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*J Immunol* 2006; 177:925-933; doi: 10.4049/jimmunol.177.2.925

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Antigen-Experienced T Cells Limit the Priming of Naive T Cells during Infection with Leishmania major

Peter M. Gray,* Steven L. Reiner,‡ Deborah F. Smith,† Paul M. Kaye,‡ and Phillip Scott2*†

One mechanism to control immune responses following infection is to rapidly down-regulate Ag presentation, which has been observed in acute viral and bacterial infections. In this study, we describe experiments designed to address whether Ag presentation is decreased after an initial response to Leishmania major. Naive αβ-Leishmania-specific (ABLE) TCR transgenic T cells were adoptively transferred into mice at various times after L. major infection to determine the duration of presentation of parasite-derived Ags. ABLE T cells responded vigorously at the initiation of infection, but the ability to prime these cells quickly diminished, independent of IL-10, regulatory T cells, or Ag load. However, Ag-experienced clonal and polyclonal T cell populations could respond, indicating that the diminution in naive ABLE cell responses was not due to lack of Ag presentation. Because naive T cell priming could be restored by removal of the endogenous T cell population, or adoptive transfer of Ag-pulsed dendritic cells, it appears that T cells that have previously encountered Ag during infection compete with naive Ag-specific T cells. These results suggest that during L. major infection Ag-experienced T cells, rather than naive T cells, may be primarily responsible for sustaining the immune response. The Journal of Immunology, 2006, 177: 925–933.

Control of the intracellular protozoan Leishmania major requires a sustained T cell response, because in contrast to many acute viral and bacterial infections leishmaniasis is associated with a high parasite burden for many weeks. Moreover, after resolution of disease low numbers of parasites persist indefinitely, thus requiring the maintenance of T cell-dependent immune responses. This ongoing immune response is ongoing after infection. For example, Ag presentation following infection with Listeria monocytogenes was found to be extremely short-lived (3–5). Thus, within several days, naive Listeria-specific TCR transgenic T cells were unable to proliferate when transferred into Listeria-infected mice, despite the continued presence of bacteria. Similarly, the ability of naive T cells to respond to malaria was lost within 3 days of infection (6). In contrast, another recent study found that following influenza infections, naive TCR transgenic T cells were able to proliferate if transferred into infected animals even after the virus was eliminated, suggesting that Ag presentation was maintained throughout the course of infection and beyond (7). Furthermore, the continued recruitment of naive T cells into the viral response during infection was associated with an increased number of memory T cells, suggesting that recruitment of naive T cells may not only be important in sustaining an ongoing immune response, but important for memory T cell development. Taken together, these divergent results suggest that pathogen-specific characteristics may influence whether Ag presentation is ongoing after infection.

Infections induce several immunoregulatory mechanisms that are likely to influence the ability of both previously activated and naive T cells to respond. For example, L. major infection induces the production of IL-10 by macrophages and T cells, in particular regulatory T (Treg) cells, which can decrease Ag presentation (8, 9). Another factor that may influence how well naive T cells respond once a primary response has been induced is the degree of competition that occurs between naive and primed T cells. Effector and/or memory T cells differ from naive T cells with respect to faster kinetics in their response to Ag (10, 11), decreased requirement for costimulation (12, 13), their capacity to mediate effector functions (14), and their increased sensitivity to Ag compared with naive cells (12, 15–17). All of these characteristics might make previously activated T cells better able to respond during the course of a L. major infection than naive cells. Indeed, several studies of homeostatic induced proliferation indicate that the responsiveness of naive T cells is significantly influenced by the presence of either additional naive or activated T cells (18–20). In contrast, because naive T cells migrate through lymph nodes, while effector T cells home to sites of infection, it might be argued that there is little opportunity for competition to occur between

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Received for publication November 29, 2005. Accepted for publication April 20, 2006.

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1 This work was supported by National Institutes of Health Grants AI 35914 (to P.S.) and AI 42370 (to S.L.R.), and by grants from the Wellcome Trust (to D.F.S. and P.M.K.) and the British Medical Research Council (to P.M.K.). P.M.G. was supported by National Research Training Award Grant AI 07518.

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3 Abbreviations used in this paper: Treg, regulatory T; ABLE, αβ-Leishmania specific; DC, dendritic cell; dLN, draining lymph node; SLA, soluble leishmanial Ag.
naive and effector T cells. Competition might occur in the lymph nodes, however, between naive and central memory or lymph node-homing Ag-experienced T cells (2).

In this study, we used Leishmania-specific TCR transgenic mice, termed αβ-Leishmania-specific (ABLE) mice, to determine how responses of naive T cells are influenced by an ongoing L. major infection (21). This TCR, which was originally derived from a protective T cell clone (22), recognizes the leishmanial Ag, termed LACK (23). In BALB/c mice, a robust LACK-specific response is initiated shortly after infection with L. major, leading to a 100-fold expansion of LACK-reactive T cells by 4 days (24). By adoptively transferring naive ABLE T cells into mice at various times after L. major, we found that the ability of naive Leishmania-specific T cells to proliferate rapidly diminishes following infection, independent of IL-10, Treg, or Ag load. In contrast, previously specific T cells to proliferate rapidly diminishes following infection. Thus, our results suggest that during L. major infection Ag-experienced T cells, rather than naive T cells, may be primarily responsible for sustaining the immune response.

Materials and Methods

Mice

BALB/cByJ, C57BL/6, and C57BL/6 Thy-1.1 mice were obtained from The Jackson Laboratory. ABLE TCR transgenic mice bred onto a C57BL/6 background (21), WT15 TCR transgenic mice (provided by N. Killeen, University of Pennsylvania. CpG DNA (1826) was obtained from Coley Pharmaceuticals). Parasites and Ags

L. major parasites (MHOM/80/Friedlin) were grown in Grace’s insect medium (Invitrogen Life Technologies) supplemented with 20% heat-inactivated FBS, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Mice were infected in the hind footpad with 5×10⁵ stationary phase promastigotes. Soluble leishmanial Ag (SLA) was prepared, as previously described (22). OVA (OVA323–339) and LACK (LACK156–173) peptides were obtained from the Protein Chemistry Laboratory at the University of Pennsylvania. Cpg DNA (1826) was obtained from Coley Pharmaceutical Group. Immunization of mice was achieved by administering 50 μg of SLA along with 50 μg of Cpg DNA in 50 μl of PBS in the hind footpad. Mice were boosted with a second dose of SLA and Cpg in the same footpad.

Previously described L. major parasites expressing OVA (Leishmania. OVA: Llig15S/RNA: HASPB180VA) (27) were grown in Schneider’s medium (Gibco Life Technologies) supplemented with 20% heat-inactivated FBS, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Mice were infected in the hind footpad with 5×10⁵ stationary phase promastigotes.

Treg Cell depletion

Treg cells (CD4⁺ CD25⁺) were depleted in vivo with the administration of an anti-CD25 (PC61) mAb, as previously described (28, 29). Briefly, mice were injected i.p. with 1 mg of PC61 7 days before infection. Control mice were treated with 1 mg of an isotype control (rat IgG1) Ab. Depletion of Treg cells was assessed by flow cytometry using a mAb specific for a distinct CD25 epitope (7D4). Depletion was routinely >80% compared with mice treated with the isotype control Ab.

Adoptive transfers

Lymphocytes from the spleens and lymph nodes of mice were stained with CFSE, as previously described (30), and various doses of cells were transferred via the retro-orbital plexus into recipient mice at day 1, 7, 14, or 21 following L. major infection. Ag-experienced ABLE and DO11.10 T cells were generated, as previously described, with minor modifications (31). Briefly, ABLE or DO11.10 lymphocytes were stimulated in vitro at 8×10⁵ cells/ml in 24-well plates with 1 μg/ml LACK or OVA peptide, respectively, in the presence of 5 ng/ml rIL-12 and 10 μg/ml anti-IL-4 for 4 days. After stimulation, cells were washed three times with PBS and then replated in fresh medium at 1×10⁷ cells/ml in 24-well plates for 5 days. T cells were purified by negative selection using mouse T cell enrichment columns (R&D Systems), and 2×10⁶ purified Ag-experienced ABLE or DO11.10 cells were then CFSE labeled and adoptively transferred into mice.

Dendritic cell (DC) generation and transfer

DCs were generated, as previously described (32). Briefly, bone marrow was collected from the femurs of BALB/cByJ mice, and clusters within the bone marrow suspensions were dispersed by passing through a 20-gauge needle using a syringe. Cells were seeded into 6-well plates at ~2.5×10⁶/ml in 2.5 ml of medium (RPMI 1640; Invitrogen Life Technologies) supplemented with 10% heat-inactivated FBS (HyClone), 2 mM l-glutamine (Mediatech), 100 U/ml penicillin plus 100 μg/ml streptomycin (Mediatech), and 50 μM 2-ME (Invitrogen Life Technologies). In addition, 20 ng/ml GM-CSF (ProteoTech) was added. At days 3, 6, and 8, a further 2.5 ml of medium containing 20 ng/ml GM-CSF was added. At day 10, 60% of the cultured DCs were collected and cultured at day 10 in the absence of LPS. Adoptive transfer of DCs was performed, as previously described (33, 34). Briefly, 5×10⁶ CFSE-labeled ABLE Thy-1.1 cells were transferred into day 7 L. major-infected mice. One day later, mice were given 5×10⁶ LPS-matured DCs that had been pulsed with 5 μg/ml LACK156–173 in the same footpad that had been infected. After 3 days, DCs in non-inflamed draining lymph node (dLN) were analyzed by flow cytometry. Cell surface marker expression was compared with immature DCs that were cultured at day 10 in the absence of LPS.

Flow cytometry, intracellular cytokine staining, and analysis

Cells isolated from dLN and non-dLN were analyzed by flow cytometry directly ex vivo, as previously described (35). Briefly, cells were washed in staining buffer (PBS containing 0.1% BSA and 0.1% sodium azide) and incubated with Fc block (50 μg/ml 2.4G2 and 500 μg/ml rat IgG) before incubation with specific fluorochrome-conjugated mAbs against CD4 (RM4-5), CD44 (IM7), Thy-1.2 (53-2.1), Thy-1.1 (OX-7), or isotype control Abs (all from BD Pharmingen). Cells stained with anti-mouse DO11.10 TCR (KJ1-26) (Caltag Laboratories) were preincubated with normal mouse serum before incubation with specific mAbs. For intracellular cytokine staining, cells were stimulated with 50 ng/ml PMA, 500 ng/ml ionomycin, and 10 μg/ml brefeldin A (all from Sigma-Aldrich) for 4 h before surface staining. Fixed and surface-stained cells were permeabilized with 0.2% saponin (Sigma-Aldrich) in staining buffer before staining with specific fluorochrome-conjugated mAbs against IFN-γ (XMG1.2) (BD Pharmingen). Samples were acquired on a FACSCalibur and analyzed using CellQuest Pro (BD Biosciences).

T cells were labeled with CFSE, and the subsequent dilution of this fluorescent dye was detected by flow cytometry and used to calculate the responder frequency (number of donor T cells that divided due to stimulus) and the proliferative capacity (average number of daughter cells generated per responder), as described in detail in previous work (36).

Results

Activation of naive T cells diminishes following L. major infection

Our initial goal was to use Leishmania-specific TCR transgenic (ABLE) T cells as a monitor of ongoing Ag presentation following L. major infection, as has been done with several other pathogens (3, 6, 7). BALB/c Thy-1.1 mice were infected, and at different times following infection, CFSE-labeled ABLE T cells were transferred into the animals. After 3 days, the donor cells in the lymph nodes were analyzed by flow cytometry for proliferation and IFN-γ production. No proliferation of ABLE T cells was observed.
in mice that were not infected. When ABLE T cells were adoptively transferred into mice infected with *L. major* for 1 day, >50% of the cells diluted CFSE in the dLN, and 14% of the cells acquired the ability to produce IFN-γ (Fig. 1A). However, this robust response was significantly diminished, although still detectable, when the naive ABLE T cells were transferred into mice that were infected for 1 wk or more with *L. major*. The significance, if any, of the detected response in mice that received cells 1 wk after infection is currently unknown. Similar results were obtained with cells from another LACK-specific TCR transgenic mouse (data not shown) (25). It remained possible that the inability to optimally activate naive LACK-specific T cells was peculiar to the LACK Ag. Therefore, experiments were also performed using transgenic *L. major* parasites that expressed the model Ag, OVA (*Leishmania*-OVA). In these experiments, instead of transferring ABLE T cells, OVA-specific, DO11.10 T cells were adoptively transferred 1 or 7 days following infection with *Leishmania*-OVA. Similar to recognition by ABLE T cells of LACK Ag in wild-type parasites, the *Leishmania*-OVA experiments showed a similar phenotype in that there was a reduction in proliferation in DO11.10 cells when transferred at day 7 (6%) compared with day 1 (40%) following infection (Fig. 1B). The diminished activation of naive ABLE T cells observed after *L. major* infection was reflected in the percentage of donor cells in the dLN that responded by proliferating (responder frequency) (Fig. 1C), although an analysis of the proliferative capacity (the number of daughter cells produced by each dividing cell) (36) indicated that once cells initiated their proliferative cycle they responded similarly (data not shown). These results suggest that the activation signal received by cells 7 days following infection is similar to the signal received by cells 1 day after infection, but fewer of the cells after 7 days of infection are receiving this activation signal. The diminished ability of naive T cells to proliferate during *L. major* infection was not due to any intrinsic deficit in the T cells adoptively transferred into *L. major*-infected mice, because LACK peptide induced substantial in vitro proliferation by ABLE T that had been parked in *L. major*-infected mice from day 7 to day 10 (Fig. 1D). Because transfer of lower doses of TCR transgenic T cells has been shown to increase proliferation (37, 38), we also decreased the number of ABLE T cells transferred into infected animals. At the lowest dose of donor T cells that could still be detected in the lymph nodes, proliferation of naive TCR transgenic cells was also diminished at day 7 of infection compared with day 1 (data not shown).

One explanation of these results could be that Ag presentation has ceased because of decreased levels of Ag. To test this, mice were given a second administration of live parasites 6 days after the initial infection, and CFSE-labeled ABLE cells were adoptively transferred 1 day later. As with a single infection, the ABLE T cell response was significantly diminished at day 7 when compared with cells that were transferred into mice on day 1 (Fig. 2A). Taken together, these data indicated that during infection with *L. major* the ability to optimally activate naive T cells is a transient event.

Finally, to determine whether the diminished response by naive T cells during infection was limited to the dLN of the primary infection, we assessed the ability of naive T cells to respond in another site following a secondary infection. Mice were infected with a second dose of parasites in the contralateral footpad before the adoptive transfer of ABLE cells 7 days following primary infection. In lymph nodes draining the primary and secondary sites of infection, a lower frequency of donor cells responded by proliferating compared with mice that received ABLE cells 1 day after primary infection (Fig. 2B). This was also associated with a decrease in IFN-γ+ donor cells following secondary exposure compared with the activation of ABLE cells observed 1 day following infection. Although there is only a modest decrease in the frequency of IFN-γ+ cells at day 7 compared with day 1 (Fig. 2B) (17% compared with 21%, respectively), a comparison of all the mice tested at each time point showed a significant difference (13.1 ± 1.7 at day 7 compared with 21.6 ± 1.2 at day 1; *p* < 0.0001). These data suggest that the diminished response of naive T cells that occurs during *L. major* infection is a systemic event.
The diminished response of naive T cells in L. major-infected animals is not due to immunosuppression

Both specific and nonspecific mechanisms of immunosuppression operate during infection with L. major, and may contribute to the reduced response of naive T cells once a L. major infection is established (8, 9, 39, 40). Therefore, we tested whether naive T cells with specificity to an Ag unrelated to Leishmania exhibited decreased responses in mice with an established L. major infection. BALB/c mice infected with L. major for 6 days, or naive animals, were immunized with OVA protein. The following day, CFSE-labeled DO11.10 and ABLE Thy-1.1 transgenic T cells were adoptively transferred into the immunized mice and proliferation of the donor cells in the dLN was analyzed by flow cytometry. The proliferation of DO11.10 cells in L. major-infected mice that were immunized with OVA 6 days after infection (day 6 OVA/day 7 L. major) was similar to control mice that were immunized with OVA alone. However, the ABLE cells were unable to respond in both cases, suggesting that a global decrease in Ag presentation was not responsible for the diminished response of naive T cells in L. major-infected mice (Fig. 3). Importantly, donor cells responded to their cognate Ag in control mice that were immunized with OVA protein, infected with L. major, or immunized and infected at the same time 1 day before receiving donor cells. L. major infection is associated with a Treg response and increased production of IL-10, both of which may significantly impair T cell responses (8, 9). Therefore, we tested whether IL-10 or Treg cells might modulate the responses of naive T cells during L. major infection. IL-10−/− animals were infected and received CFSE-labeled ABLE T cells at 1 and 7 days following infection, as described in Fig. 1. Three days following transfer, the mice were sacrificed, and dLN were harvested and analyzed for proliferation by flow cytometry. In the absence of IL-10 ABLE cells exhibited slightly enhanced proliferation, but naive T cells transferred at 7 days of infection still displayed decreased proliferation compared with cells transferred at the initiation of the infection (Fig. 4A). Depletion of IL-4 also had no effect on the ability of naive T cells to respond at day 7 (data not shown). In a similar set of experiments, we depleted mice of Treg cells before infection. Depletion of Treg cells failed to enhance the ability of naive ABLE T cells to respond when transferred into mice infected for 1 wk with L. major (Fig. 4B), although the depletion of CD25+ cells also led to a more robust proliferative and IFN-γ response 1 day after infection compared with control mice. Taken together, these data demonstrate that the diminished response of naive T cells in L. major-infected mice is unrelated to IL-10, IL-4, or Treg cells.
Ag-experienced T cells are more efficiently activated during L. major infection compared with naive cells

It is widely accepted that Ag-experienced T cells have a lower threshold for activation compared with naive T cells (12, 15–17). Therefore, we considered the possibility that our results were not due to loss of Ag presentation, but competition between naive and primed T cells. To test this, we first asked whether Ag-experienced T cells responded better during an ongoing infection. Thus, ABLE transgenic T cells that had been stimulated in vitro with LACK peptide were adoptively transferred into infected mice. There were robust responses elicited in mice that received either naive or previously activated cells 1 day following infection (Fig. 5A). In mice that received Ag-experienced cells 7 days after infection, 40% of the cells had diluted CFSE, while only 10% of the naive cell counterparts had done so. Thus, while the proliferative response by primed T cells was less at day 7 compared with day 1, the response was significantly enhanced compared with naive T cells.

To determine whether polyclonal, Ag-experienced T cells would proliferate in L. major-infected mice, T cells from C57BL/6 Thy-1.1 mice that had resolved infection (>12 wk) were harvested, CFSE labeled, and then adoptively transferred into C57BL/6 mice that had been infected for 1 or 7 days. When harvested 1 wk later, ~30% of the polyclonal immune cells transferred into mice 1 day following infection had diluted CFSE in the dLN and >10% of those cells had acquired the ability to produce IFN-γ (Fig. 5B). In mice that received immune cells after being infected for 7 days, >40% of the donor cells in the dLN had diluted CFSE. In addition, the percentage of IFN-γ-producing cells was also enhanced in mice infected for 7 days compared with 1 day (Fig. 5B). However, when similar experiments were attempted with the transfer of naive polyclonal cells into mice infected for 1 or 7 days, there was no detectable proliferation in either group at 3 or even 7 days following transfer (data not shown). It is likely that this is due to the low precursor frequency of Ag-specific cells present in a naive polyclonal population. Lastly, it remained possible that the decrease in activation of ABLE and the decreased sensitivity of primed cells to this effect were specific to LACK-reactive T cells. Therefore, to discount this, naive and previously activated DO11.10 cells were
adoptively transferred into mice that were infected with *Leishmania*-OVA for 1 or 7 days. Similar to ABLE cells, in mice that received Ag-experienced DO11.10 cells 7 days after infection, 25% of the cells had dilated CFSE, while only 5% of the naive cell counterparts had done so (Fig. 5C). Taken together, these data indicate that both TCR transgenic T cells and polyclonal Ag-experienced T cells are efficiently recruited into the response even when transferred following infection, suggesting that Ag-experienced cells have an advantage compared with naive T cells in maintaining the immune response to *L. major*.

Endogenous responding cells inhibit the activation/expansion of naive Ag-specific T cells

Although our results with primed T cells indicate that Ag presentation does not cease after infection, it leaves open the question of why naive T cells are less able to proliferate when transferred to infected mice. It is well known that following *L. major* infection in BALB/c mice, a LACK-specific T cell response is initiated (24, 39). Having determined that Ag-experienced T cells are more readily activated than naive T cells after infection, we wanted to test whether competition by the endogenously responding cells was responsible for the suboptimal activation of the naive T cells. Therefore, naive ABLE T cells were adoptively transferred into DO11.10 RAG2−/− mice infected with *L. major*. Because DO11.10 RAG2−/− mice only have T cells specific for an OVA epitope, the *Leishmania*-specific endogenous response has been effectively removed. When naive ABLE cells were transferred into DO11.10-infected mice that had been infected for 1 day, >70% of the ABLE cells detected in the dLN of DO11.10 mice had dilated CFSE and almost 60% of the donor cells had acquired effector function (Fig. 6). Interestingly, in DO11.10 mice that had received donor cells at 7 days following infection, nearly 70% of the ABLE cells were CFSEdim in the dLN and 76% of the cells had gained the ability to produce IFN-γ. The expansion observed in infected animals was not due to homeostatic proliferation because none was detected upon adoptive transfer of ABLE cells into uninfected control mice. In addition, in vitro experiments were performed investigating the activation of naive ABLE T cells in the presence of previously activated ABLE T cells, and found that in the presence of primed cells, naive T cells exhibit a diminished response (data not shown). Together, these data provide evidence that naive T cells are refractory to stimulation in the presence of Ag-experienced cells and indicate that during an ongoing immune response to a given Ag, previously activated cells may preferentially respond. They also suggest that Ag presentation is unimpaired during the first week of *L. major* infection.

Naive and Ag-experienced T cells compete for access to APCs

Because naive ABLE T cells were able to respond both at the initiation and during *L. major* infection in DO11.10 mice that contained no other *Leishmania*-specific cells, and because primed T cells have a lower threshold for activation than naive T cells, it would appear that competition for APCs is a likely explanation for the diminished naive T cell response observed during infection. Therefore, to determine whether APCs were limiting after infection, we asked whether DCs could overcome the diminished naive T cell response seen during infection. BALB/c mice were infected with *L. major*, and at day 7 CFSE-labeled ABLE Thy-1.1 T cells were adoptively transferred into the infected mice. One day later, infected mice that had received ABLE cells were injected with LACK-pulsed DC. Three days after the adoptive transfer of DCs, the draining and non-dLN were harvested and analyzed by flow cytometry. Infected animals that received unpulsed DCs had limited activation of naive ABLE T cells in the dLN compared with positive control mice that were uninfected, but received Ag-pulsed DCs (Fig. 7). In contrast, injection of LACK-pulsed DCs restored the ability to effectively induce proliferation of naive T cells. This was also reflected in the acquisition of effector function (Fig. 7). These data indicate that naive and Ag-experienced cells compete for access to APCs during infection with *L. major*.

![FIGURE 6](http://www.jimmunol.org/) - The previously activated Ag-specific cells prevent the optimal activation of naive Ag-reactive cells. DO11.10 RAG2−/− recipient mice (Thy-1.2+) were infected, as described in Fig. 1. CFSE-labeled ABLE transgenic cells (Thy-1.1+) were adoptively transferred into recipient mice at day 1 or 7 following infection. The dLN of recipient mice were harvested and analyzed by flow cytometry 3 days following transfer. Dot plots were gated on donor cells in the dLN of recipient mice (Thy-1.1+). The numbers in the corners represent the percentage of events in either of the CFSEdim quadrants, and the numbers in parentheses represent the percentage of cells that produce IFN-γ that are CFSEdim. The data are representative of three experiments.

![FIGURE 7](http://www.jimmunol.org/) - Naive and Ag-experienced T cells compete for access to APCs. Naive BALB/c mice (Thy-1.2+) were either infected with 56f stationary phase promastigotes or left uninfected. Naive CFSE-labeled ABLE transgenic T cells (Thy-1.1+) were transferred into naive or day 7 infected mice. One day after transfer, LPS-matured bone marrow-derived DC pulsed with 5 μg of LACK peptide or left unpulsed were adoptively transferred directly into the recipient via the footpad. Three days following transfer of bone marrow-derived DC, cells were harvested and analyzed by flow cytometry. Histogram plots were gated on donor cells (Thy-1.1+). The number in the corner represents the percentage of donor cells that were CFSEdim in the dLN. The numbers in the corners of the dot plots indicated the percentage of donor cells that acquired the ability to produce IFN-γ in the dLN of the recipient animals. The data are representative of two experiments.
Secondary immunizations do not recruit naive T cells into the immune response

A logical extension of our findings is that the competition between naive and primed T cells that we observed during infection might also influence the ability to recruit naive T cells into an ongoing immune response induced by immunization. To assess this issue, we immunized mice with an SLA fraction and the adjuvant CpG, boosted them by administration of a second injection, and assessed the ability of transferred ABLE TCR transgenic T cells to proliferate. As expected, ABLE cells transferred into mice that had received SLA and CpG 1 day earlier exhibited a significant proliferative response (Fig. 8). However, when naive T cells were transferred into mice that were given a second administration of Ag, there was an 80% reduction in the ability of the donor T cells to dilute CFSE.

Discussion

Analysis of the proliferation of TCR transgenic T cells following adoptive transfer into infected mice can be used as an indirect measure to assess how long Ag presentation continues after infection. A recent study using this approach found that Ag presentation persists for at least as long as the pathogen survives (7), while other studies indicate that Ag presentation is rapidly down-regulated after infection (3, 4, 6, 41). Using a similar approach, we found that adoptively transferred naive TCR transgenic cells that recognize the leishmanial Ag LACK do not proliferate during an ongoing infection with L. major as well as at the initiation of the response. However, our data show that this is not due to a loss of Ag presentation, but instead due to competition between naive and Ag-experienced T cells.

Infection of BALB/c mice with L. major is associated with increased IL-10 production (42, 43) and Treg cell activity (8), but neither of these was associated with the decreased naive T cell response seen in L. major-infected mice. However, elimination of endogenous T cells was able to restore the naive T cell response to levels observed in mice infected for only 1 day, suggesting that the activated endogenous Leishmania-specific CD4+ T cells are competing with the naive donor T cell population. In other systems, T cell competition has been shown to play a role during homeostatic proliferation (18, 20, 44), as well as priming of T cells of the same (45, 46) and different (47, 48) specificities during responses to Ag. In leishmaniasis, there is a substantial expansion of LACK-reactive T cells within 4 days (24, 49), which are likely to be responsible for inhibiting the activation of naive LACK-specific TCR transgenic cells. This inhibition was specific, because there was no decrease in the response of naive T cells to OVA during L. major infection. Therefore, it appears that competition for particular class II molecules presenting LACK epitopes is responsible for the decreased naive T cell response. Consistent with this hypothesis was our ability to enhance the naive T cell response with LACK-pulsed DCs. Thus, our findings are similar to those showing that the activation of naive T cells specific for a particular epitope can be inhibited in the presence of Ag-experienced cells of the same specificity through competition for access to APCs (20, 47, 48, 50).

The inability to rescue the phenotype by the administration of more parasites or parasite-derived Ag indicates that the paucity of Ag is not responsible for the transient capacity to activate naive T cells. Rather, because adoptive transfer of Ag-pulsed DCs fully rescues the ability to prime naive Leishmania-specific T cells, it would appear that LACK-presenting APCs are limiting, as the number of Ag-specific T cells expands during infection. Given the fact that there is a limited amount of surface area on DCs with which to interact with T cells, and it has been suggested that T cells with a lower threshold for activation preferentially occupy this space, preventing the activation of T cells with a higher threshold for activation (48), it is likely that this plays a significant role in T cell activation. In addition, the ability of primed T cells to respond better than naive T cells to APCs other than DCs, such as macrophages and B cells, may also contribute to the capacity of primed T cells to respond. This hypothesis is consistent with our data especially because the ability of previously activated T cells to respond to lower doses of Ag (12, 15–17), as well as their less stringent requirements for costimulation (12, 13), may make them better able to respond when DCs are limiting compared with naive T cells.

Why Ag presentation is maintained in some infections, but not others, is unknown. In the case of Listeria, it was found that Ag presentation to CD8 T cells is short-lived due to the ability of CTLs to eliminate APCs (3, 4). This is not the mechanism operating in leishmaniasis, because primed L. major T cells proliferated after transfer to L. major-infected mice. Moreover, depletion of CD8+ T cells had no effect on the ability of naive cells to proliferate (data not shown). The most likely explanation for the difference between the Listeria and L. major infections is that the magnitude of the CD8 T cell response is less following L. major infection, although we have not directly investigated this issue. Similar to Listeria, the CD8 response to a sporozoite Ag of malaria was also found to be short-lived (6). However, in this case, whether APCs were killed due to the cytolytic activity of CD8 cells was not tested, and it is possible that competition between primed and naive T cells may have contributed to this response. In contrast, results with influenza virus suggest that competition does not always occur (7). Thus, when naive influenza-specific CD4+ T cells were transferred into mice at different times after infection, they were able to proliferate for several weeks. It is unclear why the competition that we observed with L. major was not evident in the influenza system, although it might be explained by either a lower frequency of hemagglutinin-specific T cells within the activated endogenous T cell population or differences in the affinity of the TCR-peptide interactions. Taken together, these studies indicate that the ability to recruit naive T cells into an ongoing immune response will vary, presumably depending upon specific characteristics of the infection. Similarly, adoptive transfer of naive OT-I cells into mice infected for 3 wk with OVA-transgenic Leishmania
donovani was host protective, albeit with delayed kinetics compared with transfer of previously activated OT-I cells (51).

One implication of our results is that continuous boosting with vaccines may not expand the pool of naive Ag-specific cells beyond those that were primed initially. Rather, primed cells will have a significant advantage over those cells that have not encountered Ag. Our studies with both parasites and SLA suggest that once an endogenous pool of primed T cells is established, additional injections of Ag will recruit few naive T cells into the immune response. This occurred in the lymph node given a primary infection, as well as in other lymph nodes draining secondary sites of stimulation. These data suggest that Ag-experienced T cells from the initial priming event in the recipient mouse home to a secondary lymph node upon reinfection, and compete with naive T cells that were transferred into the mice (Fig. 2). These cells may be the precursors of central memory T cells, which we have shown to be critical in maintaining immunity in the absence of persistent parasites (2). Whether this population is depleted with continued Ag administration has not yet been tested, but because few additional naive T cells are recruited into the response, it is possible that additional injections of Ag will increase the pool of short-lived effector T cells at the expense of central memory T cells. In contrast, it is possible that those few naive T cells that do respond in the face of competition by primed cells are better able to survive. In studies with L. monocytogenes, it was shown that competition delayed the attrition of CD8 T cells, and increased the percentage of cells that survived and became central memory T cells (5). Thus, those cells that received reduced stimulation were at a distinct advantage, consistent with the idea that signal strength dictates cell survival (52). Although there was no evidence of competition in studies of CD4 T cells stimulated by influenza, naive T cells that responded later in the infection, thus receiving less stimulation due to lower viral loads, were better memory T cells (7). Consequently, blocking the recruitment of those naive T cells into the response diminished immunity. Although our studies do not address this issue, they help highlight the importance of determining whether continued boosting leads to expansion of more memory T cells or depletion of a central memory T cell pool.

The finding that competition inhibited the ability of naive T cells to respond following infection was unexpected, and indicates that the use of adoptively transferred naive TCR transgenic T cells to assess Ag presentation may not always be appropriate. Based on our adoptive transfers, we might have incorrectly concluded that Ag presentation ceased after the first week of L. major infection. At the same time, however, these studies allowed us to discover that significant competition exists between naive T cells and previously primed T cells following L. major infection. Future studies will be required to determine whether this is unique to LACK, or an attribute of other leishmanial Ags. However, the data with Leishmania-OVA would suggest it is not peculiar to LACK. In either event, the current study has important implications to consider for the optimal recruitment of T cells into a response following both a natural infection and a prime and boost regimen of immunization.

Acknowledgments We thank Karen Joyce for the maintenance of the mouse colonies. We thank Nigel Killeen for providing the WT15 TCR transgenic mice. We thank members of the Scott laboratory for helpful discussions.

Disclosures The authors have no financial conflict of interest.

References


