Migration and Accumulation of Effector CD4⁺ T Cells in Nonlymphoid Tissues

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The migration of antigen-specific T cells to nonlymphoid tissues is thought to be important for the elimination of foreign antigens from the body. Here, we review the evidence that naive CD4⁺ T cells are first activated by antigen presentation in secondary lymphoid organs, proliferate, and differentiate into effector cells capable of producing antimicrobial lymphokines. These effector cells then leave the secondary lymphoid organs and use newly acquired trafficking receptors to extravasate at sites of inflammation. We argue that antigen presentation is required to retain effector CD4⁺ T cells in inflamed sites, and speculate on the antigen-presenting cells and adhesion pathways that are involved.

Keywords: adhesion; antigen presentation; lymphokines

CD4⁺ T cells play a key role in eliminating microbes from the body by producing lymphokines that regulate the antimicrobial functions of other cells—for example, antibody production by B cells, phagocytosis and microbe killing by macrophages, and lysis of infected target cells by cytotoxic T cells (1, 2). To be effective in microbe elimination during the primary response, the few relevant naive CD4⁺ T cells in the body must recognize peptide-major histocompatibility complex (MHC) II ligands derived from the infecting microbe, proliferate, and differentiate into effector cells capable of producing antimicrobial lymphokines. These cells must then migrate to the nonlymphoid tissues, where the microbes are located, and then produce their antimicrobial lymphokines in this location. Here, we review the molecular events that regulate this process.

TRAFFICKING OF NAIVE CD4⁺ T CELLS

Primary immune responses are generated from naive cells (3). Naive CD4⁺ T cells are those cells that survived the process of positive selection in the thymus and exited to the secondary lymphoid organs, but have not encountered the relevant foreign peptide-MHC II ligand. These cells survive in interphase for months by sensing signals through IL-7 receptor binding to IL-7 and weak T-cell antigen receptor (TCR) binding to self-peptide-MHC II ligands (4). Naive T cells spend their lives recirculating through the secondary lymphoid organs (lymph nodes, spleen, and mucosal lymphoid organs), but are excluded, for the most part, from other organs (5). In the case of lymph nodes and

mucosal lymphoid organs, this restricted trafficking pattern is determined by the expression of a limited set of receptors on the naive T cells and ligands on the unique high endothelial venules (HEV) in these organs. To exit blood vessels into tissues, cells must first roll on the blood vessel wall. Naive T cells accomplish this task by using CD62L or $\alpha 4\beta 7$ -integrin to bind peripheral lymph node addressin (PNAd) or mucosal addressin cell adhesion molecule-1 (MadCAM-1), which are uniquely expressed by HEV. The lack of these ligands on blood vessels in other organs prevents the extravasation of naive T cells into these sites (6). Once naive T cells roll, the chemokines, CCL19 and 21, which are displayed on the HEV lumen, engage their CCR7 receptor. CCR7 signaling then activates the leukocyte function-associated antigen-1 (LFA-1) integrin on the T cell, allowing strong binding to intercellular adhesion molecule (ICAM)-1 on the HEV. This firm adhesion allows the naive T cell to squeeze between the endothelial cells of the HEV and into the lymphoid organ (Figure 1, step 1). Naive CD4⁺ T cells then spend about a day interacting transiently with dendritic antigen-presenting cells within the T-cell zones of the lymphoid organ, searching for the relevant foreign peptide-MHC II ligand. If this ligand is not encountered, then naive T cells exit the lymph node by sensing the S1P sphingolipid using the S1P₁ receptor (7). Signals emanating from S1P₁ are required for naive T cells to migrate toward and squeeze back into the efferent lymphatic vasculature (8) that eventually carries the cells to the blood to begin the process again in a different secondary lymphoid organ.

INITIAL ACTIVATION OF NAIVE T CELLS

The recirculation process is interrupted if a naive CD4⁺ T cell finds the relevant foreign peptide-MHC II ligand. Much evidence indicates that this discovery will occur on the surface of a dendritic cell (9). Dendritic cells are the most abundant MHC II-expressing cells in the T-cell zones of secondary lymphoid organs where naive CD4⁺ T cells reside. Based on this anatomy, it was not surprising that dendritic cells in the T-cell zone were the first cells found to make tight conjugates with specific naive CD4⁺ T cells after injection of antigen (10, 11). Dendritic cells produce foreign peptide-MHC II complexes from antigens in one of two general ways (9). Soluble antigens that are deposited in tissues—for example, the dermis—can be carried rapidly in the lymph to the downstream lymph node via afferent lymphatic vessels (Figure 1, step 2). The lymph enters a labyrinth of narrow conduit tubes that run through the T-cell zones of the lymph nodes. Dendritic cells stick processes through small holes in the conduit tubes, take up antigen from the lymph inside, and produce peptide-MHC II complexes as early as 30 minutes after antigen injection (12, 13). Alternatively, dendritic cells at the injection site in the dermis take up antigen in this location, enter an afferent lymphatic vessel, and migrate down this vessel (Figure 1, step 3). Upon arrival in draining lymph nodes about 12 hours later, the dermal dendritic cells have processed the

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Figure 1. Generation and trafficking of effector CD4⁺ T cells. Naive T cells enter the lymph node via the high endothelial venules (HEV) (step 1), where they can encounter cognate antigen presented on dendritic cells, which have either picked up soluble antigen in the lymph node (step 2), or migrated from the site of antigen deposition (step 3). Antigen-specific T cells then undergo a period of clonal expansion (step 4), followed by exit into the circulation via the efferent lymphatics (step 5) and preferential migration out of the blood and back into the original site of antigen deposition (step 6). Here, antigen-specific T cells are capable of encountering antigen in the context of major histocompatibility complex class II (MHC II) molecules on various antigen-presenting cells that may play a role in their preferential retention in that tissue (step 7). The oval purple shapes represent soluble antigen molecules. The cell-associated purple circles represent peptide-MHC II ligands produced from the antigen molecules.

antigen and display large numbers of peptide-MHC II complexes.

Naive CD4⁺ T cells that interact with dendritic cells displaying foreign peptide-MHC II ligands (9) become activated and begin down the path to becoming effector cells. Activation is initiated by ligation of the TCR and CD28 molecules on the naive CD4⁺ T cells by peptide-MHC II and CD80 or CD86 molecules on the dendritic cell, respectively (3). The cytokines, IL-1 and IL-12, produced by dendritic cells or other cells, also play an important role in maximizing T-cell proliferation and differentiation to the effector cytokine-producing state (3) (Figure 1, step 4). The fact that CD28 ligands, in addition to IL-1 and IL-12, are produced in response to inflammatory stimuli explains why maximal effector T-cell generation only occurs when the antigen is associated with a microbe or administered with an inflammatory adjuvant, usually a Toll-like receptor ligand (14). Once activated, naive T cells induce expression of CD69, which binds to and inactivates S1P₁, preventing the cells from leaving the lymphoid tissue (15). The activated naive T cells then produce growth factors, divide, and express tumor necrosis factor receptor family molecules that transduce survival signals (3, 16). The net effect is the generation of a large number of peptide-MHC II-specific effector CD4⁺ T cells, which peaks in the lymphoid organs in which antigen presentation occurred about a week after the antigen entered the body (1, 17). Shortly after

the peak, the number of specific CD4⁺ T cells in the lymph nodes falls as the cells regain S1P₁ and leave the lymph nodes, moving into the efferent lymph and then the blood (Figure 1, step 5).

T-CELL ACTIVATION CHANGES THE EXPRESSION OF TRAFFICKING MOLECULES

The activation process is linked to the acquisition of new adhesion molecules that allow effector CD4⁺ T cells to migrate into nonlymphoid tissues (18). Naive T cells that are activated by peptide-MHC II-displaying dendritic cells in the presence of IL-12 express the enzyme, fucosyl transferase VII, which fucosylates CD62P ligand-1 (PSGL-1). Fucosylated PSGL-1 is able to bind to CD62P or CD62E, which are expressed on the luminal surfaces of blood vessels in inflamed tissues, especially skin. Blood vessels in inflamed skin also display elevated levels of the chemokine, CCL17, and the LFA-1 ligand, ICAM-1 (19). Furthermore, T cells found in skin express fucosylated PSGL-1 and CCR4, the receptor for CCL17. This expression pattern is consistent with the possibility that effector CD4⁺ T cells that are generated in the presence of IL-12 leave the skin-draining lymph nodes expressing fucosylated PSGL-1, CCR4, and LFA-1, allowing them to roll, bind, and extravasate through blood vessels in inflamed skin displaying CD62E/P, CCL17, and ICAM-1 (Figure 1, step 6). This possibility is supported by the findings that the

migration of effector T cells into inflamed skin is impaired in mice lacking CCR4, CD62E, and CD62P, or fucosyl transferase VII.

In contrast to effector T cells generated from skin draining dendritic cells, those that are generated by peptide–MHC recognition on dendritic cells in mucosal lymphoid organs, such as the Peyer's patches, acquire elevated expression of the $\alpha 4\beta 7$ -integrin and CCR9, the receptor for CCL25 (18). These changes may explain why these effector CD4⁺ T cells migrate preferentially to the gut lamina propria after leaving the mucosal lymphoid organs, as the blood vessels in the lamina propria display the $\alpha 4\beta 7$ ligands MadCAM-1 as well as CCL25. Interestingly, the capacity of dendritic cells from mucosal lymphoid organs to impose these changes on effector T cells depends on production of retinoic acid from vitamin A (20). Thus, the existence of a special dendritic cell subset in the mucosal lymphoid organs that is capable of retinoic acid production may explain why effector T cells generated from mucosal dendritic cells migrate to gut, whereas those generated from peripheral skin-draining lymph node dendritic cells do not.

Effector CD4⁺ T cell trafficking to the lung is less well understood. It has been shown that, during an influenza infection, IFN- γ -producing effector T cells that migrate to the lungs have downregulated the lymph node homing molecules, CD62L and CCR7, and that the antigen-specific T-cell population declines there as virus is cleared (21). The role of CCR7 in effector T-cell retention in the lung was further elucidated using a model of allergic airway inflammation in which both CCR7⁻ and CCR7⁺ CD4⁺ T cells were able to enter the inflamed lung, but only CCR7⁺ T cells were capable of exiting (22). It is notable that this role for CCR7 in peripheral tissue egress is not confined to the lung, as a similar function has been shown for egress from cutaneous sites (23). Additionally, a role for an $\alpha\beta_1$ -VCAM-1 interaction during a *Mycobacterium* infection has been implicated, but not conclusively demonstrated (24).

Although the aforementioned findings provide clues as to how effector T cells are imprinted to find their way back to sites of antigen deposition in the skin and gut, an analogous imprinting mechanism for the lung has not been established.

RETENTION OF EFFECTOR T CELLS AT NONLYMPHOID SITES OF ANTIGEN DEPOSITION

The capacity of effector CD4⁺ T cells to pass through blood vessels into nonlymphoid organs is probably determined by selectins, chemokine receptors, and integrins, not by TCR recognition of peptide–MHC II ligands. Evidence for this idea comes from studies in which two anatomically distinct inflamed sites on the skin, one containing a foreign antigen, and one without, are placed simultaneously on the same individual (25). Several days after proliferating and differentiating in the lymph nodes draining the antigen injection site, antigen peptide–MHC II-specific effector CD4⁺ T cells began to appear in equal numbers in both sites, indicating that initial entry was not determined by peptide–MHC II recognition on the vascular endothelium. However, over the next several weeks, the number of antigen peptide–MHC II-specific CD4⁺ T cells increased dramatically in the antigen-containing cutaneous site, but fell in the non-antigen-containing site. The specific T cells that accumulated in the antigen-containing site showed evidence of many rounds of prior cell division, but no signs of proliferation at the site itself. Together, these results are consistent with the possibility that effector T cells that had undergone many cell divisions in the draining lymph nodes entered both sites at the same initial rate, but were retained in the antigen-containing site via antigen peptide–MHC II recognition. This conclusion is supported by

the finding that, although the antigen peptide–MHC II-specific effector CD4⁺ T cells within the antigen-containing site were not proliferating, many were producing IFN- γ (25).

These results raise the question—how could antigen presentation in nonlymphoid tissues retain specific effector CD4⁺ T cells? One possibility is that the interaction between the TCR on the effector T cells and antigen peptide–MHC II ligands on cells in the nonlymphoid tissues provides sufficient adhesion. This is an unlikely possibility, as TCRs generally have a relatively low affinity for their antigen peptide–MHC II ligands (26). Alternatively, TCR signaling could indirectly promote adhesion of effector CD4⁺ T cells to the extracellular matrix by converting relevant integrins from an inactive to an active conformation. It has been reported that the T cells in certain nonlymphoid organs express high levels of the very late antigen (VLA)-1 and VLA-2 integrins, which bind to collagen and fibronectin (27, 28). Thus, an attractive possibility is that effector CD4⁺ T cells interact with antigen-presenting cells in the nonlymphoid tissue that display the relevant antigen peptide–MHC II ligand, resulting in TCR signaling, VLA-1 or VLA-2 conformational change, and tight adhesion to the extracellular matrix.

The antigen-presenting cells in nonlymphoid tissues that are responsible for the retention of specific effector CD4⁺ T cells are unknown. Lemos and colleagues (29) showed that mice that express MHC II molecules only in CD11c⁺ cells, presumably dendritic cells, were able to control a subcutaneous parasite infection. Macrophages at the skin infection site were capable of producing antimicrobial mediators that are triggered by lymphokines from effector CD4⁺ T cells. These results indicate that dendritic cells may not only be required for activating naive T cells in secondary lymphoid organs, but also effector T cells in nonlymphoid organs, at least in the case of this parasite infection. However, these results do not rule out an ancillary role for other antigen-presenting cell types in this immune response, and a primary role in others. Thus, mast cells, monocytes, neutrophils, and tissue macrophages, all of which are capable of expressing MHC II molecules, may present antigen peptide–MHC II ligands to effector T cells, retaining them in nonlymphoid tissues during certain immune responses (Figure 1, step 7). The nature of the antigen-presenting cells and their unique cytokines and costimulatory molecules could have a major influence on the character of the effector T-cell response in different tissues. For example, interactions between effector CD4⁺ T cells and mast cells in the lungs would almost certainly result in different T-cell functions than those induced in the skin by interactions between effector CD4⁺ T cells and macrophages. Therefore, identification of the antigen-presenting cells in nonlymphoid tissues may set the stage for therapeutic enhancement or inhibition of T-cell activation in these sites.

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