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CD4+ T Cell Persistence and Function after Infection Are Maintained by Low-Level Peptide:MHC Class II Presentation

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CD4+ memory--phenotype T cells decline over time when generated in response to acute infections cleared by other components of the immune system. Therefore, it was of interest to assess the stability of CD4+ T cells during a persistent Salmonella infection, which is typical of persistent phagocytic infections that are controlled by this lymphocyte subset. We found that CD4+ T cells specific for Salmonella peptide:MHC class II (MHCII) ligands were numerically stable for >1 y after initial oral infection. This stability was associated with peptide:MHCII-driven proliferation by a small number of T cells in the secondary lymphoid organs that harbored bacteria. The persistent population consisted of multifunctional Th1 cells that induced PD-1 and became exhausted when transferred to hosts expressing the specific peptide:MHCII ligand in all parts of the body. Thus, persistent infection of phagocytes produced a CD4+ T cell population that was stably maintained by low-level peptide:MHCII presentation. The Journal of Immunology, 2013, 190: 2828–2834.

E xpanded populations of Ag-specific T and B cells that persist long after pathogen clearance are responsible for immunological memory. These cells are useful to the host, providing protective immunity to subsequent challenge by the original microbe. The memory cell paradigm has been established most clearly for CD8+ T cells. CD8+ T cells expressing TCRs specific for microbe peptide:MHC class I (p:MHC) ligands proliferate extensively, producing a peak number of effector cells about a week after acute infection. About 90% of these effector cells die by apoptosis, leaving a population of memory cells that is stably maintained by recurrent IL-15–driven homeostatic proliferation. Central memory CD8+ T cells recirculate through secondary lymphoid organs, producing new memory cells during secondary responses, whereas effector memory T cells located in nonlymphoid organs are immediately cytotoxic during secondary responses.

It is less clear whether the concept of stable immune memory applies to CD4+ T cells. Naive CD4+ T cells expressing TCRs specific for microbe peptide:MHC class II (p:MHCII) ligands proliferate extensively to produce a peak number of effector cells about a week after acute infections with lymphoctic choriomeningitis virus (LCMV) or Listeria monocytogenes (1, 2). As in the case of CD8+ T cells, ~90% of the effector cells die, leaving a population of memory-phenotype cells: about half are Th1 cells, and the other half are follicular helper cell-like central memory T cells (3). Al-

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Abbreviations used in this article: 129, 129SvJ; B6, C57BL/6; CD45.1, B6.SJL-PtprcPep3b/BoyJ; C211, F1, (CD45.1 × C57BL/6)(F1)3/211; IFN-γ, interferon-γ; LCMV, lymphocytic choriomeningitis virus; MLN, mesenteric lymph node; Nramp1-, Nramp1-resistant; OmpC, outer membrane porin C; p:MHCII, peptide:MHC class I; p:MHCII, peptide:MHCII class II; ST, Salmonella enterica serovar Typhimurium: ST-2W, ST strain SL1344 OmpC-C-2W.

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Materials and Methods

Mice

C57BL/6 (B6), B6.SJL-PtprcPep3b/BoyJ (CD45.1) mice, and 129SvJ (129) female mice were purchased from The Jackson Laboratory. (B6 × 129) F1, (CD45.1 × 129) F1, and (Act-2W × 129) F1 mice were bred in...
our facilities. All mice were housed in specific pathogen-free conditions at the University of Minnesota, and all experiments were conducted in accordance with institutional and federal guidelines.

**Infections**

Mice were pretreated by 12-h food deprivation or intragastric gavage with 100 μl 5% sodium bicarbonate solution [pH 9] prior to infection. They were then given 106 ST strain SL1344 or ST strain SL1344 OmpC-C2W (ST-2W) by intragastric gavage. Enrofloxacin was included in the drinking water (2 mg/ml) in some cases.

**Production of the ST-2W strain**

ST was tagged chromosomally with the 2W peptide (EAWGALANWA-VDSA), as described by Uzzau et al. (17). Briefly, primers were designed with extension arms homologous to the 3’ portion of the OmpC gene, deleting the stop codon, and extending downstream from it. Additionally, a single FLAG sequence was included, for blotting purposes, before reintroduction of the stop codon, whereas kanamycin resistance was introduced downstream of the newly incorporated stop codon. PCR products were generated by amplification from a template plasmid (pJM1) encoding the 2W peptide and FLAG epitope. PCR products were used directly for electroporation into ST containing the temperature-sensitive pKD46 plasmid, carrying arabinose inducible bacteriophage λ red genes. Bacterial suspensions in 10% glycerol were mixed with 0.5–1 μg PCR product and incubated on ice for 30 min before transferring to a chilled 0.2-cm cuvette. Cuvettes were subjected to a single pulse of 12.5 kV/cm. After incubation on ice for 30 min before transferring to a chilled 0.2-cm cuvette, Cuvettes were subjected to a single pulse of 12.5 kV/cm. After recovering for 1 h at 37˚C in Super Optimal broth with Catabolite repression medium, bacteria were plated on Luria–Bertani agar plates supplemented with 50 μg/ml kanamycin. DNA sequencing was used to verify recombination.

**Western blotting**

Wild-type and ST-2W bacteria were grown overnight in Luria–Bertani Broth. Bacteria were centrifuged, and pellets were suspended in 1 ml 1× Celllytic B cell lysis reagent (Sigma) containing 0.2 mg/ml lysozyme (RPI), 50 U/ml Benzonase Nuclease (Sigma), and 25 μl Protease Inhibitor Cocktail (Sigma). Cell extracts were centrifuged, and supernatants containing soluble proteins were removed for SDS-PAGE analysis. Separated proteins were transferred to a nitrocellulose membrane and then blocked in 5% BSA for 1 h. Membranes were probed with mouse anti-FLAG M2 mAb (Sigma) at 1 μg/ml in 1% BSA for 1 h at room temperature with gentle agitation. The blot was washed and then probed with 1 μg/ml secondary goat anti-mouse Ab conjugated to Alexa Fluor 680 (Invitrogen) for 1 h at room temperature. The blot was analyzed on an Odyssey Imaging System (Li-Cor) at 700 nm.

**Cell enrichment and flow cytometry**

Spleen and lymph node cells were harvested and made into single-cell suspensions. Ten percent of the cell suspensions was removed for plating bacterial CFU with the addition of 0.1% Triton. The remaining 90% of the samples were stained for 1 h at room temperature with 2W1-Aβ–streptavidin–allophycocyanin tetramer, enriched for tetramer bound cells, and then probed with 1 μg/ml secondary goat anti-mouse Ab conjugated to Alexa Fluor 680 (Invitrogen) for 1 h at room temperature. The blot was analyzed on an Odyssey Imaging System (Li-Cor) at 700 nm.

**BrdU incorporation studies**

BrdU (Sigma) was dissolved in PBS at a concentration of 10 mg/ml. In vivo BrdU labeling was performed by i.p. injection of 1 mg BrdU on day 50 of ST-2W infection. Twelve hours later, spleens and mesenteric lymph nodes (MLN) were harvested individually, and BrdU incorporation into DNA was detected by intracellular staining, according to the manufacturer’s specifications (BD Biosciences).

**Lymphokine production**

ST-2W–infected mice were injected with 100 μg 2W peptide. Two hours later, spleens and MLN cells were harvested in media containing 10 μg/ml brefeldin A. The resulting cell suspensions were fixed, permeabilized, and stained with IFN-γ, TNF-α, and IL-2 Abs, as described previously (2).

**Cell-transfer experiments**

CD4+ T cells were isolated from spleens and lymph nodes of (CD45.1 × 129) F1 mice infected 50 d earlier with ST-2W and purified using Miltenyi CD4 isolation kits. The cells were then washed in EHAA media, suspended to a concentration of 5 × 107 cells/ml, incubated with 5 μM CFSE (Invitrogen) at 37°C for 10 min, and washed with prewarmed EHAA media before i.v. injection into (B6 × 129) F1 hosts. For cytokine experiments, purified CD4+ T cells were washed and injected i.v. without CFSE labeling. To control for experiment-to-experiment variability, the numbers of parked cells were normalized for the input number of donor-derived CD4+ T cells.

**Statistical analysis**

Statistical differences between normally distributed data sets were assessed in most cases using paired or unpaired two-tailed Student t tests with Prism software (GraphPad). A Mann–Whitney U test was used in one case in which the data were not normally distributed (Fig. 4E).

**Results**

**Generation of Salmonella expressing an immunodominant CD4+ T cell epitope**

Before monitoring the stability of CD4+ T cells during persistent ST infection, it was necessary to identify a relevant epitope. Although epitopes consisting of I-Aβ molecules complexed with peptides from the FlgC and SseJ proteins of ST have been described (19), the CD4+ T cell populations that recognize them are very small (18). The largest naive CD4+ T cell population identified is specific for a peptide called 2W (18), which is a variant of MHC class II I-E α-chain peptide 52–68 (20). Therefore, we used the red-mediated recombination system to insert the 2W peptide–coding sequence at the 3’ end of the OmpC gene in the ST chromosome (17). The resulting organisms, referred to hereafter as ST-2W, express an OmpC-2W fusion protein (Fig. 1A). As shown in Fig. 1B, oral inoculation of ST-2W bacteria into Nramp1R 129 mice resulted in an infection that peaked between 2 and 3 wk in the MLN and spleen and then declined to an undetectable level in the spleen by day 50; however, it persisted at low levels in MLN for hundreds of days, as described by Monack et al. (16) for wild-type ST organisms. Therefore, ST-2W organisms were capable of producing a persistent infection that presumably was controlled by CD4+ T cells.

**FIGURE 1.** Intragastric ST-2W infection is controlled by CD4+ T cells and persists in the MLN. (A) Western blot of ST bacteria genotypically tagged to express the 2W epitope on OmpC (42 kDa). Wild-type ST bacteria do not express this peptide and are shown as a negative control. BAP is a FLAG-fusion protein of 47 kDa that served as a positive control. (B) ST-2W bacterial CFU in the MLN (●) or spleens (○) of 129 mice. Horizontal lines represent mean values at the indicated times after intragastric ST-2W infection.
The CD4+ T cell response to intragastric ST-2W infection

We next measured the number of 2W:1-Aβ–specific cells over time postinfection using a p:MHCII tetramer–based cell-enrichment approach (21). Cells from MLN and spleens of infected 129 mice were stained with fluorochrome-labeled 2W:1-Aβ tetramer and anti-fluorochrome–labeled magnetic beads and enriched on magnetized columns, as previously described (18). 2W:1-Aβ tetramer–binding cells were detected by flow cytometry among the CD4+ cells that bound to the column.

Uninfected 129 mice each contained ∼100 2W:1-Aβ–specific CD4+ T cells in MLN and ∼300 in spleen. The majority of the cells in both locations were CD44low, as expected for naive T cells (Fig. 2A). A similar number and phenotype of 2W:1-Aβ–specific CD4+ T cells were observed in these tissues in 129 mice infected with ST organisms not expressing the 2W peptide (Fig. 2A). In contrast, 2W:1-Aβ–specific CD4+ T cells increased dramatically in number and CD44 expression in MLN and spleen after ST-2W infection (Fig. 2A). 2W:1-Aβ T cells in the spleens (●) and MLN (○) over the first 552 d after intragastric infection with ST-2W bacteria (mean ± SEM; n = 3 for each time point).

Salmonella p:MHCII-specific CD4+ T cells are numerically stable during persistent infection

We next studied the kinetics of 2W:1-Aβ–specific T cells following intragastric infection of 129 mice to determine whether a stable cell population was produced. The naive 2W:1-Aβ–specific CD4+ T cells present on days 6 and 60 after ST-2W infection were observed in these tissues in 129 mice infected with ST organisms not expressing the 2W peptide (Fig. 2A). In contrast, 2W:1-Aβ–specific CD4+ T cells increased dramatically in number and CD44 expression in MLN and spleen after ST-2W infection (Fig. 2A). 2W:1-Aβ–specific T cells present on days 6 and 60 after ST-2W infection expressed large amounts of CD44 (Fig. 2A) and the Th1 lineage–defining transcription factor T-bet (Fig. 2B). Therefore, intragastric ST-2W infection of Nramp1R mice induced an early homogeneous Th1 population, some of which survived into the persistent phase of the infection.

Salmonella p:MHCII-specific CD4+ T cells are stable during persistent infection

We next studied the kinetics of 2W:1-Aβ–specific T cells following intragastric infection of 129 mice to determine whether a stable cell population was produced. The naive 2W:1-Aβ–specific CD4+ T cell population that was ∼400 cells proliferated to produce 3 × 10^5 cells in MLN and 2 × 10^6 cells in the spleen 2 wk postinfection (Fig. 2C). The number of cells in both locations then underwent an 80% reduction that stabilized around day 40. Thereafter, the number of 2W:1-Aβ–specific CD4+ T cells remained consistent at 3 × 10^4 cells in MLN and 3 × 10^5 cells in the spleen. The cells in both locations were CD44high T-bethigh cells (Fig. 2B). These results show that ST p:MHCII-specific memory-phenotype cells are stably maintained during persistent ST infection.

A BrdU-labeling experiment was then performed to determine whether proliferation within chronically infected tissues could account for CD4+ T cell stability during the persistent phase of infection. 129 mice were injected with BrdU on day 50 after ST-2W infection, when viable bacteria were detected only in the MLN.
(Fig. 1B), and lymphoid organs were analyzed for BrdU incorporation 12 h later as a measure of cellular proliferation. This short labeling period was chosen to maximize the chance that cells were labeled in the location where they divided. As shown in Fig. 3B, 5–10% of the 2W:I-A\(^{b}\)-specific CD4\(^{+}\) T cells located in the MLN labeled with BrdU, whereas only 2–3% of cells in the spleen were labeled. All of the BrdU\(^{+}\) cells expressed the proliferation-associated protein Ki67. Therefore, the stability of the 2W:I-A\(^{b}\)-specific population correlated with a higher rate of proliferation in the persistently infected MLN.

**CD4\(^{+}\) T cell maintenance is dependent upon persistent p:MHCII presentation**

If the 2W:I-A\(^{b}\)-specific population was maintained by proliferation of a subset of cells in persistently infected MLN, then clearance of the ST-2W bacteria would be expected to result in decline of the population. ST-2W-infected mice were cleared of infection by treatment with enrofloxacin antibiotic for 40 d, beginning at day 28 after ST-2W infection, as a test of this hypothesis. As shown in Fig. 4B, mice not treated with antibiotic had about twice as many 2W:I-Ab–specific T cells in their lymphoid organs on day 68 of ST-2W infection as did mice that were treated with antibiotic.

We next determined whether p:MHCII recognition was the aspect of persistent infection that was required for the stability of the 2W:I-A\(^{b}\)-specific population. CFSE-labeled 2W:I-A\(^{b}\)-specific cells from the spleens and lymph nodes of persistently infected (CD45.1\(^{-}\)129) F\(_{1}\) mice were transferred into (B6 \(\times\) 129) F\(_{1}\) mice with time-matched ST-2W or wild-type ST infections. The number of donor-derived CD45.1\(^{+}\) 2W:I-A\(^{b}\)-specific CD4\(^{+}\) T cells was enumerated 1, 7, or 50 d after transfer to assess the stability of the transferred cells. ST-2W bacteria were not transferred along with the T cells, as evidenced by the fact that the 2W:I-A\(^{b}\)-specific cells of endogenous origin in wild-type ST-infected mice receiving 2W:I-A\(^{b}\)-specific memory-phenotype cells neither proliferated nor increased CD44 expression (Fig. 4C). As shown in Fig. 4D, the numbers of transferred 2W:I-A\(^{b}\)-specific cells were similar between days 7 and 50 in ST-2W–infected hosts, but they were significantly lower on day 50 than on day 7 in hosts infected with wild-type ST bacteria lacking the 2W peptide.

The stability of 2W:I-A\(^{b}\)-specific cells in ST-2W–infected hosts was associated with proliferation. 2W:I-A\(^{b}\)-specific cells residing in ST-2W– or wild-type ST–infected hosts for 50 d underwent one to three divisions, probably in response to IL-15 (2). However, donor-derived 2W:I-A\(^{b}\)-specific cells that resided in ST-2W–infected hosts for 50 d also contained cells that had divided more than seven times (Fig. 4C). Although these cells accounted for 70% of the population 50 d after transfer, the fact that they doubled at least seven times during that period means that they arose from <1% of the initial transferred population. These results are consistent with the possibility that periodic proliferation by a

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**FIGURE 4.** Numerical stability of CD4\(^{+}\) T cells is dependent on pMHCII-induced proliferation. (A) Plots of CD4\(^{+}\) T cells at day 68 after ST-2W infection from untreated mice (top panel) or mice that were treated with enrofloxacin for 40 d beginning at day 28 postinfection (bottom panel). (B) Number of 2W:I-A\(^{b}\)-specific cells in spleen and lymph nodes at day 68 after ST-2W infection in untreated (●) or enrofloxacin-treated (○) mice. (C) CD4\(^{+}\) T cells from ST-2W–infected mice were transferred into ST-2W (left panels) or ST (right panels) infection-matched hosts 50–75 d postinfection. Plots of 2W:I-A\(^{b}\)-enriched CD4\(^{+}\) T cells 1 d (top panels) or 50 d (bottom panels) after transfer. Day-1 gates indicate donor- (CD45.1\(^{+}\)) or host-derived (CD45.1\(^{-}\)) 2W:I-A\(^{b}\)-specific cells. Day-50 gates indicate frequency of CFSE fully (left gate) or partially (right gate) diluted, donor-derived (CD45.1\(^{+}\)) 2W:I-A\(^{b}\)-specific cells. (D) Frequency of donor-derived 2W:I-A\(^{b}\)-specific cells at 7 or 50 d after transfer into ST-2W (●) or ST (○) infection-matched hosts, normalized for day-1 park rates in independent experiments. The p values are from a comparison of day 7 and 50 values from the ST-2W– or ST-infected recipients. (E) Number of CFSE fully diluted, donor-derived (CD45.1\(^{+}\)) 2W:I-A\(^{b}\)-specific cells at 50 d after transfer into ST-2W (●) or ST (○) infection-matched hosts.
small number of cells in persistently infected MLNs accounted for the long-term numerical stability of the 2W-I-A\(^{b}\)-specific cell population.

**ST-2W-induced Th1 cells are multifunctional**

We then investigated whether persistent ST-2W infection affected the function of 2W-I-A\(^{b}\)-specific T cells. Mice infected with ST-2W 14, 45, or 100 \(d\) earlier were injected i.v. with 100 \(\mu\)g of 2W peptide to elicit lymphokine production by 2W-I-A\(^{b}\)-specific T cells, which was detected by direct ex vivo intracellular staining. 2W-I-A\(^{b}\)-specific T cells increased CD69 following 2W peptide injection and produced IFN-\(\gamma\) as expected based on their high expression of T-bet. Although IFN-\(\gamma\) is essential for controlling persistent ST infection (16), the best correlates of a protective Th1 response for other infections are CD4\(^{+}\) T cells capable of simultaneously producing IFN-\(\gamma\), TNF, and IL-2 (triple\(^{+}\)) (22, 23). Interestingly, almost all of the 2W-I-A\(^{b}\)-specific cells also expressed TNF, and a fraction of these cells also produced IL-2 (Fig. 5A). Thus, 2W-I-A\(^{b}\)-specific T cells were maintained in a highly multifunctional state during persistent ST-2W infection.

**Ag abundance affects CD4\(^{+}\) T cell function**

We next tested whether the low-level, localized p:MHCII presentation in the MLN of ST-2W-infected mice that maintained CD4\(^{+}\) T cell numbers also preserved their function. We found that the overall composition of single (IFN-\(\gamma^{+}\)), double (IFN-\(\gamma^{+}\), TNF\(^{+}\)), and triple (IFN-\(\gamma^{+}\), TNF\(^{+}\), and IL-2\(^{+}\)) lymphokine-producing cells was similar between donor-derived cells that resided for 50 \(d\) in ST-2W-infected or uninfected hosts (Fig. 6C). These results indicate that periodic stimulation by 2W-I-A\(^{b}\) complexes during the persistent phase of infection did not significantly affect the cytokine-production potential of the 2W-I-A\(^{b}\)-specific cells.

This result was surprising in light of studies indicating persistent stimulation by p:MHCII complexes results in functional exhaustion of CD8\(^{+}\) T cells (24). It was possible that 2W-I-A\(^{b}\)-specific cells avoided exhaustion in mice with persistent ST-2W infection because infected APCs were too rare. We tested this possibility by assessing the fate of 2W-I-A\(^{b}\)-specific memory-phenotype cells after transfer into (Act-2W\(^{129}\)) F1 transgenic mice that display 2W-I-A\(^{b}\)-specific cells induced by ST-2W infection completely lost the capacity to produce lymphokines in response to peptide challenge after transfer and residence for 50 \(d\) in ST-2W–infected (Act-2W×129) F1 transgenic mice. This loss of function was accompanied by induction of surface PD-1, which is a marker of T cell exhaustion (Fig. 6A) (26). Therefore, 2W-I-A\(^{b}\)-specific cells became functionally exhausted when exposed to systemic 2W-I-A\(^{b}\) complexes.

**Discussion**

Previous studies of acute *L. monocytogenes* and LCMV infections reached the conclusion that p:MHCII-specific CD4\(^{+}\) memory–phenotype T cells slowly decline after infections are cleared (1, 2). The numerical decline of murine CD4\(^{+}\) memory–phenotype T cells without their relevant Ag is at odds with the remarkable stability of CD8\(^{+}\) memory–phenotype T cells, IgM\(^{+}\) memory B cells, and plasma cells (1, 27, 28), and it challenges the classical definition of immune memory. The stability of the population of 2W-I-A\(^{b}\)-specific T cells in mice with persistent ST-2W infection could result from the periodic proliferation of a few memory-phenotype CD4\(^{+}\) T cells to produce short-lived effector cells coupled with the slow death of nonstimulated memory-phenotype cells. Numerical stability would be achieved in this case, despite the fact that neither the short-lived effector cells nor the quiescent memory-phenotype cells are perfectly stable. A similar scenario was reported for CD8\(^{+}\) T cells induced by chronic LCMV infection (29).

The numerical decline of CD4\(^{+}\) memory–phenotype T cells without p:MHCII presentation may be less surprising when considering the relationship between immune memory and protection. Immune memory is useful to the host only insofar as it contributes to immune protection. CD4\(^{+}\) T cells are not essential for immunity to acute *L. monocytogenes* and LCMV infections, which are controlled by CD8\(^{+}\) T cells specific for abundant p:MHCII ligands generated by these cytosolic intracellular microbes (5, 6). Thus, the decline of CD4\(^{+}\) T cells comes at no cost to the host in these cases because these cells do not provide a protective advantage. In contrast, in a case in which CD4\(^{+}\) T cells play an essential role in controlling a persistent phagosomal ST infection, p:MHCII-specific CD4\(^{+}\) memory–phenotype T cells were numerically stable. The fact that this stability depended on persistent infection and p:MHCII presentation indicates that TCR stimulation is the
key signal needed for maintenance of CD4+ T cells. Therefore, the classical idea of memory cell stability without Ag does not have to apply to CD4+ T cells in cases where they are protective because the phagosomal infections that they control are persistent, thus providing a “constant TCR reminder” to maintain the T cell population.

In the cases of chronic LCMV, hepatitis C, and HIV infections, prolonged p:MHC1 Ag stimulation eventually results in the exhaustion of CD8+ T cells (30). The fact that persistent ST-2W infection and p:MHCII presentation was required to maintain CD4+ T cells without exhaustion is probably related to the low level of p:MHCII Ag presentation during persistent ST infection. This is supported by the recent demonstration of CD8+ T cell function in the face of chronic HSV infection in the sensory ganglia (31). The restricted anatomical location of infection could also influence CD4+ T cell function during persistent ST infection. After the first 2 mo after oral ST infection, the MLN were the primary site in the body to harbor viable bacteria. Thus, it is conceivable that CD4+ T cells periodically migrate into sites of low-level persistent infection, proliferate in response to p:MHCII ligands displayed by persistently infected phagocytes, and then migrate to other body sites to recover. This process could produce numerical stability by balancing the death of cells in the population and prevent the cells from becoming exhausted. These ideas are supported by our observations that MLN were preferential sites of CD4+ T cell proliferation in persistently infected mice, and exposure to systemic p:MHCII complexes resulted in CD4+ T cells that could not produce lymphokines and expressed high levels of PD-1, the canonical marker for exhausted cells (30). Although the lack of cytokine production by these cells could be due to an indirect mechanism like regulatory T cell suppression, it more likely relates to the direct mechanism of T cell exhaustion.

Persistent low-level p:MHCII presentation by phagocytes during ST infection was also associated with homogenous production of Th1 cells, unlike transient p:MHCII presentation during acute infections, which generates a mixture of Th1 cells and follicular helper cells (3, 32). The reason why follicular helper cells were not maintained during persistent ST infection could relate to lack of p:MHCII presentation by B cells (33).

Given the impressive lymphokine-production capacity of 2W:I-A\(^{b}\)-specific cells in the face of persistent ST-2W infection, it was possible that periodic TCR stimulation was actually required to maintain multifunctional Th1 cells with maximal IFN-\(\gamma\) production. However, our results demonstrate that T-bet expression and IFN-\(\gamma\) production capacity were maintained at a high level in CD4+ memory–phenotype T cells that were parked in Ag-free hosts. However, a decrease in the number of highly functional
Th1 cells would be expected to reduce the capacity of CD4+ T cells to control a subsequent phagosomal ST infection. Such a loss could explain the concomitant immunity phenomenon studied in Leishmania-infected individuals. In this case, IFN-γ–producing Th1 cells control the infection within phagocytes at the initial site of infection and prevent it from spreading to other parts of the body. Oddly, however, the Th1 cells never eliminate the microbes from the initial site. Indeed, persistent infection at the original site is required for the Th1 cells to eliminate bacteria from other body sites after a second infection (11). Our results indicate that localized persistent infection and pMHCII presentation are required to maintain a numerically stable CD4+ T cell population with the maximal IFN-γ production capacity needed to control the infection at the initial site and eliminate it from other sites.

The fact that CD4+ T cells control persistent ST infection in phagocytes and presumably depend on pMHCII presentation by these cells to be maintained, but yet cannot eliminate the infection, is perplexing. One possibility is that regulatory T cells within MLN restrain microbial activities of CD4+ effector T cells in the same location (34, 35). Alternatively, persistently infected phagocytes may produce IL-10, which can ameliorate some of the phagocyte-activating effects of IFN-γ made by CD4+ T cells engaging in cognate interactions.

Our results suggest that a CD4+ T cell–dependent vaccine for a phagosomal pathogen will have to produce a local and long-lasting depot of bacterial Ag to maintain the protective CD4+ T cell population, which would otherwise decline. The failure to maintain protective CD4+ T cells could explain why the effectiveness of the bacillus Calmette-Guérin vaccine against tuberculosis wanes after the vaccine organisms are cleared from the body. To improve on this situation, it may be necessary to produce even longer-lasting Ag-delivery systems, perhaps along the lines studied in...