial immunity

To determine whether the Th1 cytokines that exacerbate bacterial burden in the IFN-γR$^{-/-}$ mice were having a direct impact on epithelial cell control of *Chlamydia* infection, we developed an in vitro assay to measure the effects of cytokines on the growth of *C. trachomatis* within cultured cells. NR1 cells were stimulated and skewed as previously described. After 7 days of culture under conditions that skew to each phenotype, we harvested the supernatant from the skewed cells and applied it to monolayers of C57BL/6 MEF-TAg cells. The next day, the cells were infected with *C. trachomatis* L2. We found that supernatants from Th1- and Th0-activated NR1 cells were able to enhance epithelial cell control of *C. trachomatis* infection (Fig. 5). In contrast, supernatants from Th2 and Th17 cultures did not enhance epithelial cell control of *C. trachomatis*, nor did they exacerbate bacterial burden (Fig. 5 and data not shown). In addition, the MEF-TAg cell control of *C. trachomatis* seen after treatment of the cells with the Th1 supernatant is entirely dependent on IFN-γ given that neutralization of the IFN-γ completely blocked the protective effect of the Th1 supernatant.

Discussion

In this study, we demonstrate that *Chlamydia*-specific Th1 cells can differentiate in the absence of host production of IFN-γ or ability to respond to IFN-γ. Furthermore, we show that the presence of a preskewed Th2 immune response, or a Th1 immune response in which the host is unable to respond to IFN-γ causes an increase in bacterial burden. We confirm that IFN-γ is the key mediator of the protective Th1 response to *C. trachomatis*.

Previously, it had been shown that protective immunity against *Chlamydia* infection requires the production of endogenous IFN-γ and the ability to respond to this cytokine (Refs. 3 and 8; reviewed in Ref. 7). Mice deficient in IFN-γ generally develop Th2 immunity when challenged with pathogens that would normally engender a Th1 response (17, 18). Therefore, we sought to determine the importance of host-derived IFN-γ in the induction of Th1 immunity. We demonstrate that naive wild-type *Chlamydia*-specific CD4$^+$ T cells are primed, proliferate, and secrete Th1 cytokines when transferred into wild-type, IFN-$\gamma^{-/-}$, or IFN-γR$^{-/-}$ mice. However, recent models of *Listeria monocytogenes* infection suggest that it is possible that the IFN-γ produced by the transferred T cells might act in an autocrine manner to promote Th1 immunity (19). To address this possibility, Harring et al. infected IFN-γR1/2$^{-/-}$ mice with *L. monocytogenes* and demonstrated the development of an endogenous Th1 response in the

absence of IFN-γ signaling (12). Our findings using a *C. trachomatis* model support the IFN-γ-independent nature of Th1 cell development.

Transfer of *C. muridarum*-specific clonal T cell lines producing either Th1 or Th2 cytokine profiles has previously demonstrated protective capacity by only the Th1 cell line (6, 16). Hawkins et al. demonstrated that their Th2 clone did not confer protection or accumulate in the genital tract. However, they saw equivalent accumulation of Th1 and Th2 *C. muridarum*-specific clones in the draining lymph nodes and other peripheral tissues. In agreement with this, our data suggest that Th2 cells do not protect against *Chlamydia* infection; moreover we find that they exacerbate bacterial load. We also observed that whereas Th1 cells accumulate in the genital tract and peripheral lymphoid organs, Th2, Th17, and Th0 cells were eliminated from the host. In addition, we found that a large proportion of the skewed cells that remain in the animals now expressed IFN-γ, regardless of how they were skewed before transfer. The ability of skewed cells to switch from one Th profile to another has been extensively examined and found not to occur once cells are strongly polarized (20). Therefore, we believe the accumulation of IFN-γ-producing cells in the Th2, Th17, and Th0 populations is an enrichment for those cells which were producing IFN-γ before adoptive transfer. *Chlamydia* infection is highly polarizing, eliciting IFN-γ secretion from innate (NK and dendritic cells) as well as adaptive immune cells. IFN-γ signaling of naive T cells causes a down-regulation of IFN-γR2 and an up-regulation of IL-12Rβ2 (21, 22). Thus, early in activation IFN-γ helps reinforce the Th1 bias of the immune response. However, constitutive IFN-γ signaling is highly toxic to $CD4^+$ cells (23, 24). As a result, the non-Th1-skewed cells that express IFN-γR2 are susceptible to apoptosis (25, 26). Moreover, when expression of IFN-γR2 is forced, it confers a reduction in Th1 cytokine secretion (27). When *Chlamydia*-specific Th2 cells were transferred to IFN-$\gamma^{-/-}$ mice, we found that accumulation of Th2 cells in the uterus was equivalent to that of Th1 (data not shown). Thus, in addition to promoting Th1 development via up regulating IL-12R expression, IFN-γ induces a blockade of non-Th1 *Chlamydia*-specific cells.

This cross-regulation works in both directions. The adoptive transfer of *Chlamydia*-specific cells preskewed toward Th2 directly interferes with the development of the endogenous Th1 immune response by altering the cytokine milieu of the host (28). In our model, the time from infection to assessment of IFUs is too short for the Th2 cells to influence endogenous adaptive immunity. In addition, we demonstrated that Th2 cytokines did not exacerbate bacterial load in an in vitro epithelial cell infection model. Therefore, we would propose that the supernatant from Th2 cells has a negative impact on the innate immune response and leads to reduced ability to clear bacteria. This is consistent with a recent report showing IL-4-mediated inhibition of *Mycobacterium* clearance from macrophages (29).

A recent report by Li et al. (30) demonstrated that after infection with *C. muridarum* the transferred polarized $CD4^+$ cells are able to confer protection in IFN-$\gamma^{-/-}$ but not IFN-γ$R^{-/-}$ mice. We confirm and extend these findings using a model of *C. trachomatis* infection. *C. trachomatis* clearance is highly dependent on IFN-γ production, whereas *C. muridarum* has been shown to be capable of evading IFN-γ-mediated effects in mice (4, 9, 31). We have found that when mice are infected in the uterus with *C. trachomatis*, some inflammation-induced pathologies associated with human disease manifest in the murine genital tract (D. C. Gondek and M. N. Starnbach, unpublished observations). Previously, we reported that transfer of *Chlamydia*-specific $CD8^+$ T cells was capable of conferring protection only if the host was IFN-γ competent (32). Unlike the $CD8^+$ T cells, we show here that transfer of Th1-skewed $CD4^+$ cells were fully capable of conferring protection to a naive or IFN-$\gamma^{-/-}$ host challenged with *Chlamydia*. In contrast to the findings of Li et al., we observed that transferred Th1 cells exacerbate disease in IFN-γ$R^{-/-}$ mice (30). This highlights the critical nature of IFN-γ in conferring protection and suggests that other Th1 cell factors (either secreted or surface bound) are deleterious to the host following *Chlamydia* challenge. Several non-IFN-γ Th1 cytokines, such as IFN-α, IFN-β, IL-1, and TNF-α have been implicated in causing inflammation leading to both increased bacterial dissemination and enhanced pathology (4, 33–36). Limiting the action of these non-IFN-γ factors might be beneficial in promoting *C. trachomatis* clearance and minimizing inflammation-induced pathology.

Collectively, these data demonstrate the critical role of IFN-γ in the protective immune response to *C. trachomatis*. *Chlamydia*-specific $CD4^+$ T cells alone are capable of establishing protective immunity, highlighting the promise of T cell-based vaccines targeting this bacterium. Clearly, vaccine candidates are most promising when designed to elicit protection while minimizing the expression of deleterious cytokines during vaccination or subsequent infection.

Disclosures

The authors have no financial conflict of interest.

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