



Published in final edited form as:

J Immunol. 2009 July 15; 183(2): 1313–1319. doi:10.4049/jimmunol.0900295.

T cell responses in the absence of IFN γ exacerbate uterine infection with *Chlamydia trachomatis*

David C. Gondek, Nadia R. Roan, and Michael N. Starnbach

Department of Microbiology and Molecular Genetics, Harvard Medical School, 200 Longwood Avenue, Boston, Massachusetts 02115, USA.

Abstract

Infection with the obligate intracellular bacterium *Chlamydia trachomatis*, is controlled primarily by IFN γ and T helper type 1 (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 antigen specific CD4⁺ cells that were pre-skewed towards TH1 confer protection, whereas cells pre-skewed towards T helper type 2 (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 γ R^{-/-} 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 γ R expression, both TH1 and TH2 cells lead to increased burden of *C. trachomatis*.

Introduction

Chlamydiae are obligate intracellular bacteria and the etiologic agent 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 and thus a better understanding of protective versus 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 (2)). This protection has been shown to be dependant upon secretion of IFN γ by these populations. Although the role IFN γ plays in conferring protection against *C. trachomatis* has been described, the role of IFN γ in priming antigen specific immunity has not been explored. Similar to lymphopenic hosts, both IFN γ ^{-/-} and IFN γ R^{-/-} 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, nitric oxide 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 γ R expression are required for development of TH1-mediated immunity to *C. trachomatis* (3,8,9). Moreover the re-orientation and ligation of the IFN γ receptor on naïve 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 which demonstrated the development of *Listeria*-specific TH1 cells in IFN γ receptor deficient mice (12). 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 pre-skewed 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 γ R^{-/-} 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-*IFN γ ^{tm1Agt}* (IFN γ ^{-/-}), and B6.129S7-*IFN γ R^{tm1Agt}*(IFN γ receptor 1^{-/-}) were purchased from The Jackson Laboratory (Bar Harbor, ME). NR1 mice were described previously (13) and are maintained and cared for within the Harvard Medical School Center for Animal Resources and Comparative Medicine. 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, Grand Island NY) supplemented with 10% FCS, 1.5 g/L sodium bicarbonate, 0.1M 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) and thawed immediately prior to use.

Flow cytometry

Tissues were mechanically disaggregated and immediately stained for activation markers or stimulated for five hours 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 pre-incubated with anti-FcR γ (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 phycoerythrin-cychrome 7 (Biolegend) anti-CD62L allophycocyanin-Alexa 750 (Ebioscience) and anti-CD25 allophycocyanin (BD Bioscience). For intracellular staining the following antibodies were used: anti-IFN γ phycoerythrin or Alexa 700, anti-IL2 phycoerythrin or allophycocyanin, anti-IL4 phycoerythrin or allophycocyanin, anti-IL10 phycoerythrin, anti-IL17 phycoerythrin or Alexa 647, and anti-TNF α phycoerythrin or phycoerythrin-cychrome 7 (BD Biosciences). Cells were permeablized with the Cytotfix/Cytoperm Plus kit according to manufacturers instructions (BD Bioscience). Data were collected on a modified FACSCalibur (Cytex Development) or an LSRII (BD Bioscience) and analyzed using Flow Jo (Tree Star Industries).

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

Prior to transfer, NR1 cells were isolated from peripheral lymphoid tissues and labeled with 5 μM CFSE (CFDA, SE, Invitrogen) in serum free media. Recipient mice were injected intravenously with either 10^6 or 10^7 *C. trachomatis* (Cta1₁₃₃₋₁₅₂) specific CD4⁺ T cells (13). To infect the genital tract, mice were treated with 2.5 mg of medroxyprogesterone acetate subcutaneously and then infected one week later in the uterine horns with 10^6 IFU of *C. trachomatis* L2. Five days post infection lymph nodes, spleen, and uterus were collected. The uterus was digested with 1 mg/ml of type XI collagenase (Sigma) and 50 Kunitz/ml of DNase (Sigma) 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 negative isolation kit (Dyna, Invitrogen) per the manufacturers directions. The T cells were cultured in RPMI-1640 (Invitrogen) supplemented with 10% FCS, L-glutamine, HEPES, 50 μM 2-mercaptoethanol, 50 U/ml penicillin, and 50 $\mu\text{g/ml}$ streptomycin. To stimulate the T cells, irradiated feeder splenocytes were pulsed with 5 μM of Cta1₁₃₃₋₁₅₂ peptide and cocultured with the CD4-enriched NR1 cells at a stimulator to 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 of IL-12 (Peprotech) and 10 $\mu\text{g/ml}$ of anti-IL4 (Biolegend); for TH2 polarization the T cells were incubated with 10 ng/ml of IL-4 (Peprotech), 10 $\mu\text{g/ml}$ of anti-IL12/23 (Biolegend), and 10 $\mu\text{g/ml}$ of anti-IFN γ (Biolegend); for TH17 polarization the T cells were incubated with 30 ng/ml of IL-6 (Peprotech), 10 ng/ml of TGF β (Peprotech), and 10 $\mu\text{g/ml}$ of anti-IFN γ (Biolegend); for non-specific polarization TH0, T cells were incubated with peptide alone. Cells were stimulated for five to seven days prior to CFSE labeling and transfer into naïve CD90.2⁺ host mice.

In vivo activation and protection assay

NR1 cells were transferred into mice and twenty-four hours later mice were either challenged with 10^7 IFU of *C. trachomatis* L2 intravenously or 10^6 IFU instilled in the uterus. For activation experiments, tissues were harvested five days after infection whereas for protection experiments spleens were harvested three 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 hours after infection.

In vitro protection assay

NR1 cells were skewed as detailed above for 5 days and centrifuged. The supernatants were applied to semi-confluent monolayers of SV-40 large T antigen immortalized C57BL/6 MEF cells (MEF-TAg - a kind gift from Richard Flavell) for 18 hours. The supernatants were then removed and the tissue culture wells were washed with SPG. *C. trachomatis* was applied at an MOI of 0.5:1, or 1000 IFU/well, and centrifuged at $1928 \times g$ 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 γ receptor 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 antigen presenting cells is required to facilitate T cell skewing towards TH1 (15). Therefore, we tested both the impact of host-derived IFN γ production as well as 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 γ ^{-/-}, or IFN γ R^{-/-}. Mice were then challenged in the uterus with 10⁶ IFU of *C. trachomatis* L2 and five days later the spleen, draining (iliac) lymph nodes, and uterus were examined for NR1 cell activation and cytokine secretion. As shown in Figure 1A, regardless of whether the recipient animal could produce or respond to IFN γ , the antigen 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 (Figure 1A and data not shown). NR1 cells accumulated to a greater extent in the draining lymph nodes of mice deficient in IFN γ and IFN γ R (Figure 1B). However, cells accumulated to an equivalent extent in the spleen and uterus (Figure 1 C,D). These data demonstrate that in order 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 pre-skewing of NR1 transgenic cells, we preactivated NR1 cells under conditions designed to skew the cells towards a TH1, TH2, TH17, or TH0 phenotype. After 7 days of stimulation the cells were assessed for cytokine production to confirm their skewed phenotype (Figure 2A, B). For each phenotype, a total of 10⁶ skewed cells were transferred into recipient mice. The following day the recipient mice were challenged in the uterus with 10⁶ 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 (Figure 2C, D, E). Despite differences in cellular accumulation, all cells showed a similar level of CFSE dilution and activation marker expression (data not shown). However, following 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 prior to transfer (Fig 2F). Thus, only NR1 cells which are polarized towards the IFN γ -secreting TH1 phenotype are able to persist following 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 pre-activated antigen 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). In order to compare the protective effect of unskewed cells with cells skewed towards TH1, TH2, TH17, we adoptively transferred 10⁷ NR1 cells of each phenotype into naive B6 mice. Mice were then challenged intravenously with 10⁷ IFU of *C. trachomatis* L2 and assessed three days later for the number of IFU in the spleen. Consistent with published results (16), TH1 skewed cells are capable of conferring protection (p<0.001) (Figure 3). Interestingly, TH2 cells were found to exacerbate infection. Mice receiving the TH2 cells had 2-fold higher IFU in the spleen than naïve recipients, and ten-fold more IFU than recipients that had received TH1 cells (Figure 3). Mice receiving the TH17 cells had IFU in the spleen comparable to mice that did not receive a transfer, whereas mice receiving the TH0

cells had significantly higher recoverable IFU than naïve mice. Transfer of 10^7 naïve *Chlamydia*-specific cells also did not impart protection (data not shown). From these data we concluded that the presence of TH2 skewed *Chlamydia* antigen 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 $\gamma^{-/-}$ mice were injected with 10^7 pre-skewed cells and challenged the next day with 10^7 IFU of *C. trachomatis*. As shown in figure 4, despite higher bacterial burden in the IFN $\gamma^{-/-}$ mice, TH1 skewed NR1 cells were able to confer protection. Thus, the protective 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 IFN $\gamma R^{-/-}$ mice were not only unprotected, but 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 two fold higher numbers of recoverable IFU (figure 4). Bacterial burden increase was not due to deficit of TH1 cell infiltration into the uterus as NR1 cells were readily recruited to the site of infection in an IFN $\gamma R^{-/-}$ 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, and moreover, non-IFN γ cytokines produced by the TH1 NR1 appear to be deleterious to host immunity.

Production of IFN γ is directly protective while TH2 and non-gamma TH1 cytokines do not impact innate epithelial immunity

To determine if the TH1 cytokines which exacerbate bacterial burden in the IFN $\gamma R^{-/-}$ 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 following 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 (Figure 5). In contrast, supernatants from TH2 and TH17 cultures did not enhance epithelial cell control of *C. trachomatis*, nor did they exacerbate bacterial burden (Figure 5 and data not shown). In addition, the MEF-TAg cell control of *C. trachomatis* seen following treatment of the cells with the TH1 supernatant is entirely dependent upon IFN γ since 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 pre-skewed TH2 immune response, or a TH1 immune response where 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 (3,8) (reviewed in (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 naïve wild type *Chlamydia* specific CD4⁺ T cells are primed, proliferate, and secrete TH1 cytokines when transferred into wild type, IFN γ ^{-/-}, or IFN γ R^{-/-} mice. However, recent models of *Listeria monocytogenes* infection suggest 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 γ R1/2^{-/-} 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 *Chlamydia 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 suggests that TH2 cells do not protect against *Chlamydia* infection, moreover we find that they exacerbate bacterial load. We also observed that while 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 which remain in the animals now expressed IFN γ , regardless of how they were skewed prior to transfer. The ability of skewed cells to switch from one T-helper 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 prior to adoptive transfer. *Chlamydia* infection is highly polarizing, eliciting IFN γ secretion from innate (NK, DC) as well as adaptive immune cells. IFN γ signaling of naïve T cells causes a down regulation of IFN γ R2 and an up regulation of IL-12R β 2 (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 γ R2 are susceptible to apoptosis (25) (26). Moreover, when expression of IFN γ R2 is forced, it confers a reduction in TH1 cytokine secretion (27). When *Chlamydia* specific TH2 cells were transferred to IFN γ ^{-/-} mice we found accumulation of TH2 cells in the uterus was equivalent to 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 pre-skewed towards 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 IFU 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.* demonstrated that following infection with *C. muridarum* the transferred polarized CD4⁺ cells are able to confer protection in IFN γ ^{-/-} but not IFN γ R^{-/-} mice (30). 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 (DCG and MNS, unpublished observations). Previously we reported that transfer of *Chlamydia* specific CD8⁺ T cells were 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 naïve or IFN γ ^{-/-} 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.

References

1. Starnbach MN, Roan NR. Conquering sexually transmitted diseases. *Nat Rev Immunol* 2008;8:313–317. [PubMed: 18309315]
2. Roan NR, Starnbach MN. Immune-mediated control of Chlamydia infection. *Cell Microbiol* 2008;10:9–19. [PubMed: 17979983]
3. Johansson M, Schon K, Ward M, Lycke N. Genital tract infection with Chlamydia trachomatis fails to induce protective immunity in gamma interferon receptor-deficient mice despite a strong local immunoglobulin A response. *Infect. Immun* 1997;65:1032–1044. [PubMed: 9038313]
4. Perry LL, Su H, Feilzer K, Messer R, Hughes S, Whitmire W, Caldwell HD. Differential Sensitivity of Distinct Chlamydia trachomatis Isolates to IFN- γ -Mediated Inhibition. *J Immunol* 1999;162:3541–3548. [PubMed: 10092812]
5. Cain TK, Rank RG. Local Th1-like responses are induced by intravaginal infection of mice with the mouse pneumonitis biovar of Chlamydia trachomatis. *Infect Immun* 1995;63:1784–1789. [PubMed: 7729886]
6. Hawkins RA, Rank RG, Kelly KA. A Chlamydia trachomatis-specific Th2 clone does not provide protection against a genital infection and displays reduced trafficking to the infected genital mucosa. *Infect Immun* 2002;70:5132–5139. [PubMed: 12183563]
7. Rottenberg ME, Gigliotti-Rothfuchs A, Wigzell H. The role of IFN-gamma in the outcome of chlamydial infection. *Curr Opin Immunol* 2002;14:444–451. [PubMed: 12088678]
8. Jupelli M, Guentzel MN, Meier PA, Zhong G, Murthy AK, Arulanandam BP. Endogenous IFN-gamma production is induced and required for protective immunity against pulmonary chlamydial infection in neonatal mice. *J Immunol* 2008;180:4148–4155. [PubMed: 18322226]
9. Ito JI, Lyons JM. Role of Gamma Interferon in Controlling Murine Chlamydial Genital Tract Infection. *Infect. Immun* 1999;67:5518–5521. [PubMed: 10496942]
10. Maldonado RA, Irvine DJ, Schreiber R, Glimcher LH. A role for the immunological synapse in lineage commitment of CD4 lymphocytes. *Nature* 2004;431:527–532. [PubMed: 15386021]
11. Whitmire JK, Benning N, Whitton JL. Cutting edge: early IFN-gamma signaling directly enhances primary antiviral CD4⁺ T cell responses. *J Immunol* 2005;175:5624–5628. [PubMed: 16237051]
12. Haring JS, Badovinac VP, Olson MR, Varga SM, Harty JT. In vivo generation of pathogen-specific Th1 cells in the absence of the IFN-gamma receptor. *J Immunol* 2005;175:3117–3122. [PubMed: 16116201]

13. Roan NR, Gierahn TM, Higgins DE, Starnbach MN. Monitoring the T cell response to genital tract infection. *Proc Natl Acad Sci U S A* 2006;103:12069–12074. [PubMed: 16880389]
14. Howard L, Orenstein NS, King NW. Purification on renografin density gradients of *Chlamydia trachomatis* grown in the yolk sac of eggs. *Appl Microbiol* 1974;27:102–106. [PubMed: 4855645]
15. Johnson LM, Scott P. STAT1 expression in dendritic cells, but not T cells, is required for immunity to *Leishmania major*. *J Immunol* 2007;178:7259–7266. [PubMed: 17513775]
16. Igietseme JU, Ramsey KH, Magee DM, Williams DM, Kincy TJ, Rank RG. Resolution of murine chlamydial genital infection by the adoptive transfer of a biovar-specific, Th1 lymphocyte clone. *Reg Immunol* 1993;5:317–324. [PubMed: 8068534]
17. Wang ZE, Reiner SL, Zheng S, Dalton DK, Locksley RM. CD4+ effector cells default to the Th2 pathway in interferon gamma-deficient mice infected with *Leishmania major*. *J Exp Med* 1994;179:1367–1371. [PubMed: 7908325]
18. Graham MB, Dalton DK, Giltinan D, Braciale VL, Stewart TA, Braciale TJ. Response to influenza infection in mice with a targeted disruption in the interferon gamma gene. *J. Exp. Med* 1993;178:1725–1732. [PubMed: 8228818]
19. Wakil AE, Wang ZE, Ryan JC, Fowell DJ, Locksley RM. Interferon gamma derived from CD4(+) T cells is sufficient to mediate T helper cell type 1 development. *J Exp Med* 1998;188:1651–1656. [PubMed: 9802977]
20. O'Garra A, Murphy K. Role of cytokines in determining T-lymphocyte function. *Curr Opin Immunol* 1994;6:458–466. [PubMed: 7917115]
21. Foulds KE, Rotte MJ, Paley MA, Singh B, Douek DC, Hill BJ, O'Shea JJ, Watford WT, Seder RA, Wu C-Y. IFN- γ Mediates the Death of Th1 Cells in a Paracrine Manner. *J Immunol* 2008;180:842–849. [PubMed: 18178823]
22. Szabo SJ, Dighe AS, Gubler U, Murphy KM. Regulation of the Interleukin (IL)-12R beta 2 Subunit Expression in Developing T Helper 1 (Th1) and Th2 Cells. *J. Exp. Med* 1997;185:817–824. [PubMed: 9120387]
23. Dalton DK, Haynes L, Chu C-Q, Swain SL, Wittmer S. Interferon γ Eliminates Responding CD4 T Cells during Mycobacterial Infection by Inducing Apoptosis of Activated CD4 T Cells. *J. Exp. Med* 2000;192:117–122. [PubMed: 10880532]
24. Refaeli Y, Van Parijs L, Alexander SI, Abbas AK. Interferon γ Is Required for Activation-induced Death of T Lymphocytes. *J. Exp. Med* 2002;196:999–1005. [PubMed: 12370261]
25. Pernis A, Gupta S, Gollob KJ, Garfein E, Coffman RL, Schindler C, Rothman P. Lack of interferon gamma receptor beta chain and the prevention of interferon gamma signaling in TH1 cells. *Science* 1995;269:245–247. [PubMed: 7618088]
26. Zhang Y, Apilado R, Coleman J, Ben-Sasson S, Tsang S, Hu-Li J, Paul WE, Huang H. Interferon γ Stabilizes the T Helper Cell Type 1 Phenotype. *J. Exp. Med* 2001;194:165–172. [PubMed: 11457891]
27. Tau GZ, von der Weid T, Lu B, Cowan S, Kvatyuk M, Pernis A, Cattoretti G, Braunstein NS, Coffman RL, Rothman PB. Interferon gamma signaling alters the function of T helper type 1 cells. *J Exp Med* 2000;192:977–986. [PubMed: 11015439]
28. O'Garra A, Murphy K. Role of cytokines in determining T-lymphocyte function. *Current Opinion in Immunology* 1994;6:458–466. [PubMed: 7917115]
29. Harris J, De Haro SA, Master SS, Keane J, Roberts EA, Delgado M, Deretic V. T Helper 2 Cytokines Inhibit Autophagic Control of Intracellular Mycobacterium tuberculosis. *Immunity* 2007;27:505–517. [PubMed: 17892853]
30. Li W, Murthy AK, Guentzel MN, Seshu J, Forsthuber TG, Zhong G, Arulanandam BP. Antigen-specific CD4+ T cells produce sufficient IFN-gamma to mediate robust protective immunity against genital *Chlamydia muridarum* infection. *J Immunol* 2008;180:3375–3382. [PubMed: 18292563]
31. Coers J, Bernstein-Hanley I, Grotzky D, Parvanova I, Howard JC, Taylor GA, Dietrich WF, Starnbach MN. *Chlamydia muridarum* evades growth restriction by the IFN-gamma-inducible host resistance factor Irgb10. *J Immunol* 2008;180:6237–6245. [PubMed: 18424746]
32. Lampe MF, Wilson CB, Bevan MJ, Starnbach MN. Gamma interferon production by cytotoxic T lymphocytes is required for resolution of *Chlamydia trachomatis* infection. *Infect Immun* 1998;66:5457–5461. [PubMed: 9784557]

33. Qiu HY, Fan AG, Joyee S, Wang X, Han H, Bai L, Jiao N, Van Rooijen, Yang X. Type I IFNs Enhance Susceptibility to Chlamydia muridarum Lung Infection by Enhancing Apoptosis of Local Macrophages. *J Immunol* 2008;181:2092–2102. [PubMed: 18641348]
34. Nagarajan UM, Prantner D, Sikes JD, Andrews CW Jr, Goodwin AM, Nagarajan S, Darville T. Type I IFN signaling exacerbates Chlamydia muridarum genital infection in a murine model. *Infect. Immun.* 2008IAI.00629–00608.
35. Hvid M, Baczynska A, Deleuran B, Fedder J, Knudsen HJ, Christiansen G, Birkelund S. Interleukin-1 is the initiator of Fallopian tube destruction during Chlamydia trachomatis infection. *Cell Microbiol* 2007;9:2795–2803. [PubMed: 17614966]
36. Williams DM, Magee DM, Bonewald LF, Smith JG, Bleicker CA, Byrne GI, Schachter J. A role in vivo for tumor necrosis factor alpha in host defense against Chlamydia trachomatis. *Infect. Immun* 1990;58:1572–1576. [PubMed: 2341167]

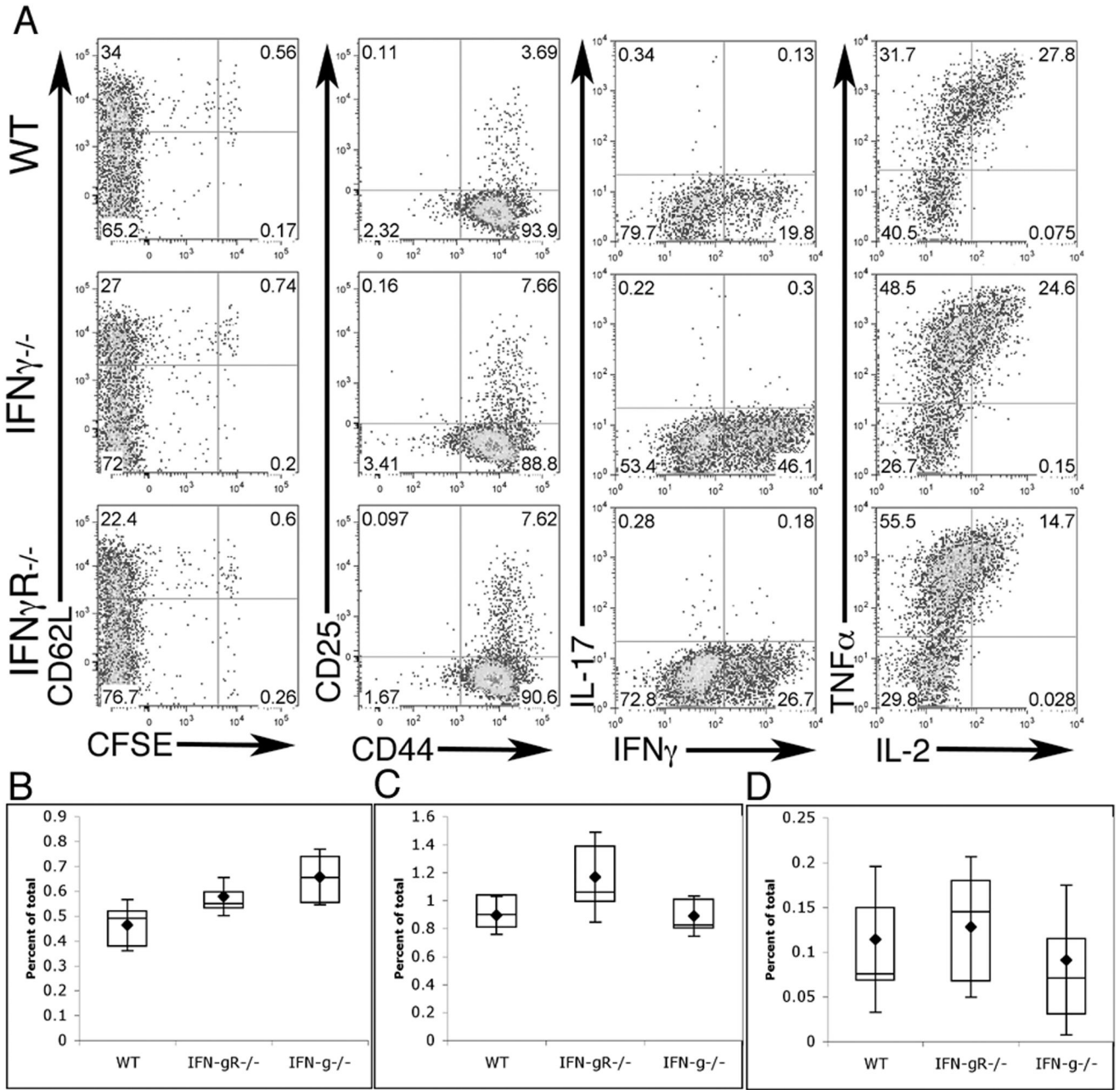


Figure 1. CD4⁺ T cells specific for *C. trachomatis* can be stimulated in IFN γ ^{-/-} or IFN γ R^{-/-} mice
 IFN γ ^{-/-}, IFN γ R^{-/-}, or WT C57BL/6 mice were injected with naïve NR1 cells and challenged the following day with *C. trachomatis*. On day 5 post-infection flow cytometry was used to analyze cells from the uterus, draining lymph node, and spleen (A). Cells were assessed for activation markers (left two panels) or restimulated for 5 hours with PMA/ionomycin and assessed for intra-cellular cytokine staining (right two panels). Flow cytometry data were first gated on live, CD4⁺, CD90.1⁺ cells and are representative of three independent experiments. Percentage of total live cells was calculated for draining lymph node (B), spleen (C), and uterus (D). Statistical analysis performed via Student's T test, * = p<0.05, ** = p<0.01.

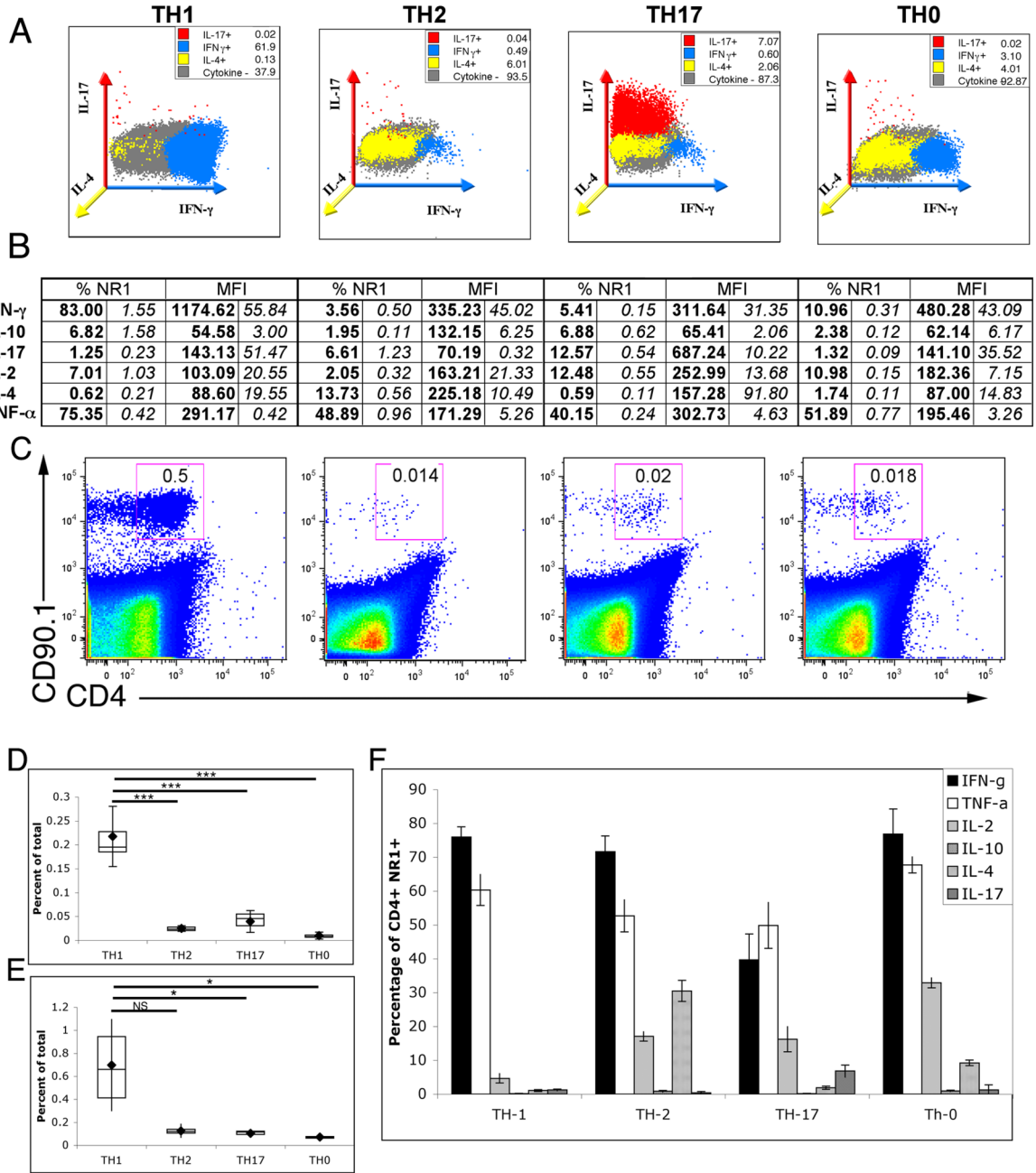


Figure 2. TH1 cells preferentially survive following *C. trachomatis* infection

CD4⁺ cells purified from naïve NR1 mice were stimulated for 5–7 d in conditions that left the cells unskewed or which skewed the cells towards TH1, TH2, or TH17. Cells were assessed for their cytokine profile via simultaneous stain (A - polyvariate plot) or via singular stains with phycoerythrin conjugated antibodies (B). Shown is mean percentage of NR1 cells or Mean Fluorescence intensity (MFI) in bold with standard deviation in italics. Skewed cells were injected into naïve mice prior to infection with *C. trachomatis*. Five days post-infection, the percentage of NR1 cells that were skewed was assessed in the uterus (C), lymph node (D), and spleen (E). Cells were restimulated for five hours with PMA/Ionomycin and stained for intracellular cytokines (F). The gates shown were pre-set on CD4⁺, CD90.1⁺ cells, and data

are representative of four independent experiments. Statistical analysis of the box-and-whisker plots was conducted using a Student's T test, * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.005$.

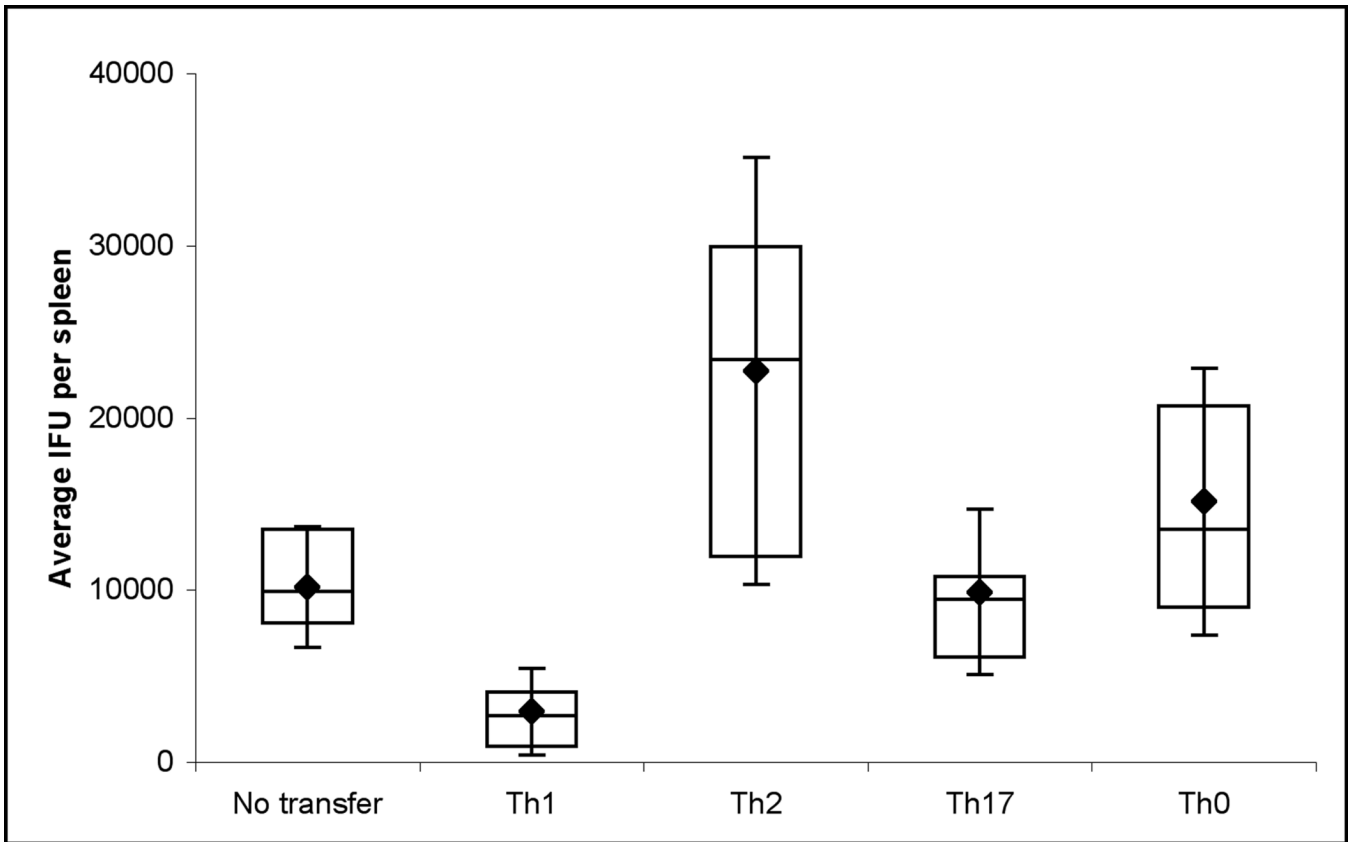


Figure 3. TH1 cells protect, while TH2 cells exacerbate infection with *C. trachomatis*
 Skewed NR1+ cells were adoptively transferred into mice followed by infection with 10^7 IFU *C. trachomatis* i.v.. Three days post infection spleens were assessed for recoverable IFU. Data is representative of three independent experiments. Statistical analysis of box-and-whisker plots was done via Student's T test. By pair wise analysis, no transfer vs TH17 was not significant, no transfer vs TH0 = $p < 0.05$, all other groups were significant greater than $p < 0.01$.

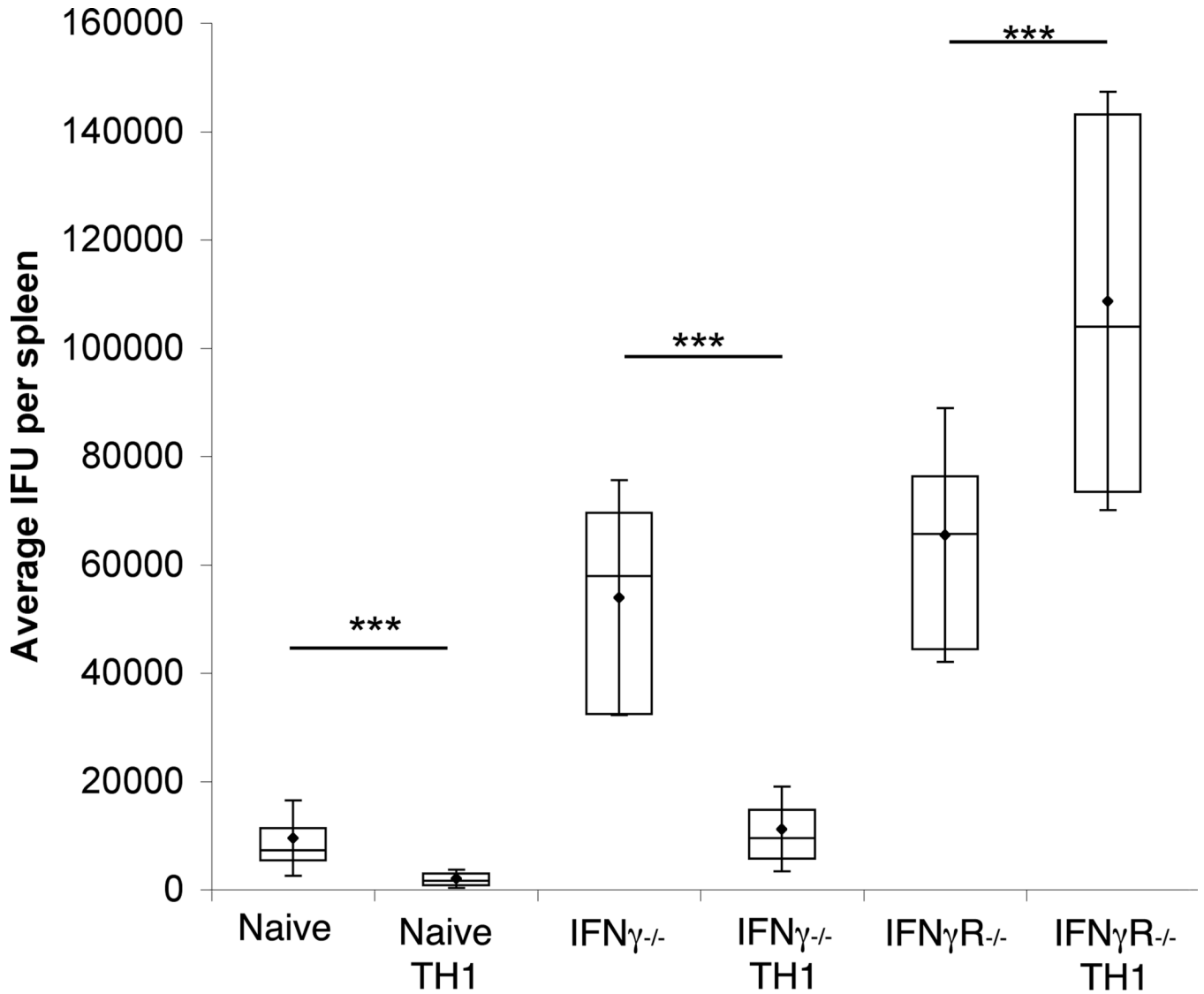


Figure 4. IFN γ receptor expression but not production of endogenous IFN γ is required for host protection

NR1⁺ cells skewed toward the TH1 phenotype were transferred into naïve IFN γ ^{-/-}, IFN γ R^{-/-}, or wild type mice. Mice were then challenged with 10⁷ IFU of *C. trachomatis*. Three days after infection the spleens were assessed for *C. trachomatis* IFU. Data are representative of two independent experiments. Statistical analysis of box-and-whisker plots was conducted using a Student's T test, * = p<0.05, ** = p<0.01, and *** = p<0.005.

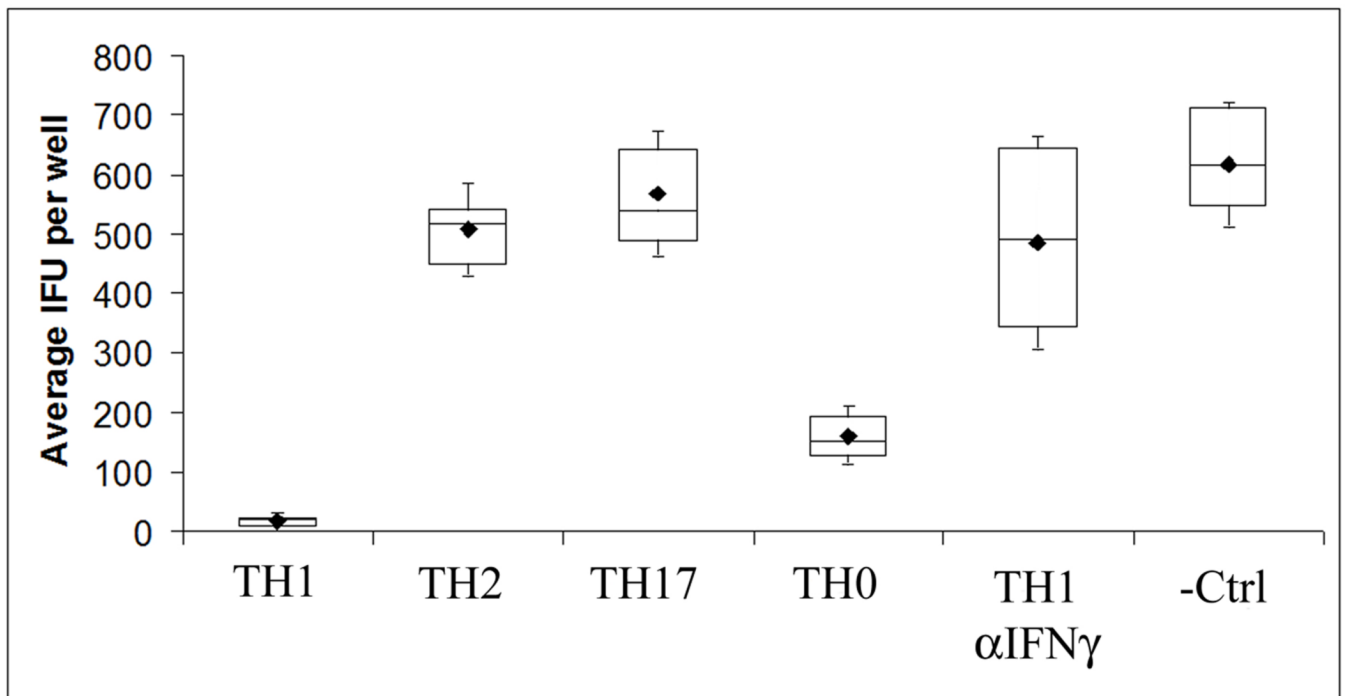


Figure 5. Of the cytokines secreted by TH1 cells, IFN γ alone is able to limit *C. trachomatis* infection in epithelial cells

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 IFU of *C. trachomatis* and incubated for an additional 30 h before enumeration of inclusions.