Antigen-Specific Lymphocyte Sequestration in Lymphoid Organs: Lack of Essential Roles for $\alpha_L$ and $\alpha_4$ Integrin-Dependent Adhesion or $G\alpha_i$ Protein-Coupled Receptor Signaling

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Selective lymphocyte sequestration was described over 30 years ago as the transient withdrawal of Ag-specific lymphocytes from the circulation as a result of their activation in secondary lymphoid organs. We used a TCR-transgenic adoptive transfer system to further characterize the Ag and adjuvant dependence of this process in mice. In addition, we examined the contribution of the $\alpha_L$ and $\alpha_4$ integrin chains as well as $\text{G}_i$ protein-coupled receptor signaling to the retention of Ag-specific T cells in peripheral lymph nodes. Our results demonstrate that selective lymphocyte sequestration is T cell autonomous and adjuvant independent, and that the duration of sequestration is not controlled by the continued presence of Ag in secondary lymphoid organs. This process is not critically dependent on the $\alpha_L$ and $\alpha_4$ integrin chains or $\text{G}_i$ protein-coupled receptor signaling. Selective lymphocyte sequestration may be mediated by redundant mechanisms and/or controlled by novel or nonclassical adhesion or trafficking molecules. The Journal of Immunology, 2004, 173: 866–873.

Over 30 years ago, Sprent et al. (1) described, in mouse, the phenomenon of selective lymphocyte sequestration whereby Ag-specific cells transiently disappear from the circulation as a result of their activation in secondary lymphoid organs. During this period, lymphocytes contact cognate Ag on the surface of APCs and undergo proliferation and effector differentiation before returning to the circulation ~72 h after immunization (1). The redistribution of Ag-specific T cells from the blood to lymphoid tissues has been observed not only in mice, but also, in rats (2, 3), sheep (4), and humans (5), under a wide range of immunogenic stimuli. The ubiquity of this process suggests that it has a fundamental and conserved role in T cell immunity, perhaps by ensuring a minimal temporal window for T cell activation and effector differentiation to occur within an optimal microenvironment.

The precise mechanism by which Ag profoundly alters the recirculation of T cells has not been determined. One possibility is that mature, Ag-loaded dendritic cells retain naive T cells in secondary lymphoid organs via stable adhesive interactions. Clusters of Ag-specific T cells and CD11c$^+$ APCs can be physically isolated from Ag-primed lymph nodes of mice, suggesting that these two cell types are indeed capable of forming strong, long-lasting interactions in vivo (6). However, the more recent observation that T cells interact with APCs in three distinct phases, only one of which involves the formation of stable conjugates, suggests that Ag-specific T cell sequestration may be mediated by mechanism(s) in addition or alternative to prolonged adhesion to dendritic cells (7).

Chemokines and chemokine receptor signaling function critically in the entry and microenvironmental localization of T cells in secondary lymphoid organs (8). Although the role of chemokine receptor signaling in T cell retention and exit from lymph nodes has not been extensively studied, the transient loss or acquisition of chemokine receptor activity could modulate the trafficking of T cells through secondary lymphoid organs during their activation. Chemokine receptor-mediated control of tissue retention is exemplified by immature Langerhans cells, which, only upon maturation and concomitant up-regulation of CCR7, exit the skin via afferent lymphatic vessels and migrate into draining lymph nodes (9). Within the lymph node, dendritic cell-derived chemokines may retain recently activated T cells in the vicinity of Ag presentation and thereby prevent or delay the re-entry of T cells into the circulation (10, 11).

We sought to characterize the mechanism by which Ag-specific T cells become sequestered in Ag-primed peripheral lymph nodes. To this end, we assessed the contribution of Ag and adjuvant, as well as molecules that function in T cell adhesion and chemotaxis, to Ag-specific T cell withdrawal from the circulation in vivo. Our results demonstrate that selective lymphocyte sequestration is T cell autonomous and adjuvant independent, and that the duration of sequestration is not strongly influenced by the continued presence of Ag in secondary lymphoid tissues. Blocking Abs to the $\alpha_L$ and $\alpha_4$ integrins, which comprise the $\alpha$-chain subunits of LFA-1 and VLA-4, respectively, fail to modulate the extent and duration of selective lymphocyte sequestration in vivo. Pertussis toxin, which inhibits $\text{G}_i$ protein-coupled receptor signaling, also fails to reverse Ag-induced T cell sequestration. These observations suggest that selective lymphocyte sequestration is mediated by redundant mechanisms that are not dependent on Ag or adjuvant.
mechanisms and/or controlled by novel or nonclassical adhesion or trafficking molecules.

Materials and Methods

Mice

Thy1.1, DO11.10, and DO11.10xThy1.1 mice were bred and housed at the Veterans Affairs Palo Alto Health Care System (Palo Alto, CA). BALB/c and JHD mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and Taconic Farms (Germantown, NY), respectively. All animal experiments were performed in accordance with institutional guidelines established by Stanford University and the Veterans Affairs Palo Alto Health Care System.

Adoptive transfers

BALB/c mice, 6–8 wk old, received 5–25 × 10⁶ RBC-depleted splenocytes from age- and sex-matched DO11.10 mice by retro-orbital injection. Where indicated, mice received a mixture of splenocytes from DO11.10 and Thy1.1 mice. In some experiments, mice received CD4⁻ cells enriched from splenocytes using a magnetic bead separation method (Miltenyi Biotech, Gladbach, Germany).

Immunizations

For i.p. immunization, mice were injected in the peritoneal cavity with 500 μg chicken egg OVA (Sigma-Aldrich, St. Louis, MO) and 100 μg LPS (Escherichia coli serotype O55:B5; Sigma-Aldrich). For s.c. immunization, mice were injected under the skin covering the lower abdomen with 500 μg OVA alone or with 1 μg cholera toxin (CT⁺; Sigma-Aldrich).

Treatments

Mice were injected i.p. with 250 μg each of blocking mAb against α₄ (TIB213; American Type Culture Collection, Manassas, VA) and α₁ (PS/2; American Type Culture Collection) every 6 h from 12 to 42 h or 36 to 66 h after s.c. immunization with 500 μg OVA. Mice received a single i.p. injection of 7.5 μg pertussis toxin (Sigma-Aldrich) on day 1 or 2 after s.c. immunization with 500 μg OVA. When blood samples were harvested on the same day that a treatment was administered, the treatment followed tissue collection.

Cell isolation

Splenocytes were prepared by disaggregating spleens between frosted microscope slides in wash buffer (HBSS containing 2% bovine calf serum). The cell suspensions were filtered through 40-μm tissue strainers (Fisher Scientific, Hampton, NH), incubated in RBC lysis buffer (Sigma-Aldrich), and washed. For blood collection, mice were anesthetized and a 25-μl sample was collected from the retro-orbital cavity, using heparinized capillary tubes (Fisher Scientific), into a solution of EGTA and dextran (Sigma-Aldrich) that was incubated at 37°C to precipitate RBCs. The supernatant was incubated in RBC lysis buffer and washed. For thoracic duct lymph (TDL) collection, an ~2–3-cm² section of skin was removed from anesthetized mice. A small incision in the periosteal lining was made and the left kidney and spleen were separated to reveal the cisterna chyli, which was pierced without puncturing the aorta. Lymph was collected in 2-μl increments into wash buffer. Lymph node cell suspensions were prepared in wash buffer by pushing intact nodes through 40-μm tissue strainers (Fisher Scientific) incubated in RBC lysis buffer (Sigma-Aldrich), followed by PE-anti-DO11.10 TCR, PerCP-anti-CD4, and allophycocyanin-anti-CD4. The percent migration of KJ1.26 CD4⁺ and Thy1.1 CD4⁺ cells was normalized to the percentage of each cell subset in the input population as previously described (12).

Statistical analysis

Values are reported as averages ± SEM or range as indicated. Multiple comparisons were performed with Bonferroni corrections where appropriate. Statistical significance was set at p < 0.05.

Results

Characterization of selective lymphocyte sequestration

In the original description of selective lymphocyte sequestration, Sprent et al. (1) transferred TDL from immunized mice to naive recipients. The capacity of TDL to transfer responsiveness to a sensitizing Ag in recipient animals was specifically reduced if it was collected on days 1 or 2 after immunization of the donor. Sprent et al. (1) interpreted this “period of unresponsiveness” as a transient withdrawal of Ag-specific lymphocytes from the circulation following immunization. To further characterize this process, we used an assay to directly track the distribution of T cells of known antigenic specificity during their activation in vivo. Because T cells of a given antigenic specificity represent a small fraction of the normal T cell repertoire, we transferred CD4⁺ splenocytes isolated from DO11.10 mice, which are specific for an OVA-derived peptide presented in the context of I-A⁺, to BALB/c mice (13, 14). This provided a small but detectable population of Ag-specific T cells that we could monitor in the blood of immunized animals over time.

To determine the effect of systemic immunization on the distribution of Ag-specific T cells, we immunized adoptive transfer recipients with a single i.p. injection of OVA and LPS and measured the percentage of Ag-specific CD4⁺ T cells in the total pool of blood CD4⁺ T cells over time. Within 24 h, the proportion of Ag-specific CD4⁺ T cells decreased ~10-fold in the pool of peripheral CD4⁺ T cells, almost reaching the limit of detection by 48 h (Fig. 1A). This marked withdrawal of Ag-specific T cells from the circulation continued until 72 h postimmunization, at which

* Abbreviations used in this paper: CT, cholera toxin; TDL, thoracic duct lymph; SLP, sphingomytin 1-phosphate.

The subiliac lymph nodes of individual mice were collected on the indicated days after immunization. Single-cell suspensions in cRPMI (RPMI 1640 supplemented with 10% heat-inactivated FBS, penicillin/streptomycin, sodium pyruvate, glutamate, and 2-ME; Sigma-Aldrich) were incubated at 37°C in 5% CO₂ for 4 h. The cells were adjusted to 5 × 10⁶ cells/ml of temperature- and pH-equilibrated cRPMI. Five × 10⁶ cells were added to the top chamber of a 5-μm pore Transwell (Costar, Cambridge, MA) and allowed to migrate for 1.5 h to 100 nM CCL21 (Peprotech, Rocky Hill, NJ), 50 nM CXCL12 (R&D Systems, Minneapolis, MN), 50 nM CCL22 (Peprotech), 100 nM CXCL10 (Peprotech), 300 nm CXCL13 (R&D Systems), or medium. A fixed number of 15-μm latex beads was added to an aliquot of migrated cells to calculate the overall percent migration to each chemokine. The remaining input and migrated cells were stained with PE-anti-DO11.10 TCR, PerCP-anti-Thy1.1, and allophycocyanin-anti-CD4. The percent migration of KJ1.26 CD4⁺ and Thy1.1 CD4⁺ cells was normalized to the percentage of each cell subset in the input population as previously described (12).

Statistical analysis

Values are reported as averages ± SEM or range as indicated. Multiple comparisons were performed with Bonferroni corrections where appropriate. Statistical significance was set at p < 0.05.
point they became reproducibly detectable in the blood of immunized mice (Fig. 1A). We also examined the effect of s.c. immunization on the extent and duration of Ag-specific and polyclonal T cell withdrawal from the circulation. As a source of polyclonal T cells, we used splenocytes from nontransgenic Thy1.1 mice, which comprised similar proportions of naive (CD45RB<sup>high</sup>CD44<sup>low</sup>) and memory (CD45RB<sup>low</sup>CD44<sup>high</sup>) cells as splenocytes from age-matched DO11.10 mice (data not shown). We cotransferred equal numbers of CD4<sup>+</sup> cells enriched from DO11.10 and Thy1.1 splenocytes to BALB/c mice and immunized the recipients on the lower abdomen with OVA and CT (Fig. 1B). As with the systemic i.p. immunization, this resulted in withdrawal of nearly all KJ1.26<sup>+</sup>CD4<sup>+</sup> T cells from the circulation (Fig. 1B). In contrast, the percentage of control polyclonal T cells in the pool of blood CD4<sup>+</sup> T cells was not significantly altered at any time after immunization (Fig. 1B).

Having established an assay for tracking selective lymphocyte sequestration in vivo, we next determined the effect of varying the Ag dose and use of adjuvant on T cell withdrawal from the circulation following s.c. immunization. As predicted by the work of Sprent and Miller (15), the extent of Ag-specific T cell sequestration was dependent on the dose of Ag administered (Fig. 1C). At both high and low Ag doses, however, adjuvant had little or no effect on the sequestration of Ag-specific T cells (Fig. 1C).

Given the Ag dose dependence of selective lymphocyte sequestration, we thought it was possible that the duration of sequestration was controlled by the clearance of Ag from secondary lymphoid organs. However, we found that Ag-specific T cells re-entered the circulation despite a second injection of OVA 2 days after the first injection of Ag (Fig. 1D). This result was also observed in B cell-deficient JHID mice, indicating that the second dose of Ag was not simply neutralized by the humoral immune response (data not shown). In these experiments, we also transferred a “second wave” of Ag-specific T cells, which were distinguishable from the “first wave” of DO11.10 cells by their expression of the Thy1.1 marker, at the time of the second immunization. As the percentage of the first wave of Ag-specific cells in the peripheral CD4<sup>+</sup> T cell pool increased on days 3 and 4 after the first immunization, the Thy1.1<sup>+</sup>KJ1.26<sup>+</sup> cells were efficiently sequestered out of the blood and in the draining lymph nodes (Fig. 1D and data not shown). The percentage of second-wave cells in the peripheral CD4<sup>+</sup> T cell pool increased on day 5 (i.e., day 3 after the second immunization) and by day 6 was equivalent to the percentage of second-wave cells in the blood of mock-immunized mice, which served as a control for determining the efficiency of the second cell transfer (Fig. 1D). The ability of recently activated T cells to effectively ignore a second dose of Ag, while Ag-inexperienced cells respond and become sequestered by it, suggests that the duration of selective lymphocyte sequestration is T cell autonomous and not regulated by transient changes in accessory cells or lymphoid tissue structure.

**Ag-specific T cells do not recirculate during selective lymphocyte sequestration**

To confirm that the disappearance of Ag-specific T cells from the blood reflects the retention of T cells in inductive lymphoid tissues, we determined the distribution of Ag-specific and polyclonal CD4<sup>+</sup> T cells in the blood, TDL, Ag-draining subiliac lymph nodes, and nondraining mesenteric lymph nodes before and after s.c. immunization with OVA and CT. Relative to control polyclonal T cells, the percentage of Ag-specific T cells in the CD4<sup>+</sup> T cell pool was selectively reduced in both the blood and TDL on day 1 postimmunization (Fig. 2A). On day 2 postimmunization, polyclonal T cells were detected in the blood and TDL (Fig. 2A) as well as in Ag-draining and nondraining lymph nodes (Fig. 2B). In contrast, Ag-specific T cells remained sequestered out of the blood and TDL (Fig. 2A) and accumulated in the Ag-draining lymph nodes (Fig. 2B) where they were dispersed throughout the paracortex (data not shown). The skewed tissue distribution of Ag-specific T cells, coupled with their absence from the TDL, demonstrates that...
they do not recirculate during their activation in Ag-draining lymphoid organs.

Effect of blocking Abs against α4 and α4 on selective lymphocyte sequestration

We examined the effect of in vivo treatment with a blocking mAb against the α-chain subunit of LFA-1 (16) on selective lymphocyte sequestration to test the hypothesis that Ag-specific T cells are retained in Ag-draining lymphoid tissues through LFA-1-dependent adhesion to APCs. This treatment was combined with a blocking mAb against the α4 integrin chain (17), which can pair with β1 to form a receptor for VCAM-1 and fibronectin. We reasoned that combined treatment with blocking mAb against both αL and α4 would allow us to test not only the idea that sequestration is mediated by T cell adhesion to APCs, but also the hypothesis that activated T cells are retained in secondary lymphoid organs by adhesion to extracellular components of the lymph node microenvironment (5, 18).

We injected adoptive transfer recipients in the peritoneal cavity with 250 μg each of blocking mAb against αL and α4 every 12 h from 12 to 48 h after s.c. immunization with OVA. PBLs and subiliac lymph nodes were collected 48 h after immunization and stained with a mAb specific for rat IgG2a, the isotype of both mAb used for treatment. Total CD4+ T cells collected from the blood and Ag-specific and total CD4+ T cells isolated from the subiliac lymph nodes of control mice were not bound by anti-rat IgG2a (shaded histogram in top panel of Fig. 3A and data not shown). In contrast, CD4+ T cells in the blood and both Ag-specific and total CD4+ T cells collected from the subiliac lymph nodes of mAb-treated mice were bound by high levels of anti-rat IgG2a, (shaded histogram in bottom panel of Fig. 3A and data not shown). Incubating the cells collected from mAb-treated mice with anti-αL and anti-α4 mAb followed by incubation with anti-rat IgG2a did not result in higher levels of anti-rat IgG2a binding (unfilled histogram in bottom panel of Fig. 3A), indicating that surface αL and α4 were saturated by the injected mAb in vivo. Surprisingly, the extent of sequestration, measured in terms of the percentage of Ag-specific T cells in the peripheral CD4+ T cell pool, was not significantly different between control and mAb-treated mice at the 48-h time point (Fig. 3B).

When we calculated the absolute number of KJ1.26+ CD4+ cells in the blood of mice, we did observe a statistically nonsignificant increase in the number of Ag-specific T cells in mAb-treated animals (Fig. 3C). However, this increase did not correlate with a decrease in the absolute number of Ag-specific T cells in the subiliac lymph nodes of treated mice (data not shown). These data strongly suggest that the treatment did not dislodge Ag-specific T cells from the lymph nodes of immunized mice. In addition, using CFSE analysis, we observed that the Ag-specific T cells in the blood of treated animals were CFSEhigh/CD69+ small lymphocytes, whereas the Ag-specific T cells in the subiliac lymph nodes of treated mice were CFSElow/CD69low blasts (Fig. 3D and data not shown), indicating that the Ag-specific T cells in the blood of treated mice had not been activated.

The inability of our mAb treatment to significantly alter the blood-lymph node distribution of Ag-specific T cells, despite causing the redistribution of coinjected polyclonal Thy1.1+ CD4+ cells from lymphoid tissues to the blood (Fig. 4), confirms that Ag-specific T cells do not recirculate for the duration of selective lymphocyte sequestration. Furthermore, it strongly implies that the retention of Ag-specific T cells in inductive lymphoid tissues is not mediated by adhesion through the αL and α4 integrin chains. To address the possibility that an inhibitory effect of mAb treatment on retention could have been occluded by a defect in the ability of “released” cells to exit lymph nodes, we treated adoptive transfer recipients with anti-αL and anti-α4 mAb every 6 h from 36 to 66 h after s.c. immunization. Ag-specific T cells were able to re-enter the circulation at 72 h postimmunization (Fig. 3E), despite being covered with blocking mAb (data not shown). In fact, the absolute number of Ag-specific T cells in the blood of treated mice was greater than that in control animals (data not shown), most likely due to the blocking effect of mAb treatment on αL+ and α4+-dependent re-entry of T cells into lymphoid and peripheral tissues (8, 19). Collectively, these observations demonstrate that the retention of Ag-specific T cells in secondary lymphoid tissues during selective lymphocyte sequestration is not solely dependent on the αL and α4 integrin chains.

Changes in chemotaxis and effect of pertussis toxin on selective lymphocyte sequestration

Changes in T cell chemotaxis could be among the alterations in motility that contribute to selective lymphocyte sequestration. To investigate this hypothesis, we compared the chemotactic properties of Ag-specific and polyclonal CD4+ T cells isolated from the subiliac lymph nodes before and after s.c. immunization with OVA and CT. The polyclonal T cells served as an internal control to quantify the changes in T cell chemotaxis caused by selective sequestration. Based on the ability of dendritic cell-derived CCL22 (10) and CXCL10 (11) to attract recently activated T cells in vivo, we thought that these ligands may function to retain Ag-specific T cells in the draining lymph nodes on days 1 and 2 postimmunization. However, Ag-specific T cells did not acquire...
detectable responsiveness to these chemokines until day 3 postimmunization (Fig. 5, C and D, respectively). The response of Ag-specific T cells to CXCL13, as well as their migration toward B cell follicles, where CXCL13 is constitutively expressed (20), were also delayed until day 3 postimmunization (Fig. 5 E and data not shown).

Given the delayed acquisition of chemotaxis to CCL22, CXCL10, and CXCL13, these ligands are not likely to mediate the early sequestration of Ag-specific T cells on days 1 and 2 postimmunization. To determine whether chemotaxis to other untested chemokines retains Ag-specific T cells in inductive lymphoid tissues, we treated mice with pertussis toxin on day 1 postimmunization. This treatment ablated lymphocyte chemotaxis in an ex vivo Transwell assay (Fig. 6A), but had no effect on the percentage of Ag-specific T cells in the peripheral CD4+ T cell pool on day 2 postimmunization (Fig. 6B). Pertussis toxin treatment did cause a statistically nonsignificant increase in the absolute number of Ag-specific T cells in the blood relative to control animals on day
2 postimmunization (Fig. 6C). This was likely due to the accumulation of nonactivated cells in the blood of pertussis toxin-treated mice, as was observed for the anti-α5- and anti-α4-treated mice (Fig. 3D and data not shown). To more precisely determine whether pertussis toxin treatment dislodges Ag-specific T cells from inductive lymphoid sites, we compared the absolute number of Ag-specific and coinjected polyclonal CD4+ T cells in the blood and subiliac lymph nodes on day 2 postimmunization in mice that were treated with saline or pertussis toxin on day 1 postimmunization. Whereas pertussis toxin shifted the distribution of bystander Thy1.1 CD4+ T cells from lymph nodes toward the circulation, Ag-specific T cells responding to local Ag were retained in the activating lymph nodes (Fig. 7). This conclusively demonstrates that activated T cells are not selectively retained in secondary lymphoid organs via a positive chemotactic stimulus.

In contrast to the delayed acquisition of chemotaxis to CCL22, CXCL10, and CXCL13 by only Ag-specific T cells, both Ag-specific and polyclonal CD4+ T cells exhibited strong chemotactic responses to CCL21 and CXCL12 before immunization (Fig. 5, A and B, respectively). However, on days 1 and 2 postimmunization, the percent chemotaxis of Ag-specific T cells to CCL21 and CXCL12 dropped dramatically and was reduced >50% relative to polyclonal T cells (Fig. 5, A and B, respectively). By day 3 postimmunization, the percent chemotaxis of Ag-specific T cells to CCL21 remained lower than that of polyclonal T cells (Fig. 5A), whereas the response to CXCL12 recovered and was equal to the response of polyclonal T cells (Fig. 5B). To determine whether the reacquisition of CXCR4 or another Goi-mediated chemotactic activity was responsible for T cell exit from lymph nodes, we treated mice with pertussis toxin on day 2 postimmunization. This treatment did not prevent Ag-specific T cells from re-entering the peripheral CD4+ T cell pool (Fig. 6B). In fact, it caused a significant increase in the absolute number of Ag-specific T cells in the blood on day 3 postimmunization (Fig. 6C), consistent with the requirement for Goi protein-coupled receptor signaling during T cell extravasation from the blood into lymphoid and peripheral tissues (8). This demonstrates that the exit of T cells from lymphoid tissues can occur independently of chemokine receptor and other Goi-mediated signals.

**Discussion**

Although the phenomenon of selective lymphocyte sequestration was described many years ago, the precise mechanism by which Ag-specific T cells transiently suspend their recirculation through the blood and secondary lymphoid organs has not been elucidated.

We characterized the process of selective lymphocyte sequestration by directly tracking T cells of known antigenic specificity in the blood of living animals over time. Our results confirm and extend Sprent’s initial observations (1, 15). First, they demonstrate that the duration of Ag-specific T cell withdrawal from the circulation is highly conserved following multiple routes of immunization. Second, our results show that sequestration is dependent on the ability of T cells to recognize Ag and not on indirect effects of adjuvant on the lymphoid microenvironment or motility of lymphocytes. This distinguishes selective lymphocyte sequestration from “cell shutdown,” which was defined as a nonspecific drop in total cellular output from lymph nodes shortly after local stimulation (21, 22). Third, in the second-wave experiments, we found that although recently activated T cells effectively ignore a second dose of Ag to re-enter the circulation, a second population of Ag-inexperienced cells respond and become sequestered by it. This suggests that the duration of selective lymphocyte sequestration is controlled on a T cell-autonomous level.

A plausible mechanism for selective lymphocyte sequestration is that T cells transiently adhere to APCs or other components in the lymph node microenvironment following activation. Because LFA-1 participates in the formation and stabilization of T cell-APC conjugates (23), we tested the effect of in vivo treatment with a blocking mAb against the α-chain of LFA-1 on the extent and duration of selective lymphocyte sequestration. We combined this treatment with a blocking mAb against α4, which pairs with β2 to bind VCAM-1 and fibronectin, both of which may participate in T cell adhesion to components of the lymph node extracellular matrix (5, 18). Our results strongly suggest that the retention of Ag-specific T cells in secondary lymphoid organs is not solely dependent on adhesion through the αL and/or α4 integrins, which is in
The number of KJ1.26−/H11001−CD4+ cells was calculated for mock- (□) and pertussis toxin-treated (▦) mice as described in Materials and Methods. Bars represent the average and SEM for four to five mice per group.

Because chemokines are essential for the entry and localization of T cells in lymph nodes (8) and for the exit of dendritic cells from peripheral tissues (9), we reasoned that transient changes in chemokine receptor expression or activity by recently stimulated T cells could contribute to their sequestration in secondary lymphoid organs. However, the results of our pertussis toxin experiments preclude a requirement for Gαi protein-coupled receptor signaling in both the sequestration and subsequent exit of Ag-specific T cells from secondary lymphoid tissues. Chemokines, such as CCL22 and CXCL10, may function to retain Ag-specific T cells in lymphoid tissues only at later stages of T cell differentiation (10, 11). Similarly, CXCL13 may retain helper T cells in B cell follicles for effective T-B collaboration days after the initial trapping of Ag-specific T cells (20). The activated T cells that effectively ignore these signals to re-enter the peripheral circulation on day 3 postimmunization may represent a population of "first responders" that migrate to peripheral sites of Ag deposition and tissue injury. Indeed, Ag-specific T cells rapidly acquire tissue-specific homing receptors and the potential to produce effector cytokines during their sequestration in secondary lymphoid organs (27).

Although our data indicate that the retention of activated T cells in inductive lymphoid tissues is not solely dependent on the α4 and αL integrin chains or Gαi protein-coupled receptor signaling, other ligand-receptor pairs could modulate the motility of T cells during their activation. Of particular interest is sphingosine 1-phosphate (S1P), which has been implicated in regulating T cell recirculation through secondary lymphoid tissues (28). Interestingly, T cells down-regulate the S1P receptor, S1P-1, shortly after TCR stimulation in vitro (29) and in vivo (28). Thus, changes in S1P sensitivity could suspend the ability of recently activated T cells to transit secondary lymphoid organs.

In our own studies, the potent S1P receptor agonist FTY720 was the only agent that effectively blocked the exit of sequestered Ag-specific T cells into the circulation (data not shown). However, FTY720 prevents the exit of nearly all T and B lymphocytes, regardless of their antigenic specificity, from secondary lymphoid organs (28, 30). Although FTY720 is currently being used in clinical trials for the prevention of renal transplant rejection (30), a complete understanding of the mechanism of selective lymphocyte sequestration might lead to the development of novel therapeutics that target the egress of only recently activated effector T cells from lymphoid organs to grafted tissues or sites of inflammation.

Our studies exclude several possible mechanisms for Ag-specific T cell retention in secondary lymphoid organs and lay the foundation...
for further investigation of how SIP receptors or other mechanisms control the important process of selective lymphocyte sequestration.

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References