

Chapter 3

LYMPHOCYTE TRAFFICKING

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It is difficult to overstate the importance of cell migration to the immune system. It is discussed repeatedly in this volume under the guises of inflammation, differentiation, adhesion, recruitment, or cell contact. This chapter serves as a basic introduction to the processes and concepts involved. It is divided into three sections: the cell biology and molecular basis of migration; an overview of the odysseys taken by immune cells; and a synthesis of clinical issues to which migration is particularly important.

CELL BIOLOGY AND THE MOLECULAR BASIS OF MIGRATION

Why cell motility is essential in the immune system

Almost all cells in an adult are anchored and remain relatively sessile. It is striking, therefore, that many cells in the immune system, such as lymphocytes, are mobile throughout an adult's life. This motility is essential to the function of the immune system and reflects its intrinsic design. The necessity for this motility in the immune system is twofold.

The first reason motility is so necessary relates to the ongoing development and differentiation that occurs in the immune system even in the adult. The motility in the immune system has little parallel in the adult, except perhaps in wound healing. In contrast, it has very strong parallels with the processes that occur during embryogenesis. This parallel is a profound one. Alone among organ systems, the immune system is continuously undergoing development throughout life. The process of ongoing differentiation requires movement within tissues (such as the multiple sequential cellular interactions that occur in the thymus; see

Chapters 2 and 10) and between tissues (for example, to the thymus for initial positive or negative selection of T cells, then into lymph nodes for the first encounter with antigen, and then into sites of inflammation for effector function).

The second necessity relates to the requirement that the immune system serve in the defense of the *whole* organism. Some resources of the immune system are centralized and anatomically fixed (such as the primary and secondary lymphoid organs). Other resources are widely distributed but stationary, such as the γ δ T cells, which are found in skin, gut, and other mucosal surfaces. They are interpreted to be fixed sentinels capable of rapidly responding to a defined range of environmental antigens.^{1,2} But the majority of the resources are maintained in continuous movement to enable rapid deployment to any site where they are needed. The power of this strategy is important at multiple levels. For example, naive T cells of the specificity required to combat a given virus are rare; therefore, incessant movement of all cells is necessary to ensure that the rare relevant cell rapidly reaches the site of viral antigen display. Thereafter, the antigen-reactive T cells expanded at that site recirculate

KEY CONCEPTS

Cell Biology of Migration

1. Motility is fundamental to the function of the immune system.
2. Immune cells use dozens of molecules to adhere to and move through tissue.
3. Cell adhesion occurs via multistep sequential molecular interactions referred to as "adhesion cascades."
4. Rapid regulation of adhesion (in seconds or minutes) is essential to recruitment, migration, and recognition.
5. Longer term activation and differentiation (hours to days) also profoundly influence patterns of migration and adhesion.
6. Cells of the immune system exhibit cycles of motility and rest.

*In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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rapidly to the bloodstream so that they are available for defense elsewhere.

Modes of motility

The term "recirculation" refers to emigration of a lymphocyte from blood into tissue and its return via lymph to the blood.³ The magnitude of recirculation is amazing. The number of lymphocytes entering the blood from lymph each day is 10 times the total circulating lymphocyte pool; in other words, the circulating lymphocyte pool is "replaced" 10 times a day with cells mobilized from a vastly greater tissue pool.

During recirculation, immune cells use two fundamentally different modes of movement (Fig. 3-1). One is crawling,⁴ which requires them to adhere to their surroundings. This is how they move within a tissue; crude calculations suggest that it would take 4 days for a crawling lymphocyte to move 1 cm. The other is in fluid phase, which requires that they *not* adhere. This is the way lymphocytes travel great distances in the blood and lymph and by our calculation is at least 10^4 -fold faster. Therefore, it is often faster for a lymphocyte to exit via lymphatics, travel in the blood, and reenter nearby tissue via endothelial binding and transmigration than to crawl a millimeter within tissue.

Cell trafficking depends on and is closely related to a range of biologic processes: adhesion, signal transduction, motility. The process of adhesion and the molecules that mediate it are discussed in detail in Chapter 11 but need to be introduced briefly here in conceptual terms. During most of their lives, lymphocytes are in a tissue, adhering either to other cells or to extracellular matrix (or typically both). Like other cells, immune cells express a large number (dozens) of "adhesion" molecules that mediate this adhesion.⁵ However, on the whole, lymphocytes express molecules that mediate rapidly regulatable adhesion rather than those that mediate relatively permanent adhesion. For example, the integrin family of adhesion molecules is of extraordinary importance in mediating lymphocyte adhesion. Lymphocytes typically have on their surface five or more members of this family. As would be expected for adhesion receptors, each integrin binds specifically to ligands in the extracellular matrix and/or on apposing cell surfaces. But the feature of integrins that is most important for their role in motility is their capacity to undergo rapid changes in adhesive function.⁶ In contrast, the cadherins, another large and important family of cell adhesion proteins, appear to be virtually absent from immune cells. The simplest explanation is that cadherins tend to be involved in stable, long-term associations between cells, which are atypical of immune cells.

Adhesion cascades

During the process of immunosurveillance, migrating T cells are arrested by their contact with an apposing cell displaying antigen. When the T cell antigen-specific receptor is engaged, the T cell rapidly increases the adhesive function of its integrins and thereby forms a strong contact with the apposing cell.⁷ This may be relatively short (less than 15 min), as in the case of a cytotoxic T cell killing an apposing target. Alternatively, when this is an interaction between a T helper cell and a B cell, a series of signals

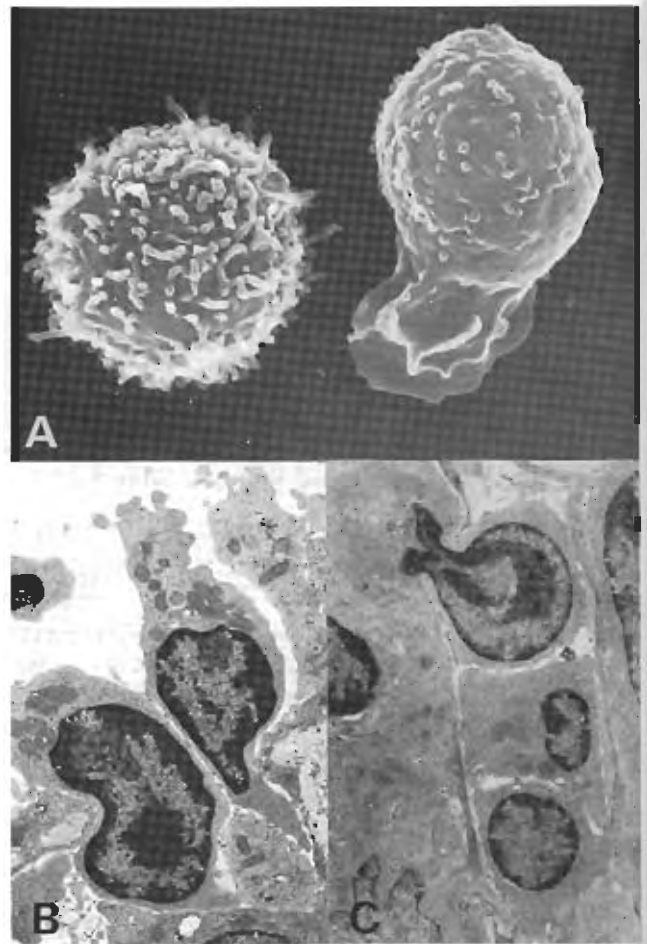


Fig. 3-1. Images depicting locomotion of lymphocytes: **A**, Contrasting SEM morphology of resting (round) vs. motile (with extended lamellipodium) cell in vitro. **B** and **C**, Morphology of motile (constricted nucleus) and resting (round nucleus) lymphocytes in vivo. In **B** two lymphocytes are both trying to crawl into the LN cortex from a cortical lymphatic sinus. In **C** a lymphocyte negotiates the abluminal junctions of HEV cells to join its colleagues resting in the fibroblastic reticular cell sheath.

exchanged between the T and B cells results in prolonged adhesion (at least 24 h).

Both T cell help and cell-mediated cytotoxicity illustrate the important concept of regulation of T cell adhesion by the T cell receptor. However, much (perhaps most) regulated adhesion of T cells occurs without participation of the T cell receptor; to signify this distinction we refer to it as antigen-independent adhesion. The best understood example is that of leukocyte binding to endothelium.⁸ Since the concepts involved are particularly important, we describe T cell binding to endothelium (Fig. 3-2); the analogous process of granulocyte binding to endothelium is described in Chapter 11. Strong adhesion to endothelium is mediated by integrins on the leukocyte (Fig. 3-2, *fourth panel*). However, the integrins on circulating T cells are turned off functionally. Therefore, the leukocytes need to receive a triggering stimulus from the endothelial cell to activate their integrins, just as they do from an antigen-positive cell.

Since binding of T cells to endothelium (and subsequent

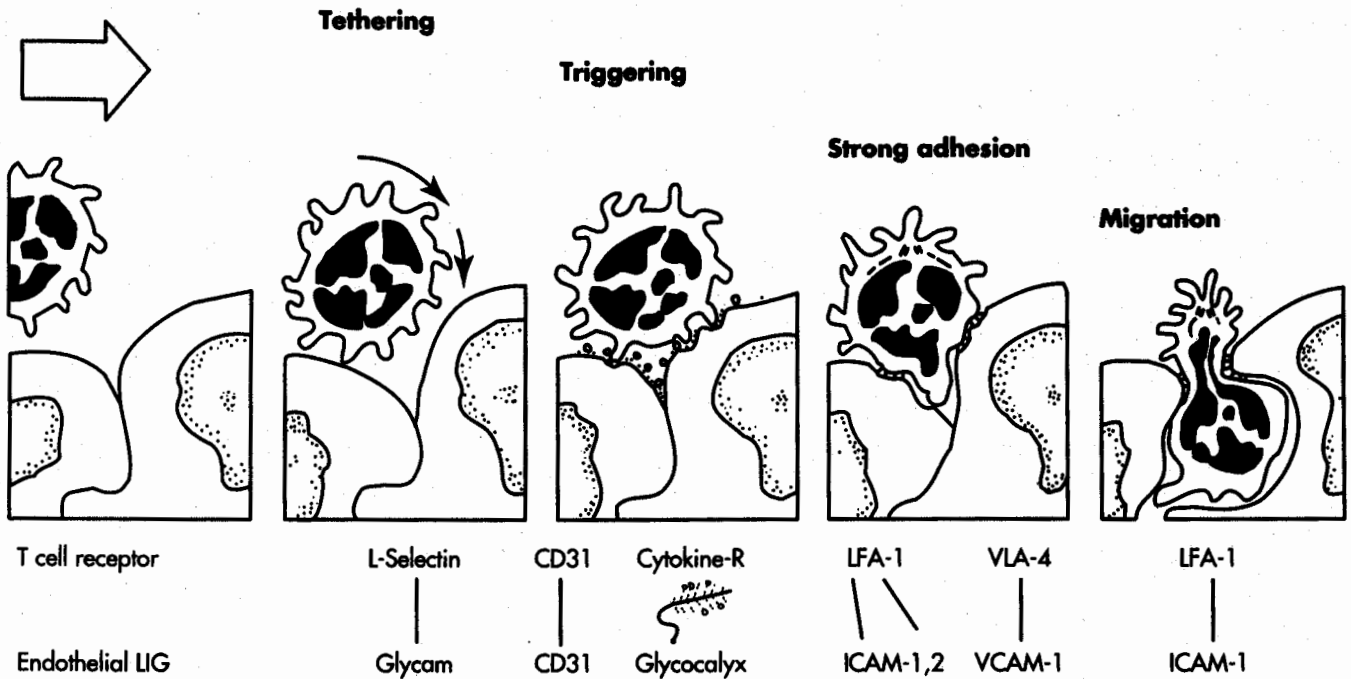


Fig. 3-2. Consensus model of T cell adhesion cascade for binding to LN high endothelial venules. See text for description. (Adapted from Anderson AO, Shaw S: *Semin Immunol* 5:271, 1993.)

recruitment into tissue) occurs regardless of the antigen specificity of the T cell, receptors other than the T cell receptor must be involved in triggering adhesion. Until recently, the identity of these signaling molecules has been unknown. Now emerging evidence strongly implicates a range of inflammatory cytokines, particularly members of the chemokine family,^{9,10} in “turning on” the lymphocyte integrins. Included in the two chemokine subfamilies are about a dozen structurally related, soluble mediators including IL-8, MIP-1 β , and Rantes. Chemokines are typically produced by a variety of cell types that have been stimulated by a particular inflammation or stressful conditions. Such chemokines produced by endothelium or reaching the endothelial cell surface from underlying tissue are “presented” to passing leukocytes (Fig. 3-2, *third panel*).¹¹ Because each chemokine preferentially acts on distinct subsets of leukocytes, only those subsets respond by turning on their integrins and binding to the endothelium. For example, naive T cells respond better to MIP-1 β than do memory T cells.

Before the triggering, the leukocyte is loosely tethered to the endothelial wall in a fashion that allows it to roll (Fig. 3-2, *second panel*). This facilitates the subsequent two steps in two ways: (1) by establishing intimate (albeit still reversible) contact with the endothelial surface and (2) by prolonging such membrane contact. This tethering-rolling is mediated by members of the selectin family of molecules interacting with specific carbohydrates on the apposing cell.

Taken together these three steps are referred to as an adhesion cascade, similar in concept to the complement cascade or the coagulation cascade. The cascade contributes to the exquisite specificity of cell recruitment into tissue. The recruitment is selective and generally adaptive because

of the precise regulation of receptors and ligands on each side. Initially the specificity was attributed to unique receptor-ligand pairs for each interaction of each subset of cells. It is now understood that the selectivity results from combinatorial requirements for the receptor-ligand pairs involved in each step.^{12,13} In this manner, endothelial cells can specify which of the chaotic mix of circulating cells they will recruit.

Endothelial cells: Importance of postcapillary venules and their specialization

Endothelial cells in different parts of the vascular tree have distinct phenotypes and functions. Postcapillary venules (PCV) are the vessels relevant for binding circulating cells and recruiting them into tissue. This is explained in part by the favorable hemodynamic conditions of slow flow and relatively high surface area. Equally important, evolution has endowed PCV endothelium with a phenotype optimized for cell recruitment. The epitome of effective PCV endothelium is the so-called high endothelial venule (HEV) endothelium, which mediates the torrential recruitment of lymphocytes into lymph nodes (Fig. 3-3). First, HEV cells express a unique molecule called “glycam,” which is the ligand for leukocyte L-selectin and thereby increases the efficiency of leukocyte binding to HEV (Chapter 11). Second, HEV endothelium is ideally designed to receive and display proadhesive cytokines, which are believed to trigger leukocyte adhesion to endothelium (see above). They receive cytokines from an underlying conduit system, which is believed to transport cytokines rapidly to HEV cells from the underlying tissue and from afferent lymph.¹⁴ Furthermore, HEV cells have a thick glycocalyx on their luminal surface, which appears especially suited to

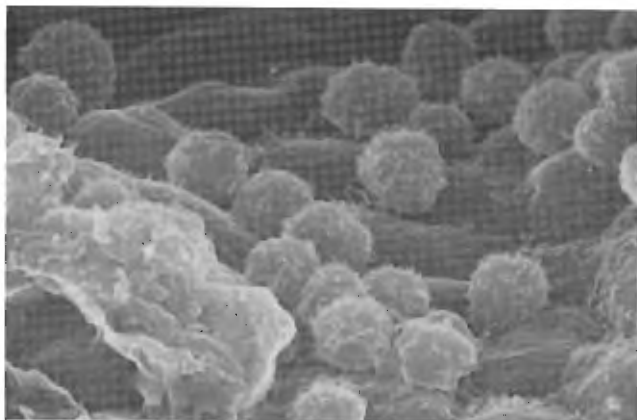


Fig. 3-3. High endothelial venules with plump protruding endothelial cells (as revealed by SEM) are a major portal of entry for lymphocytes involved in recirculation. Note the adherent lymphocyte, whose microvilli have redistributed (*bottom right*) proximate to migration between endothelial cells. (Modified from Anderson AO. In Oppenheim JJ, Shevach E, editors, *Immunophysiology, the role of cells and cytokines in immunity and inflammation*. London, 1990, Oxford University Press.)

retain cytokines for presentation to leukocytes.¹¹ Third, there are specialized junctions between HEV endothelial cells, which facilitate the egress of recruited cells and the ingress of the relevant cytokines.^{3,14} Postcapillary venules in other tissues serve the same general function as lymph node HEV but differ in detail. It is differences in details of ligand expression that determine the exact cell type recruited to a given tissue.

Positioning or ecotaxis

The examples above illustrate regulated adhesion of immune cells to apposing cells. In concept, similar mechanisms regulate immune cell adhesion to extracellular matrix (ECM). Both T cell receptor occupancy and exposure to proadhesive cytokines induce T cells to adhere to extracellular matrix components such as laminin or fibronectin. Thus, retention of cells at a site in tissue usually involves coordinated regulation of adhesion to ECM and other cells.¹⁵

Immune cells must position themselves appropriately within a tissue. For example, B cells must find follicles within lymph nodes and neutrophils must find the microbes within an inflamed tissue. How is this positioning achieved? In concept, one search involves random movement and the other directed migration. The case of a B cell is illustrated. It might migrate randomly and only stop when it encountered signals upon chance encounter with the follicle. Alternatively, it might respond to gradients either of soluble factors (cytokines) or extracellular matrix components.¹⁶ Although there is much that remains unclear in this area, cytokines are expected to play a prominent role, either in a soluble form (chemotaxis) or when immobilized on ECM or cell surfaces (haptotaxis). Immunohistologic studies (Fig. 3-4) strongly suggest that directed migration must play an important role because recirculating B cells enter lymph nodes in the midst of T cell areas and purposefully move to B cell areas.

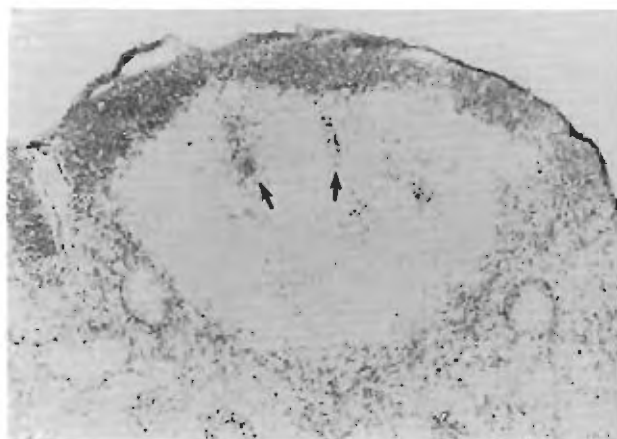


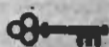
Fig. 3-4. Immunohistochemical staining for surface IgD in LN shows ecotaxis of B cells (*arrows*) marching across the T-dependent cortex from the HEV to join their comrades in the B-dependent cortex and follicles above.

Mechanics of migration

Recognition events mediated by adhesion are seemingly trivial matters of sticking or not sticking. In contrast, migration involves a formidable task of regulating adhesion in time and in space and coordinating that with contraction of the cell's molecular muscles. Nevertheless, the same concepts in adhesion regulation pertain. When analyzed in vitro, cell migration begins with the extension of a leading edge (or lamellipod) in the intended direction of motion; effective migration occurs when adhesion of the leading edge is coordinated with anteroposterior contraction of cortical microfilaments and release of the cellular components (uropod) at the trailing edge. This appears to involve orchestrated interactions of at least three fundamental processes: (1) regulated assembly (at the leading edge) and disassembly (at the trailing edge) of an actin-based cytoskeleton; (2) regulated anchorage of this cytoskeleton to ECM (or apposing cells) via adhesion molecules, typically integrins; and (3) drawing the cellular components along the cytoskeletal framework by myosin-based interactions (contraction wave). In effect it resembles the motion of an earthworm advancing through successive waves of extension followed by contraction. The cortical cytoskeletal framework of the cell is its muscular motor. Its integrin anchorage to the ECM functions like the worm's sticky surface, which transiently links it to its surroundings. In an extended-attached condition, waves of myosin-dependent contraction pull the cell forward as muscles do for the earthworm. The worm repeats the cycle by detaching, extending, and repeating the procedure; the cell disassembles some of its actin at the rear, pushes forward its leading edge, and assembles new actin and anchorage there.

ODYSSEYS OF IMMUNE CELLS

There are too many odysseys of immune cells to detail them all. Instead we focus on important and informative examples. Begin by considering an individual's first skin sensitization to poison ivy.^{17,18} Following initial contact the

KEY CONCEPTS**Odysseys of Immune Cells**

1. Maturation of immune cells typically occurs via multiple episodes of tissue entry, differentiation, and exit.
2. Expression of adhesion molecules is actively regulated during immune cell differentiation.
3. The tissue microenvironment in which a lymphocyte differentiates influences its subsequent migration.

epidermis is penetrated by a mix of chemicals, which includes the specific antigen urushiol, together with chemical irritants (which are, in effect, adjuvants). In the midepidermis there are specialized antigen-presenting cells (APC) called Langerhans' cells. These APC are triggered by the chemical irritant, pick up antigens from their epidermal milieu, detach themselves, crawl into the draining lymphatics, and are carried via the afferent lymphatics to the regional lymph node. They penetrate the lymph node tissue and become resident there. In short, Langerhans' cells *transport* antigen to lymph node.¹⁹

Among the billions of naive lymphocytes that emerge from the thymus, fewer than 1 in 10,000 are likely to have the "right" T cell receptor specificity to recognize the urushiol foreign antigen. Statistically, 10,000 irrelevant T cells must encounter these APC to ensure that the single relevant T cell has the opportunity to be activated. Secondary lymphatic tissue is designed to facilitate such a high frequency of encounters. Specifically, there is a virtual torrent of T cells that migrate into the lymph node to mediate surveillance of antigen presented there. When a relevant T cell encounters this APC it stops migrating and undergoes activation-proliferation for several days. Thereafter, the memory cell progeny of the naive cell exit into the blood via the efferent lymphatics; during their differentiation into memory cells they acquire a new set of cell surface adhesion molecules to facilitate their subsequent preferential migration into skin.

During this initial stage of naive T cell response in the regional lymph node, no specific T cell-mediated response is occurring at the site of antigen contact. However, that area is undergoing changes to prepare it for the next phase. The endothelial cells are responding to local stimuli by the expression of new (or increased levels of) adhesion molecules as a result of a cytokine cascade initiated by the chemical irritant. As soon as antigen-specific memory cells appear in blood, their new ensemble of adhesion receptors ensures that they are among the memory cells recruited into that site. When these T cells encounter poison ivy antigen they produce additional cytokines that rapidly amplify the inflammatory response.

Finally, the Langerhans' cells need to be replenished in the epidermis. This too occurs by cell motility. There is continuous seeding of Langerhans' cell precursors into skin and further differentiation there. The precursor emigrates from bone marrow into blood and thence into skin, where residency time is on the order of 1 to 9 weeks, depending upon whether or not they encounter antigen or stimulants.¹⁹

The foregoing example illustrates some of the power of

the mobility of the cells in mediating the response. Motility (1) brings immune cells to new locations for their differentiation; (2) allows transport of antigen; (3) enables encounter between cells collaborating in the generation of an immune response; and (4) brings effector cells to sites at which they are needed. This is a typical example of a concerted response of multiple cell types. A more detailed consideration of the migration of cells of individual lineages, T cells, B cells, and progenitor cells, follows.

T cell development

A complex ensemble of cell movements and morphogenetic interactions between lymphocytes and stromal elements gives rise to the thymus (and other lymphoid organs). Although comprehending this process increases understanding of certain immunodeficiency syndromes (discussed later in this chapter), the details are beyond the scope of this chapter. Instead, the movement of maturing lymphocytes through developed thymus and secondary lymphoid structures is emphasized.

Pre-T cells migrate (from the site of hematopoiesis) via the blood to enter the thymus and undergo extensive education and differentiation there (Chapter 10). The T cell receptor for antigen occurs in two heterodimeric forms: $\alpha\beta$ and $\gamma\delta$. Early in thymic development, several waves of $\gamma\delta$ cells emerge and populate epithelial and mucosal sites.^{1,2} As mentioned previously, the $\gamma\delta$ T cells reside there (relatively permanently) and are thought to act as sentinel cells responding to particular prevalent environmental insults. The specific functions of these cells are presently an active and diverse area of research. For example, these cells are believed to secrete immunoregulatory cytokines, mediate cytotoxicity, and participate in tolerance induction.

$\alpha\beta$ T cells preponderate during the remainder of thymic ontogeny and through the rest of life. These thymic emigrants are designated "naive cells." In the description of skin sensitization, it has been noted that naive T cells selectively go to secondary lymphoid tissue (lymph nodes and spleen). When activated by specific antigen at those sites, they proliferate and undergo profound differentiative changes. Of particular importance for the present discussion, they radically change their cell surface expression of molecules involved in adhesion (Table 3-1).¹⁸ Not surprisingly then, when they emerge as memory cells, their pattern of migration is markedly different. As memory cells, they preferentially home to nonlymphoid tissues such as skin or gut (rather than secondary lymphoid tissue). This fits reasonably well with the increases seen in expression of adhesion molecules. Memory CD4 cells express more VLA-4 and LFA-1 and have increased capacity to bind to their ligands VCAM-1 and ICAM-1, whose expression is increased at sites of inflammation.

The foregoing paragraph describes the differentiation of a naive T cell in an imaginary "generic" secondary lymphoid tissue. In fact, secondary lymphoid tissues draining distinct tissues differ from each other and therefore influence maturation of T cells differently (Fig. 3-5).^{18,20} If lymphocytes are activated in lymph nodes that drain skin they become specialized to migrate preferentially into skin and serve the immunologic needs of skin. Conversely, if lymphocytes are

Table 3-1. Molecules differentially expressed on resting CD4⁺ naive and memory cells

Molecule	Other names	Approximate memory: naive ratio	Comments
Integrin family			
LFA-1	CD11a, CD18	2:1	Receptor for ICAM-1 and ICAM-2; mediates leukocyte adhesion and signaling; CD18 is common two chain
VLA	CD29	4:1	Common 1 chain of VLA subfamily integrin; ? signaling
VLA-3 α	CD49c	6:1	Ligands collagen, fibronectin and laminin
VLA-4 α	CDw49d	3:1*	Ligands fibronectin and VCAM-1; and roles in T cell homing and signaling
VLA-5 α	FNR ³ , CDw49e	3:1	Ligand fibronectin, signaling
VLA-6 α	CDw49F	3:1	Ligand laminin, signaling
Immunoglobulin supergene family			
ICAM-1	CD54	3:1	Ligand for LFA-1; leukocyte adhesion and signaling
CD2	T11	2:1	Receptor for LFA-3; T cell adhesion and signaling
LFA-3	CD58	10:1	Ligand for CD2; T cell adhesion and signaling
Other adhesion or homing-related receptors			
CD44	Hermes, Pgp-1	1.5:1	T cell homing, adhesion to erythrocytes, and signaling
CD26	DPP IV	2:1*	? Ligand fibronectin or collagen
HECA-452	CLA	4:1*	SLE ^a , SLe ^x , ligand ELAM-1, skin-associated homing
Control			
CD3	TCR	0.9:1	Antigen-specific T cell receptor complex
CD45	LCA, T200	1.2:1	Invariant part of CD45 molecule; cytoplasmic portion tyrosine phosphatase

*Clear heterogeneity with CD45R0⁺ memory cells.

SLe^x, Sialyl Lewis-x; VCAM, Vascular cell adhesive molecule.

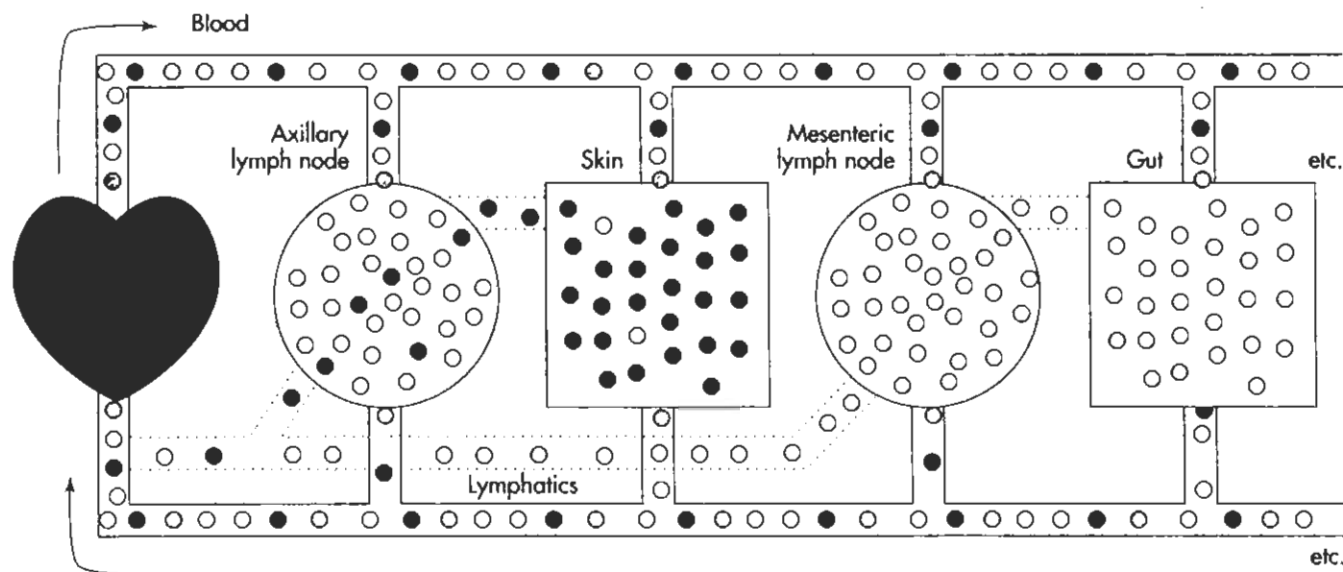


Fig. 3-5. Specialization of lymphocytes depending on their site of primary activation. Solid lines demarcate the blood vessels and dotted lines demarcate lymphatics. Naive cells (*open circles*) migrate preferentially to all secondary lymphoid tissue. Naive cells activated in skin-draining lymph nodes become memory cells with a distinctive phenotype (*filled circles*), which enables them to migrate preferentially into skin. In contrast, naive cells activated in gut-draining lymph nodes become memory cells with a distinctive phenotype (*stipled circles*) that enables them to migrate preferentially into gut.

activated in lymph nodes draining mucosal sites, they become specialized to migrate preferentially into gut and serve its immunologic needs. What makes mucosal and peripheral lymphoid tissue different and what differences do they imprint on the lymphocytes differentiating therein? The re-

sulting differences in the lymphocytes are beginning to be understood. Differentiation in mucosal lymph nodes results in the induction of integrins, which should predispose T cells to interact with gut endothelium and therefore migrate into the gut: included are both the integrins $\alpha\epsilon\beta 7$ for CD8

cells and $\alpha\beta 7$ for CD4 cells. Conversely, T cells maturing in skin-draining lymph nodes preferentially retain expression of L-selectin and the cutaneous lymphocyte associated (CLA) antigen, both of which may facilitate subsequent entry into the skin. These differences in the phenotype of emerging T cells must reflect local differences in microenvironment. Such differences have not been defined clearly; however, TGF- β has been proposed to be better expressed in mucosal sites and to contribute to the distinctive phenotype.

There are qualitative differences in immune responses between skin-draining and gut-draining lymphoid tissues. The previous paragraph illustrates concepts that are fundamental to regional immunity. There is increasingly strong evidence that immune responses at different sites are markedly different in ways which allow adaptation of the general capabilities of the immune response to the particular needs of that microenvironment.

B cell development

B cell development also entails a coordinated sequence of migratory and differentiative events (Chapter 9).²¹⁻²³ The original definition of B cells was based on their development in the bursa of Fabricius in chickens. In humans, the anatomy has been more difficult to define. However, it is increasingly clear that the first step in mammals is also migration of pre-B cells from the marrow via blood to a bursa-like structure (the distal ileal Peyer's patch in the sheep and the appendix in rabbits).^{24,25} In those sites pre-B cells expand rapidly and undergo diversifying mutations in the genes encoding the antigen binding sites during VDJ rearrangement (Chapter 4).²³ They are then subject to positive selection to generate an Ig repertoire biased toward antigens prevalent in the environment; progeny that do not bind antigen in this milieu undergo apoptosis. Survivors leave the follicles in efferent lymph and enter the blood, where they circulate as small precursor ($\mu\delta$) B cells; these are the preponderant recirculating B cell. They migrate continuously into all secondary lymphoid tissue. Their subsequent differentiation depends on whether they are first activated in mucosal sites (such as Peyer's patches) or in nonmucosal lymphoid tissue (lymph nodes and spleen) where they are influenced by prevalent cytokines (e.g., IL-4, -5, -6 in Peyer's patches and IL-2, IFN- γ in lymph nodes) and unique stromal and accessory cell populations.^{26,27}

Some precursor B cells, by chance, will be first activated in spleen or lymph node. In that process they are programmed toward one of the IgG isotypes (rather than IgA). Daughter cells from this clonal expansion may leave the lymph node in the efferent lymph, return to the blood, and seed the spleen, bone marrow, sites of inflammation, and other lymph nodes with plasma cells and "memory" B lymphoblasts.²²

Other precursor B cells, by chance, will be first activated in a Peyer's patch. Those cells become committed to rearrange their immunoglobulin heavy chain genes to express IgA (rather than IgG isotypes noted above); despite this commitment having been made, the switch to IgA is often delayed for several days. This cell leaves in the efferent lymph, passes through the mesenteric lymph node (where

it may be subject to immunoregulation by T cells it encounters there), and returns to the blood. These cells selectively migrate to the spleen, which serves as an auxiliary site for clonal expansion prior to dissemination of the daughter cells via the blood into mucosal lamina propria. This process takes 5 to 7 days, at least four trips in the blood, and two to three cell generations.

Odysseys of progenitor cells

In the embryo hematopoietic stem cells originate in the connective tissue of dorsal mesentery of the aorta, close to areas of major blood vessel development.²⁸ Before the stem cells arrive at their "normal" site of expansion in bone marrow, they undergo a remarkable odyssey of development at multiple sites. First, they expand in blood islands in the yolk sac, derived from endoderm.²⁹ Second, they migrate to and expand in liver, also an endodermal derivative. Third, they migrate to and expand in spleen. Finally, they relocate to bone marrow as soon as blood vessels invade provisionally calcified cartilage. Although this odyssey is irrelevant to understanding hematopoiesis in the normal adult, it helps to understand the sites at which extramedullary hematopoiesis may occur pathologically and where neoplastic lymphoid cells may lodge if they revert to expression of "hematopoietic" adhesion molecules.

CLINICAL RELEVANCE OF MOTILITY

Because of the fundamental importance of lymphocyte trafficking, perturbations thereof are relevant to many diseases. Some of these are discussed in this section. Many others are addressed in detail in the specific chapters relevant to individual diseases.

Conditions that alter the frequency of circulating lymphocytes

There are many diseases in which the number of circulating lymphocytes (or composition of subsets) is altered. Indeed there is an extensive differential diagnosis for both of these clinical manifestations.^{15,30} Only two are discussed here. Given how much work has been done studying circulating lymphocytes, it is remarkable that so few diseases are manifested by marked changes in circulating lymphocytes. Why? It is suspected that there are at least two reasons. First, there appear to be strong homeostatic mecha-

KEY CONCEPTS



Clinical Relevance of Migration

1. Motility is fundamental to the function of the immune system.
2. Homeostasis in circulating lymphocyte counts makes that determination a poor indicator of many diseases.
3. Peripheral tolerance induction is particularly dependent on lymphocyte traffic patterns.
4. Much of the adjuvant effects critical to immunization relate to migration.
5. Therapies aimed at modifying migration or adhesion seem promising in a variety of diseases.

nisms that maintain leukocyte counts in a normal range. The molecular mechanisms for such homeostasis are unknown. Because of it, however, changes in lymphocyte number are seen only very late in disease processes. Second, many circulating cell populations are present at low abundance and major fluctuations in these small populations most likely go unnoticed.

A topical example of lymphocytopenia is HIV, which is notorious for selective depletion of circulating CD4 cells. It was an enigma for years why depletion occurred without a high frequency of infected cells in circulation. Recently it has been discovered that infection changes the adhesive-migratory phenotype of CD4 cells, causing them to be sequestered in secondary lymphoid tissue, where infected cells abound.^{31,32}

In contrast, lymphocytosis is induced by certain bacterial infections, particularly pertussis infection. Clinically, the disease is associated with marked lymphocytosis. The apparent mechanism is a conceptually interesting one in which pertussis toxin blocks intracellular signaling pathways essential for the normal emigration of the lymphocytes. Specifically, pertussis toxin causes ADP ribosylation of a G-protein that is essential for the triggering step of the adhesion cascade outlined previously.³³ Furthermore, it has recently been observed that the toxin has regions of strong homology with the carbohydrate binding domains of L-selectin. Given the molecular mechanism, it is not surprising that the accumulation of lymphocytes in blood is accompanied by depletion of lymphocytes in tissue.

Localized processes resulting in attenuated systemic immunity

Because of the mobility of cells, many relatively localized processes have systemic implications. Anergy provides a useful illustration. In some situations, antigen-reactive T cells are sequestered at the site of an inflammatory lesion; in that case, the frequency of antigen-reactive cells in blood (and moving through other sites) is diminished, resulting in temporary anergy. For example, in granulomatous lesions such as miliary tuberculosis or leprosy, anergy coincides with active disease but reactivity reappears during recovery. At least a part of this appears to be due to sequestration.

A more profound case is systemic tolerance induction. Systemic tolerance can be achieved by a localized mechanism of clonal deletion (or anergy induction) provided it operates in a location with high volume lymphocyte traffic. Such traffic ensures that all relevant antigen-reactive T cells encounter the deletion-inducing process. A provocative example is the systemic tolerance that can be induced by administering antigen into the anterior chamber of the eye. Following such administration a limited number of antigen-bearing cells migrate via the blood to the spleen and apparently induce tolerance by this kind of mechanism.³⁴ TGF- β in the eye appears to be critical in generating this tolerance. Gut-induced systemic tolerance is being explored as a therapeutic modality to attenuate a variety of unwanted immunologic interactions.^{35,36} Among the disease processes currently being treated experimentally with orally administered toleragen are multiple sclerosis (with myelin basic

protein), arthritis (with type II collagen), and even hypersensitivity to poison ivy. Indeed, Native Americans appear to have been the first to use poison ivy leaves orally to induce tolerance in themselves.

Conditions in which facilitation of lymphocyte migration would be beneficial

The success of immunization depends on lymphocyte migration. One important means of facilitating strong immune responses is to increase the rate of migration of lymphocytes through the draining lymphoid tissue, as well as into the site of antigen injection. One mechanism of action of adjuvants is to provide stimuli for such a rate increase. For example, Freund's adjuvant, poly I:C-lysine, avridine, and muramyl dipeptides all increase the rate of migration of lymphocytes across the high endothelial venules, typically threefold to fivefold (Fig. 3-6). The mechanisms for such increases are diverse:^{37,38} (1) increased blood flow, (2) increased expression of adhesion molecules on the endothelium, (3) soluble factor delivery to the endothelium to facilitate recruitment, or (4) proliferation of HEV to enlarge effective surface area for sustained increased recirculation.

Some of these mechanisms are illustrated in Fig. 3-6A, which summarizes data on the kinetics of entry, accumulation, and exit of lymphocytes trafficking through a regional lymph node after subcutaneous inoculation of antigen-adjuvant.^{37,38} The inoculation causes an initial rapid increase in entry of lymphocytes from the blood. This parallels a rapid change in blood flow to the node and is associated with a brief reduction in exit of lymphocytes into efferent lymph. There is a prolonged increase of lymphocytes exiting into efferent lymph after 72 hours, which includes cells specific for the antigen inoculated. This and release of dendritic cells ensures dissemination of immunologic memory to the spleen and other lymph nodes.

Continuing improvement in immunization strategies will depend on improved exploitation of these physiologic mechanisms. Of course, adjuvants have many other effects, such as triggering cytokine cascades; facilitating the function of antigen-presenting cells; and increased division, dissemination, and differentiation of daughter effector lymphoid cells and antigen-bearing cells.³⁷

A second rather different clinical challenge in which optimization of lymphocyte migration is essential is the new modality of tumor therapy: tumor infiltrating lymphocytes (TIL).³⁹ The premise is that tumor-specific cytotoxic T lymphocytes expanded *in vitro* and reinfused will home to tumor sites and kill the tumor cells. For this therapy to be effective, the TIL must "home" to the tumor. Under laboratory conditions the rate of migration into tumor may be somewhat higher than that into adjacent tissue, but it is still extremely low. Since TIL have been cultured *in vitro*, their adhesion phenotype is markedly altered; methods to restore them to a more appropriate phenotype for migrating to tumor are currently being explored.

Therapeutic reductions in lymphocyte migration

Increasingly in developed countries, disease results from an excessive or inappropriate immune response rather than

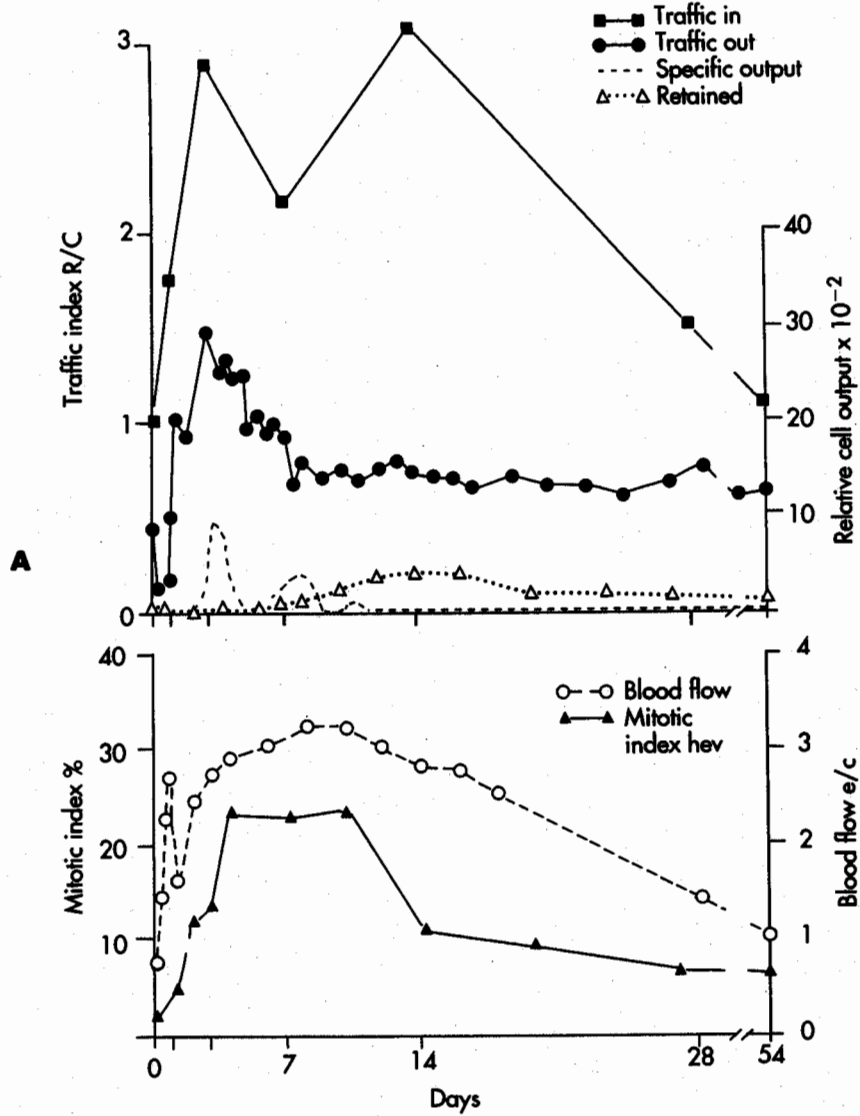
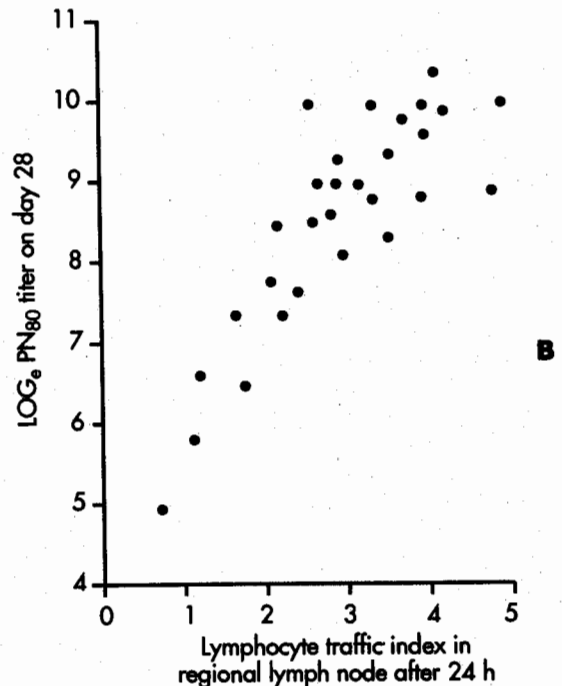


Fig. 3-6. Importance of cell traffic in adjuvant action. Inoculation of an antigen with adjuvant initiates a coordinated sequence of physiologic changes in the regional lymph node. **A**, These changes (top curves) occur in two phases, early (3-72 h) and late (72 h up to 50 days) after inoculation. The early changes in cell traffic correspond to initiation of cytokine cascades and other mediators that effect blood flow and the rate of attachment and emigration of lymphocytes at HEV (bottom curves). The later sustained increase in traffic is associated with expansion of the HEV network through endothelial proliferation and differentiation. The waves of antigen-specific cell output punctuate the beginning of the late phase of traffic increase. Without the early adjuvant stimulated increase in traffic the specific cell output might not have been detectable. **B**, A positive correlation between the traffic index (cpm regional/cpm contralateral 24 h after iv infusion of radiolabeled thoracic duct lymphocytes) and subsequent antibody titer on day 28 was found after inoculation of a single dose of viral vaccine with six doses of adjuvant. Each point represents data paired according to adjuvant dose from an experiment using two sets of five rats for each of six doses of adjuvant with single dose of vaccine. One set was analyzed for traffic and the other for antibody response; $n = 60$. (A modified from Cahill RN et al: *J Exp Med* 143:870, 1976; *J Exp Med* 143:707; and Anderson AO: *Int J Immunother* 1:185, 1985. B modified from Levy HB, Lvosky E, Riley F et al: *Ann NY Acad Sci* 350:33, 1980.)



from deficient immune responses. Therapies for these diseases, typically referred to as autoimmune, are directed at reducing immune responses. Furthermore, transplantation rejection constitutes an unwanted immune response. There is considerable excitement about a possible role for antiadhesion therapies to reduce the influx of inflammatory cells in both autoimmune disease and transplantation.^{8,40}

Some existing therapies are effective, at least in part, by virtue of their reduction of lymphocyte migration. For example, corticosteroids block early cytokine cascades, which thereby blunts the induction of adhesion ligands on endothelium; large doses of parenteral corticosteroids also result in acute transient depletion of the circulating pool with sequestration in the bone marrow. Similarly, gold therapy of rheumatoid arthritis decreases the expression of E-selectin in synovial endothelium.⁴¹

New therapies are being developed to interfere specifically with leukocyte recruitment. The first wave of these are based on monoclonal antibody (mAb) inhibition. Effects have been dramatic for acute injury from neutrophils (Chapter 11). However, even in more chronic lymphocyte-mediated diseases success has been reported. Experimental allergic encephalomyelitis, an animal model of multiple sclerosis, has been used to show that antibodies to VLA-4 reduce T cell infiltration into the brain and slow disease progression.⁴² Similarly, antibodies to ICAM-1 show promise in the prevention of graft rejection and in the treatment of rheumatoid arthritis⁴³; it is not yet clear how much of this effect is due to direct effects on migration.

mAb are not good candidates for therapy of chronic disease because they must be given repeatedly by parenteral routes. Therefore drugs that are more readily produced and administered are being developed. Because of the importance of carbohydrates (in selectin-mediated tethering and other less well-defined roles in migration), carbohydrates are being explored. Because of the importance of defined peptides such as the tripeptide arginine-glycine-aspartic acid (RGD) in integrin-mediated strong adhesion to endothelium, peptide-based therapies are also being investigated.

CONCLUDING REMARKS

The immune system, like the nervous system, is designed to serve the entire organism. The nervous system has adopted one strategy, namely fixed structural elements that span the organism. The immune system has adopted a different strategy, movable elements that traverse the entire organism. The seemingly effortless movement of lymphocytes belies the amazing feats of molecular engineering that make it possible. Based on the importance of migration in immune function, new therapeutics designed to modify adhesion-chemotaxis are likely to prove valuable.

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