Cutting Edge

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Cutting Edge: Redundant Roles for MHC Class II–, CD1d-, and MR1-restricted T Cells in Clearing *Bartonella* Infection

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The importance of unconventional T cells for mucosal immunity is firmly established but for systemic bacterial infection remains less well defined. In this study, we explored the role of various T cell subsets in murine *Bartonella* infection, which establishes persistent bacteremia unless controlled by antibacterial Abs. We found that αβ T cells are essential for Ab production against and clearance of *B. taylorii*, whereas MHC class I (MHC-I)- or MHC class II (MHC-II)-deficient mice eliminated *B. taylorii* infection with normal kinetics. Similarly, animals lacking either CD1d or MR1 suppressed bacteremia with normal kinetics. Interestingly, mice with a combined deficiency of either MHC-II and CD1d or MHC-II and MR1 failed to clear the infection, indicating that the combination of CD1d- and MR1-restricted T cells can compensate for the lack of MHC-II in this model. Our data document a previously underappreciated contribution of unconventional T cells to the control of systemic bacterial infection, supposedly as helper cells for antibacterial Ab production. ***The Journal of Immunology*****, 2024, 213: 553–558.**

Besides conventional αβ T cells, which recognize peptide ligands presented on MHC class I (MHC-I) and MHC class II (MHC-II), unconventional T cells (UTCs) have entered the spotlight for their roles in tissue repair and antimicrobial immunity (1–3). UTCs represent an evolutionarily more ancient form of the immune system and are mostly studied for their interactions with the microbiota (1). They comprise γδ T cells recognizing phosphorylated metabolites (commonly referred to as phosphoantigens) and NKT cells responding to carbohydrate-linked lipids in a CD1-restricted manner. In mice, unlike in humans, the latter type of T cell consists solely of CD1d-restricted cells, comprising chiefly invariant NKT cells but also others, such as γδ T cells (4). MR1-restricted T (MR1T) cells include mucosal-associated invariant T (MAIT) cells, representing a third type of UTC that recognizes microbial metabolites (5, 6), but also γδ T cells can bind MR1 (7, 8). Both CD1-restricted T cells and MR1T cells use a limited repertoire of TCR chains (9). Importantly, they are potent cytokine producers and can provide cognate help to B cells, thereby supporting protective Ab responses (4, 6, 10–12). CD1d-restricted iNKT cells were shown to both enhance Th–B cell interaction in a noncognate manner (13) and to provide cognate help directly to the B cell, inducing Ab production, class-switch, and germinal center reactions (14, 15). Interestingly, both CD1d-restricted iNKT and MR1-restricted MAIT cells were shown to mediate Ab production in the absence of conventional MHC-II–restricted T cells (10, 16). In contrast, the lack of either CD1d or MR1 alone leads to an increased susceptibility to bacterial, viral, and fungal infections in mice (17–19), supporting an important role for both cell types in the control of infections. However, most studies on the role of UTCs focus on their function in mucosal tissues, and little is known about their importance in systemic infections.

Bartonella spp. are Gram-negative facultative intracellular pathogens that infect a wide variety of mammalian hosts, including humans, and can result in a broad spectrum of symptoms ranging from a subclinical course of infection to life-threatening disease (20, 21). Clinically relevant infections are caused by human-specific species, such as *B. bacilliformis*, the causative agent of life-threatening Carrion disease, and *B. quintana*, which causes trench fever (20, 21). Zoonotic infection with *B. henselae* manifests as cat scratch disease (22).

Bartonellae are transmitted by blood-sucking arthropods and cause a long-lasting intraerythrocytic bacteremia in their natural host (23). Inside RBCs the bacteria are shielded from the host's immune system, enabling their replication and persistence. We have recently explored the role of specific B cells and Abs in clearing bacterial infection in the well-established *B. taylorii* (*Btay*) mouse model (24, 25). Neutralizing Abs preventing bacterial

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Abbreviations used in this article: β2M−/−, β-2 microglobulin−/−; *Btay*, *Bartonella taylorii*; EAI, erythrocyte adhesion inhibition; MAIT, mucosal-associated invariant T; MHC-I, MHC class I; MHC-II, MHC class II; MR1T, MR1-restricted T; WT, wild-type.

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attachment to the surface of RBCs represent a mechanism of protection and operate independently of Fcγ receptors or complement (24). In line with a prominent role for Abs, cognate T cell help seems key for the control of *Btay* infection. Specifically, mice lacking CD40L (24) failed to clear the infection, which was associated with their failure to mount a durable Ab response.

In this study, we explored the role of individual T cell subsets in clearing *Btay* infection in C57BL/6 mice. We report that in the absence of conventional MHC-II–restricted CD4 T cells, the combination of unconventional CD1d-restricted Th and MR1T helper cells is required for *Btay* clearance, whereas MHC-I–restricted CD8 T cells are dispensable. The ability of CD1d-restricted T and MR1T cells to compensate for the lack of MHC-II–restricted T cell responses suggests the importance of UTCs in systemic bacterial infection and may extend beyond their well-studied role in mucosal tissues.

Materials and Methods

Bacterial growth conditions

Bartonella strains [*B. taylorii* IBS296 *rpsL*, LSB001 (24) and *B. taylorii* IBS296 GFP$^+$, LSB115 (24)] were grown as previously described (24).

Mouse experimentation

All animal work was approved by the Veterinary Office of the Canton Basel City (license no. 1741 and no. 2665). Animals were housed at specific pathogen-free conditions; adult mice (>5 wk) of both sexes were used for experiments.

Female BALB/cJRj and C57BL/6JR mice were obtained from Janvier Labs. β-2 microglobulin$^{-/-}$ (β2M$^{-/-}$) (26), KbDb$^{-/-}$ (27), sIgM$^{-/-}$ AID$^{-/-}$ (24), Rag1$^{-/-}$ (28), TCRβδ$^{-/-}$ (29), CD40L$^{-/-}$ (30), and MHC-II$^{-/-}$ (31) mice were bred at the Laboratory Animal Science Center (University of Zurich, Zurich, Switzerland) and at the ETH Phenomics Center. CD1d$^{-/-}$ (32) and MR1$^{-/-}$ (33) mice were bred at the University of Basel (Basel, Switzerland). MR1$^{-/-}$MHC-II$^{-/-}$ and CD1d$^{-/-}$MHC-II$^{-/-}$ mice were intercrossed from the respective single-knockout strains. TCRβ$^{-/-}$ and TCRδ$^{-/-}$ were backcrossed to single knockouts from the respective double knockout using C57BL/6 mating partners.

Btay mouse infections, bacteremia assessment, and serum collection were performed as previously reported (24). *Btay*-immune and nonimmune control sera were injected i.v. in 100 μl on days 14 and 35 postinfection. A total of 250 μg of mAb LS5G11 (24) was injected in 100 μl.

For Lymphocytic choriomeningitis virus infection, clone 13 expressing the Lymphocytic choriomeningitis virus strain WE GP was administered a dose of 2×10^6 PFUs i.v. (34).

Cytokine concentrations in mouse serum samples were analyzed by Eve Technologies (multiplex Mouse Cytokine/Chemokine Discovery Assay Array).

Erythrocyte adhesion inhibition assay

The erythrocyte adhesion inhibition (EAI) assay was performed as described previously (24). EAI titer was calculated using end point titer determination as described previously (35). In brief, GFP-expressing *Btay* was incubated with naive or immune serum for 1 h. Murine erythrocytes were added and after 24-h incubation, the bacterial adhesion to erythrocytes was determined by flow cytometry (24).

Data analysis

Graphs were generated using GraphPad Prism 9.1.0.

Results

Ab responses are necessary and sufficient to clear Bartonella *bacteremia*

Infection of C57BL/6 mice with *Btay* strain IBS296 results in intraerythrocytic bacteremia lasting for ~40 d (34), thus lending itself as a robust model for the investigation of immune control mechanisms in the natural murine host (24, 25, 36). Unlike in wild-type (WT) mice, Rag$^{-/-}$ mice, lacking B and T cells, exhibit lifelong persistent bacteremia (24, 25, 37). Similarly unchecked bacteremia occurs also in sIgM$^{-/-}$AID$^{-/-}$ mice that have a mature B cell compartment that responds to infection but fails to secrete Abs (24) (Fig. 1A), providing unambiguous evidence that Ab defense is necessary for *Bartonella* control in mice.

To test whether *Bartonella*-specific Abs are sufficient for bacterial load control when endogenous T and/or B cells are lacking, we performed passive Ab therapy experiments in Rag$^{-/-}$ and sIgM$^{-/-}$AID$^{-/-}$ mice. For treatment, we used either *Btay*-immune serum or *Btay*-neutralizing mAb LS5G11 (24). Both types of Ab treatment afforded a consistent transient suppression of bacteremia. More importantly, 3 of 10 Rag$^{-/-}$ mice and 1 of 6 sIgM$^{-/-}$AID$^{-/-}$ mice receiving *Bartonella*-specific Abs were abacteremic by the end of the experiment, whereas control mice given naive serum exhibited uniformly high-level bacteremia (Fig. 1B–E). These findings indicated that

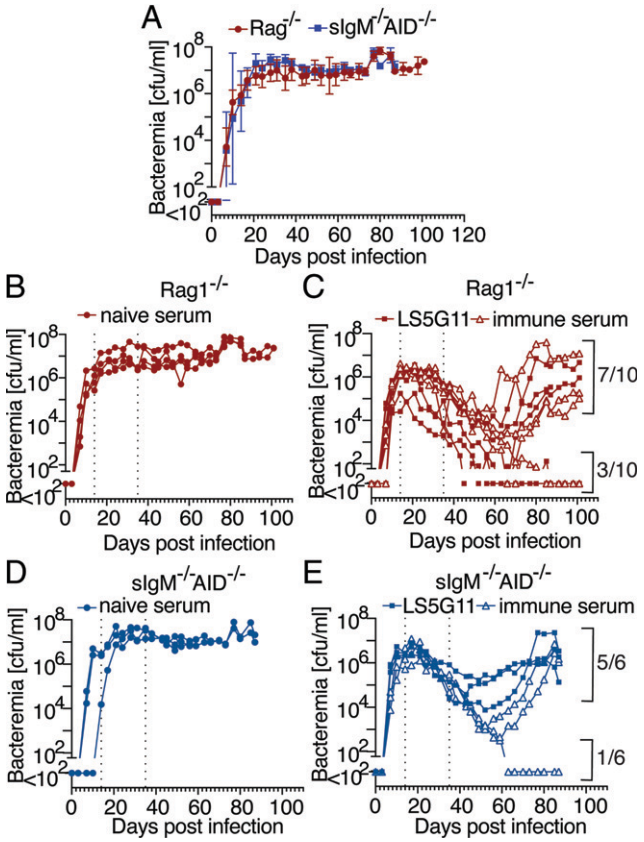

FIGURE 1. Ab responses are necessary and sufficient to clear *Bartonella* bacteremia. Mice were infected with *Btay*, and bacteremia was determined. *Btay*-immune and nonimmune control serum or mAb LS5G11(24), respectively, were administered i.v. (**A**) Bacteremia in Rag1$^{-/-}$ mice (n = 4) and sIgM$^{-/-}$AID$^{-/-}$ mice (n = 3). (**B** and **C**) Serum transfer and LS5G11 transfer into infected Rag1$^{-/-}$ animals. Naive serum (n = 4, B), immune serum (n = 5, C), or mAb (n = 5, C) was administered on days 14 and 35 postinfection (vertical dotted lines). The number of bacteremic and nonbacteremic animals at the final time point is indicated. (**D** and **E**) Serum and LS5G11 transfer into infected sIgM$^{-/-}$AID$^{-/-}$ animals. Naive serum (n = 3, D), immune serum (n = 3, E), or mAb (n = 3, E) was administered as in (B) and (C). The number of bacteremic and nonbacteremic animals at the final time point is indicated. Representative data are from at least two independent experiments. Data are reported as mean ± SD in (A). Single animals are shown in (B) and (E).

Btay-specific Abs can suffice, in principle, to suppress bacteremia to less than detectable levels even when hosts are devoid of T and B cells. Moreover, the noninferiority of *Btay* clearance kinetics and clearance rates in Rag$^{-/-}$ as compared with sIgM$^{-/-}$AID$^{-/-}$ mice suggested that T cell functions other than help to B cells are not essential for bacterial elimination (Fig. 1B–E).

Control of Btay *bacteremia depends on TCR*αβ *T cell help*

These findings raised the question which T cell subset(s) provide the necessary help in *Bartonella* infection. We explored a range of targeted mutant mice lacking defined TCR types and Ag presentation molecules. Unlike in WT mice (Fig. 2A), animals completely devoid of T cells (TCRβδ$^{-/-}$) remained bacteremic throughout the observation period of 60 d postinfection (Fig. 2B). Next, we tested the corresponding single-mutant TCRβ$^{-/-}$ and TCRδ$^{-/-}$ mice, which lack TCRαβ or TCRγδ T cells, respectively. Persistent bacteremia was observed in mice lacking TCRαβ T cells, whereas animals lacking TCRγδ cells cleared the infection comparably with WT controls (Fig. 2C, 2D).

In light of their importance for *Bartonella* control (24), we determined also EAI Abs in the infected animals. As expected, WT mice exhibited EAI Ab titers from 14 d postinfection onward (Fig. 2A). Alongside unimpaired bacterial load control, also TCRδ$^{-/-}$ mice mounted EAI Ab responses, albeit with some delay (Fig. 2D). In stark contrast, EAI Ab titers in TCRβδ$^{-/-}$ and TCRβ$^{-/-}$ noncontroller mice remained at around detection limits; they were only intermittently detected and subsided at later stages of the infection (Fig. 2B, 2C).

These observations extended earlier findings (24, 37) on the importance of T cells for *Bartonella* control by identifying TCRαβ, but not TCRγδ, T cells as essential. The tentative correlation of bacterial clearance with the occurrence of EIA Abs was in line with the concept that T cells were important as helper cells for efficient B cell responses.

Mice lacking classical MHC-I or MHC-II clear Bartonella *infection*

An alternative role for T cells could have consisted in MHC-I–restricted cell-mediated cytotoxicity. Studying *Btay* bacteremia in mice devoid of classical MHC-I molecules, as well as of CD1d (38, 39), because of targeted disruption of β2M$^{-/-}$, we found clearance kinetics indistinguishable from WT controls (Fig. 3A, 3B). Bacterial clearance was also achieved in MHC-I–deficient mice carrying mutant H-2K^b and H-2D^b alleles (K^{b}D$^{b-/-}$ mice; Supplemental Fig. 1), altogether arguing against a substantial contribution of MHC-I–restricted T cells to *Btay* control. Thus, we infected mice lacking MHC-II (MHC-II$^{-/-}$), which serve as a model devoid of classical CD4 Th cells. To our surprise, also MHC-II$^{-/-}$ animals controlled *Bartonella* infection with clearance kinetics comparable with WT mice (Fig. 3C). In marked contrast with WT and β2M$^{-/-}$ mice, however, which mounted robust and durable Ab titers from day 14 onward, EAI serum titers in MHC-II$^{-/-}$ mice were intermittently detected on day 7 postinfection, then subsided and reappeared concomitantly with bacterial clearance, albeit at titers that were lower than in WT controls and only marginally greater than technical detection limits (Fig. 3A, 3C). Unimpaired bacterial clearance in MHC-II$^{-/-}$ animals contrasted with our recent report on CD40L$^{-/-}$ mice, which failed to mount robust EIA Ab responses and developed lifelong bacteremia (24). Taken together, these observations raised the possibility that CD40-CD40L–dependent T help is required for robust Ab-mediated suppression of *Btay* bacteremia, yet that such T help cannot only be supplied by classical MHC-II–restricted CD4 T cells.

CD1d- and MR1-restricted defense can compensate for MHC-II–restricted T help to enable Bartonella *control*

We hypothesized that unconventional CD1d-restricted Th and/or MR1T helper cells may compensate for the lack of MHC-II–restricted T cells to enable *Btay* control. Both CD1d$^{-/-}$ and MR1$^{-/-}$ mice cleared *Bartonella* bacteremia similarly to WT animals (Fig. 4A). In keeping with the earlier results in CD1d-deficient β2M$^{-/-}$ mice, these observations indicated that deficiencies in iNKT cells, MR1T cells, and/or γδ T cells did not preclude *Bartonella* control. The earlier findings did not, however, exclude a potentially substantial contribution of these cells that was redundant with the one

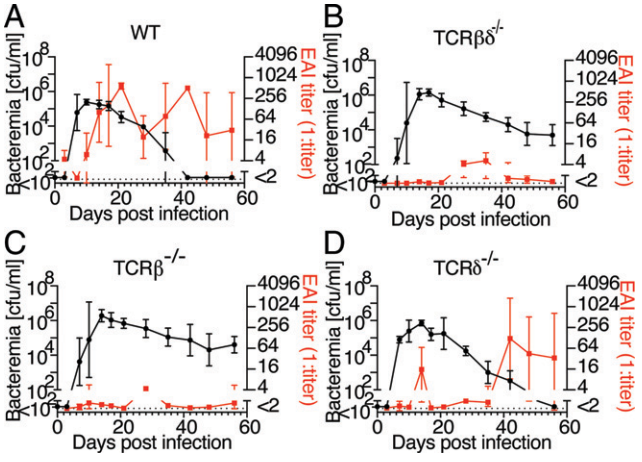

FIGURE 2. TCRαβ T cells are required for clearing *Bartonella* bacteremia. Mice were infected with *Btay*, and bacteremia (CFUs/ml blood) was determined. Bacteremia and EAI titers are shown for (**A**) WT, (**B**) TCRβδ$^{-/-}$, (**C**) TCRβ$^{-/-}$, and (**D**) TCRδ$^{-/-}$ mice. Data were collected from three mice (A and B) or four mice (C and D) per group and are represented as mean ± SD. Data are representative of at least two independent experiments.

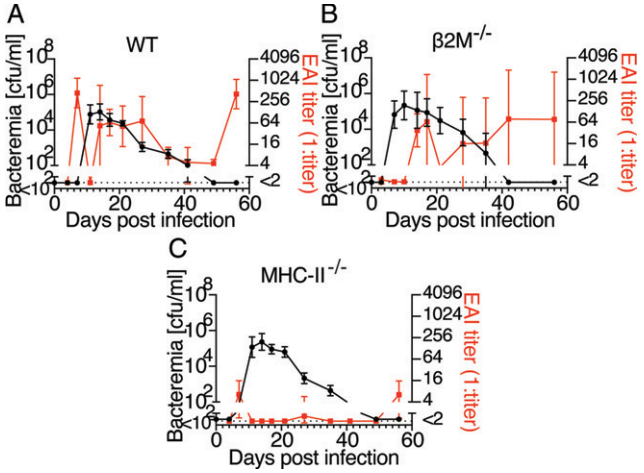

FIGURE 3. Mice lacking MHC-I or MCH-II alone clear *Bartonella* infection. Bacteremia and EAI titers of *Btay*-infected (**A**) WT, (**B**) β2M$^{-/-}$, and (**C**) MHC-II$^{-/-}$ mice. Data were collected from three (B) and five mice (A and C), respectively. Data are representative of at least two independent experiments. See also Supplemental Fig. 1.

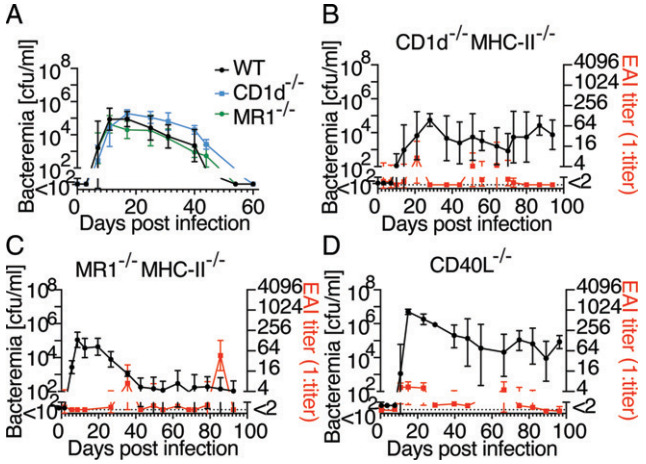

FIGURE 4. Prolonged bacteremia in MHC-II$^{-/-}$ mice additionally lacking MR1 or CD1d. (**A**) Bacteremia of *Btay*-infected WT ($n = 5$), CD1d$^{-/-}$ ($n = 4$), and MR1$^{-/-}$ ($n = 5$) mice. (**B–D**) Bacteremia and EAI titers of *Btay*-infected CD1d$^{-/-}$MHC-II$^{-/-}$ mice ($n = 4$, B), MR1$^{-/-}$MCH-II$^{-/-}$ mice ($n = 5$, C), and CD40L$^{-/-}$ mice ($n = 5$, D). Representative data from two independent experiments are shown. Data show means ± SD.

of MHC-II–restricted T cells. Thus, we crossed MHC-II$^{-/-}$ mice to either CD1d$^{-/-}$ (CD1d$^{-/-}$MHC-II$^{-/-}$) or MR1$^{-/-}$ (MR1$^{-/-}$MHC-II$^{-/-}$) mice. *Bartonella* infection of CD1d$^{-/-}$ MHC-II$^{-/-}$ mice resulted in persistent bacteremia throughout the observation period of almost 100 d, analogous to TCRβδ$^{-/-}$ and TCRβ$^{-/-}$ mice (Fig. 4B, compare Fig. 2B, 2C), albeit with considerable interindividual variability (Supplemental Fig. 2A–C). EAI Abs were only intermittently detected at low titers and were not sustained (Fig. 4B, Supplemental Fig. 2A–C). *Btay* control in MR1$^{-/-}$MHCII$^{-/-}$ mice exhibited substantial interindividual variability, ranging from virtually permanent bacteremia for 100 d over transient clearance with relapsing bacteremia to normal clearance kinetics (Fig. 4C, Supplemental Fig. 2D–F). EAI Abs were detected only intermittently and in some animals, but not others, preceded an at times transient decline in bacteremia. Interestingly, partial or intermittent control of bacteremia in CD1d$^{-/-}$ MHC-II$^{-/-}$ and MR1$^{-/-}$MHCII$^{-/-}$ mice with similarly intermittent EAI Ab responses resembled the course of *Btay* infection in CD40L$^{-/-}$ mice (Fig. 4D, Supplemental Fig. 2G, 2H) (24). Alternatively and not mutually exclusively, UTCs may provide help to B cells by secreting cytokines. A time-course analysis of several cytokines and chemokines in the serum of *Btay*-infected mice documented, however, that such responses were not detectable at the systemic level (Supplemental Fig. 3). This precluded us from investigating differences in serum cytokine levels as a potential surrogate of UTC helper cell function in CD1d$^{-/-}$MHC-II$^{-/-}$ and MR1$^{-/-}$MHCII$^{-/-}$ mice. Taken together, these observations indicated that UTCs could compensate for MHC-II–restricted T cell activity in controlling *Btay* infection, and that one likely role of UTCs in *Bartonella* control may consist of them providing CD40L-mediated help.

Discussion

Neutralizing Abs interfering with bacterial adhesion to RBCs can efficiently clear *Btay* from the bloodstream, and cognate CD40-CD40L–dependent T help is essential for the robust induction of such responses (24). In this study, we show that conventional MHC-II–restricted Th cells are key for early and sustained high-titer EAI Ab responses, but they are dispensable for bacterial clearance. CD1d-restricted iNKT and/or γδ T cells, as well as MR1-restricted MR1T cells, respectively, can at least partially compensate for MHC-II–restricted T help to enable bacterial elimination.

We propose that an important role of CD1d- and MR1-restricted UTCs consists in the provision of cognate help to B cells producing protective anti-*Bartonella* Abs. Incomplete control of *Bartonella* bacteremia in CD40L$^{-/-}$ mice but unimpaired bacterial elimination in MHC-II$^{-/-}$ animals suggests that cells other than classical MHC-II–restricted T cells can provide CD40/CD40L-dependent help to *Bartonella*-specific B cells. In conjunction with incomplete control of *Bartonella* bacteremia in CD1d$^{-/-}$MHC-II$^{-/-}$ and MR1$^{-/-}$MHC-II$^{-/-}$ mice, these findings support a scenario in which CD1d-restricted and MR1-restricted UTCs represent sources of CD40L-mediated help. Accordingly, we hypothesize that in the absence of classical peptide/MHC-II–specific T help, lipid- and bacterial metabolite-specific T cells, restricted by CD1d and MR1, respectively, provide help in a CD40/CD40L-dependent manner to support protective Ab production and enable bacterial clearance.

Notably, the imperfect correlation of EAI Ab responses with bacterial control in mice lacking MHC-II does not contradict our proposition that UTCs control *Bartonella* infection by providing help to B cells. This study relies on the EAI assay (24) representing the only currently available methodology for the reliable quantification of Abs against *Btay* and for the assessment of functional Abs in particular. It seems likely, however, that Ab defense against *Btay* comprises a broad range of molecular effector mechanisms and corresponding targets. Abs directed against bacterial cell-wall components such as LPS may, for example, rely on opsonization and complement activation for their protective effects, thus extending well beyond the activity detectable in EAI assays. We consider it likely that UTCs provide help to B cells responding to such target Ags, many of which are nonproteinaceous. Accordingly, we acknowledge that the types of Ab detected by the EAI assay represent a limitation of our study. We are also aware that our data do not exclude alternative and/or additional roles of UTCs, such as the CD40L-dependent activation of bacterially infected APCs (17–19, 40). T cell–mediated restriction of bacterial growth within macrophages as classically described for *Mycobacteria* (40) is, however, less likely of relevance for *Bartonella* infection. Despite the ability of *Bartonella* to infect dendritic cells and macrophages (41, 42), endothelial cells and erythrocytes are the bacterium's main cellular reservoir (23). Although persisting bacteremia in CD1d$^{-/-}$MHC-II$^{-/-}$ and MR1$^{-/-}$MHC-II$^{-/-}$ double-deficient mice indicates that both CD1d-restricted T and MR1T cells are necessary for *Bartonella* control when MHC-II–restricted T cells are missing, our experiments were not designed to evaluate whether the earlier UTCs are sufficient for *Bartonella* control in MHC-II$^{-/-}$ mice.

UTCs are known to be important for the clearance of several microbial pathogens (43, 44), as well as for the regulation and composition of the microbiome (1). In the context of influenza A virus, as well as of *Pseudomonas aeruginosa* infection, CD1d-restricted iNKT cells play a crucial role by interacting with and stimulating antimicrobial innate immune cells (17, 18). Also, MR1-restricted MAIT cells were shown to restrict the intracellular

growth of *Mycobacteria* and *Francisella* by directly interacting with infected macrophages or by secreting cytokines (19, 40). Different NKT subsets are defined by their transcription factor and cytokine profile, exhibit distinct effector functions, and can be assigned differential roles in infection control. Some of these NKT cell subsets also provide help to B cells, promoting efficient Ab production (5, 11, 12, 15). Oftentimes this helper function is redundant with classical Th cell activity (10, 16). In *Vibrio cholerae* infection, for example, $CXCR5^+$ T follicular helper cell-MAIT cells promote B cell differentiation and IgA production inside mucosal immune compartments (10). Similarly, CD1-restricted T cells were shown to assume a Tfh-like phenotype and to provide help to B cells via CD40-CD40L interactions (13, 14, 16). Interestingly, the resulting Ab responses were short-lived, which is reminiscent of the observations made here (16).

Thus far, however, the investigation of CD1- and MR1-restricted UTCs in infection has generally been limited to mucosal tissues. In this article, we report on their contribution to the control of a systemic blood-borne infection. Although *Bartonella* infection as a relevant disease model is optimally suited to investigate the role of UTCs in systemic Ab production and bacterial control, the lack of immunological tools for the study of *Bartonella*-specific T cell responses has precluded corresponding analyses and represents a clear limitation of our study.

The lack of a detectable serum cytokine response to *Bartonella* infection is not unexpected. *Btay* is uniquely adapted to its natural murine host and can establish lifelong high-level bacteremia in mice without any disease manifestation (24, 25). The latter is possible only because of several stealthing mechanisms that allow the germ to almost completely avoid a systemic inflammatory reaction, which otherwise would be detrimental to the host (23). For example, the bacterium is equipped with a sophisticated armamentarium to prevent the secretion of proinflammatory cytokines by infected innate immune cells (42).

Taken together, our observations document the ability of CD1d- and/or MR1-restricted UTCs to substitute for classical MHC-II–restricted T cells in the control of *Bartonella* infection, supporting an important role for UTCs in the defense against a systemic bacterial disease.

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Disclosures

The authors have no financial conflicts of interest.

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