Immunity
Article

Toll-like Receptor and Inflammasome Signals Converge to Amplify the Innate Bactericidal Capacity of T Helper 1 Cells

Hope O'Donnell,[1,2] Oanh H. Pham,[1] Lin-xi Li,[1] Shaikh M. Atif,[1] Seung-Joo Lee,[1] Marietta M. Ravesloot,[1] Jessica L. Stolfi,[1] Sean-Paul Nuccio,[3] Petr Broz,[4] Denise M. Monack,[4] Andreas J. Baumler,[3] and Stephen J. McSorley[1,*]
[1]Center for Comparative Medicine, Department of Anatomy, Physiology, and Cell Biology, School of Veterinary Medicine, University of California, Davis, Davis, CA 95616, USA
[2]Microbiology, Immunology, and Cancer Biology Graduate Program, University of Minnesota Medical School – Twin Cities, Minneapolis, MN 55455, USA
[3]Department of Medical Microbiology and Immunology, School of Medicine, University of California, Davis, Davis, CA 95616, USA
[4]Department of Microbiology and Immunology, School of Medicine, Stanford University, Stanford, CA 94305, USA
*Correspondence: sjmcsorley@ucdavis.edu
http://dx.doi.org/10.1016/j.immuni.2013.12.013

SUMMARY

T cell effector functions can be elicited by noncognate stimuli, but the mechanism and contribution of this pathway to the resolution of intracellular macrophage infections have not been defined. Here, we show that CD4$^+$ T helper 1 (Th1) cells could be rapidly stimulated by microbe-associated molecular patterns during active infection with *Salmonella* or *Chlamydia*. Further, maximal stimulation of Th1 cells by lipopolysaccharide (LPS) did not require T-cell-intrinsic expression of toll-like receptor 4 (TLR4), interleukin-1 receptor (IL-1R), or interferon-γ receptor (IFN-γR) but instead required IL-18R, IL-33R, and adaptor protein MyD88. Innate stimulation of Th1 cells also required host expression of TLR4 and inflammasome components that together increased serum concentrations of IL-18. Finally, the elimination of noncognate Th1 cell stimulation hindered the resolution of primary *Salmonella* infection. Thus, the in vivo bactericidal capacity of Th1 cells is regulated by the response to noncognate stimuli elicited by multiple innate immune receptors.

INTRODUCTION

Pathogen-specific lymphocytes recirculate at low frequency between the blood and secondary lymphoid tissues and undergo rapid expansion in response to infection (Kwok et al., 2012; Moon et al., 2007). As clonal expansion occurs, responding T cells integrate local instructional stimuli to acquire effector functions tailored to combat different pathogen types (Obar and Lefrançois, 2010; Zhu et al., 2010). The expansion and functional maturation of individual T cell clones are tightly regulated by pathogen-specific T cell receptors (TCRs) that recognize microbial peptides in the context of host major histocompatibility complex (MHC) molecules. Thus, the adaptive immune response to infection produces a large population of antigen-specific effector T cells with appropriate functional activities to combat invading microbes.

Although the initial activation and expansion of pathogen-specific T cells are controlled by TCR ligation, the subsequent signals for inducing T cell effector functions are incompletely understood. In a noninfectious context, the elicitation of effector functions by tissue-resident CD4$^+$ T cells requires TCR recognition of cognate antigen presented by local antigen-presenting cells (McLachlan et al., 2009). However, a lower threshold for stimulating activated effector T cells might be advantageous when the host confronts a replicating pathogen, especially one that can manipulate host MHC expression (Griffin and McSorley, 2011). Indeed, inflammatory cytokines, notably interleukin-12 (IL-12) and IL-18, cause noncognate stimulation of effector CD8$^+$ T cells (Beadling and Slifka, 2005; Berg et al., 2002; Freeman et al., 2012). During bacterial infections, the production of inflammatory cytokines can be initiated by host recognition of conserved molecular patterns via multiple innate immune receptors (Broz and Monack, 2011). Thus, bacterial flagellin can efficiently drive noncognate stimulation of CD8$^+$ memory T cells in a process that involves dendritic cell sensing of cytosolic flagellin by NLR family CARD-domain-containing protein 4 (NLRC4) (Kupz et al., 2012). However, the role of toll-like receptor (TLR) and inflammasome signaling in the elicitation of T cell effector functions is currently unclear. Such noncognate stimulation pathways might allow T cell effector functions to be induced rapidly in an inflammatory context and provide an evolutionary advantage for the host in combating bacterial pathogens.

The efferent phase of the CD4$^+$ Th1 cell response to an intramacrophage pathogen has the potential to be relatively nonspecific, given that it consists of macrophage activation by locally produced interferon-γ (IFN-γ). Although cytokine secretion might be restricted to the synapse during cognate (antigen receptor agonist) stimulation, CD4$^+$ Th1 cells can activate macrophages in the absence of cognate stimuli and also provide cross-protection against unrelated coinfecting microbes (MacKaness, 1964; Müller et al., 2012; Poo et al., 1988). Even though Th1 cell secretion of IFN-γ can be induced by cognate antigen and MHC class II molecules presented on infected phagocytes, it can also occur in the presence of cytokines (Robinson et al., 1997; Takeda et al., 1998) or TLR ligation (Caramalho

et al., 2003; Reynolds et al., 2010). However, the signals that drive noncognate stimulation of CD4+ Th1 cells and the contribution of this pathway to bacterial clearance have not been clearly defined in vivo.

In this study, we examined the mechanism and contribution of noncognate T cell stimulation to the resolution of intramacrophage infection. Expanded T-bet+CD4+ T cells in *Salmonella*- and *Chlamydia*-infected mice were induced to secrete IFN-γ by brief in vivo exposure to ligands for TLR2, TLR4, and TLR5, and this required T-cell-intrinsic expression of the adaptor protein MyD88 and IL-18R, as well as IL-33R, in this amplification of Th1 cell responses. Generating an inflammatory environment favoring noncognate stimulation of Th1 cells required host expression of TLR and inflammasome components, which together enhanced concentrations of circulating IL-18. CD8+ T cells were able to respond in a similar manner but made a smaller contribution to bacterial clearance. Mice lacking T-cell-intrinsic expression of MyD88 had impaired ability to control primary *Salmonella* infection, demonstrating the importance of noncognate responses to the resolution of an intramacrophage infection. Overall, these data show that noncognate stimulation of T cells can occur in response to innate inflammatory cues and contribute to defense against intramacrophage pathogens.

RESULTS

CD4+ and CD8+ T Cells in Infected Mice Can Secrete IFN-γ in Response to Innate Receptor Stimuli

Salmonella infection of C57BL/6 mice induced the expansion of splenic CD44hiCD4+ and CD8+ T cell populations that persisted as a major fraction of the T cell pool until bacterial clearance occurred, approximately 5–8 weeks later (Figure 1A) (Srinivasan et al., 2004). The majority of CD4+ T cells responding to *Salmonella* infection expressed the transcription factor T-bet (Figure 1B), consistent with a requirement for Th1 cells in the resolution of intramacrophage infections (Griffin and McSorley, 2011). A small population of CD4+ (<5%) or CD8+ (<2%) T cells in the spleen of *Salmonella*-infected mice were found to be actively secreting IFN-γ; these were part of the expanded CD44hi population and were undetectable in uninfected mice (Figure 1C, "no stim"). However, intravenous injection of infected mice with ultrapure lipopolysaccharide (LPS) induced IFN-γ secretion from approximately 30%–50% of CD4+ T cells and 5%–20% of CD8+ T cells within 4 hours, whereas a response to innate receptor stimuli was not detected in uninfected recipients (Figure 1C). Among CD4+ T cells, LPS-induced IFN-γ production was confined to T-bet+ cells, and typically 50%–70% of all T-bet+CD4+ T cells participated in this innate response at the peak of infection (Figures 1D and 1E). As *Salmonella* infection resolved around day 35 (Srinivasan et al., 2004), the proportion of T-bet+CD4+ T cells able to respond to innate stimuli correspondingly decreased (Figure 1E). However, a small population (5%–10%) of T-bet+CD4+ T cells retained the ability to respond rapidly to innate stimuli for at least 24 weeks after primary infection (Figure 1E). A similar response was detected among activated CD8+ T cells, but the magnitude was lower than that among CD4+ T cells at each time point (Figure 1E). There were no other major splenic cell populations that produced IFN-γ in response to innate stimuli (Figure S1, available online), indicating that CD4+ and CD8+ T cells are the major IFN-γ-producing cells in this model.

To determine whether innate amplification of CD4+ Th1 cell effector function was a process unique to *Salmonella* infection, we examined C57BL/6 mice infected with *Chlamydia muridarum*, another common intramacrophage pathogen (Farris and Morrison, 2011). T-bet+CD4+ T cells in the spleen of C57BL/6 mice infected with *Chlamydia muridarum* produced IFN-γ rapidly in response to LPS stimulation (Figures 2A and 2B). LPS injection also caused a small population of CD8+ T cells to secrete IFN-γ (Figure 2B). As in the *Salmonella*-infection model, little IFN-γ secretion was detectable in CD4+ T cells of uninfected mice injected with LPS or in *Chlamydia*-infected mice prior to LPS injection (Figures 2A and 2B). Next, we examined whether other TLR ligands had the capacity to induce IFN-γ production from T-bet+CD4+ T cells by injecting *Salmonella*-infected mice with LPS (recognized by TLR4), flagellin (TLR5), Pam3CSK4 (TLR1 and TLR2), CpG DNA (TLR9), or Imiquimod (TLR7). Injection of LPS, flagellin, or Pam3CSK4 amplified the production of IFN-γ from T-bet+CD4+ T cells in *Salmonella*-infected mice, and LPS acted as the most effective inducer (Figures 2C and 2D). A small percentage of CD8+ T cells also produced IFN-γ in response to each of these stimuli (Figure 2D). In contrast, the injection of CpG DNA or Imiquimod failed to induce IFN-γ production over baseline production detected in CD4+ and CD8+ T cells from *Salmonella*-infected mice (Figures 2C and 2D). LPS stimulation of T-bet+CD4+ and CD44+CD8+ T cells was greatest when 25 μg of LPS was used; however, a rapid innate response was also detectable when as little as 100 ng of LPS was injected (Figure 2E). Together, these results demonstrate that innate stimulation of CD4+ and CD8+ T cells can occur rapidly in response to a variety of microbial stimuli and that this capability is a common feature of the immune response to intramacrophage infections.

T Cells Require Expression of MyD88, but Not TLR4, for Innate Amplification

Mixed bone marrow (BM) chimeric mice were generated to define the T-cell-intrinsic requirements for innate amplification of CD4+ and CD8+ T cell effector functions. Congenically marked (CD90.2+CD45.1+) mice were irradiated and reconstituted with a 1:1 mixture of BM from wild-type (WT) mice (CD90.1+CD45.2+) and a variety of gene-deficient (CD90.2+CD45.2+) mice (Figure 3A), allowing direct comparison of WT and gene-deficient T cells within a single *Salmonella*-infected host. As expected, CD4+ and CD8+ T cells in *Salmonella*-infected, nonchimeric, TLR4-deficient mice were unable to produce IFN-γ in response to innate stimulation with LPS (Figure S2). However, in *Salmonella*-infected chimeras containing a 1:1 mix of both deficient and WT cells, both WT and TLR4-deficient CD4+ and CD8+ T cells responded equally well to LPS stimulation (Figure 3B and Figure S3A). Next, we utilized mixed BM chimeras to examine the requirement for MyD88, an essential component of most TLR and IL-1-like receptor (IL-1R) signaling. CD4+ and CD8+ T cells lacking expression of MyD88 were unable to respond to LPS injection, whereas WT CD4+ and CD8+ T cells in the same mouse generated robust IFN-γ responses (Figure 3C and Figure S3B). Thus, both CD4+ and CD8+ T cells require expression of MyD88, but not TLR4, in order to rapidly respond to innate stimuli.

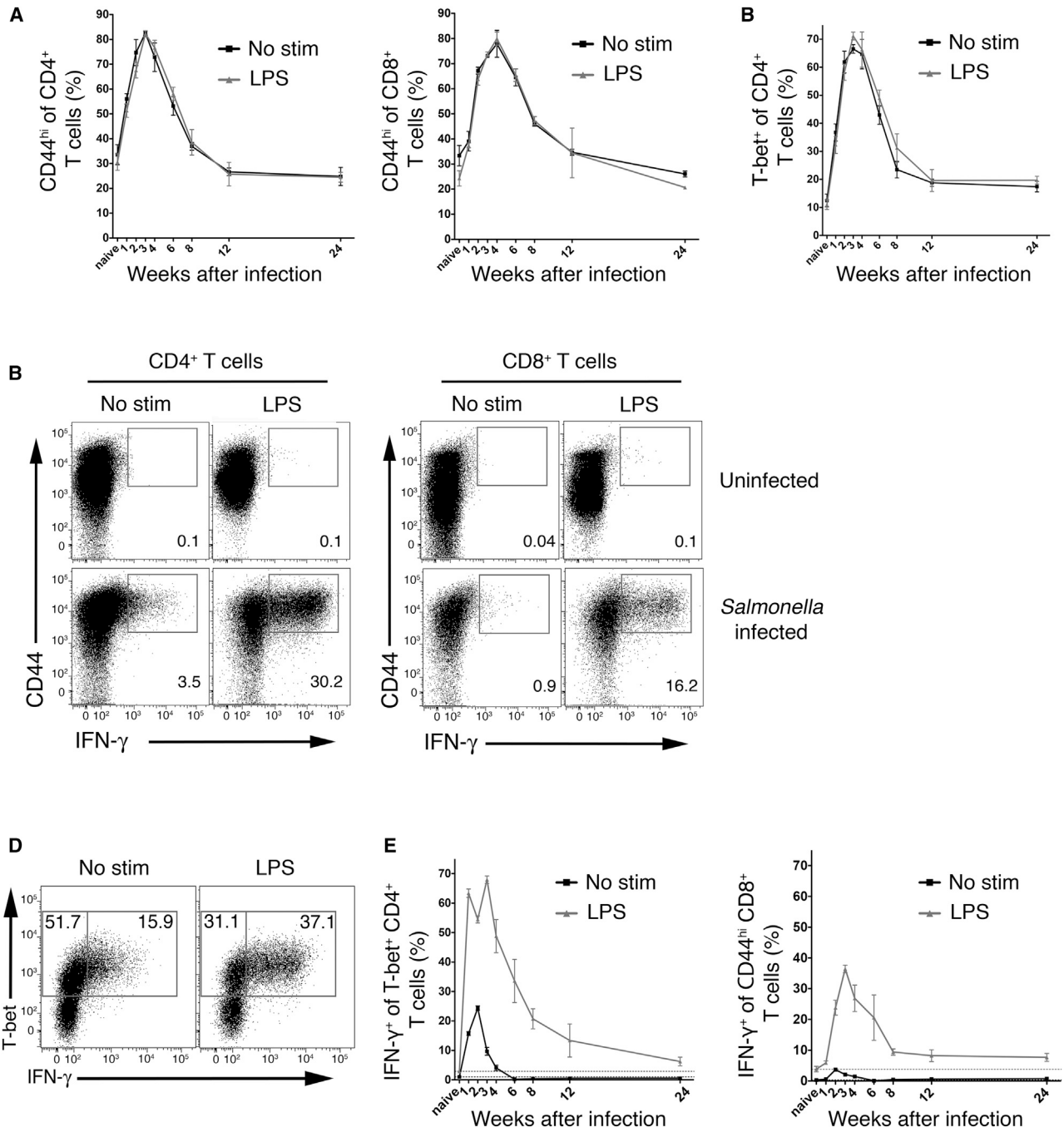


Figure 1. Expanded CD4+ Th1 and CD8+ T Cells Acquire the Ability to Respond to Innate Stimulation

C57BL/6 mice were infected intravenously (i.v.) with 5 × 10^5 *Salmonella* (BRD509) and at various times later, the proportion of spleen CD4+ and CD8+ T cells expressing CD44 and IFN-γ (as well as CD4+ T cells expressing T-bet) was assessed by flow cytometry. Four hours prior to analysis, mice were injected i.v. with 10 μg LPS or received no additional stimulus ("no stim").

(A, B, and E) Graphs show the change in the percentage of $CD44^{hi}$ (A), T-bet+ (B), or IFN-γ+T-bet+ (Th1 cells producing IFN-γ) (E) CD4+ T cells and $CD44^{hi}$ (A) and IFN-γ+$CD44^{hi}$ (activated CD8+ T cells producing IFN-γ) (E) CD8+ T cells in the spleen. Error bars represent the mean ± SEM.

(C and D) Representative flow cytometry plots showing the production of IFN-γ at day 14 after infection of CD44+CD4+ and CD8+ T cells (C) or T-bet+CD4+ T cells (D). Numbers show the percentage of IFN-γ+ or IFN-γ− T cells within the boxed gates. Experiments contained at least three mice per group at each time point, and data are representative of at least three experiments.

See also Figure S1.

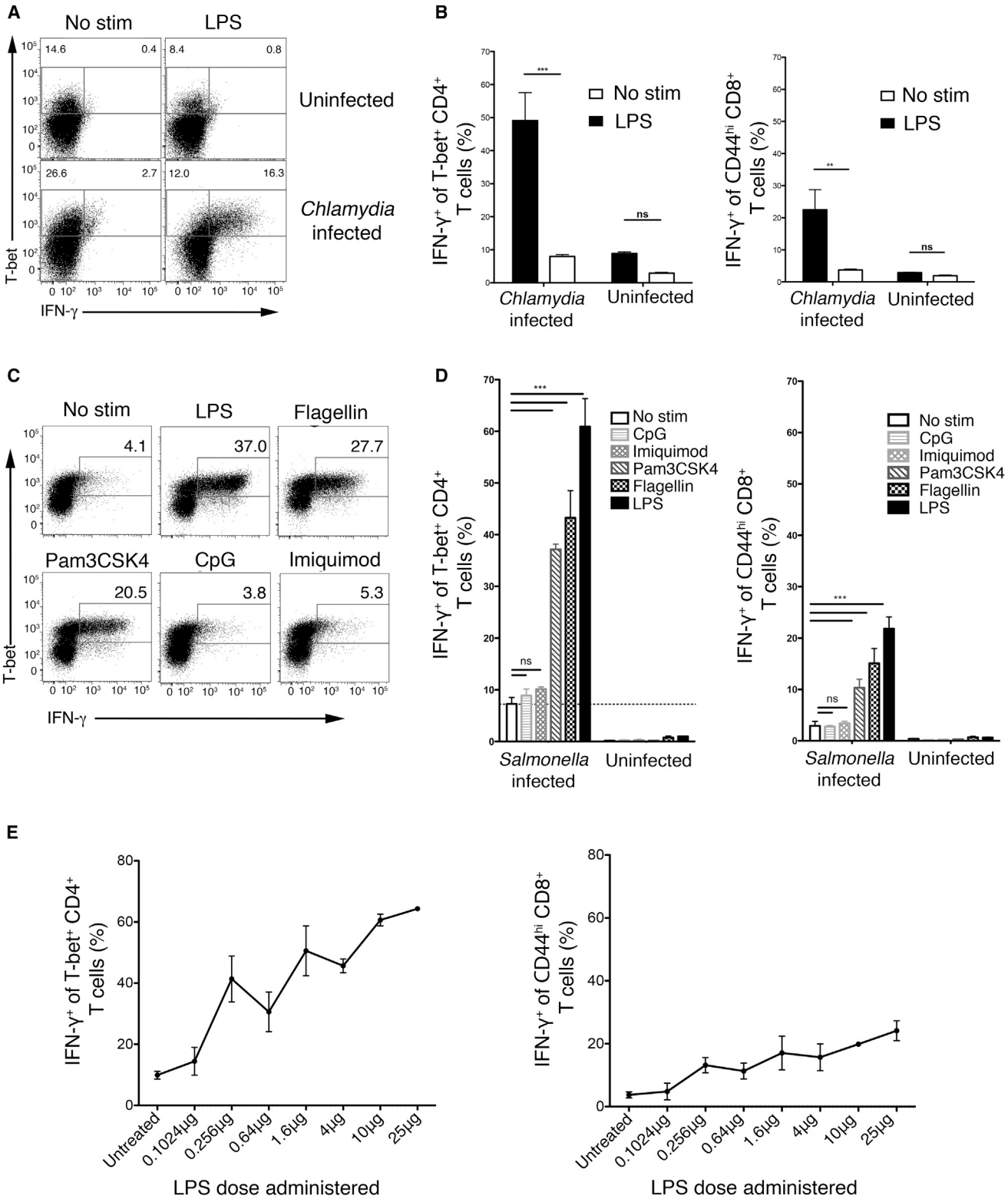


Figure 2. Amplification of CD4+ Th1 and CD8+ T Cell Responses Occurs with Multiple Innate Ligands and in Multiple Intramacrophage Infections

C57BL/6 mice were infected i.v. with 1 × 10^7 *Chlamydia muridarum* EBs or 5 × 10^5 *Salmonella*. One (*Chlamydia*) or 2 (*Salmonella*) weeks later, infected or uninfected mice were injected i.v. with LPS, flagellin, CpG DNA, Imiquimod, or Pam3CSK4 and spleens were harvested 4 hours later for determining IFN-γ production.

(legend continued on next page)

CD4+ T Cell Expression of IL-18R and IL-33R Is Required for Maximal Innate Responses

Given the requirement for MyD88 expression in T cells, we generated mixed BM chimeras to examine various cytokine receptors that utilize this adaptor molecule in downstream signaling (IL-1R, IL-18R, IL-33R, and IFN-γR). Loss of IL-1R or IFN-γR expression had no effect on the innate CD4+ or CD8+ T cell responses to LPS in *Salmonella*-infected mice (Figures 4A and 4B and Figures S3C and S3D). In marked contrast, CD4+ or CD8+ T cell expression of IL-18R was essential for rapid amplification of IFN-γ production in response to LPS stimulation (Figure 4C and Figure S3E). Interestingly, although IL-33 is usually associated with Th2 cell responses (Lloyd, 2010), T-bet+ CD4+ T cells lacking the expression of IL-33R demonstrated consistently lower IFN-γ responses to LPS (Figure 4D). Together, these data indicate that effector CD4+ Th1 cells respond directly to IL-18 and IL-33 in order to maximize IFN-γ production in the presence of innate stimuli.

LPS Induction of IL-18 Requires Inflammasome Activity in *Salmonella*-Infected Mice

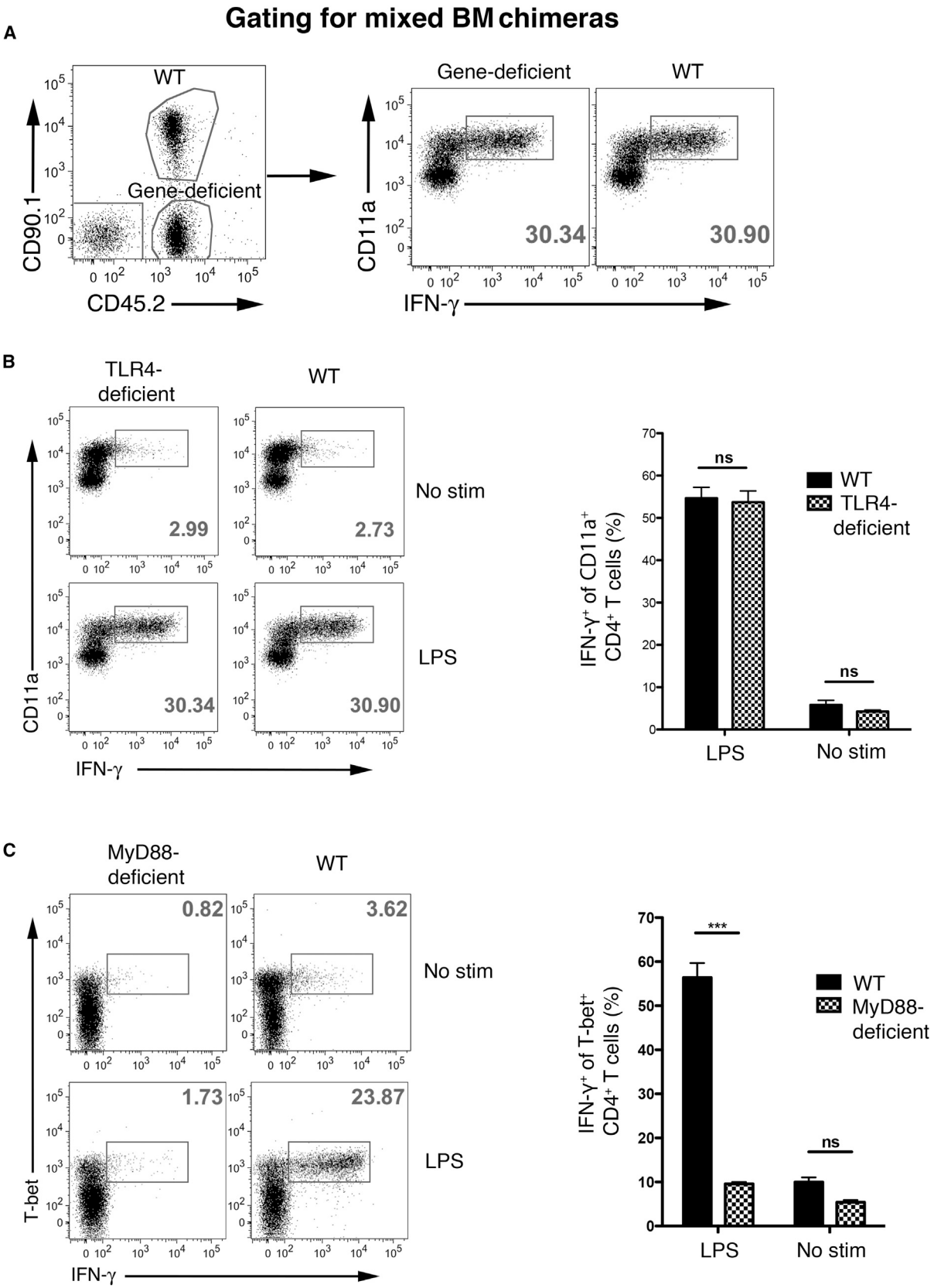
Given the requirement for T cell expression of IL-18R and IL-33R, we examined cytokine production in the spleen and liver during innate stimulation with LPS. By quantitative RT-PCR (qRT-PCR) analysis, a modest increase in IFN-γ mRNA was detected in the liver and spleen of uninfected mice that had been injected with LPS (Table 1). However, LPS injection of uninfected mice had little effect on IL-12, IL-18, or IL-33 mRNA in the spleen and only modestly increased these mRNAs in the liver (Table 1). In contrast, injection of LPS into *Salmonella*-infected mice (day 14 postinfection) caused a notable increase in spleen and liver IFN-γ, IL-12, IL-18, and IL-33 mRNA, and the IL-33 increase was particularly prominent in the liver (Table 1). At the protein level, injection of LPS into *Salmonella*-infected mice caused a rapid 300-fold increase in IFN-γ and a 35-fold increase in IL-18 in serum (Figures 5A and 5B). In contrast, IL-12 and IL-33 concentrations were below the level of detection in the sera of *Salmonella*-infected mice, even after injection with LPS (data not shown), suggesting that the production of these cytokines is restricted to infected tissue or subject to additional posttranscriptional controls. Injection of flagellin or Pam3CSK4 into *Salmonella*-infected mice also caused a marked increase in serum IFN-γ and IL-18, whereas injection of Imiquimod did not (Figures 5C and 5D), supporting the observation in Figure 1 that T cells can respond to LPS, flagellin, or Pam3CSK4 stimulation, but not Imiquimod stimulation.

A requirement for NLRC4 in flagellin-mediated noncognate stimulation of CD8+ T cells has previously been reported (Kupz et al., 2012), suggesting that direct recognition of injected flagellin by inflammasome components occurs. Because NLRC4 and NLRP3 play redundant roles in the recognition of *Salmonella* (Broz et al., 2010), we examined whether NLRC4 and NLRP3 were required for noncognate stimulation of CD4+ T cells by LPS or flagellin. Whereas the injection of WT mice with flagellin or LPS induced a rapid increase in serum IL-18, this same response was markedly lower in mice deficient in both NLRC4 and NLRP3 (Figure 5E). This reduced IL-18 production in response to flagellin or LPS correlated with a severely curtailed innate CD4+ T cell response to either stimulus in mice lacking NLRC4 and NLRP3 (Figure 5F). A similar requirement for NLRC4 and NLRP3 in the induction of IFN-γ by CD8+ T cells was also observed (Figure S4). A requirement for NLRC4 and NLRP3 in the innate response of CD4+ T cells to LPS suggested that inflammasome stimulation by live bacteria is required for maximal CD4+ T cell responses to occur. However, CD4+ and CD8+ T cells in mice infected with flagellin-deficient *Salmonella* responded normally to innate stimulation by ultrapure LPS (Figure 5G and Figure S4B), indicating that bacterial ligands other than flagellin can also drive the inflammasome activation required for innate T cell stimulation. Together, these data indicate that rapid production of IL-18 in vivo is a critical regulatory checkpoint for noncognate CD4+ and CD8+ T cell responses to inflammatory stimuli and that this process requires TLR recognition of bacterial ligands and NLR activation in infected mice.

Noncognate Stimulation of T Cells Contributes to Bacterial Clearance

In *Salmonella*-infected mice, depletion of CD4+ T cells severely limited bacterial clearance from the spleen and liver (Figures 6A and 6B), confirming the importance of CD4+ T cells to host protective immunity (Nauciel, 1990). Depletion of CD8+ T cells also hindered bacterial clearance (Figures 6A and 6B), consistent with a secondary role for CD8+ T cells in host protection (Lee et al., 2012a; Nauciel, 1990). Although both CD4+ and CD8+ T cells participate in *Salmonella* clearance, the contribution of cognate versus noncognate stimulation is currently unknown. In order to assess the in vivo relevance of noncognate T cell activation, we examined the course of *Salmonella* infection in loxP-*Myd88* mice expressing Cre-recombinase driven by the *Lck* promoter (*Myd88*$^{fl/fl}$ *Lck-cre* mice). Early clearance of *Salmonella* was unaffected in *Myd88*$^{fl/fl}$ *Lck-cre* mice (data not shown), consistent with the dependence of early control on the innate immune compartment (Griffin and McSorley, 2011). However, beginning around 3 weeks postinfection, approximately 1 in 6 *Myd88*$^{fl/fl}$ *Lck-cre* mice succumbed to primary *Salmonella* infection (Figure 6C). Furthermore, at 5 weeks postinfection, the spleens of the remaining *Myd88*$^{fl/fl}$ *Lck-cre* mice displayed

(A and B) Representative flow cytometry plots (A) and bar graphs (B) of combined data show CD44 or intracellular T-bet and IFN-γ staining in *Chlamydia*-infected mice after gating on CD4+ or CD8+ cells as indicated.
(C and D) Representative flow cytometry plots (C) and bar graphs (D) of combined data show CD44 or intracellular T-bet and IFN-γ staining in *Salmonella*-infected mice after gating on CD4+ or CD8+ cells as indicated.
(E) Graph showing the percentage of IFN-γ+ cells after intravenous LPS administration at various doses. CD4+ cells are shown as a percentage of the total T-bet+CD4+ cells producing IFN-γ, and CD8+ cells are shown as a percentage of the total CD44^{hi}CD8+ cells producing IFN-γ+. Error bars represent the mean ± SEM.
(B and D) Statistical significance was determined by two-way ANOVA with a Bonferroni posttest. ***$p < 0.005$, **$p < 0.01$, or $p > 0.05$ (ns). All experiments contained at least three mice per group and were conducted at least twice. Error bars represent the mean ± SEM.
See also Table S1.



(legend on next page)

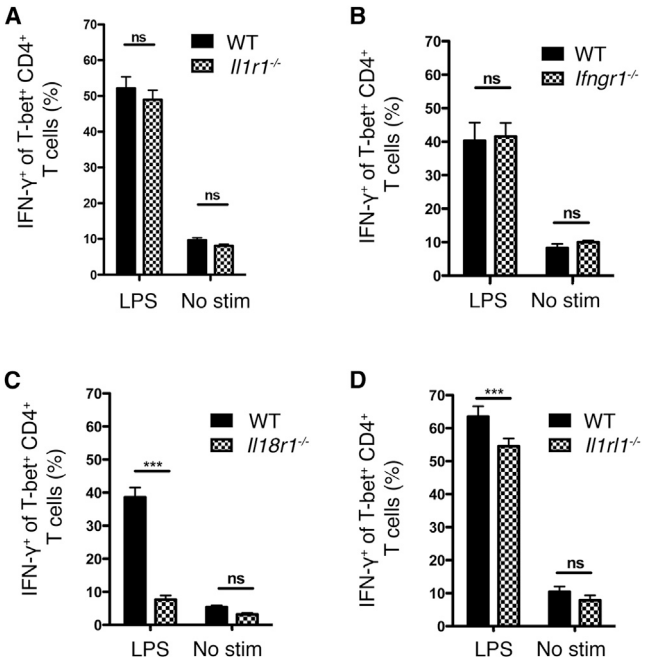


Figure 4. Maximal Th1 Cell Stimulation by LPS Requires T-Cell-Intrinsic Expression of IL-18R and IL-33R

Mixed BM chimeras were generated, infected, and stimulated as described in Figure 3. A graph showing the percentage of IFN-γ+ Th1 cells in WT versus gene-deficient CD4+ T cells is shown for *Il1r1*$^{-/-}$ (A), *Ifngr1*$^{-/-}$ (B), *Il18r1*$^{-/-}$ (C), and *Il1rl1*$^{-/-}$ (IL33R) (D) mixed BM chimeras. All experiments included at least three mice per group and were performed at least twice with similar results. Statistical analyses were performed by two-way repeated-measures ANOVA with a Bonferroni posttest. Error bars represent the mean ± SEM. ***$p < 0.005$, **$p < 0.01$, *$p < 0.05$, and $p > 0.05$ (ns).

See also Figure S3.

Table 1. LPS Stimulation Leads to Increased mRNA Expression of IFN-γ, IL-12, IL-18, and IL-33

	Effect of LPS (Relative to That on Unstimulated Controls)			
C57BL/6 Mice	IFN-γ	IL-12	IL-18	IL-33
Spleen				
Uninfected	1	1	1	1
Uninfected + LPS	8.67	0.99	1.30	1.60
Salmonella-infected	1	1	1	1
Salmonella-infected + LPS	22.02	4.52	2.26	5.35
Liver				
Uninfected	1	1	1	1
Uninfected + LPS	11.07	1.47	1.96	6.16
Salmonella-infected	1	1	1	1
Salmonella-infected + LPS	27.9	5.36	2.6	16.2

C57BL/6 mice were infected i.v. with 5 × 10^5 *Salmonella*, and 2 weeks later, infected or uninfected mice were injected i.v. with LPS. Spleens and livers were harvested 4 hours later, and qRT-PCR was performed on extracted mRNA. Numbers shown are normalized to *Gapdh* expression and indicate the fold-change increase over unstimulated mice. See also Table S2.

significantly higher bacterial loads than did WT and heterozygous littermate controls (Figures 6D and 6E). Thus, the ability of T cells to respond to MyD88-dependent signals is an important component in resolving infection with an intramacrophage pathogen.

DISCUSSION

Approximately 50 years ago, George MacKaness reported that *Brucella*-infected mice display transient cross-reactive protection against other intramacrophage pathogens and that this effect correlates with the induction of a cellular immune response (MacKaness, 1964). The mechanistic basis of this cross-bacterial protection is usually understood to derive from the indiscriminate killing activity of activated macrophages via reactive oxygen and nitrogen species (Fang, 2004). A lack of target specificity in the efferent phase of host defense against intramacrophage pathogens is likely to have evolved to combat superinfection or coinfections and is held in check by TCR-mediated clonal expansion and effector development (Jenkins et al., 2001). However, it has become apparent that after clonal expansion, effector T cells can be activated by a variety of noncognate stimuli (Beadling and Slifka, 2005; Berg et al., 2002; Freeman et al., 2012; Guo et al., 2012; Kupz et al., 2012; Soudja et al., 2012), suggesting that cognate stimulation might not be required in infected tissues. However, the role of noncognate T cell stimulation in defense against intramacrophage pathogens is poorly understood.

Several prior studies have documented the expansion of a large population of activated CD4+ T cells in mice infected with *Salmonella* (Mittrücker et al., 2002; Srinivasan et al., 2004; Srinivasan et al., 2007). Although antigen-specific T cells can be visualized in this infection model with the use of tetramers (Lee et al., 2012b; McSorley et al., 2002; Moon et al., 2007), these populations account for a tiny fraction of the overall polyclonal Th1 cell response to *Salmonella*. Our data show that a large population of T-bet+CD4+ T cells expands and contracts in concert

Figure 3. Innate Th1 Cell Stimulation by LPS Requires T-Cell-Intrinsic Expression of MyD88

CD45.1+ C57BL/6 mice were irradiated and reconstituted with a mixture of BM from wild-type (WT) (CD90.1+CD45.2+) and TLR4- or MyD88-deficient (CD90.2+CD45.2+) mice. After immune reconstitution, BM chimeras were infected i.v. with 5 × 10^5 *Salmonella* and response to LPS was determined in the spleen. (A) Gating strategy used for examining donor WT and gene-deficient CD4+ T cells in mixed BM chimeras. Splenocytes were gated as live, singlet, and CD4+CD8− cells and then gated as shown in the left panel for congenic markers. Each gated cell population was assessed for IFN-γ production and either CD11a (TLR4) or T-bet (MyD88).

(B and C) Representative flow cytometry plots and a graph of combined data are shown for TLR4-deficient (B) and MyD88-deficient (C) chimeras. All experiments included at least three mice per group and were performed at least twice with similar results. Statistical analyses were performed by two-way repeated-measures ANOVA with a Bonferroni posttest. Error bars represent the mean ± SEM. ***$p < 0.005$, **$p < 0.01$, *$p < 0.05$, and $p > 0.05$ (ns).

See also Figure S2.

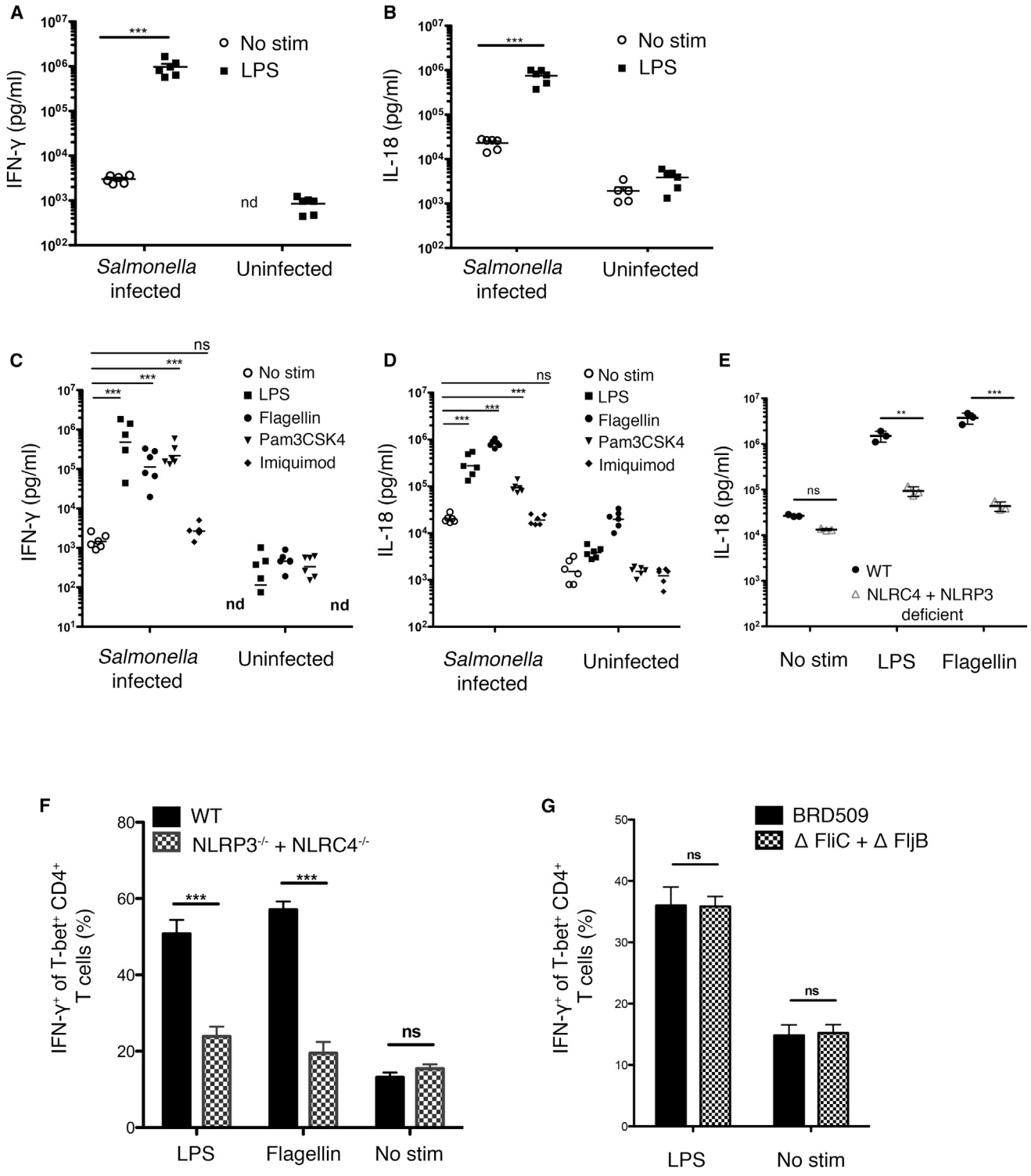


Figure 5. Production of IL-18 and Amplification of CD4+ Th1 Cell Responses Requires Inflammasome Components

WT or mice deficient in NLRP3 and NLRC4 were infected i.v. with 5 × 10^5 *Salmonella* (BRD509 or flagellin-deficient BRD509), and 2 weeks later, the splenic response to LPS, flagellin, Pam3CSK4, and Imiquimod injection was determined 4 hours after stimulation.

(A–E) Plots show serum cytokine concentrations as determined by ELISA for IFN-γ (A and C) and IL-18 (B, D, and E). Data shown were pooled from two experiments. nd, none detected.

(F) IFN-γ production by Th1 cells in *Salmonella*-infected WT mice or mice lacking NLRP3 and NLRC4 after administration of LPS or flagellin. Data were pooled from two experiments each with more than three mice per group. Error bars represent the mean ± SEM.

(legend continued on next page)

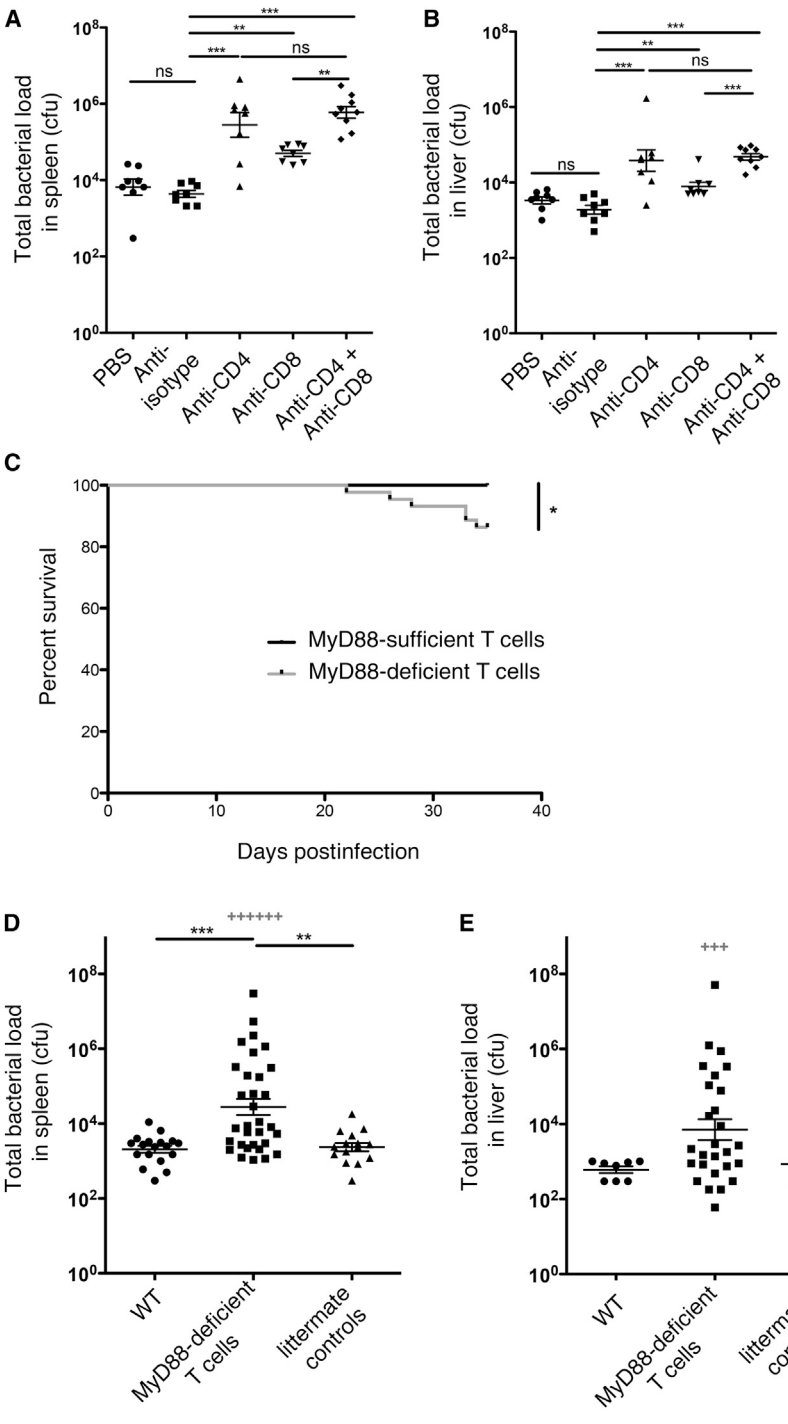


Figure 6. Mice Lacking T Cell Expression of MyD88 Are More Susceptible to *Salmonella* Infection

T-cell-depleted (A and B) or WT, *Myd88*$^{fl/fl}$ *Lck-cre* homozygous, and *Myd88*$^{fl/WT}$ *Lck-cre* heterozygous (C–E) mice were infected i.v. with 5 × 10^5 *Salmonella*.

(A and B) Mice given PBS, anti-CD4, anti-CD8, both, or an isotype control antibody were sacrificed at day 30 postinfection, and bacterial loads in the spleen (A) and liver (B) were determined. For *Myd88*$^{fl/fl}$ *Lck-cre* and *Myd88*$^{fl/WT}$ *Lck-cre* mice, survival (C) or bacterial burdens in the spleen (D) and liver (E) were monitored 5 weeks after infection. Error bars represent the mean ± SEM.

(C) Survival curve after *Salmonella* infection. The graph shows combined data from at least three experiments with at least ten mice per group. WT and littermate control *Myd88*$^{fl/WT}$ *Lck-cre* mice are grouped together. Statistical significance was observed by the log-rank (Mantel-Cox) test.

(D and E) Bacterial loads in the spleen (D) and liver (E) of individual mice 5 weeks postinfection with BRD509 in *Myd88*$^{fl/fl}$ *Lck-cre*, *Myd88*$^{fl/WT}$ *Lck-cre*, and WT mice. Gray plus signs show the number of mice that became moribund prior to assessment of bacterial burdens at the 5-week time point. Statistical significance was determined on log-transformed data by one-way ANOVA. Error bars represent the mean ± SEM.

***$p < 0.005$, **$p < 0.01$, *$p < 0.05$, and $p > 0.05$ (ns).

with tissue bacterial loads and that these CD4+ T cells are required for protective immunity. Furthermore, these expanded Th1 cells transiently gain the ability to secrete IFN-γ when the host is confronted with innate stimuli such as bacterial LPS, flagellin, or Pam3CSK4. The fact that Imiquimod and CpG failed to induce effector responses in this model could mean that cell-surface TLRs are more efficient at amplifying the Th1 cell response; however, it is also possible that endosomal TLRs can mediate the same effect if these ligands are internalized, as could occur during infection. Indeed, given the data above, it seems likely that numerous TLR ligands will be capable of amplifying CD4+ T cell responses and that the individual microbe-associated molecular patterns (MAMPs) involved will vary depending on the infection model. The ability of LPS to stimulate CD4+ T cells during *Chlamydia* infection also supports the concept that noncognate T cell stimulation is common to intramacrophage infection models.

An important feature of this CD4+ response to innate stimuli is that it is maximally effective during the period of active infection. Therefore, during active *Salmonella* infection, effector CD4+ Th1 cells have a reduced threshold for stimulation and IFN-γ

(G) IFN-γ production by Th1 cells after LPS stimulation of mice infected with BRD509 (flagellin-expressing) or a flagellin-deficient mutant of BRD509 (ΔfliCΔfljB). Data are representative of at least two experiments with at least three mice per group. Statistical significance was determined by two-way ANOVA with a Bonferroni posttest. Error bars represent the mean ± SEM.

***$p < 0.005$, **$p < 0.01$, *$p < 0.05$, and $p > 0.05$ (ns). See also Figure S4.

production can be elicited immediately when Th1 cells encounter the cytokine milieu in inflamed tissues. The ability to induce effector functions without a requirement for recognizing cognate antigen and MHC on an infected cell has probably evolved to enhance the efficiency of the adaptive response to infection. This might well be a common feature of the T cell response to many microbial pathogens. Surprisingly, the heightened response of $CD4^+$ T cells to LPS-induced inflammation during infection required the expression of NLRC4 and NLRP3. The most likely explanation for this finding is that inflammasome sensing of intracellular bacterial components synergizes with TLR recognition of MAMPs to induce cytokine production. Indeed, the MAMPs that efficiently induce IFN-γ production from $CD4^+$ and $CD8^+$ T cells are also able to rapidly increase concentrations of circulating IL-18. This model of T cell stimulation is distinct from that in a previous study (Kupz et al., 2012), which suggested that injected flagellin has to be transported into dendritic cells for cytosolic recognition by NLRC4 to thus induce noncognate stimulation of memory $CD8^+$ T cells. Indeed, our data demonstrate that flagellin expression by *Salmonella* is not essential for noncognate T cell stimulation to occur, although this finding might also reflect other differences between these two models.

Having a reduced threshold for effector T cell stimulation is likely to provide an important evolutionary advantage when the host combats rapidly dividing pathogens such as *Salmonella*. It also suggests that activated $CD4^+$ T cells might provide cross-reactive immunity against coinfections, although we have not yet examined this possibility directly. Although our model required the injection of MAMPs to uncover the mechanism of noncognate T cell stimulation, this same pathway most likely contributes to the endogenous production of IFN-γ in infected tissues. Indeed, the fact that *Myd88*$^{fl/fl}$ *Lck-cre* mice had difficulty resolving primary infection with *Salmonella* supports an important role for this noncognate pathway in primary clearance. Further studies will be required for determining whether noncognate stimulation can provide protection during coinfections or, conversely, whether this same innate inflammatory mechanism plays a role in immune-mediated pathology (Raetz et al., 2013).

Various inflammatory cytokines and TLR ligands are known to activate effector T cells in vitro and in vivo (Beadling and Slifka, 2005; Berg et al., 2002; Freeman et al., 2012; Guo et al., 2012; Kupz et al., 2012; Soudja et al., 2012). Indeed, a role for MAMP-induced IL-18 has previously been described in the stimulation of $CD8^+$ T cells and natural killer cells in mouse models of *Listeria* infection, LPS toxicity, and inflammation-induced T cell turnover (Lertmemongkolchai et al., 2001; Pien et al., 2002; Tough et al., 2001). For Th1 cells, IL-12 and IL-18 can stimulate IFN-γ production in vivo (Robinson et al., 1997; Yoshimoto et al., 1998), whereas IL-33R stimulation in Th2 cells can induce IL-13 (Guo et al., 2009). However, the contribution of these noncognate T cell responses to the resolution of infection in vivo is unclear. Our data on BM chimera show that $CD4^+$ T cell expression of MyD88, IL-18R, and IL-33R has an impact on maximal IFN-γ production but that intrinsic IL-1R or TLR4 expression is not required. Thus, $CD4^+$ Th1 cells most likely respond to a mix of IL-18 and IL-33 in infected tissue, given that mRNA for both of these cytokines was increased in the liver and spleen after LPS injection. Although IL-33R makes a modest contribution to the splenic Th1 cell response, this might be because at high concentrations of IL-18R signaling, this pathway becomes largely redundant and thus masks the proinflammatory role of IL-33. Future experiments will determine whether IL-33 can play a more prominent role in response to other TLR ligands or under physiological conditions.

Together, our data provide a mechanistic framework for understanding noncognate stimulation of $CD4^+$ and $CD8^+$ T cells and the contribution of this response in defense against intramacrophage pathogens. Greater understanding of this pathway might lead to the development of effective immunomodulatory therapeutics for the treatment of persistent infection, as well as highlight potential mediators of immunopathology for more targeted immunosuppressive interventions.

EXPERIMENTAL PROCEDURES

Mice

C57BL/6, B6.SJL-Ptprc^{a}Pepc^b/BoyJ, B6.PL-Thy1^a/CyJ, TLR4-deficient, and IL-18R-deficient mice were purchased from The Jackson Laboratory or the National Cancer Institute at 6–8 weeks of age. MyD88-, IL-1R-, and IFN-γR-deficient C57BL/6 mice were obtained from Dr. Jenkins (University of Minnesota) and Dr. Way (University of Cincinnati) and bred in our animal facility. IL-33R-deficient C57BL/6 mice were kindly provided by Dr. Bryce (Northwestern University). Mice deficient in NLRC4 and NLRP3 were maintained at Stanford University (Broz et al., 2010). T-cell-specific MyD88-deficient mice were generated by a cross between *Lck-cre* mice and *Myd88*$^{fl/fl}$ mice purchased from The Jackson Laboratory. Mice were genotyped by PCR according to protocols provided by The Jackson Laboratory. All animal procedures were approved by the Institutional Animal Care and Use Committee (16612) at the University of California, Davis.

Bacterial Strains and Infection

Salmonella strains utilized in this study are listed in Table S1. C57BL/6 mice were infected intravenously (i.v.) with 5×10^5 BRD509 Δ*aroAD* strain of *Salmonella enterica* serovar Typhimurium or an aflagellate BRD509 mutant SPN529 that was constructed as described in the Supplemental Experimental Procedures. *Salmonella* were grown overnight in static Luria-Bertani broth cultures at 37°C before being washed and resuspended in PBS. Bacterial concentration was estimated by spectrophotometry at OD600 for preparation of the challenge inoculum. The actual dose administered to mice was determined by serial dilution and plating on MacConkey agar plates. *Chlamydia muridarum* strain Nigg II was purchased from ATCC and cultured in HeLa 229 cells in Dulbecco's modified Eagle's medium (Life Technologies) supplemented with 10% fetal bovine serum. Elementary bodies (EBs) were purified by discontinuous density gradient centrifugation as previously described and stored at −80°C (Scidmore, 2005). The number of inclusion-forming units of purified EBs was determined by infection of HeLa 229 cells and enumeration of inclusions stained with anti-*Chlamydia* MOMP. A fresh aliquot was thawed and used for every infection experiment. C57BL/6 mice were injected i.v. with 1×10^7 *C. muridarum*.

Determination of Bacterial Loads

Mice were euthanized, and the indicated organs were collected in Hank's balanced salt solution (HBSS) or PBS (both GIBCO) on ice, homogenized, and reconstituted in a known volume of HBSS. Samples were mixed thoroughly, and serial 1:10 dilutions were plated on MacConkey agar plates (Becton Dickinson), incubated overnight at 37°C, and enumerated the following day for calculation of the number of colony-forming units in the total organ.

BM Chimeras

Mixed BM chimeras were generated by initial irradiation of 45.1$^+$ congenic mice (B6.SJL) (1,000 rads irradiation by cesium source). The following day, BM was isolated from congenic CD90.1$^+$ (B6.PL) and genetically deficient mouse

femurs and tibias and cells were counted by hematocytometer and combined at a 1:1 ratio before being administered i.v. (4×10^6 total cells) in 200 μl 1× PBS. After BM transfer, chimeras were maintained on antibiotics for at least 4 weeks, and blood was collected 4 weeks later for flow cytometric analysis of immune reconstitution. Chimeras were infected 6–8 weeks after administration of BM and at least 2 weeks after discontinuation of antibiotic treatment.

In Vivo Stimulation with TLR Ligands

Ultrapure LPS from *E. coli* strain EH100Rα (Alexis, TLRgrade), purified bacterial flagellin, endotoxin-free Pam3CSK4, CpG DNA (ODN1585), and Imiquimod (Invivogen) was diluted in 1× PBS (UltraPure PBS, GIBCO) and administered i.v. to mice. Spleens and livers were harvested from infected or uninfected mice 4 hr or less after the administration of MAMPs. Flagellin was purified from an LPS-deficient X4700 strain of *S.* Typhimurium according to a modified acid-shock protocol and passed multiple times through Detoxi-Gel columns (Thermo Scientific) for removal of residual endotoxin, as previously described (Salazar-Gonzalez et al., 2007).

Flow Cytometry

For flow cytometry, cells were prepared as a single-cell suspension of between 1×10^6 and 8×10^6 cells/ml and stained with various antibodies from eBioscience, Becton Dickinson (BD), or Tonbo Biosciences for 30 min to 1 hr in Fc block on ice. Intracellular cytokine or transcription factor staining was performed with the Foxp3 intranuclear staining kit from eBioscience, as recommended by the manufacturer. Stained cells were analyzed with a FACSCanto, FACSAria, or Fortessa (BD) with appropriate compensation controls, and flow cytometry data were analyzed with FlowJo software (TreeStar).

RNA Preparation and qRT-PCR

RNA was extracted from cryopreserved spleens and livers according to the manufacturer's instructions with the use of TRIzol reagent (Ambion) and was quantified with a Nanodrop spectrophotometer (Thermo Scientific). RNA was DNase treated with a DNA-free kit (Ambion), and a SensiFAST SYBR Hi-ROX One-Step Kit (Bioline) was used for both cDNA synthesis and qPCR reactions (100 ng total RNA per reaction). qRT-PCR was performed on a 7900HT Fast Real-Time PCR System (Applied Biosystems) in a 96-well plate, and triplicate data were analyzed in Microsoft Excel by the comparative cycle-threshold (Ct) method (Applied Biosystems) with GAPDH as the control. Data are expressed as the fold change of stimulated over unstimulated ΔCt. Primer sequences and references are listed in Table S2.

Serum Cytokine ELISAs

For serum preparation, blood was collected from retro-orbitally exsanguinated, anesthetized mice that had been infected or stimulated as indicated, incubated on ice to allow clotting, and then centrifuged, serum harvested, and stored at −20°C. Cytokine ELISAs for IFN-γ, IL-12, and IL-33 were performed according to the instructions provided by the Ready-Set-Go kit (eBioscience), and concentrations were determined by the protein standard provided. For the IL-18 ELISA, the same standard protocol was followed with the use of capture and detection antibodies (Medical and Biological Labs) as recommended by the manufacturer with rmIL-18 as a standard (R&D). After substrate was added, plates were read at 450 nm with a microplate reader (Spectra Max M2, Molecular Devices) and analyzed in Microsoft Excel.

Antibody Depletion of CD4+ and CD8+ T Cells

Loss of CD4+, CD8+, or CD4+ and CD8+ T cells was examined in vivo by depletion of cells with indicated rat anti-mouse monoclonal antibodies (BioXCell). Antibodies were administered intraperitoneally twice per week starting at day 7, and depletions were maintained until euthanization. The initial doses (days 7 and 10) contained 200 μg of antibody per mouse, and all subsequent doses contained 300 μg per mouse. Maintenance of depletion was monitored by flow cytometry on blood collected once per week from the lateral tail vein.

Statistical Analyses

All statistical analyses were performed as described in the figure legends with GraphPad Prism version 5. All error bars represent the mean ± SEM. ***$p < 0.005$, **$p < 0.01$, *$p < 0.05$, or $p > 0.05$ (ns).

SUPPLEMENTAL INFORMATION

Supplemental Information include Supplemental Experimental Procedures, four figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.immuni.2013.12.013.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health (NIH) to S.J.M. (AI055743, AI076278, and AI056172), A.J.B. (AI076246), and D.M. (AI095396 and AI089722). H.O. was supported by an NIH T32 training grant (AI60555), and O.P. was supported by a Vietnam Education Foundation fellowship. The authors would like to thank Rebecca A. Elsner for her feedback on this manuscript and helpful discussions.

Received: August 22, 2013
Accepted: December 23, 2013
Published: February 6, 2014

REFERENCES

Beadling, C., and Slifka, M.K. (2005). Differential regulation of virus-specific T-cell effector functions following activation by peptide or innate cytokines. Blood *105*, 1179–1186.

Berg, R.E., Cordes, C.J., and Forman, J. (2002). Contribution of CD8+ T cells to innate immunity: IFN-gamma secretion induced by IL-12 and IL-18. Eur. J. Immunol. *32*, 2807–2816.

Broz, P., and Monack, D.M. (2011). Molecular mechanisms of inflammasome activation during microbial infections. Immunol. Rev. *243*, 174–190.

Broz, P., Newton, K., Lamkanfi, M., Mariathasan, S., Dixit, V.M., and Monack, D.M. (2010). Redundant roles for inflammasome receptors NLRP3 and NLRC4 in host defense against Salmonella. J. Exp. Med. *207*, 1745–1755.

Caramalho, I., Lopes-Carvalho, T., Ostler, D., Zelenay, S., Haury, M., and Demengeot, J. (2003). Regulatory T cells selectively express toll-like receptors and are activated by lipopolysaccharide. J. Exp. Med. *197*, 403–411.

Fang, F.C. (2004). Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. Nat. Rev. Microbiol. *2*, 820–832.

Farris, C.M., and Morrison, R.P. (2011). Vaccination against Chlamydia genital infection utilizing the murine C. muridarum model. Infect. Immun. *79*, 986–996.

Freeman, B.E., Hammarlund, E., Raué, H.P., and Slifka, M.K. (2012). Regulation of innate CD8+ T-cell activation mediated by cytokines. Proc. Natl. Acad. Sci. USA *109*, 9971–9976.

Griffin, A.J., and McSorley, S.J. (2011). Development of protective immunity to Salmonella, a mucosal pathogen with a systemic agenda. Mucosal Immunol. *4*, 371–382.

Guo, L., Wei, G., Zhu, J., Liao, W., Leonard, W.J., Zhao, K., and Paul, W. (2009). IL-1 family members and STAT activators induce cytokine production by Th2, Th17, and Th1 cells. Proc. Natl. Acad. Sci. USA *106*, 13463–13468.

Guo, L., Junttila, I.S., and Paul, W.E. (2012). Cytokine-induced cytokine production by conventional and innate lymphoid cells. Trends Immunol. *33*, 598–606.

Jenkins, M.K., Khoruts, A., Ingulli, E., Mueller, D.L., McSorley, S.J., Reinhardt, R.L., Itano, A., and Pape, K.A. (2001). In vivo activation of antigen-specific CD4 T cells. Annu. Rev. Immunol. *19*, 23–45.

Kupz, A., Guarda, G., Gebhardt, T., Sander, L.E., Short, K.R., Diavatopoulos, D.A., Wijburg, O.L., Cao, H., Waithman, J.C., Chen, W., et al. (2012). NLRC4 inflammasomes in dendritic cells regulate noncognate effector function by memory CD8+ T cells. Nat. Immunol. *13*, 162–169.

Kwok, W.W., Tan, V., Gillette, L., Littell, C.T., Soltis, M.A., LaFond, R.B., Yang, J., James, E.A., and DeLong, J.H. (2012). Frequency of epitope-specific naive CD4(+) T cells correlates with immunodominance in the human memory repertoire. J. Immunol. *188*, 2537–2544.

Lee, S.J., Dunmire, S., and McSorley, S.J. (2012a). MHC class-I-restricted CD8 T cells play a protective role during primary Salmonella infection. Immunol. Lett. *148*, 138–143.

Lee, S.J., McLachlan, J.B., Kurtz, J.R., Fan, D., Winter, S.E., Baumler, A.J., Jenkins, M.K., and McSorley, S.J. (2012b). Temporal expression of bacterial proteins instructs host CD4 T cell expansion and Th17 development. PLoS Pathog. *8*, e1002499.

Lertmemongkolchai, G., Cai, G., Hunter, C.A., and Bancroft, G.J. (2001). Bystander activation of CD8+ T cells contributes to the rapid production of IFN-gamma in response to bacterial pathogens. J. Immunol. *166*, 1097–1105.

Lloyd, C.M. (2010). IL-33 family members and asthma - bridging innate and adaptive immune responses. Curr. Opin. Immunol. *22*, 800–806.

MacKaness, G.B. (1964). The Immunological Basis of Acquired Cellular Resistance. J. Exp. Med. *120*, 105–120.

McLachlan, J.B., Catron, D.M., Moon, J.J., and Jenkins, M.K. (2009). Dendritic cell antigen presentation drives simultaneous cytokine production by effector and regulatory T cells in inflamed skin. Immunity *30*, 277–288.

McSorley, S.J., Asch, S., Costalonga, M., Reinhardt, R.L., and Jenkins, M.K. (2002). Tracking salmonella-specific CD4 T cells in vivo reveals a local mucosal response to a disseminated infection. Immunity *16*, 365–377.

Mittrücker, H.W., Köhler, A., and Kaufmann, S.H. (2002). Characterization of the murine T-lymphocyte response to Salmonella enterica serovar Typhimurium infection. Infect. Immun. *70*, 199–203.

Moon, J.J., Chu, H.H., Pepper, M., McSorley, S.J., Jameson, S.C., Kedl, R.M., and Jenkins, M.K. (2007). Naive CD4(+) T cell frequency varies for different epitopes and predicts repertoire diversity and response magnitude. Immunity *27*, 203–213.

Müller, A.J., Filipe-Santos, O., Eberl, G., Aebischer, T., Späth, G.F., and Bousso, P. (2012). CD4+ T cells rely on a cytokine gradient to control intracellular pathogens beyond sites of antigen presentation. Immunity *37*, 147–157.

Nauciel, C. (1990). Role of CD4+ T cells and T-independent mechanisms in acquired resistance to Salmonella typhimurium infection. J. Immunol. *145*, 1265–1269.

Obar, J.J., and Lefrançois, L. (2010). Memory CD8+ T cell differentiation. Ann. N Y Acad. Sci. *1183*, 251–266.

Pien, G.C., Nguyen, K.B., Malmgaard, L., Satoskar, A.R., and Biron, C.A. (2002). A unique mechanism for innate cytokine promotion of T cell responses to viral infections. J. Immunol. *169*, 5827–5837.

Poo, W.J., Conrad, L., and Janeway, C.A., Jr. (1988). Receptor-directed focusing of lymphokine release by helper T cells. Nature *332*, 378–380.

Raetz, M., Hwang, S.H., Wilhelm, C.L., Kirkland, D., Benson, A., Sturge, C.R., Mirpuri, J., Vaishnava, S., Hou, B., Defranco, A.L., et al. (2013). Parasite-induced TH1 cells and intestinal dysbiosis cooperate in IFN-γ-dependent elimination of Paneth cells. Nat. Immunol. *14*, 136–142.

Reynolds, J.M., Pappu, B.P., Peng, J., Martinez, G.J., Zhang, Y., Chung, Y., Ma, L., Yang, X.O., Nurieva, R.I., Tian, Q., and Dong, C. (2010). Toll-like receptor 2 signaling in CD4(+) T lymphocytes promotes T helper 17 responses and regulates the pathogenesis of autoimmune disease. Immunity *32*, 692–702.

Robinson, D., Shibuya, K., Mui, A., Zonin, F., Murphy, E., Sana, T., Hartley, S.B., Menon, S., Kastelein, R., Bazan, F., and O'Garra, A. (1997). IGIF does not drive Th1 development but synergizes with IL-12 for interferon-gamma production and activates IRAK and NFkappaB. Immunity *7*, 571–581.

Salazar-Gonzalez, R.M., Srinivasan, A., Griffin, A., Muralimohan, G., Ertelt, J.M., Ravindran, R., Vella, A.T., and McSorley, S.J. (2007). Salmonella flagellin induces bystander activation of splenic dendritic cells and hinders bacterial replication in vivo. J. Immunol. *179*, 6169–6175.

Scidmore, M.A. (2005). Cultivation and Laboratory Maintenance of Chlamydia trachomatis. Curr. Protoc. Microbiol. *Chapter 11*, 1.

Soudja, S.M., Ruiz, A.L., Marie, J.C., and Lauvau, G. (2012). Inflammatory monocytes activate memory CD8(+) T and innate NK lymphocytes independent of cognate antigen during microbial pathogen invasion. Immunity *37*, 549–562.

Srinivasan, A., Foley, J., and McSorley, S.J. (2004). Massive number of antigen-specific CD4 T cells during vaccination with live attenuated Salmonella causes interclonal competition. J. Immunol. *172*, 6884–6893.

Srinivasan, A., Salazar-Gonzalez, R.M., Jarcho, M., Sandau, M.M., Lefrancois, L., and McSorley, S.J. (2007). Innate immune activation of CD4 T cells in salmonella-infected mice is dependent on IL-18. J. Immunol. *178*, 6342–6349.

Takeda, K., Tsutsui, H., Yoshimoto, T., Adachi, O., Yoshida, N., Kishimoto, T., Okamura, H., Nakanishi, K., and Akira, S. (1998). Defective NK cell activity and Th1 response in IL-18-deficient mice. Immunity *8*, 383–390.

Tough, D.F., Zhang, X., and Sprent, J. (2001). An IFN-gamma-dependent pathway controls stimulation of memory phenotype CD8+ T cell turnover in vivo by IL-12, IL-18, and IFN-gamma. J. Immunol. *166*, 6007–6011.

Yoshimoto, T., Takeda, K., Tanaka, T., Ohkusu, K., Kashiwamura, S., Okamura, H., Akira, S., and Nakanishi, K. (1998). IL-12 up-regulates IL-18 receptor expression on T cells, Th1 cells, and B cells: synergism with IL-18 for IFN-gamma production. J. Immunol. *161*, 3400–3407.

Zhu, J., Yamane, H., and Paul, W.E. (2010). Differentiation of effector CD4 T cell populations (*). Annu. Rev. Immunol. *28*, 445–489.