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The microanatomy of T-cell responses

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Summary: The priming of a T cell results from its physical interaction with a dendritic cell (DC) that presents the cognate antigenic peptide. The success rate of such interactions is extremely low, because the precursor frequency of a naive T cell recognizing a specific antigen is in the range of $1{:}10^5$–10^6. To make this principle practicable, encounter frequencies between DCs and T cells are maximized within lymph nodes (LNs) that are compact immunological projections of the peripheral tissue they drain. But LNs are more than passive meeting places for DCs that immigrated from the tissue and lymphocytes that recirculated via the blood. The microanatomy of the LN stroma actively organizes the cellular encounters by providing preformed migration tracks that create dynamic but highly ordered movement patterns. LN architecture further acts as a sophisticated filtration system that sieves the incoming interstitial fluid at different levels and guarantees that immunologically relevant antigens are loaded on DCs or B cells while inert substances are channeled back into the blood circulation. This review focuses on the non-hematopoietic infrastructure of the lymph node. We describe the association between fibroblastic reticular cell, conduit, DC, and T cell as the essential functional unit of the T-cell cortex.

Keywords: *lymph node, anatomy, conduit, stroma*

Adaptive immunity depends on secondary lymphatic tissues

Innate immune cells are constantly patrolling the body. Within minutes of local infection, they are recruited to the site of damage where they exert their defense function. The speed of such responses is possible because the innate immune cells are always available: some are resident within the tissue and some are quickly recruited from the bloodstream, where they are either freely circulating or patrolling along the endothelial lumen (1–4). As there are only few types of specialized innate immune cells, the copy number of each type is high, and supply is always guarantied. Adaptive immune responses are fundamentally different, because the number of specialized cell types is in the range of many millions, as lymphocytes of different specificity are randomly generated during somatic development. Naturally, the copy numbers of each specific cell type are extremely low. It was recently demonstrated that for an average antigen, the number of specific T cells is in the range of

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a few hundreds (5, 6). Hence, the few specialized cells that recognize an antigen have to be expanded before they can effectively invade any tissue. The principle of lymphocyte activation/expansion is based on stochastic encounters between the antigen-presenting cells of the innate immune system, the dendritic cells (DCs), and T cells. Once the T cell physically contacts the DC presenting its cognate antigen, it is activated and expanded to a population size that is now sufficient to effectively invade the infected sites.

Secondary lymphatic organs are the solutions to solve the 'needle in the haystack' problem of identifying and expanding rare T cells within reasonable time frames. The prototypic example of a secondary lymphatic organ is the lymph node (LN), which we discuss in this review. LNs are compact immunological projections of the patch of peripheral tissue that they drain. DCs that collected information and antigens in the periphery migrate via the afferent lymphatic vessels into the draining LN. Once in the T-cell area, the immigrated DCs display, based on the information they received in the periphery, the antigen in a context that instructs the T cell to differentiate into the adequate effecter type (7). T cells recirculate through the LNs with the bloodstream that they leave via high endothelial venules (8). Within the T zone, the necessary high encounter rates between DCs and T cells are maintained by rapid cell locomotion that is effectively organized by chemokines (9). The big advantage of this system is that instead of scanning the whole periphery, naive T cells just visit the LN and receive all the instructions they need. Indeed, the adaptive immune system is so highly dependent on its organization centers that in the absence of secondary lymphatic organs, adaptive immunity is abolished (10).

Upon closer inspection, the schematic concept of immigrating DCs being the representation of the periphery is too simplified, and the reality is more complex. LNs are not only receiving DCs with the afferent lymph but also the whole stream of interstitial fluid with all its contents: soluble antigens, intact microbes, microbial particles, and locally produced extracellular signaling molecules like cytokines and chemokines. LNs are fluid filters that are providing the infrastructure to collect, select, and load soluble antigens on DCs that are permanently residing in the T zone. The filter function also physically prevents that replicable pathogens spread systemically via the blood circulation. LNs are also 'information hubs' that are quickly relaying soluble signals from the periphery toward the T cells in the blood circulation. Finally, it seems likely that LNs are critical regulators of the fluid balance between interstitium and blood.

The anatomy of LNs is complex, extremely dynamic, and until 2001, it was largely ignored by immunologists. Only the rise of intravital imaging technologies within the last years brought structural aspects of the lymphatic system back into the mind of the immunologist. Numerous excellent reviews are available that summarize the emerging knowledge of leukocyte motility patterns within LNs (11–13). However, the focus was largely on the hematopoietic fraction of the LN that is relatively easy to visualize. The mesenchymal infrastructure of the LN that supports and directs the blood cells is still poorly appreciated. Closely connected to the mesenchymal anatomy is the question of fluid dynamics within the lymphatic system. Although a detailed physiological knowledge of particle and solute transport will be necessary to understand the kinetics of infections at the organismic level, many aspects of these processes are not investigated.

In this review, we describe the non-hematopoietic infrastructure of the lymphatic system in the functional context of cell migration and fluid drainage. Our focus is the T-cell response, as the B-cell compartment has been reviewed recently (14), but we mention the B cells when necessary for comparative purposes. We use the skin and the draining LN as an example. For two reasons we also include an overview over the dermal microenvironment. First, almost every immune response is initiated in the periphery, and the dermal fluid and cell drainage system is critically involved in the initiation of adaptive immunity. Second, molecular composition and physiological characteristics of the dermal interstitium are well investigated and serve as a model to further discuss LN physiology that follows the same principles but is still an open field.

Circulation patterns of fluids

While cells and some microbes have the possibility to move autonomously, extracellular substances within vertebrates are either immobilized to cells or molecular components of the extracellular matrix (ECM) or they move with the bulk flow of fluid by convective forces, meaning that they are flushed (15). The interstitial fluid of vertebrates appears in several different forms while it is recirculating through the body. The plasma fraction of the blood is the most accessible variant. In the capillary bed, plasma is filtrated from the vasculature and becomes interstitial fluid, which is slowly percolating through the interstitium into the afferent lymphatic vessels (16). From there on it is termed afferent lymph. The afferent lymph is filtered through the draining LN, and once it has exited the LN is termed efferent lymph. In larger vertebrates and humans

(but not in mice), the efferent lymph is filtered through a hierarchy of second- and third-order LNs before it enters the cisterna chyli and is further actively transported via the thoracic duct back into the venous blood circulation (17, 18). All particles will passively follow the convective drag of the interstitial fluid if they are not immobilized or taken up by cells, trapped by binding to ECM, or filtered at a barrier due to size reasons. As a consequence of the different filtration steps, plasma, interstitial fluid, afferent lymph, and efferent lymph have quantitatively different molecular compositions, although they are in continuous exchange (19) (*Fig. 1*).

The peripheral tissues: dermis

After breaking the barrier of the epidermis, extracellular pathogens either spread laterally, grow into deeper areas, or distribute systemically by accessing the blood vasculature. The molecular architecture and the hydrostatic conditions within the dermis dictate that the fluid and particle stream is strictly directed from the blood vessel into the lymph vessel (16, 19). This convective stream counteracts entry into the blood circulation by flushing particles away from blood vessels. Furthermore, the small pore size of the interstitium acts as a sterical barrier for active spreading. We start by describing general features of the dermal compartment, with focus on the questions of how molecular anatomy determines the transport of solutes, particles, and immune cells. The dermal stroma consists of two components: the stroma cell (typically a fibroblast) and the ECM that the stroma cell produces. The dermis is characterized by a wide extracellular space that is scaffolded by dense arrays of biopolymers. The fibroblasts are scattered within the tissue without forming cell–cell contacts. They are rather embedded as single cells into the surrounding ECM. Owing to the spacing, fibroblasts themselves do not form any significant barrier for solutes, cells, or particles (20). The ECM can be functionally divided into two categories: the fibrillar fraction that consists of arrays of fibrillar collagen bundles, elastic fibers, and microfibrils, and the non-fibrillar fraction that consists mainly of long-chained aminosugars (glycosaminoglycans) and proteoglycans. The fibrillar fraction is mechanically stable and counteracts the tension produced by the fibroblasts that contract the extracellular fibers via acto-myosin forces (21). The interplay between fibroblasts and fibers determines the mechanical characteristics of the skin. The non-fibrillar fraction fills the volume between the fibers. Unlike the fibrillar proteins, these extremely large sugars (chains of hyaluronic acid can reach a length up to 5 μm and a molecular weight of several million Daltons) are not tightly interconnected, and biophysically they rather behave like a very viscous fluid (22). Glycosaminoglycans are highly negatively charged and therefore bind ions and, as a consequence, attract and immobilize water. By sucking water, they act as 'expansion elements' that counteract the tensile force of the fibrillar ECM fraction (16).

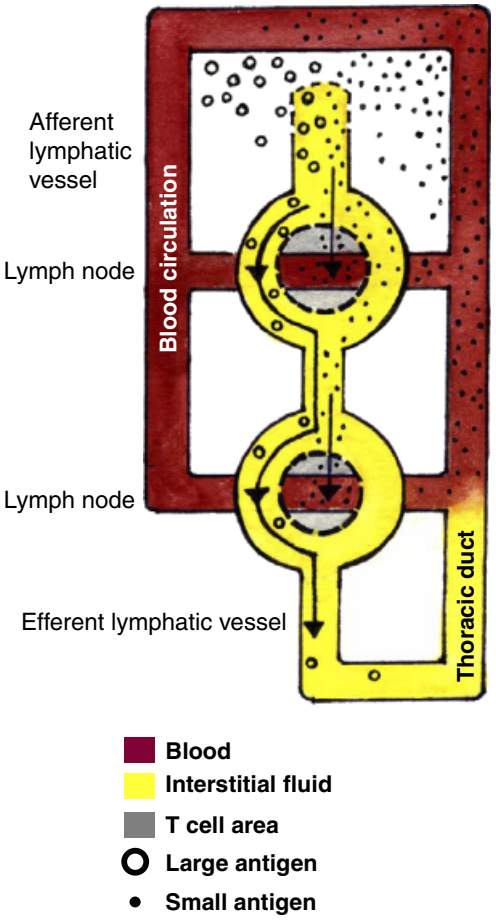

***Fig. 1.* Circulation patterns of fluids.** Small particles (small dots) become filtrated from the blood stream (red) in the periphery, enter the afferent lymphatic vessel (yellow), and are transported into the draining LN. Here, they are directly reabsorbed into the bloodstream. Large particles (large, open dots) also enter the afferent lymphatic vessel but percolate through the sinus system of the LN and eventually (if they are not trapped in the sinus) are channeled via the efferent lymphatic vessel and the thoracic duct back into the blood circulation.

Solute transport within the dermis

What are the driving forces of interstitial flow? The flow is a function of Starling's forces that set the capillary filtration rate: the local hydraulic pressure difference (blood pressure–interstitial pressure) across the blood vessel wall minus the colloid osmotic pressure difference between blood and interstitium (23). The hydraulic pressure of the dermal interstitium is actively kept low by the suction force of the lymphatic vessels that pump fluid away from the tissue and into the draining LN.

Under normal conditions the interstitial flow is slow, because the hydraulic conductivity of the interstitium is low. Conductivity is set by the pore size (the hydraulic radius) of the interstitium that is determined by the surface area of glycosaminoglycans and fibrillar ECM that is filling the interstitium. In the dermis, this radius is in the range of 100 nm, so that the interstitium is a sterical barrier for bigger particles like viruses or bacteria (24). The low mobility of interstitial water that is caused by the charged glycosaminoglycans prevents quick shifts of interstitial fluid upon pressure changes and restricts the diffusive or convective distribution of soluble molecules, even under mechanical stress. Only in the situation of peripheral lymphedema is the glycosaminoglycan fraction saturated with water. This dilution leads to the effect that the excess water moves freely (16, 25, 26). Therefore, edematous tissue can be easily 'squeezed out,' which is used as a simple clinical test: pushing into edematous skin but not into healthy skin leaves a pit. To our knowledge, the consequences of edema for particle transport have not been investigated systematically, but it is a clinical fact that peripheral edema predisposes for local infection (27) (*Fig.* 2).

There are two important implications for particle and solute transport within the dermis. (i) In principle, solutes and small molecules can freely diffuse, but their transport is dominated by the convection caused by the bulk fluid movement that is directed from the capillaries into the lymphatic vessels. The dermis is in a state of constant fluid secretion, and although this is frequently stated, re-absorption of fluid and solutes in the efferent capillary bed or across venules does not occur normally. (ii) For larger particles, the pore size of the interstitium is a sterical barrier. They 'become filtered,' and the only way to spread is by autonomous movement, digestion of the ECM, and subsequent increase of the pore size, or by 'hitchhiking' with cells.

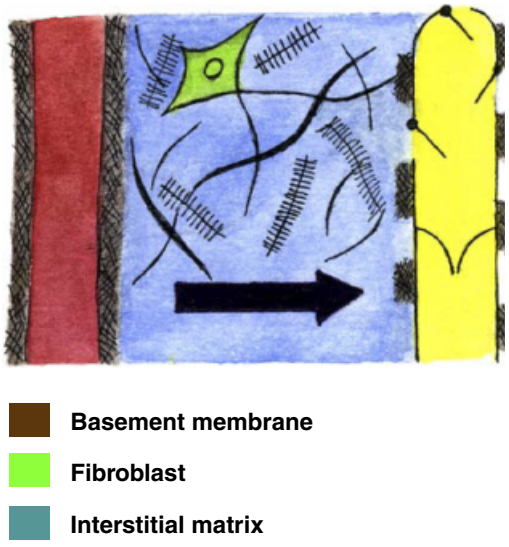

***Fig.* 2. Interstitial flux within the dermis.** Plasma becomes filtrated from the blood vessel (red). The endothelium and the endothelial basement membrane (brown) form a semi-permeable membrane. The interstitial flux (arrow) is strictly directed from the blood vessel toward the lymphatic vessel (yellow) that has no continuous basement membrane but flap valves opening toward the lumen. The interstitial matrix (blue) consists of collagen fibers (thick lines), microfibrils (thin lines), and glycosaminoglycans (brush like structures). Fibroblasts (green) associate with the interstitial matrix.

Cell migration within the dermis

A soluble molecule or a particle can reach the draining LN via two principal routes: passively with the interstitial flow or carried by cells. The usual cell type that carries antigens into the LN is the DC. In the steady state, DCs are forming an area-wide network that constantly samples the tissue for internal (damage) or external (microbes) danger signals (7). Within the skin, there are two populations of resident DCs: the Langerhans cells of the epidermis that reside between the keratinocytes above the epidermal basement membrane (28, 29) and the dermal DCs that are scattered within the interstitium (30). Upon danger sensing, DCs undergo so-called maturation. DCs transiently become phagocytotic and subsequently acquire a state of high motility (7, 31). This metamorphosis from a rather static cell that adheres to the ECM via integrins to a very motile cell is associated with the upregulation of the chemokine receptor 7 (CCR7) that guides the cells toward the afferent lymphatics that are expressing the CCR7 ligand CCL21 (31). How do DCs migrate through the interstitial microenvironment? Considering the textbook paradigm that cell migration in metazoans relies on integrin-mediated coupling of cytoskeletal force to the ECM (32), it seems rather paradoxical that we and others found that upon maturation, DCs functionally inactivate their integrins (28, 29). However, we recently learned that DC migration in the interstitium is completely integrin independent. We rather found that DCs move through the fibrillar scaffold of the interstitium by a protrusive flowing of the actin cytoskeleton and by occasional contractions of the trailing edge that facilitates squeezing of the non-elastic nucleus through narrow areas (T. Lämmermann *et al.*, unpublished observations). Proteolytic digestion of fibrillar matrices is not required for this very quick mode of movement, and the spacing of the fibers is obviously large enough for the cells to squeeze through (33). Hence, leukocyte migration through the interstitium happens without causing permanent damage by remodeling or rearrangement of ECM; the cells move along the path of least resistance (34). It is currently

unclear how cells handle the tight network of glycosaminoglycans that fills the space between the fibers. It is likely that these polymers are just pushed away. This is possible because the glycosaminoglycan network is not tightly crosslinked and is rather fluid.

The basement membrane as a specialized ECM

Although the interstitial matrix is not allowing unrestricted movement of particles, there is a variant of the ECM of much lower permeability: the basement membrane. Basement membranes underlie epithelia and endothelia and surround axons, fat, and muscle cells. Rather than a loose scaffold like the interstitial matrix, basement membranes are dense sheets of tightly interconnected glycoproteins of the laminin and the non-fibrillar collagen IV families that are coupled together by bridging molecules like nidogens (35). Basement membranes do not only serve mechanical functions but also act as molecular sieves, anchoring structures and polarity signals for polarized cells, pathways or barriers for migrating cells, and, importantly, they harbor heparan sulfate proteoglycans like perlecan and agrin. These sulfated proteoglycans are again serving as molecular sinks, storage sites, or presentation platforms for the many heparan sulfate-binding growth factors and chemokines (36). Basement membranes strongly vary in their molecular composition, depending on the cell type they are produced by and associated with. Sixteen different laminin isoforms with distinct tissue-specific and developmental expression patterns and distinct cell-binding and biophysical characteristics are only one example of the highly specialized and not well-explored specializations of basement membranes (37).

Role of basement membranes for solute transport

The role of basement membranes in fluid filtration is not well investigated, except in the kidney where the primary urine is filtered through the basement membrane associated with the podocyte endfeet. Here, a dual role as a sterical and a charge filter for molecules above the size of albumin has been proposed (38). Three basement membranes transect the skin in a way that they might be relevant for fluid filtration: the basement membrane of the epidermal–dermal junction, of the blood vessel endothelium, and of the lymphatic endothelium. Although no functional studies are available, it appears likely that the endothelial basement membrane contributes to the semi-permeability of the blood vessel (39). The same is possible for the epidermal basement membrane, while the basement membrane of the lymphatic endothelium appears to be functionally different. It has long been doubted that lymph vessels have at all an underlying basement membrane and lymphatic endothelial cells can be differentiated from blood endothelial cells by very low expression levels of collagen IV and laminins (40–42). Only recently it became clear that the basement membrane of lymph vessels exists but that it is very fragile and also fragmented (43, 44). This structural configuration makes it unlikely that the lymphatic basement membrane is a significant barrier to fluid movement.

The role of basement membranes for cell migration

Basement membranes are structurally dense and as such require specialized mechanisms if they should be transmigrated (45). Leukocytes can circumvent most dermal basement membranes, and the only sites where transmigration is necessary are the endothelial basement membrane of venules and the epidermal basement membrane. Cells have to penetrate these barriers to leave the bloodstream and the epidermis, respectively. Passing the epidermal basement membrane clearly requires proteolytic degradation and likely also integrin-mediated adhesion, as demonstrated for epidermal Langerhans cells and autoreactive T cells (45–48). For extravasating leukocytes that have traversed the endothelial monolayer and face the underlying basement membrane, this is less clear. Recently, it has been suggested that preformed openings (also called low expression sites as they show low levels of laminins and collagen IV) allow neutrophils to squeeze through the membrane without creating defects (49). Passing the rudimentary basement membrane of the lymphatics does not require active proteolytic mechanisms, and DCs likely enter via preformed openings (our unpublished data).

The lymphatic vessels

Entry of fluid into the lymphatic vessels

The lymphatic vessels actively pump interstitial fluid out of the tissue and thereby keep the interstitial pressure low and guarantee that flow is unidirectional away from the capillary bed. In most tissues, the lymphatic vessels are the only route of fluid absorption, and efferent capillaries and venules are not pathways of significant absorption (LNs and the gut are exceptions as we discuss below) (19). Unlike blood vessels, lymph vessels openly communicate with the interstitium. No tight endothelial barrier or continuous basement membrane restricts the movement of solutes. The suction force of the lymph vessel is generated by the interplay of four functional units: (i) the smooth muscle layer around the lymphatic collector vessels (the proximal part of the lymphatics) periodically contracts and thereby pushes fluid toward the draining LN, (ii) backflush of fluid into the periphery during contractile phases is prevented by the semilunar valves between segments

of collector vessels, (iii) the lymphatic endothelial cells of the blind ending initial (distal) part of the lymphatics are arranged in an overlapping pattern that forms flap valves that allow entry but prevent exit of fluid, and (iv) the abluminal side of the lymph vessels are anchored to the interstitial fibrillar matrix via elastic filaments. Using the tensile force of the interstitial ECM, these filaments pull the lumen of the lymphatics open, even when interstitial pressure rises during edema. Edema rather enforces the opening of the lumen as it raises the tension within the interstitial matrix (50).

Cellular entry into the lymphatic vessels

How cells enter lymphatic vessels has not been thoroughly investigated *in vivo*. Several adhesion molecules have been implicated in this process based on *in vitro* assays or pharmacological inhibition. These molecules include the cell surface receptor clever 1 (51) and the interaction between leukocyte function-associated antigen-1 (LFA1) and very late antigen 4 (VLA4) on the DC and its counter ligands vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1) on the abluminal side of the lymphatic vessels (52). However, a recent report (and our own data) suggests that like for fluid transport, for cellular trafficking, the lymphatic vessels might not be a considerable barrier. The cell–cell contacts between the lymphatic endothelial cells at the level of initial lymph vessels are discontinuous. Instead of the continuous adherens junctions that are found between blood endothelial cells, the junctions between lymphatic endothelial cells are organized as 'buttons'. Within the buttons, the same molecular connectors, platelet-endothelial cell adhesion molecule-1 (PECAM-1) and VE Cadherin, are found as in the blood vessels. The spacing between the buttons is in the range of 3 μm (53). Although not formally demonstrated, the resulting gaps would be sufficient for DCs and lymphocytes to squeeze through without using specialized mechanisms of intravasation. Such a passive model would imply that lymphatic entry is only determined by the chemokine cue that guides the cells into the vessel. Lymphatic vessels express high levels of CCL21 (54), which could result in a diffusive gradient that attracts the leukocytes into the vessel. However, the mechanism of this process is far from clear, as the continuous interstitial flux would counteract a diffusive distribution of chemokine away from the lymphatic vessel and would instead suck CCL21 into the vessel. Recently, Swartz and colleagues (55, 56) suggested an alternative model for chemotactic migration within the dermis that was termed 'autologous chemotaxis.' Many cells expressing CCR7 also express the ligand CCL19, which seems paradox at first sight. However, within the interstitium, convection will drag the secreted chemokine toward the lymph vessel and thereby create a gradient that can then be followed by the same cell. Autologous chemotaxis was demonstrated for metastatic tumor cells moving into lymph vessels and also was suggested as a driving force for DC migration into the lymphatics (55, 56). Although this concept appears appealing, it remains to be challenged *in vivo* by either testing if CCL19-deficient DCs migrate less or, more importantly, showing that even in the absence of CCL21 on lymphatic vessels DCs can move directionally into the lymph vessels.

Transport within the lymphatic vessels

It is beyond doubt that transport within the lymphatic vessels is the same for solutes and cells. It is a passive flowing with the stream of interstitial fluid that is powered by the periodic contractions of the lymphatic vessels. Cells and fluid are pumped directly into the subcapsular sinus of the node (50). Within the sinus, the sieving function of the LN starts. In the following we describe anatomy and physiology of the LN, and as in the previous section, we separately discuss the implications for the transport of solutes and the migration of cells.

The LN sinuses

Two anatomically distinct compartments of the LN are distinguished: the sinus and the cortex. Both consist of a stromal and a hematopoietic component. The most simplified view of a LN is that of an organ bathing within the lumen of a dilated lymphatic vessel. The sinuses represent the remaining lumen of the vessel, and the cortex represents the actual organ. Usually LNs mark sites where lymph vessels coming from different drainage areas meet, and therefore every LN has several afferent but most times only one efferent lymph vessel. The entry side of the LN that is close to the afferent vessels is called subcapsular sinus, and the one close to the efferent vessel is called the medullary sinus (57). As the sinus system surrounds the whole LN, the subcapsular and medullary sinuses are directly interconnected, and there is an open communication between them. Hence, cells, fluid, and soluble molecules can pass through the LN without contacting the cells of the cortex (58). In larger LNs, there are also intermediate sinuses that penetrate the parenchyma and constitute a direct route between afferent and efferent lymph (57, 59, 60). It has further been described that the deep cortex harbors a lymphatic labyrinth that is connected to the medullary sinus. These are blind ending sinuses that likely collect emigrating lymphocytes and lead them into the medulla (59, 60).

The subcapsular sinus is populated by different resident cell types. The stromal cell of the sinus is the sinus reticular cell or

retothelial cell (61–64). This cell type has morphological similarities with the fibroblastic reticular cell (FRC) of the T-cell area but is also carrying some typical surface markers of the lymphatic endothelial cell lineage (65). The retothelial cells constitute a three dimensional meshwork that is the backbone of the sinus and bridges the LN capsule with the LN cortex (60, 66). The retothelial cells are assembled around an ECM scaffold that appears different from the stroma of the periphery and already shows features of the conduit structures of the T-cell area. Bundles of fibrillar collagens transect the sinus and connect the fibrous capsule of the LN with the ECM of the conduits in the T-cell area (67). These collagen bundles are partially surrounded by a basement membrane and are completely enwrapped with stroma cells (59, 68–70). They form a structure that resembles the conduit of the T-cell stroma. Unlike in conduits, in the sinus the basement membrane is discontinuous and rather resembles rings or spirals. At some places where the basement membrane is more patchy, it takes the configuration of buttons that are connecting the cells with the interstitial matrix of the fibers (71). The functional significance of this ECM assembly has not been investigated. It appears likely that such a structure facilitates fluid drainage and at the same time gives structural support. The discontinuous nature of the basement membrane is also reminiscent of the button-like cell–cell adhesions of the initial lymphatics, with the exception that the retothelial cells build a three dimensional sponge-like structure instead of the two-dimensional sieve structure of the lymphatics. The interstitial matrix of the sinus is not qualitatively different from the interstitium of the dermis – it is just assembled in a more organized and condensed manner. The same is the case for the retothelial cells that appear like a condensed form of the dermal mesenchyme. While the dermal fibroblasts are scattered within the interstitium as single cells, the retothelial cells assemble a unique and complex type of cell–cell junctions that have been termed puncta adherentia (62).

The connective tissue of the sinus is not only populated by stroma cells, passaging lymphocytes, and DCs that wander through the sinus in search of danger; there is also a resident population of rather immobile subcapsular macrophages that settle between the retothelial cells. They constitute a big part of the cell mass of the sinus. It is unknown if subcapsular macrophages couple to the retothelial cells, if they bind to the ECM within the sinus, and if there are specific signals that localize these cells to the sinus. Interestingly, the subcapsular macrophages are not 100% restricted to the sinus but also leak into the first 10–30 μm of the cortex, which might support their function as antigen relays (72). The morphological details of the intermediate and medullary sinuses and the lymphatic labyrinth are poorly described. As there is a continuum between the subcapsular and the medullary sinus, the stroma of both areas has strong similarities. Additionally, the medullary sinus harbors antibody-secreting plasma cells.

The configuration of the sinuses is extremely flexible in inflammatory situations. It has been shown that during the course of a local LN activation by peripheral inflammation, the sinuses enlarge within hours and become more extended and also 'infiltrate the cortex.' This expansion has been interpreted as a consequence of proliferating lymphatic endothelial cells that invade the cortex and possibly make influx from the periphery more effective (73). However, how closely the retothelial cells indeed are related to the lymphatic lineage and if they respond to the same growth factors as lymphatic endothelia deserves further investigation. It is also possible that the stroma cell population within the sinus is not homogenous and that a population of lymphatic endothelial cells exists in parallel to the retothelial cells (*Fig.* 3).

A remarkable but rarely mentioned characteristic of the sinus is that it lacks blood vessels, and oxygenation has to be provided by the underlying cortex (60). Teleologically, a lack of blood vessels makes sense when viewing the sinus as the first level of particle filtration with large pore sizes. A direct connection to the blood circulation would bring along a high risk of hematogenic distribution of infectious particles. Consequently, all blood vessels in the LN are shielded from the permeable sinus and are embedded into the filtrating conduit system of the FRC network.

Solute transport within the sinus

Soluble molecules, particles, and cells periodically arrive with the pumping contractions of the lymphatic vessels. At pressure peaks, lymphatic fluid is actively pushed into the subcapsular sinus. Although direct measurements are lacking, intravital microscopy data suggest that fluid transport within the sinus is rather unrestricted (2). The hydraulic radius of the sinus is likely very large and allows not only soluble molecules but also particles and cells to float through the meshwork of resident cells. The solute distribution within the sinus can therefore be described as percolation: the resident cells are bathed within the unrestricted stream of afferent lymph. As the sinus system allows open communication between subcapsular and medullary areas, solutes can take a shortcut from afferent to efferent lymph. This route is preferentially taken by immunologically inert particles and larger molecules that are excluded from the cortex, as they do not have access to the conduit system of the T-cell cortex and further to the blood circulation.

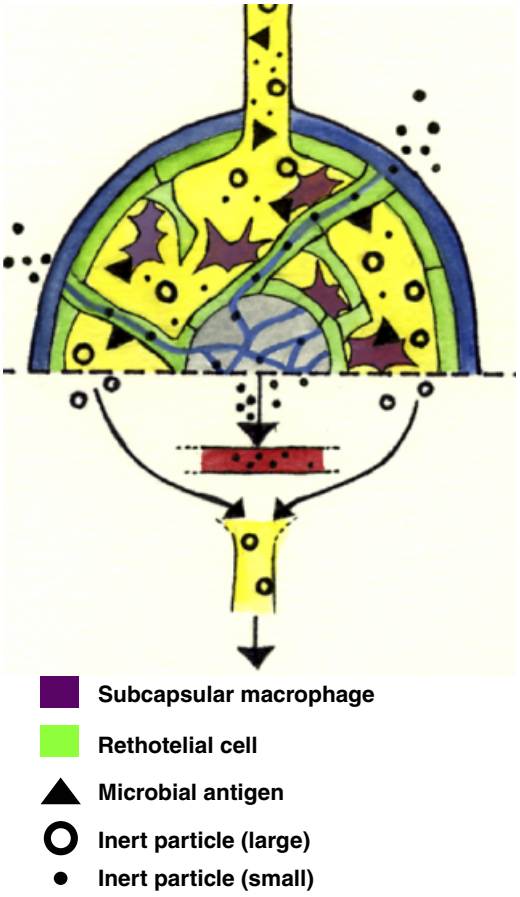

***Fig. 3.* Particle movement within the sinus.** The LN capsule consists of interstitial matrix (blue) that is continued within the sinus. Here, the matrix is surrounded by retothelial cells (green). Subcapsular macrophages (orange) are associated with the retothelial cells. Large particles percolate through the sinus and become trapped on macrophages and retothelial cells if they are immunologically relevant (triangles) and pass into the efferent lymph if they are inert (round dots). Small molecules can enter from the capsule and the sinus into the conduits of the T-cell cortex from where they access the blood.

The requirement for such a 'bypass route' becomes especially evident in the intestine, where the lymph does not only play a role in fluid drainage and immune surveillance but also transports the absorbed nutrients. Chylomicrons are collected in the initial lymphatic vessel of the villus (the lacteal), are channeled through the mesenteric nodes, and are transported via the thoracic duct into the blood circulation. Immunologically, all particles that pass through the sinus are irrelevant; they are not visible for DCs, T, or B cells if they are not trapped in the sinus. Selective trapping of solutes contained within the percolating lymph is achieved by the resident cells of the sinus. Both retothelial cells and macrophages catch soluble antigens and efficiently endocytose particles and microbes (29, 74). Accordingly, macrophages but also retothelial cells carry a rich surface repertoire of scavenger receptors (C-type lectins and many others) (65, 75) that unspecifically immobilize various microbial antigens. How the retothelial cells contribute to immune responses after antigen trapping is unclear, while it has been recently shown that subcapsular macrophages can 'hand over' antigen and even whole virus particles to B cells through the floor of the subcapsular sinus (76–78). Junt *et al.* (77) demonstrated the important concept that the filtering capacity of subcapsular macrophages serves both innate and adaptive immune functions. First, it is essential to mount effective B-cell responses to viruses that arrive with the interstitial fluid. Second, it prevents the hematogenic spreading of the virus itself. When subcapsular macrophages were depleted from the sinus, virus passed the LN without accessing the parenchyma and recirculated via the efferent lymph and the thoracic duct into the blood circulation (77).

It is a widely ignored fact that an access route for small molecules into the sinus exists that is independent of afferent lymphatics. The interstitium of the sinus is directly connected to the ECM that constitutes the fibrous capsule of the node. The capsule consists of fibroblasts, their fibrillar collagen matrix, and likely many other components of a normal interstitium. Although the capsule encases and mechanically protects the LN, it does not shield it from interstitial fluids that surround the node. It was shown that tracers injected close to the node distribute along the capsular ECM and directly drain into the sinus and most likely also further into the conduit system of the T-cell cortex. This means that the ECM of the capsule forms a fluid exchange continuum with the ECM of the sinus and the parenchyma (79).

Cell movement within the sinus

Although histologically most areas of the sinus appear rather densely packed with cells, intravital microscopy studies showed that spacing of cells and interstitium is (at least in some areas of the sinus) obviously loose enough to allow free floating of single cells (2). Especially during pressure peaks caused by the peristaltic compressions of the afferent lymphatic pump, round cells are flushed through the sinus. However, other intravital microscopic studies also showed active amoeboid migration of DCs and T cells within the sinus (80, 81). Hence, both active migration and passive flowing occur in the sinus. Exit from the sinus has not been studied intensively. One intravital microscopy study suggested that the medullary sinus has preformed openings that allow active exit from the medullary sinus into the efferent lymphatics (82). Scanning electron microscopic studies favored the lymphatic labyrinth that continuously connects to the medullary sinus as the site of massive lymphocyte exit (59, 60).

It was shown by intravital microscopy that the sinus contains a population of motile DCs that wander between the static

retothelial cells and macrophages (80). It has not been addressed if these DCs are representing a specialized subset or if they are dermal DCs and Langerhans cells in steady state transit from the periphery toward the T-cell cortex. Independent of their origin, it is likely that these DCs are foraging for antigen and danger signals that are immobilized on the retothelial cells or macrophages. Once they detect a target, they likely go through their usual program: they transiently arrest and phagocytose, subsequently become motile, and actively enter the T-cell cortex to present the antigen.

The sinus–cortex interface

The floor of the sinus is demarcated by a basement membrane that has direct connections with the conduit system of the cortex. However, this basement membrane is not continuous and gaps allow cellular traffic without the need for proteolytic degradation. The stroma cell that is directly covering the floor of the sinus has been termed, describing its position, the sinus-lining cell. It has not been addressed if this cell is a morphological variant of the retothelial cell, a form of lymphatic endothelial cell, or a separate specialized cell lineage.

Penetration of fluid and small molecules into the parenchyma

Almost 50 years ago, tracer experiments demonstrated that there is no free exchange of small molecules between the sinus and the T-cell parenchyma (70). Although not widely acknowledged in textbooks, these findings clearly argued against free percolation being the principle of interstitial flux in the T-cell area. In their seminal tracer studies, the laboratories of Anderson and Shaw (58, 83) showed that rather than percolating through the parenchyma, small molecules are channeled through the conduit system of the T-cell area. Interestingly, molecules with a molecular weight above the size of albumin (70 kDa) were excluded from the parenchyma and were exclusively detectable within the sinus (58). Most large molecules and particles eventually exit the node via the efferent lymphatic vessel, without ever becoming immunologically visible. The fraction of large molecules that is not bypassing the T zone is trapped by retothelial cells or sinus macrophages and further processed.

The communication between the sinuses and the T-cell cortex is restricted for large soluble molecules and bigger particles. Small molecules can enter the cortex but remain confined to the conduit system instead of percolating between the T cells. The morphological correlate of the size-selective sinus-cortex barrier is not known. Below we put forward the idea that a physical barrier is not required to explain the exclusion of soluble molecules from the T-cell parenchyma but that this is just a consequence of a lacking interstitium between T cells.

Passage of cells through the sinus floor

Restricted movement of soluble molecules suggests that also cellular trafficking between sinus and cortex is restricted. However, this does not seem to be the case. It has been shown by intravital microscopy that T cells can effortlessly glide into the subcapsular sinus when exposed to artificial gradients of chemokine (81). Observations of DCs penetrating the subcapsular sinus suggested the same: locomotion kinetics of cells at the sinus cortex interface did not indicate that a barrier has to be traversed (84). If the sinus lining cells are discontinuous, if they 'open the gate' on demand, or if they are traversed transcellularly is unknown. Taken together, active cellular movement between sinus and cortex is possible while the passive movement of solutes and particles is restricted. Molecules, be it foreign antigens or endogenous factors dissolved in the interstitial fluid, with a size below 70 kDa can enter the T-cell cortex via the conduit. Larger molecules are excluded and have to enter as cellular cargo on the surface or inside incoming DCs or are relayed into the B-cell follicle by macrophages.

The T-cell cortex

The stromal backbone

The anatomy of the T-cell cortex is dominated by the densely packed T cells that constitute more than 95% of the cellular mass. The remaining space is occupied by resident DCs and the T-cell stroma cells. The stroma is the infrastructure that dictates shape and organization of the T zone. It is the only stable component while T cells are in continuous motion to maintain the high contact frequencies with DCs. The T-cell stroma is condensed to a minimal volume and has an extremely unusual appearance. It forms a filigrane three-dimensional network that is highly interconnected via cell–cell contacts and structurally resembles the skeleton of a sponge (67). The cells forming this network are called FRCs, and the ECM they assemble has been termed conduit, after its function to channel small molecules through the cortex. The stromal endoskeleton of the LN is therefore not only a structural backbone but also a complex system of tubes that forms one continuously interconnected fluid exchange system with physical connections to all sinuses and all blood vessels.

The conduit

The conduit, varying in diameter from 200 nm to 3 μm, contains all known types of ECM molecules from peripheral

tissues, with the decisive distinction that they are assembled in a highly ordered manner. The center of the conduit is formed by parallel bundles of fibrillar collagens (I, III). Associated with these bundles, depending on the size of the conduit, are the typical collagen-associated stabilizing and crosslinking molecules like fibromodulin, decorin, and lumican (our unpublished data). The collagen fibers vary in number from 20 to 200 per cross-section, and the individual thickness varies around 50 nm (57, 85). They are not tightly packed. Transmission electron microscopy studies demonstrated that an unordered meshwork of 5–10 nm fibrils, morphologically resembling microfibrils, fills the space between the collagen fibers (86). The whole array of collagen bundles is embedded in a coat of microfibrils. Accordingly fibrillins, the molecular constituents of microfibrils have been detected between and especially around the collagen fibers (29). Although not been thoroughly investigated, it is most likely that, as in the interstitium of the periphery, glycosaminoglycans are filling the gaps between fibers and fibrils. In human LNs, dermatan sulfate was detected in association with the conduit ECM, but studies of other sugars are missing (87). The fibrillar core is a cell-free compartment that is tightly enclosed by a basement membrane that forms a cylindrical cover, giving the conduit the ultrastructural appearance of a tube. The cell that produces and settles on the basement membrane is the FRC. It forms a cellular sleeve around the conduit by anchoring to the basement membrane. The FRC continuously enwraps the conduit, and by forming intracellular cell–cell contacts, they seal the conduit against the T-cell-containing parenchyma. Along the longitudinal axis, the individual FRCs connect with intercellular junctions (57). The exact nature of the cell–cell junctions between the FRCs remains to be determined. Although more than 95% of the conduit is covered with FRCs, gaps remain that are covered by hematopoietic cells (29, 88, 89). These cells are mainly resident DCs that are embedded in the FRC layer and extend processes into the lumen of the conduit.

The conduit as a compacted interstitium

The conduit structure by itself appears rather unusual, as basement membranes normally form two-dimensional sheets that separate mesenchymal from epithelial compartments. However, when envisaging the conduit core as a very condensed form of a conventional interstitium, the whole arrangement appears more familiar and could be viewed as a compacted and tiny version of the peripheral interstitium that we described for the dermis. It is compacted to an extent that it appears as a system of microchannels that are bordered by a basement membrane. To appreciate the similarity between peripheral interstitium, we suggest the following thought experiment: imagine a vascularized mesenchymal tissue and extend the luminal surface of the endothelium until the interstitium almost disappears. The curvature of the endothelial lumen eventually bends backwards, and the interendothelial junctions reconnect without changing endothelial polarity. The endothelial cells would now 'internalize' their own interstitium, and by wrapping around the ECM, they will seal it against the 'luminal' surface that is in contact with the hematopoietic cells. Such a tissue would now resemble the T-cell cortex, where the blood cells occupy the labyrinthine 'lumen' formed by the FRCs, and the extravasacular space would be too tiny to contain any cells. In such a view, the T-cell cortex would rather be a specialized intravascular compartment than an extravascular space. In the T-cell cortex, FRCs take the function of both interstitial fibroblasts and endothelial (or epithelial) cells. FRCs clearly display features of epithelial and endothelial cells (apical basal polarity, cell–cell junctions, an underlying basement membrane, expression of cytokeratins) but also have characteristics of a fibroblast with myofibroblastic features (production of interstitial matrix, smooth muscle actin, expression of desmin and vimentin) (57, 90, 91).

LN anatomy during development and inflammation

The FRC network is an extreme variant of stromal differentiation that is already emerging in the sinus where a certain but not as extensive condensation of stroma and ECM occurs. This morphological continuity becomes obvious when looking at large collagen bundles that branch off the fibrous capsule, cross the subcapsular sinus (here they are loosely decorated with basement membrane patches and covered with retothelial cells), and enter the T-cell parenchyma, where they become incorporated into the conduit network. The view of a condensed interstitium is also consistent with the ontogeny of the FRC. During developmental and inflammatory lymphorganogenesis, FRCs are described as 'organizer cells' that are differentiating in a mutual crosstalk with hematopoietic 'inducer cells' that provide lymphotoxin signals. These lymphotoxin signals drive the transformation process of undifferentiated scattered mesenchymal cells into the FRC lineage (92, 93). The origin of undifferentiated mesenchyme in adult tissues is unclear, but the pericyte pool might be an interesting source. The FRC differentiation, which goes along with the formation of cell–cell junctions and polarization, resembles mesenchymal condensations or mesenchymal epithelial transitions that are well described in many other organogenetic processes like bone formation or kidney development (94).

In terms of anatomical features, the T-cell area is rather homogenous and does not show qualitatively distinct regions.

A quantitatively distinguishable area has been termed 'cortical ridge.' This is the T-cell area in close vicinity to the B-cell follicles. The cortical ridge shows a high density of high endothelial venules, and also the spacing of the reticular fibers was described as more densely packed (95).

The T-cell area shows enormous plasticity. During acute inflammation, LNs massively expand within hours, and the number of lymphocytes in the node multiplies. Theoretically, the sponge-like architecture of the T-zone stroma would allow for a certain swelling without increasing the surface area of the FRC network that could at the same time maintain structural integrity. However, mechanical expansion does not seem to be the critical factor, as it has been shown that during LN swelling the FRC surface expands. Although quantitative data on FRC spacing during LN expansion are lacking, qualitative data indicate that the FRC network grows with the increasing number of lymphocytes (96). It will be interesting to find out which factors keep the FRC to T-cell ratio constant. One possibility is that such a homeostatic balance might be regulated by factors that also regulate organogenesis like lymphotoxins.

Solute transport within the T-cell zone: entry into the conduit system

While in the periphery and in the sinus interstitial fluid and particles percolate around the cells through the interstitial space, the situation is radically different within the T zone. Large molecules are completely excluded from the parenchyma; they remain restricted to the sinus. Small molecules cannot be detected between the T cells either, but they have access to the conduit core. After subcutaneous injection, small molecular weight tracer molecules exclusively locate within the conduits, where they are sheltered from the T cells by the conduit basement membrane and the FRC layer around it (29, 57, 87). The conduit system is not a blind ending network but is highly connected with the capillaries and venules of the cortex (67). The conduits drain their fluid into the bloodstream: subcutaneously injected small molecules appear within the lumen of the high endothelial venules within minutes, demonstrating a shortcut between lymphatic fluid and blood at the level of the first draining LN (58, 83).

What is the physiological mechanism behind this size selective and directed transport? It has been proposed that flow within the conduit is active, meaning that sinus-lining cells at the sinus cortex interface (where the conduits start) pump the interstitial fluid into the conduits in a size-selective manner. Gretz *et al.* (57) suggested that a high transcytotic activity of the sinus-lining cells might support this function. However, it is entirely unclear how the conduit system could actively maintain pressure gradients that promote directed movement of fluid through such a complex and highly interconnected meshwork. A theory of active transport would also require an active exclusion mechanism that prevents entry of solutes into the T zone and a resulting percolation between the T cells. A recently published study showed that a small molecular tracer quickly distributed from the subcapsular sinus into the B-cell follicles, where it located diffusely between the lymphocytes. Interestingly, within the T zone the tracer distribution was restricted to the conduits (97). As there is clearly no anatomical barrier between T and B zone, such observations are difficult to include in a theory of active fluid transport through the conduits.

We propose that solute distribution within the T zone is a simple consequence of the biophysical characteristics of the T-zone interstitium. Instead of postulating that solutes are actively pushed into and confined within the conduit compartment, we propose that the conduit is the only space where interstitial fluid can distribute for reasons of hydraulic conductivity. As mentioned above, the FRCs are highly polarized, and on their apical side, they do not display or bind any ECM molecules (29). As T cells or DCs do not produce their own ECM, the T-cell compartment does not contain any interstitium that fills the intercellular space. This is a highly unusual situation and means that T cells are shielded from any ECM environment. Even the 'fluid phase ECM' of the blood, the serum proteins, are absent as the interstitial fluid is exclusively channeled through the conduit. This is indicated by tracer experiments where soluble molecules below 10 kDa were not visible between the T cells but restricted to the conduit (58). Hence, T cells move within a 'vacuum' and are packed at maximal density (98). As such, the T-cell compartment resembles a cell pellet after centrifugation. It is likely that the hematopoietic cells in the T zone are so tightly packed that they almost slide along each other in a membrane to membrane fashion, just separated by a layer of transmembrane proteins and cell surface glycosaminoglycans (that remain to be characterized in detail).

Although direct measurements of the hydraulic conductivity within the T-cell area are missing, it is likely that both diffusion and convection are extremely low between the T cells and that all significant fluid movements happen in the porous environment of the conduit core. Such a 'passive' view of the conduit system also explains that tracers continuously disperse from the LN capsule through the sinus into the conduit system. The continuity of the interstitial matrix allows for a free distribution within the interstitium, driven by Starling's forces that define the direction of flow.

The simple model of the conduit as a compact version of the dermal interstitium that is following the same physiological rules also implies that the B-cell area is behaving fundamentally differently, as it allows the free distribution of small soluble molecules. In this context, it is interesting that B-cell follicles show a diffuse staining for hyaluronic acid (our unpublished data) and are positive for the ECM molecule vitronectin (99). These findings open the possibility that B cells are surrounded by an interstitium with larger hydraulic conductivity than the T cells.

The driving force of solute distribution in the conduit system

In most peripheral tissues, the capillary system is in a state of continuous filtration, while the lymphatics absorb the filtered fluid volume. The situation changes in tissues where fluid enters via a separate route and creates a fluid excess. This is the case for submucosal tissues, where fluid enters across the gut epithelium, and for LNs, where interstitial fluid enters via the sinus (19). In gut and LN, Starling's forces result in an interstitial flux toward efferent capillaries and venules and cause a flow of fluid and small molecules into the blood system. Hence, LNs are in a continuous state of fluid absorption, which is reflected by measurements that show that the protein content of the afferent lymph is far below that of the efferent lymph (100–104). It is likely that absorption in the LN is as important for maintaining peripheral fluid homeostasis as the recirculation of interstitial fluid via the thoracic duct.

Unfortunately, comparative measurements of the molecular weight distribution of proteins within afferent and efferent lymph are lacking. Such data would allow to quantitatively test the 70 kDa size exclusion volume of the conduit. They might be further of pharmacological importance, as the lymph-blood shortcut at the level of the draining LN might be an important route for how subcutaneously applied substances enter the blood circulation. To understand the interstitial flow within the conduit, we further require a more detailed view of its molecular composition and biophysical properties. The extent to which sugars carrying fixed charges are embedded within the core region and the basement membrane will be required to estimate its swelling force and the hydraulic conductivity.

It will be interesting also to test the conduit function during altered physiological states like in the absence of afferent lymph drainage and elevated venous pressure. Under homeostatic conditions, the conduit system does not fill via the blood system, but this might change upon alterations of hydrostatic conditions. It has been shown that artificially increasing the venous pressure not only leads to a higher peripheral filtration rate of plasma but also causes dilution of the efferent lymph. This steeper decrease in protein content of the afferent versus the efferent lymph was due to decreased fluid absorption in the draining LN (100). High venous pressures might even lead to retrograde filling of the conduits via the blood. Studying the fluid balance of LNs in detail would tell us if the conduit system does indeed follow the rules of Starling or if active transport mechanisms, like water transport via aquaporin channels that are expressed in sinus-lining cells and LN endothelium (60), play a role.

When reflecting the physiology of fluid transport in LNs, it is interesting that the conduits of the spleen (105) and the thymic medulla (our unpublished data) fill via the blood system. In both cases, the conduits are solely connected to the blood vessels and the organs have no additional supply via lymphatics. Hence, these tissues should be in a state of continuous apillary filtration which matches the direction of interstitial flux within their conduits.

Small molecules and fluid are channeled through the conduit system of the T-cell cortex and drain into the vasculature. If access into the T-cell area is completely restricted and no solutes leak into the parenchyma, then where does the filtration take place and which sense does it make to channel all liquid through the T-cell cortex? It was demonstrated that the cell type that has best access to the content of the conduit is the resident DC that extends processes through the FRC layer into the lumen of the conduit (29, 30, 88, 106). Via this route, DCs can sample the content of the interstitial fluid like any other DC in the periphery can. Soluble antigens can be acquired and presented but also extracellular signaling molecules produced in the periphery can potentially be sensed. Via the conduit, the resident DCs of the LNs are rapidly informed about the state of the periphery. In other words, the soluble periphery is continuously projected into the conduits, and resident DCs can read out its condition. Immunologically, this principle is most effective, as the only cell that has to collect environmental information is the DC. T cells are not involved in 'decision making'; they just receive instructions from the DC (7). The anatomy of the T zone guaranties that they are not exposed to information they never need.

Functional implications of the conduit system

Even more enigmatic than these proximate aspects of conduit mechanisms is the ultimate question concerning the adaptive value of excluding large molecules from the conduit. We propose that size exclusion is a universal and efficient way to prevent that the lymph–blood shunt within the draining node

is exploited by infectious agents. By depleting subcapsular macrophages, Junt *et al.* (77) demonstrated that the filtering function of the sinus is essential for preventing the spread of virus particles via the blood circulation. In the absence of the scavenger function of the macrophages, virus arriving with the interstitial fluid quickly distributed via the thoracic duct into the blood and became systemic (77). Especially in bigger animals (and humans), many LNs are connected 'in row' (17), and it is likely that if virus is not cleared in the first node it becomes trapped in one of the following. If the conduits would not filter out large particles that have the potential to replicate, the lymph–blood shortcut would support hematogenic distribution and therefore would be a fatal distribution system for pathogens. In other words: as the LN blood vessels absorb fluid, a LN interstitium without a size exclusion volume would compromise the entire filtering concept of the LN.

We think that a larger exclusion size of the conduit would be dangerous for the organism. But what would be the consequence if the FRC network were to lack an interstitium, if the conduits would not exist? Why are they useful? The experiment to genetically eliminate the FRC interstitium has not been done, so we have to rely on indirect evidence. Immunologically, there seem to be two main functions of conduits: distribution of soluble antigens and endogenous signaling molecules toward resident DCs and information relay from the periphery into the lumen of high endothelial venules. In the absence of conduits, resident DCs would most likely have no access to soluble antigens or cytokines produced in the periphery – all the information would need to be (slowly) carried in by infiltrating DCs. Although this sounds like a dramatic change, it is far from clear what role soluble molecules have for the course of T-cell immune responses. Itano *et al.* (106) showed that soluble antigen presented on resident DCs can elicit T-cell responses. However, these responses were not sustained, and it is possible that they rather act as initiators that have to be consolidated by later incoming DCs. The only infectious model where immunity has been shown to be mainly triggered by soluble antigen is leishmaniasis (107). It remains to be shown how general such processes are and if soluble antigens are as important for the triggering of T-cell responses as they are for humoral immunity. Current data suggested that for tolerance induction, soluble antigens are not decisive, as maintaining tolerance is completely dependent on CCR7-mediated steady state migration of DCs (108). Much work has to be done on the role of soluble antigen.

The conduits as an information distribution system for resident DCs have not been addressed directly, and it would be interesting to see how resident DCs react to soluble cytokines or chemokines that arrive with the afferent lymph. Conduits as information superhighways have been studied in another context. It was demonstrated that chemokines produced in the periphery are rapidly (within seconds) channeled with the interstitial flux via the conduits into the lumen of high endothelial venules (109, 110). Here, they became immobilized to the luminal side of the endothelium, were sensed by circulating lymphocytes, and modulated their entry into the parenchyma. This system was interpreted as a remote control of the LN by the peripheral tissue and completes the picture of the LN as an immunological projection of the periphery that can rapidly adapt to peripheral threats (110).

A completely neglected context in which conduits might play a decisive role is fluid homeostasis. The fact that high amounts of fluids are resorbed into the blood circulation within the draining LN suggests that in the absence of conduits, interstitial fluid would either recirculate entirely via the efferent lymph and the thoracic duct or peripheral edema would develop. Unfortunately, data on the thoracic duct transport volume and the hydration state of peripheral tissues in mice lacking LNs for genetic reasons are not available.

Cell migration within the T-cell cortex

The functions of the FRC network are not entirely connected with the formation of conduits. As the only non-hematopoietic cells of the T-cell cortex, the FRCs are also organizing the migratory patterns of the blood cells. Cell migration patterns within the T-cell cortex were subjects of intense investigation during the last years, and many excellent reviews on this topic are available (9, 11–13). Here, we focus on the direct role of the stromal network for T-cell and DC migration. Although Gretz *et al.* (57) predicted that the FRC network plays a decisive role in guiding T cells through the LN, the reticular network was long ignored by intravital microscopists studying the swarming of T cells. The pathway taken by T cells was described as a random walk, and the encounters with DCs were regarded as random collisions (11, 57, 111). Bajenoff *et al.* (112) demonstrated that the opposite is the case: they showed that the routes of T-cell migration are not at all random but rather deterministic, as they lead preferentially along the FRC network. A theoretical model that tried to match experimental data on T-cell movement came to the same conclusion (113). By simultaneously visualizing T cells and FRCs *in vivo*, Bajenoff *et al.* (112) showed that the migration tracks of most T cells were aligned with the FRC scaffold. The migration direction of the T cells on the tracks was random. Hence, the FRCs appear to be an adhesive and chemokinetic surface that lack intrinsic

directional information (112, 114). FRCs are the producers of the homeostatic chemokines CCL19 and CCL21 that bind to the chemokine receptor CCR7 that is expressed on naive T cells (54, 115, 116). Both chemokines trigger and maintain the motile state of T cells and are essential for their swarming behavior. As CCL21 immobilizes to the FRC network, it is possible that the FRC network forms a chemokinetic surface that keeps the T cells dynamic and at the same time restricts their migration to the T-cell area. Such a system of chemokinetic surfaces has the attractive potential to generate high migration dynamics within sharp boundaries. In an independent study, the Germain laboratory (117) proved another important principle: cytotoxic T cells are able to respond quickly and flexibly to chemokines that are locally released by a successfully interacting pair of DC and helper T cell. Single cell tracking *in vivo* revealed that cytotoxic T cells actively chemotax toward the signal source (117). This finding means that the chemokinetic principle of swarming can be locally overcome by gradients that steer cells in a certain direction. If this directional migration is still guided along the FRC network or if T cells become autonomous from the chemokinetic scaffold once they follow soluble gradients is unknown. Many issues have to be resolved until a cell biological model of migration within the cortex will be available. Determining the role of soluble versus immobilized chemokines is one, while the question how T cells adhere to the FRC surface is another. It is also unclear how FRCs exactly handle the chemokines they produce. Interestingly, most of the CCL21 in the LN associates with the basement membrane of the conduit, where it likely immobilizes to the heparan sulfate residues (our unpublished data). Can the FRCs actively shuttle chemokine onto their apical surface where the T cells can sense it, and could such a step be flexibly regulated?

It is likely that DC migration within the cortex follows in principle similar rules: the population of resident DCs is partially embedded into the FRC layer, and it was shown also that antigen-carrying DCs immigrating from the periphery become incorporated into these networks (80). Like naive T cells, immigrating DCs respond primarily to CCR7 ligands (31). How the resident DCs couple to the FRC network is unknown, but it is most likely that they use cell surface receptors to interact with conduit and FRCs. One study showed that the DC population most tightly associated with conduits expresses high levels of a collagen-binding integrin (118). Importantly, migration along the FRC tracks leads the T cells directly into the DC networks and is therefore an ideal strategy to maximize encounters. However, testing the importance of these optimization strategies will require immunological methods that challenge the detection limit of the adaptive immune system. Most of the common manipulations work with high doses of antigen and high frequencies of (transgenic) T cells that easily find each other, even in the absence of sophisticated guidance systems.

The functional unit of the T zone

We reviewed the anatomical and physiological features of a LN that define its function as a filter for soluble molecules and as an organizer of cellular encounters. We described in detail how the conduit, its FRC sleeve, the DCs embedded between the FRCs and the T cells gliding along the FRC surface form the functional unit of the T-cell zone. This functional unit is the

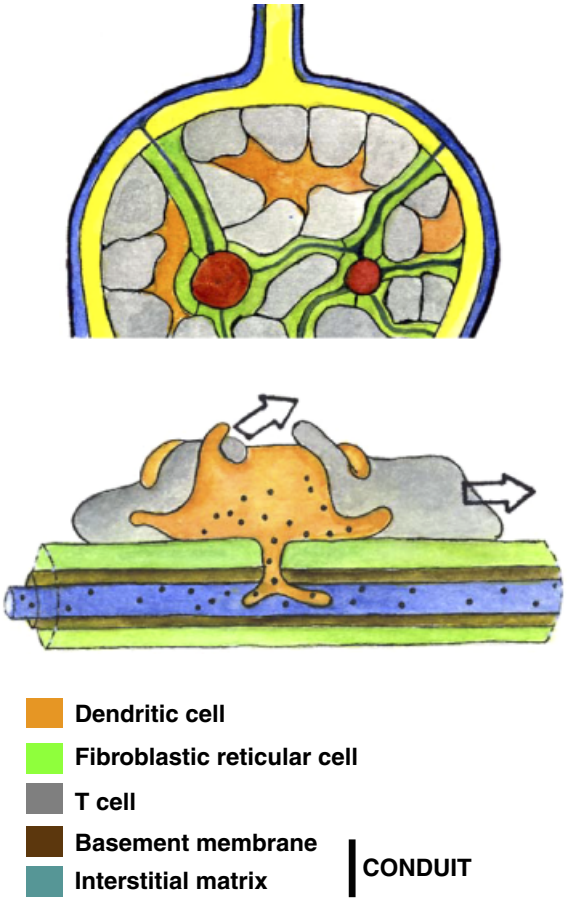

***Fig. 4.* Functional unit of the T-cell cortex.** Upper schematic: the T-cell cortex is packed with T cells (gray) and dendritic cells (orange) and is interspersed with FRCs (green) that contain interstitial matrix in their lumen and connect with the blood vessels (red). Lower schematic: FRCs enwrap the conduit that consists of interstitial matrix and a surrounding basement membrane (brown). DCs associate with the FRCs and extend processes into the conduit lumen that can take up small molecules that are flushed through the conduit toward the blood vessels. T cells move on the surface of the FRCs and collide with the DCs.

essential environment that supports the principle of the adaptive immune system, where rare cell–cell encounters have to be selected. Accordingly, the functional unit characterizes not only all secondary lymphatic organs but it forms almost everywhere in the sustained presence of lymphocytes. Likely driven by cell surface lymphotoxin, the mesenchymal condensation is triggered that ultimately leads to the assembly of conduits, FRC networks, embedded DCs, and swarming T cells that are searching their cognate antigen. Accordingly, conduit-like structures are not only found in secondary but also in tertiary lymphatic organs and even in chronically inflamed areas like the perivascular space and the parenchyma of brains of multiple sclerosis patients (119, 120) (*Fig.* 4).

Conclusion

Although LNs gained much attention during the last years of intense dynamic imaging, they are still frequently viewed as simple containers for swarming and interacting blood cells. However, they are much more, and it is beyond doubt that the LN's complex stromal infrastructure is absolutely essential for organizing the dynamic state of order required for the high encounter rates between innate and adaptive immune cells. We are still far away from an integrated physiological concept of lymph nodes as the filter stations of the interstitium. It will be necessary to collect basic physiological data on LN fluid homeostasis under normal and inflammatory conditions and integrate them into the concept of adaptive immune priming.

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