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TLR and B Cell Receptor Signals to B Cells Differentially Program Primary and Memory Th1 Responses to Salmonella enterica

Tom A. Barr,*† Sheila Brown,*† Pietro Mastroeni,‡,1 and David Gray*†,1

Protective Th1 responses to Salmonella enterica do not develop in the absence of B cells. Using chimeric mice, we dissect the early (innate) and late (cognate) contributions of B cells to Th programming. B cell-intrinsic MyD88 signaling is required for primary effector Th1 development, whereas Ag-specific BCR-mediated Ag presentation is necessary for the development of memory Th1 populations. Programming of the primary T cell response is BCR/B cell MHC II independent, but requires MyD88-dependent secretion of cytokines by B cells. Chimeras in which B cells lack IFN-γ or IL-6 genes make impaired Th1 or Th17 responses to Salmonella. The Journal of Immunology, 2010, 185: 2783–2789.

The main function of B cells is to make Abs that provide protection against potential pathogens. Sometimes, due to a failure of tolerance, B cells also make Abs that bind self-Ags and so cause autoimmune disease. For this reason, B cell depletion therapy is increasingly used in the rheumatology clinic (1). In some patients, the treatment is effective despite unchanged autoantibody titers (2), highlighting Ab-independent functions of B cells, including Ag presentation and cytokine secretion (3–5).

B cells present Ag very effectively only when they take up Ag via their BCR (6); however, their exact role as APCs in vivo remains unclear (7–12). B cells begin to contribute as APCs during the primary response (8, 11, 13); however, their major impact is thought to be in the reactivation of CD4 memory T cells (14–18). Cytokine production by B cells extends their sphere of influence to the development of lymphoid tissue microenvironments (19) and regulation of T cell responses (20–22). For instance, the secretion of IL-10 by B cells modulates T cell function (23, 24) to bring about resolution of inflammation in autoimmune disease models (25–27). Regulatory B cell activity has also been seen in infection models protecting against acute Th1 (24, 28) and chronic Th2 (29–31) inflammation.

In influencing T cell responses, B cells usually show a propensity to drive Th2 responses (32–35). Lund and colleagues (4) demonstrated that B cells can be polarized in their cytokine production in the same way as CD4 T cells, to become Be1 (36) and Be2 cells (37). More recently, cytokine production by B cells was shown to be necessary for the development of protective Ab-mediated Th2 immunity to Heligmosomoides polygyrus infection (38). As the Be2 cells were required to express IL-4R (38), this seemed to involve cross-talk between T cells and B cells rather than any exogenous pathogen-associated signal. Like other APCs, B cells express a broad range of TLR that upon ligation stimulate the secretion of cytokines (39). The direct stimulation by TLR ligands of cytokine production by B cells offers a means to influence at an early stage the differentiation of Th subsets.

To address this issue and to investigate differential roles of TCR and BCR in the programming potential of B cells, we chose an infection model in which both TLRs and BCR would be stimulated as the disease progressed and one that generates a strong Th1 response. Salmonella enterica serovar Typhimurium is a bacterium that resides within the phagosomal compartment of macrophages (40). The infection has a transient extracellular phase, during systemic dissemination (41). To combat the infection, macrophages must be activated by inflammatory cytokines (42, 43). Thus, Th1 CD4 responses are crucial for the clearance of the bacteria and for protective immunity upon reinfection (44, 45). B cells have two roles, first as producers of Abs (46, 47) and second, an Ab-independent role (48, 49). Thus, B cell-deficient (μMT) mice can clear infection; however, the Th1 response they develop is transient, and they are not protected from reinfection with a virulent strain (50).

In this study, we find that B cells contribute to the early phase of T cell programming via a MyD88-dependent mechanism and are required in a BCR-dependent process for the development of the memory T cell response. Thus, TLR activation of B cells optimizes the generation of the primary Th1 response, a process that does not require Ag presentation, but relies on B cell cytokine secretion. For the development of Th1 memory cells and hence protective immunity to Salmonella, BCR recognition and B cell Ag presentation are an absolute requirement.

Materials and Methods

Mice and generation of bone marrow chimeras

C57BL/6, B cell-deficient (μMT), MyD88−/−, BALB/c, Jp1D, and MD4 mice were aged 6–10 wk old at the start of experimental regimes. Mixed bone marrow chimeric mice were generated, as described previously (51). Briefly, host μMT mice were lightly irradiated with 8 Gy gamma-radiation. Mice were then reconstituted with 2 × 108 mixed-inoculum bone marrow cells (80% μMT marrow and 20% knockout or wild-type [WT] marrow). To control for a possible deficiency of 20% in other hematopoietic cells, we made chimeras in which 20% of all the hematopoietic
lineages are derived from gene-deficient bone marrow (by transferring 80% WT bone marrow + 20% gene-deficient bone marrow into irradiated recipients). Results from these chimeras and further validation of the chimera model are provided in Supplemental Fig. 1. MyD88 \(^2\)/\(^2\) mice (52) and TIR-domain containing adapter-inducing INF-\(\gamma\) \(^2\)/\(^2\) (53) were provided by S. Akira (Hyogo College of Medicine, Hyogo, Japan). BCR transgenic MD4 strain (54), J3D (BALB/c \(\mu\)MT) (55), \(\mu\)MT (C57BL/6 \(\mu\)MT) (56) IFN-\(\gamma\) \(^2\)/\(^2\) (57), IL-6 \(^2\)/\(^2\) (58), and I-A\(\beta\) \(^2\)/\(^2\) (59) mice have been bred in-house long-term and have been backcrossed to C57BL/6 or BALB/c (MD4 and JHD) in excess of 10 generations. Experiments were covered by a Project License granted by the U.K. Home Office. This license was approved locally by the University of Edinburgh Ethical Review Committee.

S. enterica serovar Typhimurium infection

The \(aroA\) attenuated strain of S. enterica serovar Typhimurium, SL3261, was used for all infections (60). Bacteria were grown as stationary-phase overnight cultures in Luria-Bertani broth. Animals were injected i.v. with \(1 \times 10^6\) CFU diluted in PBS.

Preparation of bacterial Ags

Bacterial Ag (C5SENaOH) was prepared, as described previously (61). Briefly, overnight cultures of the C5 strain were washed twice in PBS containing 5 mM EDTA. The resulting suspension was sonicated on ice, and cellular debris was removed by centrifugation at 13,000 \(\times\) g. The supernatant was alkali treated with 5 M NaOH for 3 h at 37\(^\circ\)C and then neutralized with HCl. The NaOH-treated Ag was then 0.22 \(\mu\)m sterile filtered and stored at \(-20^\circ\)C. Protein content was calculated using a standard bicinchoninic acid assay. Heat-killed bacteria (HKB) were prepared by pelleting from snap-frozen stock vials of SL3261, resuspending in complete IMDM, and then heating to 85\(^\circ\)C for 10 min.

Preparation and sorting of CD4\(^{+}\) ve and CD19\(^{+}\) ve lymphocytes

CD4\(^{+}\) ve and CD19\(^{+}\) ve lymphocytes for use in all in vitro restimulation assays were isolated from mice using standard magnetic sorting techniques. Following manual disruption and lysis of RBCs, splenocytes were positively sorted using anti-CD19 or anti-CD4 microbeads and LS columns, according to manufacturer’s instructions (Miltenyi-Biotec, Auburn, CA). CD4 T cells and B cells were >98% pure following two-column passes.

In vitro restimulation assays

Highly purified B cells from SL3261-infected mice were cultured (2 \(\times\) \(10^6\) cells/ml) with stimuli, including the following: HKB SL3261, Salmonella LPS (1 \(\mu\)g/ml; InvivoGen, San Diego, CA), PMA, and ionomycin (10 ng/ml and 1 \(\mu\)g/ml; Sigma-Aldrich, St. Louis, MO). Supernatants were harvested at day 5. T cell restimulation assays were set up by coculturing highly purified CD4 T cells with irradiated (30 Gy) WT, naive splenocytes at a 1:1 ratio (2 \(\times\) \(10^6\) cells/ml). Supernatants were harvested at 24 h for IL-2 and 72 h for IFN-\(\gamma\), IL-10, and IL-17 detection.

Cytokine ELISA

Paired Ab sets for IFN-\(\gamma\), IL-2, IL-6, IL-10, and IL-17 (BD Biosciences, San Jose, CA) were used for capture and detection in ELISA. Cytokine concentrations were determined by extrapolation from a standard curve. Minimum levels of detection were as follows: IL-2, 0.2 ng/ml; IL-6, 0.3 ng/ml; IL-10, 0.1 ng/ml; IL-17, 0.1 ng/ml; and IFN-\(\gamma\), 0.4 ng/ml.

FIGURE 1. Cytokine production is dependent upon both TLR and BCR reactivity. A, CD19\(^{+}\) B cells from Salmonella-infected MyD88 \(^{-/-}\) and MD4 mice were isolated from spleens at day 7 of infection and cultured for 5 d with a serial dilution of HKB, as indicated on the x-axis. In each case, black symbols represent WT controls, and open symbols knockout or transgenic mice. Data points represent mean values of triplicate cultures carried out on pooled B cells from groups of four mice, and error bars represent SEM. Data are representative of three independent experiments. Naive WT CD19\(^{+}\) B cells when stimulated with HKB maximally secreted 1–4 ng/ml IL-6, 1–2 ng/ml IL-10, and no IFN-\(\gamma\). B, Anti-Salmonella Ab titres in MD4 (left) and MyD88 \(^{-/-}\) (right) mice were determined by ELISA on sera from four groups of mice bled at time points indicated (\(n=4–6\)). Black symbols represent WT controls, and open symbols represent transgenic or knockout mice. Each data point represents an individual mouse, and bars represent mean for the group. Data are representative of three separate experiments.
Results

BCR and TLR signals are necessary for cytokine secretion by B cells in Salmonella-infected mice

We wished to investigate the relative importance of BCR and TLR stimulation in the B cell cytokine response. To address the Ag specificity of the bacteria-elicited cytokine response, we infected anti-hen egg lysozyme BCR (MD4) transgenic mice with S. enterica and then restimulated purified B cells from these mice in vitro with HKB. We found that cytokine secretion was substantially impaired in B cells unable to respond to the bacteria through their BCR (Fig. 1A). Some IL-6 and IL-10 was still secreted by the MD4 B cells, indicating only a partial dependence on BCR signals. However, B cells from S. enterica-infected MD4 mice made no detectable IFN-γ, indicating a total dependence on BCR stimulation for IFN-γ secretion (Fig. 1A). To confirm that B cells from MD4 mice could not respond via any endogenous BCR rearrangements (receptor editing), we measured anti-Salmonella Ab responses in these mice. Ab was undetectable up to 51 d postinfection (Fig. 1B). Experiments have been repeated with MD4 mice crossed onto a J HER 1.2 background (endogenous BCR rearrangements not possible), with identical results.

To address the TLR dependency of B cell cytokine production during S. enterica infection, we used MyD88-deficient mice. Thus, MyD88-deficient mice were infected with S. enterica and splenic B cells were taken for in vitro restimulation with HKB at 7 d. IL-6, IL-10, and IFN-γ secretion was negligible, illustrating total dependence upon TLR stimulation of B cells for secretion of these cytokines by B cells (Fig. 1A). Normal BCR reactivity in MyD88−/− mice was confirmed by measuring Ab responses during infection; these mice showed titres of anti-Salmonella total IgG approximately equivalent to WT controls (Fig. 1B). It was not possible to assign cytokine production to particular B cell subsets, as during Salmonella infection classical subset distinctions cannot be made (see Supplemental Fig. 2).

MyD88 signals to B cells enhance primary development of CD4 Th1 and IL-10 responses, but are dispensable for memory development

To study the role of MyD88 signaling in B cells as APCs in the development of CD4 Th1 responses during S. enterica infection, we constructed bone marrow chimeric mice in which the B cells lacked MyD88. Thus, transfer of bone marrow cell inocula from MyD88−/− mice and from μMT mice in a 20:80 ratio into irradiated μMT recipients meant that after reconstitution the B cell compartment was wholly MyD88 deficient, whereas the other hematopoietic populations were mainly WT with respect to MyD88. Further validation of this chimera model is provided in Supplemental Fig. 1. CD4 T cells were isolated from the spleens of MyD88−/− and WT chimeras at the peak of primary infection (day 7) and also after mice had cleared the infection (8 wk). These T cells were restimulated in vitro with an S. enterica Ag preparation. The B cells in these chimeras showed equivalent expression of activation markers (Supplemental Fig. 2B). Fig. 2A shows IL-2, IFN-γ, and IL-10 production by CD4+ T cells at the peak of primary infection (day 7). IFN-γ and IL-10 production are significantly impaired in the primary responses, with T cell-derived IL-10 being particularly dependent upon B cell MyD88 expression (both p < 0.001). IL-2 production from T cells was below the limit of detection during the primary response in MyD88−/− and WT chimeras. Thus, MyD88-dependent (TLR) stimulation of B cells in vivo is necessary for optimal Th1 differentiation of CD4+ T cells and also for the generation of IL-10–producing B cells.

FIGURE 2. Primary Th1 responses require B cell TLR reactivity. A, CD4+ T cells from Salmonella-infected MyD88−/− bone marrow chimeric mice were sorted by magnetic separation at day 7 (top) and week 8 (bottom), and cultured for 3 d with C5SENaOH at a range of concentrations, as indicated on the x-axis. In each case, black symbols represent WT bone marrow chimeras, and open symbols MyD88−/− chimeras. Data presented are the mean of duplicate cultures on groups of five animals (n = 5) and are representative of three separate experiments. Error bars indicate SEM. Asterisks indicate statistically significant impaired cytokine production as determined by two-way ANOVA (⁎⁎⁎p < 0.001). B, Bacterial load in spleens from infected mice were calculated at day 7 and week 5. Each symbol represents CFU from an individual mouse, and the line represents the mean for each group. Presented data are representative of three independent experiments. ND, not detected.
T cells during *S. enterica* infection. MyD88 also acts as a signaling adapter for IL-1 and IL-18; however, we feel that the effect on Th1 response development shown in this study is TLR mediated, as we see a similar impairment of the IFN-γ response in TIR-domain containing adapter-inducing IFN-β²/² knockout mice (Supplemental Fig. 3).

At week 8 postinfection when bacteria had been cleared, we harvested CD4⁺ T cells from spleens of infected chimeras to test the memory response. This revealed robust and equivalent cytokine secretion in both groups of chimeras. Thus, the memory IL-2, IFN-γ, and IL-10 responses were similar in MyD88⁻/⁻ and WT chimeric mice. Despite the significant reduction in early cytokine production, the development of Th1 and IL-10–producing memory T cells is not impaired. Indeed, the late/memory Th1 cell development was sufficiently strong to resolve the infection. Bacterial burdens in the spleen at day 7 were similar in WT, μMT, and MyD88⁻/⁻ mice. MyD88⁻/⁻ mice showed increased bacterial burden at day 7, and took longer to resolve the infection; however, all types of mice clear bacteria at an approximately similar rate (Fig. 2B).

**Development of memory, but not primary CD4 Th1 and IL-10–producing T cells is dependent on Ag-specific BCR signals to B cells**

MD4 transgenic mice expressing a hen egg lysozyme-specific BCR were used to investigate the requirement for BCR signals to B cells in their capacity to influence primary and memory T cell responses during *S. enterica* infection. CD4⁺ T cells were isolated from MD4 spleens at the peak of primary infection (day 7) and restimulated in vitro with *S. enterica* Ag. Fig. 3A shows IL-2, IFN-γ, and IL-10 secretion by T cells from MD4 mice. At day 7, T cells from MD4 mice showed largely normal cytokine production, with...
a small, but significant reduction in IFN-γ (p < 0.05). IL-10 secretion by T cells from MD4 mice was equivalent to those from WT mice. Ag-specific IL-2 secretion by T cells isolated from S. enterica-infected mice was below the limit of detection in both WT and MD4 mice.

A second cohort of animals was left to clear the infection, to quantify the development of cytokine-secreting memory T cells. T cells from week 7 postinfection were restimulated as previously. Fig. 3A shows that at week 7 there is no Ag-specific Th1 response from these T cells (no detectable IL-2 or IFN-γ; p < 0.001), and it is clear that generation of memory Th1 cells is seriously impaired in mice that cannot respond through the BCR. Interestingly, IL-10 secretion was still apparent in T cells isolated from MD4 mice 7 wk postinmunization, though was partially impaired (p < 0.05). Thus, stimulation of B cells through the BCR is necessary for generation of Th1 memory in Salmonella infection. As MD4 mice clear bacteria less efficiently (Fig. 3B) than WT mice, we also carried out experiments in which the mice were treated with antibiotic from the peak of infection for 10 d. Supplemental Fig. 4 carried out experiments in which the mice were treated with an-

Thus, stimulation of B cells through the BCR is necessary for generation of Th1 memory in

is clear that generation of memory Th1 cells is seriously impaired

was not completely absent in the cytokine chimera, so we conclude that B cell-derived cytokines play an important, but supporting role.

Memory T cell responses require Ag presentation by B cells, whereas priming does not

Our demonstration that primary T cell responses were influenced by B cells in an Ag-nonspecific manner, whereas memory T cell responses were dependent upon BCR suggests Ag presentation by B cells is dispensable at early stages, but crucial later on. To investigate this further, we generated mixed bone marrow chimeras in which B cells could not present Ag via MHC class II (MHC-II<sup>B<sup>−/−</sup></sup>). Primary T cell responses to Salmonella in these mice, as in BCR-

irrelevant mice, were found to be largely normal (Fig. 4) and were sufficient to allow bacterial clearance. In contrast, at week 8 postinfection, memory Th1 responses (IL-2 and IFN-γ secretion) were both highly dependent upon B cell presentation (Fig. 4). The same is not true of the memory IL-10 response, which is unaffected in MHC-II<sup>B<sup>−/−</sup></sup> chimeras. To test whether this failure of Th1 memory development influenced the protection, we challenged the mice, 12 wk later, with a virulent Salmonella strain and found that they survived less well than controls (Supplemental Fig. 6).

Effect of B cell cytokine deficiency on the development of T cell responses

To see whether there was a direct effect of B cell-derived cytokines on the polarization of T cell responses, we constructed bone marrow chimeras in which the B cell compartment was deficient in IL-6 or IFN-γ. In IL-6<sup>B<sup>−/−</sup></sup> chimeras, we noted no effect on CD4 T cell-derived IL-2, IL-10 (data not shown), or IFN-γ responses (Fig. 5A), but did note a significant decrease in the production of IL-17 during Salmonella infection (Fig. 5A). In IFN-γ<sup>B<sup>−/−</sup></sup> chimeras, the CD4 T cell-derived IL-2 (data not shown) and IL-10 (Fig. 5B) responses were slightly lower than in WT chimeras, but this was not statistically significant. The IL-17 response was not altered. However, the CD4 T cell-derived IFN-γ response to Salmonella was significantly impaired in the mice in which B cells could not secrete IFN-γ (Fig. 5B). In summary, B cell-derived IL-6 and IFN-γ supported development of the Th17 and the Th1 responses, respectively. T cell cytokine response was not completely absent in the cytokine chima, so we conclude that B cell-derived cytokines play an important, but supporting role.

Discussion

In this study, we have identified two distinct and functionally separable phases of the B cell contribution to T cell differentiation during a response to an ongoing Salmonella infection. The first, early phase is dependent on B cell-intrinsic MyD88 signals, most likely as a result of TLR ligation. This phase is almost wholly independent of BCR-mediated Ag uptake or presentation to CD4 T cells via MHC class II on the B cells. B cell cytokine production is largely MyD88 dependent, and we propose that the early influence of B cells on the developing CD4 T cell response is mediated by cytokines produced in response to TLR ligation. In support of this, we show that when B cells are incapable of making certain cytokines (e.g., IFN-γ or IL-6), this has measurable effects on the development of polarized effector T cell subsets (e.g., Th1 and Th17). We do not know whether the B cell-derived cytokines act directly on activated T cells or on other cell types that influence T cell differentiation. The second phase of B cell influence is during the development of CD4 T cell memory and is dependent on recognition and uptake of Ags by BCR and their subsequent presentation by MHC class II. In the absence of this cognate phase of B–T cell interaction, the protective Th1 memory cell response fails to develop.

Evidence of the importance of B cells in the development of CD4 T cell memory has accumulated over several years in studies using protein Ags and TCR transgenic models (13, 17, 62). The data presented in this work along with other recent studies (14–16, 18, 63) show, during infection, the failure to develop T cell memory in the absence of Ab-independent B cell function. We identify the expression on B cells of both an Ag-specific BCR and MHC class II as crucial for memory T cell development, presumably to enable Ag uptake and presentation. It is equally clear that these cognate molecules on B cells are dispensable for the generation of the primary CD4 T cell response. This means that other APCs (e.g., dendritic cells [DCs]) are competent and sufficient to drive the
primary response, strongly suggesting that, as the response to Salmonella Ags proceeds, the participating APC changes from the DC, initially, to B cells later on. It is not simply that B cells present Ag upon secondary exposure, but rather perform a “follow-on” APC function as DC presentation falls away. In support of this, the primary IFN-γ response we observe in MD4 (BCR-restricted) mice at day 7 is already reduced compared with control mice, and this response declines in these mice over the next 2–3 wk. Ab seems to play no significant role in enhancing Ag presentation during the primary response, as provision of anti-Salmonella Abs to the MD4 mice did not alter the magnitude or the longevity of their primary response (data not shown). As we did not identify individual Ag-specific memory T cells in this study, we can say little about memory T cell survival. Our definition of memory in this work is a functional one in which we detect enhanced responsiveness to Ag recall. Thus, we do not know whether the memory we see is mediated by bona fide memory T cells or long-lived effectors, but given that systemic CD4 T cell memory is not apparent in some mice (e.g., MD4, MHC-IIb−/− chimeras), we feel this demonstrates that functionally either or both cell types are missing. Future studies using Salmonella bacteria that express a peptide epitope detectable using MHC II tetramers will address events at a cellular level.

In previous work from this laboratory (13), we showed that Ag presentation by B cells affected the T cell response as early as day 3–4 postimmunization, somewhat earlier than we indicate in this work. The two studies differ in Ag delivery; in this study, we follow the primary response to bacterial infection, and this may lead to enhanced APC activation in comparison with protein immunization with adjuvant. More importantly, Crawford et al. (13) used a TCR transgenic model in which the frequency of naive Ag-specific T cells was artificially increased. Under these conditions, it is possible that the APC capacity of endogenous DCs was insufficient to allow optimal activation of the transferred transgenic T cells and that the “follow-on” APC function of B cells was apparent earlier. Taken together, these data indicate that in vivo the contribution of DCs is time limited and cannot lead to optimal memory cell development. Ahmed and colleagues (18) also saw rapid decline (within 3 wk) of lymphocytic choriomeningitis virus-specific CD4 T cell response in μMT and concluded that B cell involvement is in the establishment and maintenance phase of CD4 T cell memory; we would agree.

The majority of published studies that have addressed the influence of B cells on T cell differentiation have noted their involvement in Th2 responses (32–35, 38). In this study, we show a clear contribution of B cells to the development of an effective Th1 (and Th17) response to Salmonella infection. As discussed above, for Th1 memory this relates to continued APC function. However, in the primary response, B cells are, to a large extent, dispensable as APCs (MD4, BCR-restricted mice and MHC-IIb−/− chimeras exhibit relatively normal primary responses). Despite this, the presence of B cells does have a measurable effect on the development of polarized Th subsets. In MyD88−/− chimeras, the primary IFN-γ and IL-10 T cell responses are significantly reduced, indicating that TLR-induced polyclonal B cell activation during the infection elicits mediators that can act on differentiating T cells. This conclusion is supported by our recent study of Ab responses in MyD88−/− chimeras in which the IFN-γ-dependent IgG2c responses were most severely impaired (64). We investigated the cytokines, known to be secreted by B cells after TLR activation (39). In IFN-γ−/− chimeras infected with Salmonella, the primary CD4 T cell IFN-γ response was lower than in control chimeras. Recent data suggest that Th1 commitment and T-bet expression occur in a biphasic fashion, the early phase involving TCR and IFN-γ signals and the later consolidating phase requiring IL-12 (65). Our data suggest that TLR-activated B cells are a likely source of this early IFN-γ that optimizes Th1 commitment. The fact that the T cell IFN-γ response in the IFN-γ−/− chimeras is only partially inhibited may be because IL-12 has an overriding effect (65) or that there are other cellular sources of IFN-γ. It has also been shown that Salmonella can induce early innate activation of CD4 T cells that then make IFN-γ (66); whether the innate activation and IFN-γ secretion by B cells influence this T cell response is not known. In IL-6−/− chimeras, the T cell IL-17 response to Salmonella was reduced, which we think indicates a significant contribution by B cells of the IL-6 required for Th17 development (67). TNF-α expression by B cells has been shown to augment Th1 effector responses in Toxoplasma gondii infection, but we have not yet tested this in appropriate Salmonella-infected chimeras. An obvious candidate Th1-polarizing cytokine is IL-12; however, mouse B cells make very little IL-12p40 (39) and chimeras in which B cells lack IL-12 exhibit completely normal primary and memory Th1 responses to Salmonella (data not shown). We show in this study a direct effect of B cell cytokines on the development of Th1 effector T cells during infection. The worry that the use of B cell depletion therapy in autoimmune patients might have consequences for infection is only exacerbated by these data, as B cells, in addition to Ab production, also support the differentiation of inflammatory, protective Th1 and Th17 responses.

The impairment of the primary T cell cytokine responses to Salmonella in MyD88−/− chimeras indicates the importance of an innate component of the B cell response. The adaptive B cell response including APC function becomes absolutely crucial later (7–14 d) for sustaining the effector T cell response and enabling memory T cell formation. It will be intriguing to discover where these two distinct phases of the B cell-dependent T cell response occur within lymphoid tissues. We conclude that a complete and rounded CD4 T cell response is dependent on B cells.

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Disclosures
The authors have no financial conflicts of interest.

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