Distribution of Cycling T Lymphocytes in Blood and Lymphoid Organs During Immune Responses

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Proliferation of murine T lymphocytes in blood, lymph nodes, and spleen was studied in four in vivo stimulation systems, using BrdU pulse-labeling of DNA-synthesizing cells. The T cell response to the superantigen *Staphylococcus* enterotoxin B (SEB) was studied in detail. Vβ8+ T cells showed a peak of DNA synthesis 16–24 h after SEB injection, and the percentage of BrdU+ CD4 and CD8 T cells was higher in blood than in lymph nodes and spleen. DNA synthesis was preceded by massive migration of Vβ8+ cells from blood to lymph organs, in which the early activation marker CD69 was first up-regulated. SEB-nonspecific Vβ6+ cells showed minimal stimulation but, when cycling, also expressed a high level of CD69. The other systems studied were injection of the IFN-γ inducer polyinosinic-polycytidylic acid, infection by the BM5 variants of murine leukemia virus (the causative agent of murine AIDS), and T cell expansion after transfer of normal bone marrow and lymph node cells into recombinase-activating gene-2-deficient mice. In each case, a peak of T cell proliferation was observed in blood. These data demonstrate the extensive redistribution of cycling T cells in the first few hours after activation. Kinetic studies of blood lymphocyte status appear crucial for understanding primary immune responses because cycling and redistributing T lymphocytes are enriched in the circulating compartments. }

**Materials and Methods**

**Mice**

All experiments were done with 6- to 8-wk-old C57BL/6 (B6) mice bred in our own facilities or purchased from Charles River (Cléon, France). B6 recombinase-activating gene-2-deficient (RAG-2−/−) mice were obtained from (Centre de Développement des Techniques Avaucets, Orléans, France).

**In vivo T cell stimulation systems**

On day 0 of the experiments, mice received an i.p. injection of 10 μg of SEB (Sigma, St. Louis, MO), poly(I:C) (Sigma, 150 μg in 100 μl of PBS), or 0.3 ml of supernatant from the SC-1 cell clone infected by LP-BM5 MuLV and kindly supplied by H. C. Morse III (National Institutes of...
Health, Bethesda, MD). Control mice were injected with the same volume of PBS or uninfected SC-1 cell supernatant.

Lymphocyte reconstitution

Irradiated (3 Gy) RAG-2-deficient mice were injected i.v. with a mixture of mouse T cell-depleted bone marrow (BM) and total LN cells (3 × 10⁶ each) from C57BL/6 mice.

BrdU labeling

At various times (2 h to several weeks) after injection of the activating agent or cell transfer, 1 mg of BrdU (Sigma) in 100 μl of PBS was injected i.p., twice at a 1-h interval.

Thirty minutes after the second BrdU injection (90 min after the first), lymphoid organs (thymus, mesenteric lymph nodes (MLN), and spleen) and blood samples were harvested for BrdU detection. With this injection protocol, we labeled DNA-synthesizing cells only, and not their postmitotic progeny. As indicated in Results, in some experiments this standard protocol was slightly modified by using only one BrdU injection or by extending the delay between BrdU injection and detection.

Cell staining and immunofluorescence

Cell suspensions were prepared and counted, and blood samples were then centrifuged on Ficoll gradients. All suspensions were prepared in ice-cold PBS containing 4% FCS and 0.2% sodium azide (Sigma). Surface molecules were stained with the following sets of three fluorochrome-conjugated mAbs: tricolor-conjugated anti-CD4 (Caltag, San Francisco, CA), PE-conjugated anti-CD8, and biotinylated anti-TCR β-chain (clone H57/97); anti-β8.2 (clone F23.2) or Vβ6 (clone 44.22.1) TCR chain, anti-CD69 (clone H1.2F3), anti-CD44 (clone 1 M681), anti-CD62L (MEL-14), or anti-ICAM-1 (PharMingen, San Diego, CA). Most of the biotinylated Abs were prepared in our laboratory, and binding was revealed with allo-phycocyanin-conjugated streptavidin (Molecular Probes, Eugene, OR).

After surface staining, cells were fixed and permeabilized in PBS containing 1% paraformaldehyde and 0.01% Tween 20 for 48 h at 4°C. BrdU was then detected by using a published method (19) including DNase I cell treatment, with a FITC-conjugated anti-BrdU Ab (Becton Dickinson, San Jose, CA) or with the anti-BrdU Ab 76/7 (a gift from T. Ternynck, Institut Pasteur, Paris, France); revelation was done with FITC-conjugated goat anti-mouse IgG1 (Southern Biotechnology, Birmingham, AL).

Four-color immunofluorescence was analyzed in a FACScalibur cytometer (Becton Dickinson), using the Cell Quest program. Nonlymphoid cells, dead cells, and aggregates were eliminated by using an electronic gate set on forward and side light scatter.

Results

Baseline peripheral T lymphocyte proliferation in normal mice

Thirty minutes after the second BrdU injection, the percentage of labeled cells was higher in blood (0.8 ± 0.2% in CD4 T cells and 1.7 ± 0.3% in CD8 T cells) than in LN and spleen (<0.5% in all subsets). These relative frequencies do not directly reflect the absolute numbers of cycling T cells present in the different compartments, because blood contains only 2% of the total T lymphocyte pool (20). Normal peripheral cycling T cells are all CD44⁺, and have a conventional activation phenotype (A. Le Campion et al., unpublished results); they are likely to result from a recent TCR/Ag reaction that requires contact between T cells and APCs and is more likely to occur in organs than in the circulating compartment (21). We thus postulated that cycling cells found in blood might be activated cells having recently migrated from organs, and we explored the distribution of cycling T cells during experimental immune responses.

T cell response to the bacterial superantigen SEB

To study the kinetics and specificity of the proliferative T cell response, we used SEB as an in vivo stimulator. The superantigen was injected i.p. to elicit a systemic response. This system has several advantages: the response to SEB is very rapid and involves a large number of Ag-specific T lymphocytes (all those expressing the Vβ8 TCR families); the bystander response can be evaluated by measuring the proliferative response of nonspecific cells (Vβ6⁺ cells in our experiments).

![FIGURE 1. SEB-induced proliferation of T lymphocytes in lymphoid compartments. BrdU was injected twice (a 1-h interval) into mice 2 h to 6 days after SEB injection. Lymphocytes were harvested 30 min after the second BrdU injection and stained with anti-CD4-tricolor anti-CD8 PE, and biotinylated anti-Vβ8 or anti-Vβ6 plus streptavidin-allophycocyanin. BrdU was then detected after cell fixation and permeabilization, with an anti-BrdU-FITC Ab. The results are percentages of BrdU⁺ cells in gated CD4 or CD8 SP lymphocytes with strong Vβ8 or Vβ6 expression. Data are means (±SEM) of 4 to 12 determinations in 2 to 6 independent experiments (2 mice at each time point).](http://www.jimmunol.org/lookup/doi/10.4049/jimmunol.169.3.1554)
the control (4.6% at 24 h post-SEB). CD8⁺ Vβ8⁺ cell proliferation also started at 16 h and lasted longer in blood (still at the plateau on day 2). Vβ8⁺CD8 SP thymocytes showed a transient and intense wave of DNA synthesis (~20% BrdU⁺ cells at 16–24 h). In blood, Vβ6⁺ cell stimulation was stronger in the CD8 subset (8% at 24 h) than in the CD4 subset. In all four compartments tested, BrdU incorporation was thus much higher in Vβ8⁺ cells than in Vβ6⁺ cells. However, as shown in Fig. 2A, the proportion of Vβ8⁺ cells among DNA-synthesizing T cells was only 30–50%. The question arises as to whether Vβ8⁺ cell proliferation is a bystander response. Tough et al. (17) reported that injection of type I IFN induced CD8⁺ cell proliferation and defined this as bystander response because it was not accompanied by CD69 expression. We compared CD69 expression by BrdU⁺ cells detected 16 h after SEB injection by labeling blood, LN, and spleen cells with anti-CD4 (or anti-CD8) plus anti-Vβ8, anti-CD69, and anti-BrdU. The 16-h time point was preferred to 24 h because, as shown in Fig. 5, the early activation marker CD69 starts to be down-regulated by 24 h. The BrdU/CD69 dot plots of gated CD4⁺ or CD8⁺ cells were CD69⁺ in all compartments, and the proportion was only slightly lower in BrdU⁺ Vβ8⁺ T cells (78–95%, depending on the subset and compartment). SEB-induced BrdU⁺ cells all expressed a CD44 high, CD62L low, and ICAM-1 high phenotype (data not shown). These data suggest that most Vβ8⁺ cycling cells might result from a TCR-mediated cross-reaction to SEB and that bystander proliferation is minimal in this system. Similar results were obtained when we examined CD69 expression by cycling Vβ6⁺ cells, but the small number of cells obtained, particularly in blood, prevented us from obtaining quantitatively significant data. Incidentally, we observed that the percentages of BrdU⁺ cells were

**FIGURE 2.** Phenotype of SEB-induced proliferating cells in blood, MLN, and spleen. A, One day after SEB injection mice were pulsed with BrdU, and lymphocytes from blood, MLN, and spleen were stained with anti-CD4-tricolor, anti-CD8 PE, biotinylated anti-Vβ8 or anti-Vβ6 plus streptavidin-APC, and BrdU-FITC. The results of one experiment (of three performed) are presented as BrdU/Vβ8 or BrdU/Vβ6 fluorescence dot plots obtained after gating on CD4⁺ or CD8⁺ lymphocytes. Percentages of cells present in each quadrant are indicated. B, Sixteen hours after SEB injection, three mice were pulsed with BrdU. Thirty minutes after the second BrdU injection, lymphocytes from lymphoid organs and blood were pooled and stained with anti-CD4 or anti-CD8-tricolor, anti-CD69-PE, biotinylated anti-Vβ8 plus streptavidin-APC, and BrdU-FITC. CD69⁺BrdU⁺ fluorescence dot plots were obtained after gating on CD4⁺ Vβ8⁺, CD4⁺ Vβ6⁺, CD8⁺ Vβ8⁺, and CD8⁺ Vβ8⁻ cells. Percentages of CD69⁺ and CD69⁻ cells in BrdU⁺ cells are indicated. Results are for one typical experiment of three performed. The time (16 h) after SEB injection was chosen for this analysis (vs 24 h in Fig. 2A) because CD69 is rapidly down-regulated after 16 h (see Fig. 5). This difference explains why the percentages of BrdU⁺ cells are lower in Fig. 2B than in Fig. 2A.
higher among total Vβ8− cells than among Vβ6+ cells (Fig. 2A). SEB induces Vβ3+ cell proliferation (data not shown), and it is also possible that a significant proportion of Vβ8+ responding cells down-regulated TCR expression, at least during DNA synthesis.

**SEB-specific T cells synthesize DNA in blood**

We then determined the origin of cycling T cells present in blood. The period between the first BrdU injection and detection of labeled cells (90 min in our standard protocol) may be sufficient for resident cells labeled during late S phase to complete DNA synthesis, divide, and migrate from organs to blood. We therefore examined BrdU incorporation 30 min after a single BrdU injection given 24 h after SEB. The percentages of BrdU-labeled Vβ8+ cells found in all the compartments were not significantly different from values obtained with the standard two-injection protocol, and the relative distribution of cycling cells in blood and lymph nodes was the same (Fig. 3). This shows that SEB-reactive T lymphocytes synthesize DNA, and then divide, in the blood compartment. It does not mean, however, that circulating cycling cells were initially activated in blood.

**T cell redistribution during SEB-induced activation and expansion**

To evaluate cell migration and initial activation events, we investigated changes in the absolute numbers of T cells after SEB injection and studied the expression kinetics of the activation markers CD69 and CD44. Changes in the absolute numbers of CD4+ and CD8+ Vβ8+ cells in blood, MLN, and spleen during the first 72 h after SEB injection are shown in Fig. 4. In blood, we observed an immediate, sharp fall in the number of SEB-specific cells: 90% of blood Vβ8+ cells disappeared within 2 h. Their numbers showed a further increase at 24 h and then plateaued at numbers slightly higher than in controls. Similar but slower changes in SEB-specific cell numbers were observed in the spleen, in which the numbers of CD4+ and CD8+ Vβ8+ cells were reduced by 50% after 16 h. During the same period, Vβ8+ CD4+ cells transiently increased in MLN. SEB-nonspecific cells expressing Vβ6 were also depleted from blood, but much less markedly than Vβ8+ T cells; in contrast, their numbers did not change significantly in LN and spleen. The most likely interpretation for these data is that SEB-responsive cells are transiently trapped in specialized lymphoid organs (particularly LN) where the Ag is presented and then return to the circulation after activation. If this is so, activation markers might be detectable earlier in LN than in blood. We therefore compared the kinetics of DNA synthesis and CD69 and CD44 expression in the different compartments. The data obtained during the first 48 h after SEB injection are presented in Fig. 5. CD69 induction always preceded DNA synthesis, but CD69 induction was clearly more rapid in LN and spleen than in blood; 4 h after SEB injection the proportions of CD69+ cells among Vβ8+ CD4+ cells were 94% in LN and 86% in spleen but only 48% in blood. Similar differences were observed with CD8+ cells. These data confirm that SEB-specific cells are first activated in LN and spleen and then circulate in the blood. The changes in CD44 expression by LN and spleen Vβ8+ cells were much slower than the changes in CD69 expression. CD44 expression increased significantly during the second day only, after the peak of DNA synthesis. By contrast, the proportion of CD44high cells among blood Vβ8+ cells increased early and sharply. This enrichment in CD44high cells coincided with the drop in total Vβ8+ blood cell numbers; it was probably due more to preferential trapping of blood CD44− cells in LN than to activation-linked CD44 up-regulation.

The short term fate of postcycling cells was studied by comparing the frequencies of Vβ8+ BrdU+ cells found immediately (standard protocol) and 24 h after the BrdU pulse (done on day 1 after SEB injection) (Fig. 6). The half-life of injected BrdU is very short (3), and this experiment allowed us to trace the cohort of cells that
were in S phase at the time of the injection. In MLN and spleen, the percentages of BrdU<sup>1</sup> cells doubled during the 24 h following BrdU incorporation. In blood, a three- to fivefold increase in the percentage of BrdU<sup>1</sup> cells among V<sup>b</sup>8<sup>1</sup> subsets was observed; this applied even to CD4<sup>1</sup> cells, most of which had returned to a resting state on day 2 post-SEB. One day after BrdU injection, 45–59% of peripheral blood CD4<sup>1</sup>V<sup>b</sup>8<sup>1</sup> cells and 60–80% of CD8<sup>1</sup>V<sup>b</sup>8<sup>1</sup> cells were BrdU labeled. These percentages are very high for a short BrdU pulse and suggest that the vast majority of V<sup>b</sup>8<sup>1</sup>T cells circulating in blood on day 2 post-SEB had been cycling just before. Combining the data in Figs. 4 and 6, we converted these percentages into absolute numbers of BrdU<sup>1</sup> cells in each subset to evaluate V<sup>b</sup>8<sup>1</sup> cell expansion during the 24 h following BrdU incorporation. For the CD4<sup>1</sup>V<sup>b</sup>8<sup>1</sup> subset, the expansion factors were 9.1 in blood, 2.6 in MLN, and 3.25 in spleen; for the CD8<sup>1</sup>V<sup>b</sup>8<sup>1</sup> subset, the corresponding expansion factors were 120, 7.8, and 8.23. Although the moderate expansion observed in the organs could be explained by cell division only, this was not the case in blood, where CD8<sup>1</sup>V<sup>b</sup>8<sup>1</sup> cells expansion could result only from both cell division and migration from organs to blood, even if significant CD8<sup>1</sup> cell proliferation persisted in blood on day 2 post-SEB.

In the thymus, the percentages and numbers of labeled V<sup>b</sup>8<sup>1</sup>T cells increased by ~1.5-fold during the 24 h following BrdU injection, and most BrdU<sup>1</sup> cells found on day 2 in these conditions were no longer cycling (Fig. 6, compare closed and striped bars in each subset); this expansion corresponded to less than one division in situ and could be explained by our recent finding that many cycling mature thymocytes are ready to leave the thymus (13). The possible thymic origin of circulating cycling SEB-specific cells was tested by repeating the experiment described in Fig. 6 with mice thymectomized 1 mo before. The percentages and distribution of BrdU<sup>1</sup>V<sup>b</sup>8<sup>1</sup> T cells in blood, MLN, and spleen were the same as in euthymic animals (data not shown). This ruled out any major involvement of the thymus in the acute phase of SEB-specific T cell redistribution.

Globally, the results obtained with the SEB system suggest that Ag-specific T cells first encounter the Ag in organs, in which APCs are located, and then recirculate after entering the activation cascade (this includes DNA synthesis, which is readily detectable in blood).

The redistribution process described above could be SEB specific; alternatively, it could be a general rule governing immune responses. To test these possibilities, we assayed T cell proliferation in two stimulation systems excluding bacterial superantigens and during lymphoid development in mouse chimeras.
Table I. Proliferation of blood and LN CD44<sup>hi</sup> lymphocytes after poly(I:C) injection<sup>a</sup>

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<td>Day 3</td>
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<sup>a</sup> Pulse-bromodeoxyuridine incorporation was measured in control mice and 1 to 3 days after poly(I:C) injection. Results are percentages of bromodeoxyuridine cells (means of three experiments). SEs were <10% of the mean.

Poly(I:C) stimulation

Tough et al. (17) have shown that activation of lymphoid cells by virus in vivo can be mimicked by injecting the IFN-I inducer poly(I:C). We repeated this experiment and measured the labeling index in CD44<sup>hi</sup> lymphocytes in blood and MLN. As shown in Table I, BrdU incorporation was maximum on day 2 after poly(I:C) injection in both lymphocyte subsets and 2.5- to 5-fold higher in blood than in LN.

Acute LP-BM5 virus infection

The MuLV-derived LP-BM5 virus preparation used here induces a MAIDS, sharing certain aspects with human AIDS (18). We studied the kinetics of BrdU incorporation in blood, MLN, and spleen CD44<sup>hi</sup> lymphocytes during the first 2 wk after virus infection. The results are presented in Fig. 7. In blood, T cell proliferation began to increase on day 6, peaked on day 8, and returned to normal values on day 10. It was much higher in CD4<sup>+</sup>CD8<sup>+</sup> cells (16.5 ± 3.9% on day 8) than in CD4<sup>+</sup>CD8<sup>+</sup> cells (5.7 ± 0.7%). In LN and spleen, a very small increase in BrdU incorporation was observed on day 6 and was similar in CD4<sup>+</sup> and CD8<sup>+</sup> cells.

Despite the very different kinetics, blood cycling lymphocytes were highly enriched in both the poly(I:C) and LP-BM5 virus activation systems. In the latter case proliferation was restricted to circulating cells in some individual experiments.

Peripheral T cell proliferation during lymphoid reconstitution

To determine whether the distribution of cycling peripheral T cells described above is specific to responses to exogenous stimulators, we studied BrdU incorporation by LN and blood CD4 and CD8 T cells from chimeric mice prepared by transfer of normal BM cells alone or combined with syngeneic LN T cells into B6/RAG-2<sup>−/−</sup>/− recipients. The results, presented in Fig. 8, show that BrdU incorporation by blood and LN T cells increased strongly during the third week after transfer, decreased at week 4, and returned to values similar to those in normal adult B6 mice by 6 wk. LN cellularity was 30% of normal at 4 wk, and normal at 6 wk. In RAG-2<sup>−/−</sup> mice transferred with BM cells only, the first CD4<sup>+</sup> and CD8<sup>+</sup> cells appeared in the periphery on days 18 and 21, respectively, and also proliferated strongly (A. Le Campion et al., unpublished results). In this system, T cell proliferation was not higher in blood than in organs, but these data confirm that newly produced or transferred mature T cells are able to expand in the deprived periphery and that this proliferation is readily detectable in blood, as during immune responses.

Discussion

In a detailed study of acute T cell response to SEB, but also in three different superantigen-independent systems, we observed that T cell proliferation was at least as intense in blood as in lymphoid organs. In the MAIDS system, proliferation was virtually restricted to the blood compartment, values in LN and spleen being only slightly higher than in control mice. In the SEB stimulation system, proliferation was preceded by extensive and specific cell redistribution. Cell proliferation in blood, LN, and spleen appeared to be synchronous; T cell DNA synthesis peaked 24 h after SEB injection and was followed by an increase in total V<sup>B8</sup> cell numbers in blood and spleen on day 2, as observed by other authors (16). The BrdU pulse-labeling protocol used here only measures the DNA synthesis rate, and the lag between DNA synthesis and cell expansion corresponds to the time necessary for proliferating cells to complete the S + G<sub>2</sub> + M phases of the cell cycle. The increase in cell numbers ceased after 48 h, when DNA synthesis returned to baseline. V<sup>B8</sup> cell DNA synthesis and the increase in their numbers were observed in both the CD4 and CD8 populations. Involvement of the CD8 subset in the response to SEB has already been reported (16). Shortening the time between BrdU injection and detection from 90 to 30 min did not change the relative distribution of SEB-specific cycling cells in blood and LN. This demonstrates that T cells undergo DNA synthesis in the blood and rules out the possibility that T cells obligatorily complete DNA synthesis and mitosis in organs before migrating to blood. T cell responses require contacts between T cells and APCs, which occur in the specialized environment of lymphoid tissues (21).

Consequently, the enrichment in DNA-synthesizing cells in blood reflects T cell detachment from the LN stroma, following changes in adhesion molecule expression during activation (22, 23).

T cell proliferation in blood was not a bystander, lymphokine-induced process. Even in the CD8 blood subset, the proliferative response of V<sup>B8</sup> cells was much stronger than the response of V<sup>B6</sup> cells. Tough et al. (17) have suggested that during antiviral responses, most proliferating cells are not Ag specific and are stimulated by lymphokines (a bystander response). Limit dilution analysis-based calculations of the frequencies of Ag-specific responder cells have given very low values in several different systems (24–26). The notion of a prominent bystander response has recently been challenged by a series of reports in which the specificity of the response in vivo was examined directly (27–31). In these studies, the calculated frequency of Ag-specific cells was at least 50–70% of all responding cells, a result coherent with our present data. Even the V<sup>B8</sup> cell response to SEB did not appear to be a pure lymphokine-induced bystander process, because most cycling V<sup>B8</sup>−, and particularly V<sup>B6</sup>− cells, were CD69<sup>hi</sup>. However, more cells incorporated BrdU in the total V<sup>B8</sup>− population than in the V<sup>B6</sup>− population. Two possible explanations for this difference can be forwarded: SEB also stimulates V<sup>B3</sup> cells; and a large proportion of SEB-responding cells might down-regulate TCR surface expression.

The kinetics of T cell proliferation varied in the different stimulation systems, peak DNA synthesis occurring on day 1 with SEB, on day 2 with poly(I:C), and on day 8 with LP-BM5 MuLV. These differences are probably related to different modes of Ag presentation: poly(I:C) directly induces IFN production; and SEB presentation is direct and occurs with minimal Ag processing compared with LP-BM5 viral peptides. Hayden et al. (32) have shown that injection of Mtv-7-positive (Mls-1a) cells also induces a proliferative response by specific (V<sup>B8</sup>) cells; in this system, BrdU was continuously infused, and only 2% of CD4<sup>+</sup>V<sup>B8</sup>− LN cells were labeled on day 1; this percentage increased to 40% on day 3 and to 70% on day 4 (cumulative labeling). The 1-day delay observed relative to the SEB system probably corresponds to Ag processing and presentation.

Superantigen-induced apoptosis was not measured in our study. Working with C3H (I-E<sup>+</sup>) mice, d’Adamio et al. (33) suggested...
that SEB-induced expansion of LN V\(\beta\)8\(^+\) cells is preceded by significant depletion. However, Renno et al. (34), combining cumulative BrdU labeling and apoptotic cell detection, observed that only postmitotic cells underwent apoptosis. It has recently been reported (35) that although Fas and apoptosis-inducing genes were rapidly induced after SEB injection, apoptosis-preventing gene products (particularly bag-1) were transiently up-regulated on the first day, simultaneously with LN and spleen V\(\beta\)8\(^-\) cell blasto- genesis and before the appearance of apoptotic cells. These recent data strongly suggest that SEB-specific cell proliferation precedes apoptosis.

We found that the highest percentage of cycling cells was in blood, at a time when the global number of specific cells was strongly reduced in this compartment. The initial depletion in blood probably reflects specific cell trapping in peripheral lymphoid organs, as observed for V\(\beta\)6\(^+\) cells accumulating in the

FIGURE 7. T cell proliferation after BM5 virus infection. Percentages of BrdU\(^+\) cells among CD44\(^{hi}\) T cells were determined in control mice (day 0), and in mice infected 1 to 14 days previously with the BM5 MuLV virus mixture (A). As shown in B, all cycling cells were CD44\(^{hi}\) on day 8.
draining LN after local injection of mammary tumor virus (36). With the soluble superantigen SEB, T cell accumulation in LN is very transient, and proliferating cells recirculate in the blood rapidly after superantigen presentation and activation. Thymic SEB-reactive cells (mainly Vβ8+ CD62SP cells), which are also stimulated, could also emigrate to blood after starting DNA synthesis, as we have recently demonstrated in normal, noninfected mice (13), but their participation appears quantitatively marginal, given that the global process described here was not modified by adult thymectomy.

The total number of peripheral blood T lymphocytes represents a low proportion (~2%) of the total T cell pool (20), and preferential recirculation of Ag-specific cycling cells will result in a strong increase in their frequency in the blood. In this respect, blood appears to be a sort of “magnifying glass” for following the immune response. Recently, Pantaleo et al. (37) showed that during primary HIV infection, HIV-specific clones accumulated preferentially in blood and were determined at various times after infection. Means ± SEM of two to five determinations.

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References