The Early Generation of a Heterogeneous CD4+ T Cell Response to *Leishmania major*

Sara L. Colpitts and Phillip Scott

*J Immunol* 2010; 185:2416-2423; Prepublished online 12 July 2010; doi: 10.4049/jimmunol.1000483

http://www.jimmunol.org/content/185/4/2416

**References** This article cites 64 articles, 40 of which you can access for free at: http://www.jimmunol.org/content/185/4/2416.full#ref-list-1

**Why The JI?** Submit online.

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

*average

**Subscription** Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions** Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts** Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
The Early Generation of a Heterogeneous CD4+ T Cell Response to Leishmania major

Sara L. Colpitts1 and Phillip Scott

CD4+ T cells are an essential component of both the primary and secondary immune response against the intracellular protozoan parasite Leishmania major. Our laboratory has previously shown that CD62L\textsuperscript{hi}IL-7R\textsuperscript{hi} central memory T (T\textsubscript{CM}) cells mediate protective immunity following secondary challenge. To determine when T\textsubscript{CM} cells develop, we examined the phenotype of Leishmania-specific CD4+ T cells in the first 2 wk following infection. As expected, we identified a population of CD4+ T cells present in the draining lymph node with the characteristics of effector T cells. However, in addition, a second population phenotypically resembling T\textsubscript{CM} cells emerged coincident with the effector population. These T cells, expressing CD62L, CCR7, and IL-7R, failed to produce IFN-\gamma, but had the capacity to give rise to IFN-\gamma-producing effector cells. Our studies also demonstrated that the degree of proliferation and the timing of lymph node entry impact T\textsubscript{CM} cell development. The early generation of T\textsubscript{CM} cells following L. major infection indicates that T\textsubscript{CM} cells may not only control secondary infections, but may also contribute to the control of the primary infection.

Abbreviations used in this paper: dLN, draining lymph node; LN, lymph node; ndLN, nondraining LN; n.s., not significant; pi, postinfection; SPL, spleen; T\textsubscript{CM}, central memory T; WT, wild type.

Received for publication February 16, 2010. Accepted for publication June 8, 2010.

This work was supported by National Institutes of Health Grant AI35914 (to P.S.).

Address correspondence and reprint requests to Dr. Phillip Scott, Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104

\textsuperscript{1}Current address: Department of Immunology, University of Connecticut Health Center, Farmington, CT.

\textcopyright{} 2010 by The American Association of Immunologists, Inc. 0022-1767/10 $16.00

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1000483
the CD62L<sup>high</sup> cells are stimulated in the draining LN (dLN) within the first few days following infection, but many of the T<sub>CM</sub> cells soon thereafter cease proliferation. These data show that the early CD4<sup>+</sup> T cell response to L. major is heterogeneous, and suggest that the generation of T<sub>CM</sub> cells is concurrent with the initiation of the primary immune response to L. major.

**Materials and Methods**

**Mice**

C57BL/6, B6.Pt- Thy1<sup>−/−</sup>CD4<sup>+</sup> T cells were transferred into congenic recipients, unless otherwise noted in figure legends. For detection of low numbers of transferred cells, the harvested and pooled together as representative peripheral LNs (also referred to as non-draining LNs [ndLNs]) from individual naive or infected mice. For infection of low numbers of transferred cells, the harvested tissues were first enriched by negative selection (MACS) for the donor marker prior to analysis by flow cytometry (24). Briefly, samples were incubated with biotinylated Abs against CD45.1, followed by incubation with antibiotin microbeads, according to the manufacturer’s instructions (Miltenyi Biotec). The CD45.2<sup>+</sup> donor cells were then negatively selected with antibiotin microbeads, according to the manufacturer’s instructions (Miltenyi Biotec). The CD45.2<sup>+</sup> donor cells were then negatively selected using LS columns. Blocking Ab against CD45.2 (MEL-14) was a gift from S. Reiner (University of Pennsylvania, Philadelphia, PA). A total of 250 μg Ab was administered i.p. at the indicated time points.

**Flow cytometry and BrdU treatment**

The following Abs used to detect cell surface markers were purchased from eBioscience (San Diego, CA): CD4, Thy1.1, Thy1.2, CD45.1, CD45.2, CD45.1, B220, CD11b, NK1.1, CD127 (IL-7R<sub>α</sub>) (PE or allophycocyanin only), and CD62L. For detection of CCR7, cells were incubated with CCR7 Ab (PE or allophycocyanin only) for 1 h at 37˚C, washed, and then stained for any remaining surface Ags, as above. For intracellular detection of cytokines, cells were stimulated with PMA, ionomycin, and brefeldin A for 4 h in vitro and fixed with 2% parafomaldehyde in PBS. Cells were then permeabilized with 0.2% saponin and stained with IL-2 allophycocyanin and IFN-γ PE-Cy7 (eBioscience). BrdU was purchased from BD Pharmingen (San Diego, CA), and 1 mg BrdU was administered i.p. every 12 h for 3 d (6 mg total per mouse). Cells were permeabilized and stained for flow cytometry, per the manufacturer’s instructions. Data were acquired on an LSR II or a FACS-Canto (BD Biosciences). Analysis was performed using FlowJo software (Tree Star, Ashland, OR). For all samples, gating was established using a combination of isotype and fluorescence-minus-one controls.

**Statistics**

Statistical analysis was performed using Prism (GraphPad, San Diego, CA). For all graphs, data are presented as the mean ± SEM. One-way ANOVA was used to establish significance in combination with the indicated posttest, and p < 0.05 was considered significant.

**Results**

**IL-7R expression on CD4<sup>+</sup> T cells defines two populations of T cells responding to L. major infection**

To monitor the Ag-specific CD4<sup>+</sup> T cell response to L. major, we adoptively transferred CFSE-labeled OTII T cells into congenic C57BL/6 mice and challenged the recipient mice with L. major parasites expressing OVA (Leish-OVA) (22). Proliferation and IL-7R expression on the Ag-specific OTII cells in the LN draining the site of infection (dLN) were assessed on days 3, 7, and 14 following infection (Fig. 1). We initially chose to focus on the expression of the IL-7R because its expression is required for the survival of memory T cells (25–27). As early as 3 d pi, there was a robust proliferative response of the OTII cells. Almost all of the proliferating cells downregulated the IL-7R, which is consistent with previous studies (25, 28–37). However, we observed two distinct populations of responding cells by 7 and 14 d pi. One group of proliferating CD4<sup>+</sup> T cells expressed a low level of the IL-7R, a phenotype suggestive of an activated effector T cell that has recently been stimulated via the TCR (38). The other population of responding cells expressed the IL-7R at levels equivalent to that of naive T cells. We also observed that by day 7, proliferated OTII cells could be detected within other peripheral LNs (ndLNs).

Most likely these T cells proliferated in the dLN and then migrated to the ndLNs. Interestingly, only the IL-7R<sup>−</sup>expressing T cells were found in ndLNs, suggesting that these two T cell populations may exhibit differences in their migratory potential or that migration away from the inflammatory site allows for the upregulation of the IL-7R. Although parasites can disseminate through the blood to distant sites (39), the lack of an IL-7R<sup>−</sup> population in the ndLNs could suggest that priming within the ndLNs is minimal or that the activation of CD4<sup>+</sup> cells under such conditions generates cells with a T<sub>CM</sub>-like phenotype. Thus, based on expression of the IL-7R, within 2 wk of L. major infection, two distinct populations of CD4<sup>+</sup> T cells are evident.

**Expression of LN-homing molecules by CD4<sup>+</sup> T cells responding to L. major infection**

The expression of CD62L and CCR7 is required for efficient entry of T cells into LNs, and their differential expression has also been used to define memory T cell subsets (1). We have previously reported that CD62L<sup>high</sup>CD4<sup>+</sup> T CM cells are present in mice that have resolved a primary infection with L. major (referred to as immune mice) (19), and we wanted to determine whether cells with a similar phenotype were present early pi. Therefore, we again adoptively transferred CFSE-labeled OTII T cells to naive recipients that were subsequently challenged with Leish-OVA and asked whether the T cells responding to infection exhibited differential expression of CD62L and CCR7. As shown in Fig. 2, a pattern similar to that observed with IL-7R expression was seen in proliferating (CFSE<sup>dim</sup>) OTII T cells. Thus, two populations of responding T cells were present early pi: one population of cells expressed CD62L and CCR7, whereas the second did not (Fig. 2A, 2C). Furthermore, the CD62L<sup>high</sup> CD4<sup>+</sup> T cells expressed uniformly low levels of the IL-7R, whereas the population of CD62L<sup>high</sup> cells contained both IL-7R<sup>−</sup> and IL-7R<sup>low</sup> cells (Fig. 2B). We confirmed these results with another TCR transgenic mouse (termed ABLE) that recognizes a Leishmania-derived peptide (LACK<sub>156-175</sub>; Leishmania homolog of receptors for activated C kinase) in the context of MHC II I-A<sup>d</sup> and found similar results (data not shown).

Recent work has demonstrated that adoptive transfer of large numbers of transgenic T cells can impact the emergence of memory T cells (40–43). Thus, to determine whether the generation of these two different cell types was due to the transfer of nonphysiologic numbers of T cells with the same specificity, we repeated the above experiment, but transferred reduced numbers of TCR transgenic cells (10<sup>5</sup>, 10<sup>4</sup>, and 10<sup>3</sup>). Due to the loss of cells following transfer, the actual numbers present in spleen and LN tissues following transfer correspond approximately to 10<sup>6</sup>, 10<sup>5</sup>, and 10<sup>3</sup>, respectively (44). We then infected the recipient mice with Leish-OVA and
isolated the dLN after 10 d. We found that regardless of the initial number of cells transferred, two populations of cells could be defined by CD62L expression, and the relative frequency of each population was the same (Fig. 3A, 3C). However, when lower numbers of cells were transferred, the extent of CFSE dilution was increased as only a small percentage of OTII cells remained CFSEbright after 10 d, and the fold increase in proliferating Ag-specific cells over input was significantly increased (Fig. 3A, data not shown). These data are consistent with previously published findings that proliferation is dramatically influenced by the frequency of T cells recognizing a particular epitope (41).

To further confirm that our results reflected the naturally occurring immune response following infection, we adaptively transferred CFSE-labeled polyclonal CD4+ T cells from naive conventional C57BL/6 mice to congenic recipients (CD45.1) that were subsequently infected with wild-type (WT) L. major parasites. This allows us to monitor naturally occurring Leishmania-specific CD4+ T cells at normal physiologic frequencies within a given pool of naive T cells based upon their proliferation and dilution of CFSE. We isolated the cells from the dLN after 2 wk and found that similar to the T cells from the TCR transgenic mice, polyclonal CD4+ T cells from conventional mice exhibited the same degree of heterogeneity, generating both CD62Llow and CD62Lhigh populations of responding CD4+ T cells (Fig. 3B, 3C). These results further support our findings that infection with L. major rapidly generates a heterogeneous population of CD4+ T cells. Thus, we can conclude that the early CD4+ T cell response to L. major infection generates at least two populations of cells that can be distinguished based on their expression of both the IL-7R and LN-homing molecules, and that one of these populations has the phenotype of TCM cells.

**Proliferative arrest generates CD62Lhigh T cells early following infection**

In addition to the differential expression of the IL-7R and molecules associated with LN homing, we noticed that the CD4+ T cells responding to infection could be characterized by how many cell divisions they had undergone with a substantial enrichment in the CD62Lhigh cells in divisions 1–5 (Figs. 2, 3). This enrichment varied, as is evident when looking at Fig. 3A and 3B. One cause for such variation could be the specificity of the monoclonal versus polyclonal cells. Alternatively, the dLNs were collected at different times (day 10 versus day 14 pi), and as the infection proceeds, one might anticipate that the CD62Lhigh cells would have an increased opportunity to be restimulated and further dilute CFSE. By focusing on these early time points, we can examine how cell division might contribute to the generation of the heterogeneous CD4+ T cell response. Because division was assessed by CFSE dilution after 7–14 d of infection, we could not determine when over the course of that time the cells in division 1–5 had actually divided. The cells in divisions 1–5 could represent the following: 1) cells that had only recently begun to divide; or 2) T cells that received a signal to proliferate early following infection, but had ceased dividing. To distinguish between these two possibilities, we transferred CFSE-labeled OTII cells into congenic recipients that were given BrdU at different times pi to assess when the CFSEhigh cells had proliferated. One group of mice was given BrdU from days 1 to 3, and a second group of
mice received BrdU from days 7 to 9. Thus, if the cells were proliferating just prior to sacrifice, the CFSE\textsuperscript{dim} OTII cells in divisions 1–5 would also be BrdU\textsuperscript{+} in mice receiving BrdU from days 7 to 9. However, we found that only when BrdU was administered during the first 3 d following Leish-OVA infection was a large percentage of BrdU\textsuperscript{+} cells detected in the dLN on day 10 pi (Fig. 4). Interestingly, many of these BrdU\textsuperscript{+} cells were also detected in the ndLNs, suggesting that these cells proliferated in the first 3 d, most likely in the dLN, but then migrated to other lymphoid tissues. When the BrdU was given just prior to the end of the experiment (days 7–9), the small percentage of cells that were BrdU\textsuperscript{+} was present in those cells that had undergone >5 divisions. Thus, through the combination of CFSE dilution and the incorporation of BrdU, we can conclude that many of the cells with a TCM phenotype that are observed early following infection received a signal to proliferate in the first 3 d following infection, but then underwent an active cessation in proliferation.

The timing of T cell arrival in the dLN influences their ability to respond

The above results indicate that only some CD4\textsuperscript{+} T cells continue to divide following infection with L. major, and those with reduced proliferation exhibit the phenotype of TCM cells. In previous studies, we found that naive T cells proliferated poorly when transferred into already infected mice, suggesting that when T cells are recruited into the immune response could influence how well they respond (45). Similarly, studies in mice immunized with peptide showed that early arriving T cells proliferated more, whereas late arriving T cells proliferated less and were directed toward a TCM fate (46). Thus, we considered the possibility that one difference between the cells that had divided >5 times and those that had ceased proliferating was when they entered the dLN. To determine whether this was the case, we assessed the response of T cells that were resident in the dLN at the time of infection by blocking the entry of additional recruited T cells into the LN using mAbs against CD62L. In one group of mice, anti-CD62L mAb was administered at the time of Leish-OVA infection (1 d after the transfer of OTII cells). A second group of mice did not receive anti-CD62L mAb treatment until 3 d pi, which allowed for transient recruitment of cells into the dLN. Because anti-CD62L mAb treatment prevents surface staining for CD62L, we used expression of CCR7 to differentiate between the two T cell populations. We found that very few cells in divisions 1–5 when LN recruitment was blocked at the time of infection (Fig. 5A). Most of the cells found in the dLN at day 10 pi had divided >5 times and expressed low levels of CCR7 (Fig. 5). However, a population of T cells in divisions 1–5 and expressing CCR7 was present in the dLNs of mice when Ab treatment was withheld until day 3. These data suggest that the T cells resident in the dLN at the time of infection are more likely to differentiate into effector...
the dLNs and spleens after 2 wk and compared the frequency of IFN-γ cells with this phenotype from naive and immune mice. We isolated subsequently challenged with adoptively transferred them into congenic recipients that were sub-
varying rounds of division. We then CFSE labeled the cells and include a mixture of Leishmania L. major mice that had been infected with CFSE, and transferred to naive congenic recipients. The recipient mice were infected with WT L. major the following day. After 2 wk, the extent of CFSE dilution within the CD4+CD45.2+CD45.1+ donor cells in the dLN was used to gate the plots shown in A, which are representative of >12 mice in more than four separate experiments. Intracellular cytokine production was detected ex vivo following a 4-h in vitro stimulation. Numbers indicate the percentage of cyto-

kine positive. B, CD4+CD62Lhigh cells from the spleens and LNs of naive mice, mice that had been infected with 2 × 10⁷ WT L. major 2 wk prior, and immune mice (>12 wk post-L. major infection) were MACS purified, CFSE labeled, and transferred to congenic recipients that were challenged with WT L. major the following day. After 2 wk, the dLN and spleen (SPL) were harvested, and cytokine production by the CD4+CD45.2+CD45.1+ donor cells was determined, as above. Plots are representative of three to four mice per group in two separate experiments. Numbers indicate the percentage of CFSEdim cells that are producing IFN-γ. SPL, spleen.

CD62L⁹ cells can become IFN-γ-producing T cells upon restimulation

The lack of IFN-γ production by TCM cells is one of the critical characteristics that was shown to distinguish TCM cells from effector cells following L. major infection (19). Therefore, we next assessed the ability of the different types of early responding CD4+ T cells to produce IFN-γ directly ex vivo. Naive polyclonal CD4+ T cells were enriched from the spleens and LNs of C57BL/6 mice, labeled with CFSE, and transferred to naive congenic recipients. The recipient mice were infected with L. major the following day, and at 2 wk pi, the dLN was isolated to determine cytokine production by responding (CFSEdim) CD4+ T cells in the dLN. Because in vitro stimulation with PMA and ionomycin can influence surface expression of CD62L, we used the extent of CFSE dilution to compare TCM cells in divisions 1–5 with the heterogeneous population of CD62L⁹ TCM and CD62L²⁰ effector T cells in >5 divisions. Although both populations had the capacity to make IL-2, IFN-γ was only produced by cells that had divided more than five times (Fig. 6A). Thus, similar to the TCM cells that we observed >12 wk pi, the early responding T cells that have ceased dividing and adopted a TCM phenotype fail to make IFN-γ. To determine whether the cells with a TCM phenotype that emerge in the early weeks following infection could differentiate into IFN-γ-producing cells upon restimulation, we purified CD4+CD62L⁹ cells from mice that had been infected with L. major for only 2 wk. Based on the results shown in Fig. 3B, this population of donor cells would include a mixture of Leishmania-specific cells that have undergone varying rounds of division. We then CFSE labeled the cells and adaptively transferred them into congenic recipients that were subsequently challenged with L. major. As controls, we also purified cells with this phenotype from naive and immune mice. We isolated the dLNs and spleens after 2 wk and compared the frequency of IFN-

γ-producing cells within the total population of CFSEdim cells. This comparison allowed us to normalize for the expected differences in Ag-specific cells that would be present in these three different pop-
ulations of donor T cells. We found that whereas a small percentage of cells derived from naive mice could produce IFN-γ after 2 wk, this percentage was increased ~2-fold when the donor cells were derived from mice that had been infected for 2 wk prior to isolation, transfer, and rechallenge (Fig. 6B). Thus, we found that at least some of the CD62L⁹ cells generated early pi had the capacity to differen-
tiate into Th1 cells, similar to the TCM cells we characterized in immune mice. Using current protocols, it is unknown whether the Th1-generating cells had undergone more or less rounds of division prior to restimulation. The increase in the percentage of IFN-γ-producing T cells by donor-derived cells from 2-wk-infected mice compared with naive donor cells, in combination with the lack of IFN-γ production by cells in divisions 1–5, suggests that whereas the CD62L⁹ cells may not produce IFN-γ directly ex vivo, some of the cells may already be predisposed along a pathway toward Th1 cytokine production. This would be consistent with our recent findings that some CD62L²⁰ cells, although not making IFN-γ, express T-bet, the transcription factor required for IFN-γ production (20).

Discussion

The hallmark of adaptive immunity is the ability to develop immunologic memory, such that following a primary infection we are
better able to respond when re-exposed to the same pathogen. Whereas immunologic memory is a critical component of the immune response, how and when memory develops is not completely understood. We previously demonstrated that following infection with L. major, C57BL/6 mice develop CD4+ TCM cells that can mediate resistance to reinfection (19). In this study, we find that CD4+ T cells with a TCM phenotype develop within the first few weeks following L. major infection, coincident with the development of CD4+ Th1 effector cells. These TCM cells expressed high levels of CD62L and CCR7, which promote migration through lymphoid tissues, and high levels of IL-7R, which is required for the long-term survival of memory T cells. Cytokine production by these early-emerging TCM cells was limited to IL-2 and required Ag-induced proliferation to give rise to IFN-γ-producing Th1 cells. Finally, we provided evidence that the phenotype of the CD4+ T cells is influenced by the timing of their arrival in the dLN and involves the proliferative arrest of Ag-specific T cells, despite the continued presence of parasites. Taken together, these results indicate that CD4+ T cells with a TCM phenotype develop during the early stages of an immune response.

There are several models that attempt to explain how memory T cells develop, and although some postulate that memory T cells slowly develop after the pathogen has been cleared, others propose that memory T cells—or their precursors—develop very soon pi (4–11). Our data indicate that CD4+ T cells with a TCM phenotype (CD62LhighCCR7highIL-7Rhigh) develop quite rapidly following infection with L. major. These cells do not immediately produce IFN-γ upon restimulation, suggesting that they develop prior to becoming effector cells. These results are consistent with our previous studies indicating that TCM cells in immune mice do not make IFN-γ and that, in the absence of IL-12, they can differentiate into Th2 cells (19, 21). Catron et al. (46) described a similar population of CD62LhighCD4+ T cells that were generated following peptide immunization, suggesting that these early-emerging TCM cells are not unique to leishmaniasis and may represent a normal component of the CD4+ T cell response. Thus, whereas cytokine-producing cells can contribute to the pool of long-lived memory T cells (47), our data suggest that progression through an effector phase is not a requirement for TCM generation. It is also possible that depending on the nature of the infection, TCM-like cells might develop both prior to and after becoming effector cells (48).

The mechanism(s) driving the heterogeneity of the early T cell response is not well understood. The initial interaction between a T cell and the APC can lead to asymmetric cell division, such that one daughter cell becomes an effector cell, whereas the other a less differentiated cell that exhibits a memory phenotype (18). Alternatively, Lanza and Sallusto (49) proposed the signal strength hypothesis as a mechanism to explain the heterogeneity of the CD4+ T cell response. Their hypothesis suggests that those cells receiving weaker signals will become TCM cells, whereas those cells receiving the strongest signals will become fully differentiated effector cells that exert their function and then die. Support for this hypothesis was provided by Wu et al. (50), who showed that acquisition of IFN-γ production by multiple rounds of stimulation with Ag led to the inability of a CD4+ T cell to survive long-term. However, more recent studies have shown that IFN-γ–producing cells can contribute to the pool of long-lived memory T cells (47). Despite their expression of the IL-7R, it is not thought that signals through the IL-7R drive TCM cell development (32, 51, 52). Also, whether a CD4+ T cell receives successive stimulation can have a direct impact on the degree of proliferation and the generation of memory (15, 53, 54). It is probable that a range of signal strengths exists with differential outcomes on individual CD4+ T cells. Therefore, the overall extent of stimulation can impact the type of memory T cell generated and depends on the cumulative signals imparted on a given cell through successive interactions with DCs, costimulation, and the inflammatory milieu (6, 40, 55).

By using OTII cells to track the Ag-specific CD4+ T cell response, we found that most of the TCM cells evident 2 wk after L. major infection have ceased proliferating, with many of them having divided only a few times. Why they fail to continue to proliferate is unknown. It is possible that T cells receive an inhibitory signal that actively inhibits proliferation (56). However, because continued TCR stimulation is required for CD4+ T cells to continue to divide (57), the simplest explanation for the proliferative arrest may be a loss of TCR signaling. Thus, the proliferative arrest might result from the lack of a positive signal rather than the presence of a negative signal. For example, it has been proposed that weaker signals are the result of a stochastic event caused by migration away from the site of Ag presentation (49, 58). Consistent with this idea are our findings that some of the TCM cells are present in ndLNs soon pi. These cells have the potential to migrate back through the dLN, where they can be stimulated once again and give rise to Th1 cells as needed to control the parasite burden. Competition among responding T cells can also have a negative impact on the Ag-specific T cell response, as indicated in several studies using TCR transgenic cells (40, 41). Similar to these studies, we found that altering the number of Ag-specific T cells influenced the extent of proliferation. However, it did not change the percentage of T cells that exhibited a TCM phenotype. Moreover, polyclonal responses—in which a diverse repertoire of Ag-specific T cells is maintained at physiologic frequencies—exhibited the same early development of TCM cells. Thus, whereas competition with non-physiologic numbers of T cells can influence the magnitude of the proliferative response, it does not appear to be responsible for the generation of TCM cells following L. major infection. In contrast, we found that when T cells arrive in the dLN, the percentage of T cells that respond such that only the cells recruited into the dLN developed a TCM phenotype. Using a peptide immunization model with similar results, it was suggested that this was due to decreasing Ag over time (46). However, following L. major infection, the parasite burden continues to increase during the first several weeks of infection, although it has also been proposed that there are two waves of Ag presentation, with the second wave commencing after 2 wk (59, 60). Thus, a more likely explanation for our findings might be the ability of Leishmania to impair Ag presentation by DCs, resulting in an overall decrease in the extent of T cell activation (61–64). By 2 wk pi, those cells that had fully diluted CFSE (>5 divisions) were a heterogeneous population containing both CD62Lhigh and CD62Llow cells.

Infection of C57BL/6 mice with L. major is associated with the development of a cutaneous lesion that resolves after several weeks due to the development of CD4+ Th1 response. However, a small number of parasites persists in these mice. The control of these remaining parasites is dependent upon the maintenance of an effective Th1 response, such that treatment of mice that have resolved a primary L. major infection with anti–IFN-γ leads to the reactivation of the disease. How this Th1 immunity is maintained is not well understood. One possibility involves the continued recruitment of naive T cells into the response, yet we have found that naive Leishmania-specific T cells respond poorly in the presence of previously activated T cells (45). Alternatively, the TCM cell population that we have described in this work may serve as an additional source of Ag-experienced T cells that can recirculate through the dLN and receive additional signals to further differentiate into Th1 effector cells as needed to help control the infection.
Certainly, if most highly activated Th1 cells eventually die, the maintenance of a population of TCM cells might be essential to control the parasites that persist for the lifetime of the animal. In summary, we have demonstrated that the early CD4⁺ T cell response to L. major infection involves the generation of both effector T cells and T cells with the characteristics of TCM cells. The heterogeneity of the response is characterized by differential expression of CD62L,CCR7, and the IL-7R, as well as the ability to produce the effector cytokine IFN-γ. These TCM cells are available as an expanded pool of Ag-specific T cells able to be restimulated and differentiate into effector cells, a function that may be critical in controlling not only secondary infections, but also the parasites that persist in L. major infections.

Acknowledgments

We thank Paul Kaye and Deborah Smith for Leish-OVA parasites, Steve Reiner for MEL-14 mAb, and the University of Pennsylvania Flow Cytometry and Cell Sorting Facility for technical assistance.

Disclosures

The authors have no financial conflicts of interest.

References


