nature immunology

The sense of place in the immune system

Michael D Cahalan[1] & George A Gutman[2]

This series of reviews examines the effect of differing tissue environments on the activity and functional capacity of cells in the immune system. From their origins as hematopoietic stem cells, throughout their development and as mature cells, cells of the immune system find themselves in distinct and highly specialized niches, and contact with antigen or inflammatory signals changes their phenotype, activity and trafficking. Two-photon microscopy has provided the first direct observations of living cells and their activation choreography in the tissue environment and will no doubt continue to provide greater understanding of cellular dynamics and immune function.

"If you don't know where you are, you don't know who you are….Migratoriness has its dangers."
The Sense of Place, Wallace Stegner (Random House, 1992).

The immune system, consisting of trillions of cells and weighing more than 1 kilogram in the adult human, is widely dispersed throughout the body. This rather chaotic arrangement and the continuing recirculation of cells facilitates rapid detection of invading pathogens and guarantees effective countermeasures in a variety of distinctly different tissue environments. Cells of the immune system are constantly in motion, migrating passively over long distances in the blood, locating and entering destination sites in lymphoid or nonlymphoid organs, and crawling actively in the tissue environment. The journey takes place during development, throughout adult life as mature cells recirculate, and during infection. Trafficking of cells is regulated by many processes that optimize antigen capture and recognition. This continual mixing of cells is particularly important for proper function in the immune system because cellular activation events often require direct cell-to-cell contact. Inappropriate migration and activation, however, may contribute to autoimmunity. So how do cells know where they are going and how do different niches 'shape' cell activity?

Molecular immunology has defined many of the intracellular signaling events that are triggered in T cells and B cells by exposure to antigen and in dendritic cells and macrophages by exposure to inflammatory 'danger signals'. In lymphocytes, a network of signaling pathways conducts intracellular signals from T cell receptors and B cell receptors at the cell surface to the nucleus and then back to the surface again as newly synthesized molecules (cytokines and antibodies) are secreted. Many of the requirements for antigen presentation, costimulatory molecules and response thresholds have been defined: T cells can function as single-molecule detectors of complexes of peptide and major histocompatibility molecules and respond with maximum effector function to a few 'tens' of such complexes presented on an antigen-presenting cell. The 'immunological synapse' as a concept describes the organization of interacting molecules in the zone of contact between T cells and antigen-presenting cells. But zooming out from the molecular interactions, what has been missing is a way to visualize dynamic cellular interactions in the tissue environment. This focus emphasizes the requirement for specialized sites where diverse functional capacities of the immune system are shown.

Tracking single cells

The ability of researchers to identify and track the movements of lymphocytes has always been limited by the technology of the time. The earliest observations of the lymphocyte were based on the appearance of living cells in lymph and lymphoid tissues, although their function remained a mystery for almost 200 years. The process of recirculation of lymphocytes between the blood and lymph through the high endothelial venules of lymph nodes was first described by Sir James Gowans, who was able to label cells with radioactive thymidine and detect them in tissue sections by autoradiography and by conventional light microscopy[1,2]. Soon after, distinct regions of lymphoid tissue populated by T cells and B cells were identified with autoradiography[3] and immunofluorescence[4]. In all those studies, observation of labeled lymphocytes required the preparation of histological sections. In special cases, it was possible to label lymphocytes directly *in situ* and monitor their subsequent localization, as, for example, by the use of transcapsular labeling of cortical thymocytes with tritiated thymidine[5] or fluorescein[6], but there, too, the labeled cells could be subsequently localized only by removal and slicing of the tissues. In 1976 Stamper and Woodruff[7] described the specific binding of lymphocytes *in vitro* to high endothelial venules, their site of entry into lymph nodes, which first 'opened the door' to biochemical analysis of the molecules responsible for lymphocyte trafficking. Within a few years, the monoclonal antibody MEL-14 was described[8], identifying the first member of the large and increasing family of selectins and addressins responsible for regulating the entry of lymphoid cells into tissues. But observing the movements of individual lymphoid cells in living tissue remained a 'holy grail' of immunopathology until recently. Within the past 5 years, two-photon microscopy has demonstrated dynamic aspects of cellular migration and interactions in the tissue environment of intact lymphoid organs maintained in culture[9,10] and in living mice[11,12]. The ability to track living cells *in vivo*

[1]Department of Physiology and Biophysics and [2]Department of Microbiology and Molecular Genetics, University of California, Irvine, California 92697, USA. Correspondence should be addressed to M.D.C. (mcahalan@uci.edu).

Table 1 Cell migration and activity in the immune system

Cell	Location (travel distance)	Mechanisms and activity	Seeking	Responses
Recirculating lymphocytes	Blood (m)	Long-distance travel ('go with the flow')	Homing receptor	Transmigration
Pre–T cells	Thymus (cm)	Rearrange TCR; random walk if + continues and death if not; egress (or death if –)	Self antigen	Calcium stop signal; chemotaxis to medulla
T lymphocyte	LN cortex (mm)	Random walk; clusters, cytokines; swarming; egress ($S1P_1R$ permitting)	Peptide antigen–MHC	Serial DC sampling; stable DC interaction; proliferation; memory, effector cells
B lymphocyte	LN follicle (mm)	Random walk; directed migration to follicle edge; continue to move	Soluble antigen; CCL21 gradient; helper T cell	Upregulate CCR7; chemotaxis; migrate as stable conjugate pair
Dendritic cell	Tissue interstitial space (cm)	Endocytosis and processing; dendritic probing; antigen presentation, costimulation	Antigen; T cell	Migrate to SLO via afferent lymphatics; death

TCR, T cell receptor; +, positively selected; –, negatively selected; LN, lymph node; MHC, major histocompatibility complex; $S1P_1$ R, sphingosine 1-phosphate type 1 receptor; SLO, secondary lymphoid organ.

has 'opened a new window' for the visualization of cell localization and movements in the tissue environments of bone marrow, thymus, lymph node and other organs.

Origins: stem cells in their niche

All cells of the immune system are derived from hematopoietic stem cells in bone marrow. Stem cells give rise to precursor populations that migrate through the blood and develop in specialized niches (such as the thymus) into mature cells that in turn undergo further trafficking in a basal pattern of migration and recirculation. In response to inflammatory conditions and the presence of antigens, the activity of single cells changes substantially.

Stem cells require a specialized microenvironment in bone marrow to show their potential. The accompanying review by Adams and Scadden[13] explores what is known regarding the interaction of hematopoietic stem cells with the local environment, indicating regulation of cell numbers (limited to a total cell count of about 10,000 in both cat and mouse), interactions with other cells (especially osteoblasts), the need for a homeostatic balance of inhibitory and stimulatory factors and the imprinting of stem cell characteristics on non–stem cells in the area. Clearly there is a great need for 'immuno-imaging' in bone marrow, which has thus far been able to visualize T cells, dendritic cells (DCs) and B cells[14,15], to visualize stem cells and osteoblasts; such experiments will need to cope with the requirement to label and locate limited numbers of stem cells.

'Ins and outs' of selection in the thymus

Pre–T cells entering the thymus find a receptive microenvironment in which T cell receptors are rearranged and cells are then evaluated by positive and negative selection to determine whether T cells bearing particular receptors will survive and enter the circulation as mature T cells. Most cells do not make it through these critical checkpoints, leading to an 'orgy' of rapid proliferation and death. Molecular events and migration routes involved in thymocyte maturation have been hotly debated for some time, and the review by Robey and colleagues[16] follows the journey by these cells during positive selection in the cortex and negative selection in the medulla. Of special note are the imaging results that have begun to define the patterns of cellular migrations[17] and the importance of calcium signaling in regulating motility[18], both during positive selection. Even though the cells that present self antigen have not yet been visualized, by tracking single thymocytes in thymic lobes, Robey and colleagues[17] have defined two very different types of migration: random 'walk' and directed motion. It remains to be determined which receptors and chemokines govern directed motion as positively selected cells 'stream down' into the medulla. In addition, further immuno-imaging studies are needed to describe cell motility and interactions in the medulla during negative selection.

Lymphoid organogenesis

One hallmark of the adaptive immune response is, indeed, its ability to adapt. An almost infinite array of specific antibodies and T cells can be produced in response to the universe of antigenic structures, and the known diversity of immune effector functions, both specific and nonspecific, continues to grow. This ability to adapt turns out to be well represented in the anatomical organization of the immune system as well, as is laid out in the review on lymphogenesis and lymphoneogenesis by Drayton *et al.*[19]

The classical view distinguishes primary lymphoid organs (bone marrow and thymus), which produce immune competent cells, from secondary lymphoid organs (lymph nodes, spleen, Peyer's patches and so on), in which immune responses to pathogens take place and effector cells of the T and B lineages are produced. Those organs are widely distributed so as to detect and respond to offending antigens wherever they may enter the body, with peripheral lymph nodes draining the skin, spleen filtering the blood, Peyer's patches monitoring the gut and so on. Their organization is specialized to facilitate the specific cell interactions required for immune responses, and substantial plasticity of these structures may be evident after the initiation of such responses.

But in addition to those conventional organs, there are accumulations of lymphoid cells in sites of chronic inflammation that may bear many of the hallmarks of classical lymphogenesis, including their anatomical organization, the appearance of endothelial venules displaying receptors for lymphocyte entry and the expression of many chemokines and chemokine receptors. Such structures have been referred to as 'tertiary' (or 'ectopic') lymphoid organs, and they greatly expand the ability of the effector limb of the immune system to execute its effects, both beneficial and harmful.

Stop-and-go traffic in the lymph node

Most T cells and B cells enter the lymph node by homing from the blood, reside in the node for about a day and then leave by entering lymphatic sinuses that communicate to efferent lymphatic vessels. Two-photon imaging has demonstrated homing events[12], migration of lymphocytes in the node[10] and transendothelial migration into lymphatic sinuses[20]. Entry at high endothelial venules is guided by specific chemokines that are sensed by CCR7 on T cells or CXCR5 on B cells[21]. However, the basal motility pattern of T cells in the diffuse cortex and B cells in the follicle consists of a random walk rather than a pattern directed by chemotaxis. T cells and B cells move continuously in an ameboid way, 'respecting' the compartmental boundary at the follicle

edge that separates them. The mechanisms that define the compartmentalization of T cells and B cells still remain to be defined but are also likely to involve chemokine receptors. Egress of lymphocytes into the sinus and then via efferent lymphatics takes place by migration through endothelial cells that at rest seem to function as open 'portals' between which lymphocytes pass freely[20]. The immunosuppressant FTY-720, in its *in vivo* phosphorylated form, interacts with sphingosine 1-phosphate type 1 receptors that are expressed on both lymphocytes and the lymphatic endothelium. Although controversial[21,22], it seems that this immunosuppressant works by closing the endothelial cell barrier, trapping lymphocytes in the node[20].

The presence of antigen considerably changes the activity of T lymphocytes and B lymphocytes in the lymph node. Newly migrated dendritic cells that carry antigen from peripheral sites into the node via afferent lymphatic vessels are able to contact 5,000 resident T cells per hour as a result of robust T cell motility and vigorous probing of DC dendrites[23]. Such contacts last only 2–3 minutes in the absence of antigen; this brief interval represents the time when T cells must 'decide' whether antigen is present. The resident dendritic cell network has also been visualized[24], and newly homed T cells probably must 'run a gauntlet' of DCs before migrating away. If antigen is present, T cell activity is altered in a complex choreography with DCs that progresses from serial engagements with several DCs in a local region, evolving to stable contacts, then to T cell dissociation from DCs and local swarming and, finally, to multiple rounds of cell division[11,25]. Meanwhile, B cells in the follicle that have captured antigen begin to migrate in a directional way toward the edge, using the CCR7 chemokine receptor along a haptotactic gradient of its ligand CCL21 (ref. 26). Once the antigen-engaged B cells reach the edge, they pair with activated helper T cells to form conjugate pairs that are highly motile[26]. The partners in such conjugate pairs are no doubt are exchanging signals. Notably, T cell–B cell conjugates are very stable and B cells always lead the way. However, partner exchange can occur, although stable threesomes are rarely found. We demonstrate here the exquisite choreography of the interaction of T cells first with dendritic cells[25] and then with antigen-engaged B cells[26] (**Supplementary Videos 1** and **2** online).

During inflammatory and antigen-specific responses, cell egress is inhibited, causing lymph nodes to swell. That swelling may serve the purpose of allowing effector cells to acquire specialized functions before they are released *en masse* several days after the initial infection. Mechanisms for antigen-induced inhibition of egress have yet to be determined, and again immuno-imaging will probably contribute to this. Further immuno-imaging will also be needed to visualize the differentiation of activated B cells in germinal centers and the mediation of effector functions by lymphocytes (such as interactions with targets) in the tissue environment.

Immune privilege

Also in this issue, Niederkorn reviews the phenomenon of 'immune privilege'[27], a term coined almost 60 years ago by Sir Peter Medawar, who observed that only limited immune responses are generated against antigens introduced into the brain and anterior chamber of the eye[28]. The existence of those and other immunologically privileged sites has been of both practical and theoretical importance. Before it was mostly superceded by the discovery of the 'nude' mouse, the cheek pouch of the hamster permitted researchers to propagate heterologous tumor cells that would otherwise be rejected in genetically dissimilar hosts, and the privileged nature of the anterior chamber of the mammalian eye has contributed to the considerable success rate of corneal transplants, the most commonly transplanted human organ.

But why do such sites exist and how is local immune reactivity limited? As for 'why', both the brain and the eye are limited in their ability to regenerate, and the inflammatory responses that accompany immune reactions might therefore have far more serious consequences in those sites than in others. In the case of the developing mammalian fetus, its privileged status serves to prevent immune rejection of this naturally occurring 'foreign' graft, obviously of compelling importance for the propagation of the species.

As for 'how', the answer (not unexpectedly) turns out to be complex. Medawar originally explained the phenomenon by noting a lack of lymphatic drainage from the relevant tissues, preventing localized antigens from being delivered to lymphoid tissues where they could initiate immune responses[28]. But Niederkorn reviews a large body of work since that time indicating involvement of local differences in the expression of a wide variety of molecular targets and regulators of immune responsiveness, both membrane associated and soluble. The mechanisms responsible for maintaining 'immune privilege' also normally inhibit immune reactivity to 'self', and their failure results in autoimmunity, with its potentially devastating consequences. So far, only one study has examined the interactions between activated effector memory cells and the cellular target during autoimmune-mediated damage, showing that myelin-reactive T cells make stable contacts with spinal cord neurons in an experimental rat model of multiple sclerosis[29]. We anticipate that single-cell immuno-imaging will progress from its present descriptive stage to provide a deeper understanding of pathogenic mechanisms and, with new optical probes, will enable diagnostic capabilities in the living tissue environment.

The ongoing cellular journey through time and space

Like holiday travelers, cells migrate over long distances propelled by the blood to destinations that are 'ticketed' by the expression of selectins. Once at their destination, cells can migrate autonomously (random walk or directed migration) in tissues and, if they find something of interest (such as antigen), 'stopovers' can be arranged for an extended period before the journey continues. We have summarized some general themes discussed in this overview and in the accompanying reviews (**Table 1**)[13,16,19,27].

Note: Supplementary information is available on the Nature Immunology website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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