Spatial and molecular organization of lymph node T cell cortex: a labyrinthine cavity bounded by an epithelium-like monolayer of fibroblastic reticular cells anchored to basement membrane-like extracellular matrix

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Abstract

Naive T cells encounter antigen-presenting cells within the cortex of lymph nodes to initiate primary immune responses. Within this T cell cortex is the reticular network (RN)—a system of collagen fibers and extracellular matrix (ECM) wrapped by fibroblastic reticular cells (FRC). We have investigated the distribution of various molecules, including ECM proteins and proteoglycans, in the T cell cortex of both human and rodent lymph node. We confirm and extend reports of matrix elements in the RN. In addition, we find that staining for the laminin-α3 chain and for tenascin reveals a ‘hollow’ reticular pattern, consistent with localization to the basement membrane-like covering of reticular fibers. In contrast, keratan sulfate is observed in a fine linear pattern within the RN, suggesting it is localized to the core of the fibers. Staining with the marker ER-TR7 indicates that FRC cover all identifiable ECM surfaces of the T cell cortex. Based on these findings and previous reports, we conclude that cortical lymphocytes migrate within a ‘labyrinthine cavity’ free of fibrillar ECM, distinguishing the T cell cortex from other loose connective tissues, and that the FRC lining of the cavity constitutes an epithelium-like boundary. We propose that this spatial organization facilitates ameboid leukocyte crawling along preformed paths of least resistance and that the basement membrane-like ECM of the FRC may facilitate fluid transport within the RN by limiting leakage from the fiber.

Introduction

The lymph node is a secondary lymphoid organ that orchestrates and optimizes immune responses (1,2). The T cell-dependent regions in the T cell cortex are among the most important sites at which antigen-specific naive T cells encounter their cognate antigen on antigen-presenting cells (APC) (3). Lymphocytes enter the cortex via high endothelial venules (HEV), vessels specialized to facilitate high volume binding and transmigration of circulating lymphocytes (4). APC, such as Langerhans-type dendritic cells from the skin, come to the lymph node in afferent lymph and enter the cortex by migrating across the floor of the subcapsular sinus (5,6). How the lymph node cortex directs and supports the subsequent interaction of lymphocytes and APC is of great significance, since it is a center-stage event in the drama of both normal and abnormal immune responses.

Historically, detailed ultrastructural studies of lymph node
micro-anatomy were performed before identification of individual molecular components was feasible. They characterized the reticular network (RN), a fundamental element of the inter- and peri-follicular stroma of the lymph node cortex (the T cell cortex) made of collagen fibers and fibroplastic reticular cells (FRC) wrapping the fibers (7). Lymphocytes were observed interspersed between the strands of ‘reticulum’, as the RN was called. A variety of individual extracellular matrix components were later discovered to be associated with human or rodent reticular fibers, including collagens I, III and IV (8), elastin (9), entactin (10), fibronectin (11), laminin-1 (12), tenascin (13), vitronectin (14), and heparan sulfate (10).

Four distinct functions have been attributed to these ECM components. First, the maintenance of overall lymph node architecture. This role has been ascribed to the collagens I and III (providing strength) (7) and elastin (providing elasticity) (9). Second, cell adhesion and migration. Because each of the ECM components are ligands for cell surface receptors, they have been postulated to mediate adhesion of FRC to the collagen fiber ‘core’, and adhesion/migration of hematopoietic cells such as lymphocytes and dendritic cells within the node (10,15). Third, influences on cell development. Many of these ECM proteins have effects on immune cell activation or differentiation (e.g. 16–19). Fourth, transport of fluid and cytokines from lymph to high endothelial venules via the reticular fiber. Tracer studies have shown that the fiber serves as a rapid ‘conduit’ of soluble material from the capsular sinus to HEV deep in the cortex (20–22).

In the belief that how the lymph node functions may be better understood by increasing our understanding of its structure, we investigated the ECM of the T cell cortex using immunohistochemical methods. Our results confirm and extend work of others on the spatial organization of the lymph node cortex, and support the emerging role of non-lymphoid elements in the generation of immune responses. We propose three hypotheses to explain how the lymph node micro-environment is designed to optimize immune responses via its unique structure. First, lymphocytes in the T cell cortex migrate between reticular fibers in a space free of organized extracellular matrix. Second, the walls of this space and the fibers running through it are covered by FRC, which form an epithelium-like monolayer separating the space from the surrounding interstitial matrix. Third, the FRC adhere to a specialized ECM having cardinal features of the basement membrane. Nearly four decades ago, Clark described the lymph node as a ‘labyrinthine vascular space’ based on results of his electron microscopic studies (23). We have independently arrived at a very similar term—the ‘labyrinthine cavity’—that we apply to the fundamental structure of the T cell cortex. We postulate that this distinctive micro-anatomy is designed to facilitate lymphocyte migration and cell inter-action.

**Methods**

**Reagents**

The antibodies used, their specificities and source are summarized in Table 1.

**Human lymph nodes and staining**

Lymph node biopsies submitted for diagnostic evaluation to the Hematopathology Section, Laboratory of Pathology, National Cancer Institute were frozen in OCT tissue mounting gel (Baxter, Deerfield, IL) in a dry ice/isopentane bath. The two biopsies evaluated in our studies were diagnosed as reactive hyperplasias; in each case normal lymph node architecture was intact. Cryo-sections (6–8 µm) were cut onto charged slides, air-dried, fixed briefly in acetone at room temperature (3–5 minutes) and stored at -20°C until stained. Slides were fixed again as before after thawing. Staining was performed by the avidin–biotin peroxidase method on the Ventana (Tucson, AZ) automated system according to its frozen section protocol, with a primary incubation time of 32 min. Primary antibodies were added manually using the titration protocol. Diaminobenzidine was used as the chromogen with hematoxylin counterstain. For glycosidase digestions, sections were hydrated in PBS and incubated at 37°C for 30 min with 0.2 U/ml chondroitinase ABC (Seikagaku) or 0.1 U/ml heparatinase (Seikagaku) before staining.

**Rat and mouse lymph nodes and staining**

All animals were kept in pathogen-free facilities and treated according to National Institutes of Health guidelines. Animals used include Lewis and Sprague-Dawley rats, and C57Bl6 mice. Fresh lymph nodes were positioned in moulds filled with OCT and then frozen in an isopentane/dry ice bath. Lymph nodes were sectioned (10 µm) on a Cryostat microtome. The orientation of the nodes in the moulds resulted in sections in a vertical plane in relation to the hilar blood vessels (24). This orientation of the nodes created the greatest number of sections in parallel to the reticular fibers in the lymph node cortex. Sections were dried on microscope slides for 1 h and rehydrated in PBS/0.1% BSA for 30 min. Antibodies were diluted in PBS/0.1% BSA and applied for 30–60 min each at room temperature in a humidified chamber. Slides were washed between and after antibody applications 3 times with PBS/0.1% BSA for 5 min each. Fluorophore or peroxidase-labeled streptavidin was applied for 15–30 min, slides were washed, fixed in 4% paraformaldehyde/0.1% glutaraldehyde/MOPS buffered Ringer’s lactate solution, washed in PBS and coverslipped with Prolong (Molecular Probes, Eugene, OR) or Fluormount (Southern Biotechnology Associates). Diaminobenzidine (Sigma) was used as the chromogen for peroxidase. The orientation of the nodes stained with sections in a vertical plane in relation to the hilar blood vessels (24). This orientation of the nodes created the greatest number of sections in parallel to the reticular fibers in the lymph node cortex. Sections were dried on microscope slides for 1 h and rehydrated in PBS/0.1% BSA for 30 min. Antibodies were diluted in PBS/0.1% BSA and applied for 30–60 min each at room temperature in a humidified chamber. Slides were washed between and after antibody applications 3 times with PBS/0.1% BSA for 5 min each. Fluorophore or peroxidase-labeled streptavidin was applied for 15–30 min, slides were washed, fixed in 4% paraformaldehyde/0.1% glutaraldehyde/MOPS buffered Ringer’s lactate solution, washed in PBS and coverslipped with Prolong (Molecular Probes, Eugene, OR) or Fluormount (Southern Biotechnology Associates). Diaminobenzidine (Sigma) was used as the chromogen for peroxidase. Lymph node sections stained with fluorophore-labeled antibodies were examined using the Zeiss 410 confocal laser scanning microscope. The images shown were compiled projections of 30–40 optical sections. Reticulin stains used the method of Gomori (25) (reagents from Sigma). Nuclear Fast Red was used as a counterstain where indicated.

The scanning electron micrograph of rat lymphocyte in fibrin clot was prepared as follows. Lewis Rats were prepared with a Bollman’s fistula according to Gowans (26). Single drops of fresh thoracic duct lymph were dropped into coated tissue culture slide chambers. The lymph clotted within 4 min and the slides were incubated for 30–60 min at 37°C. The slides were fixed by slow application of prewarmed 2% glutaraldehyde/PBS, then prepared for scanning electron microscopy (27). Lymph nodes were prepared for transmission electron microscopy according to Anderson (27).
Table 1. Summary of antibodies used

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*These antibodies bind the proteoglycan stub which is exposed after digestion with appropriate glycosidase.

Results

Spacing of the RN in lymph node cortex

The existence of the lymph node RN was visualized with silver stains nearly a century ago. Figure 1(A) illustrates collagen fibers in the T cell cortex visualized with the ‘reticulin’ stain. The fibers are scant within B cell follicles, abundant within the T cell cortex and predominantly oriented perpendicular to the capsule. The space between reticular fibers is 5–20 µm, accommodating two or three lymphocytes. We compared the RN to another fibrillar network, a fibrin gel, which has been used as a system in which to investigate lymphocyte migration. The lymphocyte shown in Fig. 1(B) is embedded within the fibrin gel. The spacing between the fibrin fibers is much closer than the collagen fiber spacing of the RN. When the reticulin-stained lymph node cortex is shown at the same magnification as the fibrin gel (Fig. 1C), it is especially apparent that the lymph node fibers are widely spaced relative to the lymphocyte diameters.

ECM proteins and proteoglycans are distributed along the RN and not identified within the intervening space

We investigated the expression of various ECM proteins for presence in and around the RN. Representative results are shown (Fig. 2) for fibronectin, laminin-1, tenascin and collagen type IV. Each stained in an interfollicular pattern with layout and spacing matching that of reticular fibers; there was a paucity of staining for these ECM proteins in B cell follicles (Fig. 2A, C and D). Each also stained vascular basement membranes. None of the stains revealed matrix protein within the space between reticular fibers, enmeshing lymphocytes in the manner illustrated by the fibrin clot (Fig. 1B). This characteristic pattern indicated that presence of the ECM proteins was restricted to the RN.

We used two reagents to detect specific laminin chains not found in laminin-1: P1E1, a mAb specific for laminin-α3, and

Fig. 1. Spacing of collagen fibers in lymph node, unlike a fibrin gel, is large relative to the diameter of a lymphocyte. (A) Reticulin stain of collagen fibers in the cortex of rat mesenteric lymph node (‘l’ indicates B cell follicle). (B) Scanning electron micrograph of a rat lymphocyte in a fibrin gel resulting from coagulation of normal rat lymph. (C) Same tissue section as (A) but shown at approximately the same scale as (B). Arrowheads indicate the single reticular fiber in this field, which runs from upper left to center bottom. Three dark spherical lymphocytes can be seen.
T cell cortex is a labyrinthine cavity bounded by FRC.

**Fig. 2.** ECM proteins are prominently expressed on the RN and restricted to it. Frozen tissue sections of human reactive lymph nodes were stained for fibronectin (A), collagen type IV (B), tenascin (C) and laminin-1 (D). Arrows indicate HEV. ‘f’ indicates B cell follicle. Empty white areas represent artifacts of the cryostat preparations.

**Fig. 3.** Laminin-α3 is expressed in reticular pattern in human and mouse lymph node. Sections from human reactive lymph nodes stained with antibodies against laminin-α3 are shown at low (A) and high (B) magnification. Sections from mouse lymph nodes stained for anti-laminin-5 components (α3/β2/γ2) labeled with FITC showing a B cell follicle and surrounding cortex at low magnification (C) and at higher magnification of cortex (D). ‘f’ indicates B cell follicle (A and C).

A polyclonal antiserum specific for laminin-5 (α3/β3/γ2) but unreactive with laminin-1 (α1/β1/γ1). Staining was observed in both mouse and human lymph node (Fig. 3). There was strong staining in a reticular pattern similar to the ECM proteins listed above, with no expression detected in the intervening space between fibers that is occupied by lymphocytes.
Sparse but strong staining in follicles was consistent with staining of vessels. Since proteoglycans constitute prominent and functionally important components of the ECM, their distribution was also examined (Fig. 4). Staining of heparan sulfate in human lymph node (Fig. 4A) resembled that of the ECM proteins described above: intense expression on the RN and vascular basement membrane, with an abrupt reduction in expression in adjacent B cell areas. In mouse lymph node, heparan sulfate was likewise present in vascular structures and the RN (Fig. 4C). Staining for keratan sulfate yielded a distinctive fine linear pattern, consistent with localization to the RN, but in a patchy distribution (Fig. 4B). Its expression on vascular structures was less pronounced than the other ECM components. It was not detected in mouse lymph node. Chondroitin sulfate was restricted primarily to the vascular structures (Fig. 4D). No proteoglycan staining was observed in the spaces between reticular fibers.

Tenasin and laminin-5 are present in a ring that surrounds the collagen fibers

Closer analysis revealed that the staining patterns for both tenasin and laminin-α3 are ‘hollow’ in murine lymph node (Fig. 5). Specifically, the fiber was surrounded by the stain, which marks the edge rather than the core in both immunofluorescent and immunochemical stains. This hollow staining contrasts with solid staining that is seen with stains for various other ECM proteins such as collagen III (Fig. 5A) or fibronectin (Fig. 2A). The hollow tubes appeared largely uninterrupted and a ring pattern was seen in sections in which the fiber can be visualized on end (Fig. 5E). This staining pattern indicates that tenasin and laminin-α3 are localized predominantly to layers surrounding the collagen fiber.

Lymphocytes contact FRC that ensheathe the collagen fiber bundle

Within sinuses lymphocytes can be packed closely and regularly together (Fig. 6A). Most of the lymphocytes in such a sinus are not in contact with a surface along which they could migrate. In contrast, within the cortex collections of lymphocytes are traversed by reticular strands so that most of the lymphocytes are in contact with the RN. We call the space occupied by lymphocytes in the T cell cortex a ‘labyrinthine cavity’. The light micrograph (Fig. 6A) shows the proximity of cells to the RN in a single plane of section; other cells contact the RN above or below this plane. The detailed spatial arrangement of the T cell cortex is more readily observed by electron microscopy (Fig. 6B). Bundles of collagen fibers course among lymphocytes, with several lymphocytes fitting between adjacent fibers.

We have previously referred to the spaces containing lymphocytes bounded by the RN as ‘corridors’ (28), because of their structural resemblance and functional similarity to an architectural pathway within a larger building. Within the corridor, only a small region of the lymphocyte surface is in contact with the RN at any given time. At the point of contact, there is generally an intervening FRC between the collagen fiber and the lymphocyte. Rarely, direct contacts between a lymphocyte and the reticular fiber are made without an intervening FRC (Fig. 6B, middle left). This image also illus-
Fig. 5. Tenascin and laminin-5 components show hollow tubular staining with reticular pattern. Mouse lymph node sections were stained with antibodies specific for collagen III (A, FITC), laminin-5 components(α3β2γ2) at low magnification (B, FITC) and high magnification (C, FITC), and tenascin (D and E, FITC; F, horseradish peroxidase). Collagen III staining is solid. In contrast, staining for laminin-5 chains and tenascin are 'hollow' in longitudinal section. (E) A high magnification confocal image of the cross-section of the fiber indicated by the arrow in (D) reveals a ring pattern of positive staining around the fiber.

Fig. 6. Lymphocytes contact the FRC which ensheathe the collagen fibers. (A) A reticulin-stained section from a rat axillary lymph node counterstained with nuclear Fast Red. This lymph node, chosen to illustrate lymphocytes packed in a sinus, was excised 2 days after immunization together with avidine adjuvant (68). Note the appearance of large numbers of packed lymphocytes within a cortical sinus on the right (marked with an 'S'), and the much smaller pockets of similarly packed lymphocytes within the T cell cortex (marked with 'LC'). (B) Transmission electron micrograph of lymphocytes packed together in a 'corridor' between collagen fibers (arrowheads) in a normal rat lymph node. The thin covering layer of reticular cells ensheathing the fiber are interposed between lymphocytes and the collagen fiber in all places except the middle left. The vertical space on the far right is a cortical sinus. Note the similarity between the design of the RN on the left, and design of the cell and ECM layers in the wall of the sinus on the right.

trates the fundamental similarity between the RN and the boundary between cortex and sinus. The collagen fiber and cell-covered boundary on the right is the wall of a sinus, which is similar in design to the reticular fiber on the left that subdivides two ‘corridors’ within the labyrinthine cavity of the cortex.

To more precisely understand the relationship between FRC and the underlying interstitial matrix, we performed double staining with anti-collagen III and the antibody ER-TR7, which stains a molecule produced by FRC (29). There is a continuous lining of EK-TR7 reactivity at the boundary of the cortex and the subcapsular sinus as well as at the HEV (Fig. 7A). A higher magnification image (Fig. 7B) demonstrates that the HEV is surrounded by a continuous layer of ER-TR7-stained pericytes. The higher magnification view of a large reticular fiber (Fig. 7C) likewise reveals a continuous stain of ER-TR7 along its surface.

Discussion

Concept of cortex as a ‘labyrinthine cavity’

Tissues such as skin are referred to as ‘loose’ connective tissues to distinguish them from ‘dense’ connective tissues, such as bone. Loose connective tissue, containing collagen fibers and associated fibroblasts, may at first glance appear similar to lymph node cortex, which contains collagen fibers and associated FRC. However, evidence suggests that the lymph node cortex is fundamentally different from conventional loose connective tissue. Our investigation of many ECM components found none to be present in the space between reticular strands. Similar findings were observed in multiple previous studies of the distribution of organized ECM components in lymph node (e.g. 8,10,11,13–15,30–32). Even the highly hydrated matrix proteoglycan hyaluronan is reticular in distribution (33). In addition, there is no ultrastructural evidence of matrix between the fibers (7,23,34,35). We conclude, therefore, that this space contains no organized interstitial matrix. Such a lack of organized matrix in a relatively large space is characteristic of internal body cavities or lumens of ducts, glands and vessels, and never found in the interstitium of loose connective tissue. We believe these spaces, or ‘corridors’ (28), are interconnected into a single ‘labyrinthine cavity’ within a cortical lobule, based on our understanding of its topology.

A metaphor may be helpful to convey the topology of the labyrinthine cavity. Imagine a cortical lobule as a subterranean cave just below the surface of the ground. Lymph in the subcapsular sinus is like water in a shallow lake over it. The water does not pour down into the cave because of the thin rock ceiling over the cave, which is the floor of the sinus. The cave has many vertical rock columns, the reticular fibers, which run from the ceiling to its base, like stalactites joined to stalagmites. The cave swarms with spherical creatures—lymphocytes—which enter the cave by tunneling out of enclosed aqueducts close to the floor—the HEV—and move by crawling on surfaces (walls, ceiling, columns) to reach any point within the cave.

The concept of a labyrinthine cavity in cortex is not just a semantic one; we consider it critical in understanding how
The cortex efficiently orchestrates cell migration and cell–cell interactions. In vitro studies of cell migration through ECM have demonstrated how profoundly ECM influences cell migration (36,37) and lymphocyte interaction with dendritic cells (38). The ease with which a lymphocyte migrates will depend on the structural organization of the microenvironment through which it migrates (37). The spacing and orientation of fibers is of paramount importance: the maximum rate of lymphocyte migration has been reported to be 12–28 µm/min (A. O. Anderson, unpublished observations and P. Friedl, pers. commun. and 39), but this rate is dependent on the spacing of the fibers through which it is crawling. Consider the lymphocyte depicted in Fig. 1(B) in a fibrin gel. The migration rate through the mesh decreases once the fibers become too closely spaced (P. Friedl, pers. commun. and 37,40).

The subterranean metaphor can be extended to help visualize this situation. The spherical creatures (lymphocytes) in the cave can crawl on any surface with minimal resistance. Contrast this to loose connective tissue, which is like earth through which the creatures must tunnel, providing continuous resistance. Practical experience with lymphocyte isolation is consistent with this view: protease digestion is usually required to recover lymphocytes in skin or infiltrating tumors, whereas lymphocytes are easily expelled from sectioned lymph node into liquid suspension by gentle pressure. Additionally, scanning electron microscopy indicates that lymphocytes in this space are round and often have microvilli, similar to their appearance in lymph or blood, and unlike their non-spherical appearance and lack of microvilli when transmigrating across endothelium or within connective tissue (34,35). We believe, therefore, that lymphocytes in lymph node cortex are loosely adherent to the surfaces of a cavity; in other tissues they are lodged within an interstitial matrix that is woven into a closely spaced mesh of fibers oriented in different directions.

The spacing and orientation of the fibers within the labyrinthine cavity are consistent with a design that optimizes lymphocyte migration. The spacing of fibers within the T cell cortex is narrow enough for most lymphocytes to remain in contact with the fibers, but wide enough that there is minimal obstruction to migration. Prominent reticular fibers run mostly perpendicular to the capsule, forming a relatively direct route between the HEV and the subcapsular sinus. This is significant because the overall direction of movement of lymphocytes in vitro tends to follow the orientation of fibers (41). Thus the RN may provide a preferred directionality for lymphocyte migration from HEV towards the sinus, guiding T cells to contact with antigen-presenting dendritic cells, which migrate into the labyrinthine cavity from the sinus after arrival in afferent lymph.

**FRC form an epithelium-like cell layer lining the labyrinthine cavity**

Our observations lead us to believe that the cavity is an enclosed space. The boundary of the labyrinthine cavity is the thin layer of reticular cells that form sleeves around the fibers and flattened linings on the ceiling and walls. This lining has been previously noted in ultrastructural studies, and was highlighted in our studies by concurrent staining of FRC and subjacent collagen III (Fig. 7). The lining cells are present in three forms. Where the cavity abuts the sinus there is a triple boundary made of a thin layer of collagenous ECM sandwiched between sinus lining cells and the reticular cells that cover the ceiling of the labyrinthine cavity (Fig. 6B). Within the labyrinth space, the reticular fibers are wrapped by FRC. Around the HEV, the fibrillar matrix surrounding vessels is covered by cells often called pericytes, which are ultrastructurally indistinguishable from FRC. Thus, the FRC in the labyrinthine cavity are analogous to mesothelium within the abdominal cavity—it is a continuous lining covering the surface of all fixed structures. Cells enter this cavity as they do other cavities, by transmigrating across the cellular and matrix barriers that enclose it, either from the HEV (42) or from the sinus (43).

Epithelium has been defined as ‘the tissue that covers the free surfaces of the body, from the exposed external surface
to the smallest free facets within the internal organs’ (44). The cell lining created by the FRC within the labyrinthine cavity has many features of epithelium. FRC connections to each other are characterized by tight junctional complexes (45). Furthermore, FRC are anchored to an ECM that is characteristic of basement membrane (10 and discussion below). Finally, although molecular expression does not define epithelial cells, cytokeratins are often used as markers to distinguish them, and it has been found that FRC express cytokeratins 8 and 18. This expression pattern has previously been considered anomalous (46–48). FRC expression of these keratins, which are most frequently found in simple epithelium, is consistent with the view that they may be epithelial cells. Epithelial layers can be derived from non-epithelial cells and do not necessarily require growth from a pre-existing epithelium. For example, during embryogenesis excretory tubules in kidney develop by a direct mesenchyme-to-epithelium transition (49). The FRC which enclose reticular fibers in lymph node have been reported to arise by a transition from mesenchymal cells in the early postnatal period in the rat (50). It may thus be appropriate, if somewhat unconventional, to consider the FRC lining to be a specialized type of epithelium such as mesothelium or endothelium; since some definitions of epithelial cells require that they be of ectodermal or endodermal origin, we refer to FRC as epithelium-like. It should be noted that epithelial cells may be primary lymphoid organ, the thymus. However, in the thymus, epithelial cells arise by budding from pre-existing epithelium during embryogenesis (2). As thymic epithelium is essential to thymocyte differentiation, so FRC may be essential to lymphocyte differentiation in lymph node cortex.

During the evolution of our thoughts on the labyrinthine cavity, we discovered that Clark had arrived at a very similar interpretation independently, based on ultrastructural studies (23).

“The reticular fibers which traverse dense lymphoid tissue and surround sinuses and blood vessels form a presumably continuous network; thus the reticular sheathing them also form a very complex, but probably unbroken sheet... [which] constitute a continuous simple epithelium, with reticular fibers against one surface and free cells next to the other. Instead of speaking of the reticular fibers as enclosed, one may reverse the point of view and consider the free cells as lying in something like a vascular space, enclosed and separated from connective tissue by a sort of endothelium... [I am] describing the lymph node as a labyrinthine vascular space, in which free lymphoid cells lie crowded together but separated from connective tissue.”

We have chosen the term ‘cavity’ to make explicit the concepts of enclosure and continuity of the space. The space is walled off from adjacent sinuses and blood vessels: this is most evident in studies of soluble tracers (20). Despite numerous obstacles (RN, HEV and follicle), available evidence indicates that within a cortical lobule the space is continuous. Moreover, ‘vascular space’ connotes a lumen with directional flow of fluid, which has not been found within the labyrinthine cavity.

**Localization of ECM components within the RN and proposed functions**

As noted above, the ECM components detected within the T cell cortex are localized to the RN and excluded from the labyrinthine cavity. The RN itself has at least four concentric layers to which ECM can be localized.

(i) The core. At the center is a ‘core’ layer composed of collagen fibers and intervening matrix.

(ii) The basement membrane. Surrounding the core is a layer understood to represent the basement membrane, which includes a lamina densa (e.g. 51).

(iii) The reticular cell. Anchored to this basement membrane via its ‘basal’ surface is the FRC.

(iv) The FRC glycocalyx. Exposed to the labyrinthine cavity is the ‘apical’ surface of the FRC, covered by its glyocalyx. This surface includes integral membrane proteins on the apical surface [such as VCAM-1 (52)] as well as proteins that may be tethered to the apical surface [such as hyaluronate (53)].

The ECM of the RN has distinctive molecular components: collagen IV, laminin, fibronectin, entactin and heparan sulfate, which are recognized constituents of basement membrane. This basement membrane likely plays a role in anchoring the FRC to the reticular fiber. Collagen III, not a classic ‘basement membrane’ protein, has been localized to the fiber core by immunoelectron microscopy; in contrast, laminin-1 and collagen IV have been localized to the FRC basement membrane layer (51). By light microscopy, this correlates with a distinctive ring-like staining around the fiber core (10).

Our studies identified a similar ring staining pattern of tenascin and laminin-α3 chain, which is likewise suggestive of localization to the basement membrane layer in murine lymph node.

Tenascin is a constituent of the epithelial basement membrane in tissues such as uterus and bronchus (54,55). Our light microscopic analysis indicates predominant localization of tenascin outside the fiber core in lymph node reticular fibers; immunoelectron microscopy indicates that tenascin is present within reticular fibers in human fetal but not adult spleen (56). Tenascin is essential in epithelial cell migration during development and also in wound healing (57,58). Its presence in the FRC basement membrane may facilitate migration of reticular cells during the dynamic remodeling which is part of the structural plasticity of lymph nodes in the immune response. In vitro studies showing that tenascin can strongly inhibit TCR-mediated T cell activation (17,59) underscore the importance of understanding its localization in vivo.

Laminins are a family of trimeric extracellular matrix proteins, which can assemble in many combinations, including laminin-1 (α1/β1/γ1) and laminin-5 (α3/β3/γ2) (60). Various heterotrimeric forms are characteristically present in basement membrane. Hollow or ring staining for laminin-1 has previously been reported and confirmed to be located in the basement membrane of the RN (10,51). Ring staining of laminin-α3 suggests that there is at least one other laminin family member within the basement membrane in addition to laminin-1. Reticular staining in lymph node of laminin-α3, but not γ2, has been reported (61). The absence of staining for laminin-γ2 implies that the laminin isoform present in reticulum is most likely either laminin-6 or laminin-7, isoforms which include α3 but not γ2 (60). It is possible that staining of the reticular cell layer could also contribute to the ring staining pattern, since there have been reports of intracellular staining.
for both tenascin and for laminin-5 chains, primarily in tumors (62,63). It remains to be determined whether the laminin-α3 in lymph node cortex influences T cell development there, as it has been reported to in the thymus apparently as a laminin-5 heterotrimer (18,19).

There is a growing body of evidence that the reticular fiber acts as a conduit for bulk flow of fluid and solute from the subcapsular sinus to the HEV (20–22,28). Tracer studies show that fluid in the conduit does not leak freely into the labyrinthine cavity, indicating that there is barrier to lateral leakage from the fiber. Previously, the FRC itself has been interpreted to be the primary barrier to leakage, since most of the fiber surface is coated the cell body of FRC. Yet there are rare areas of the fiber—‘gaps’ in the FRC covering—that are not covered by FRC (64). The barrier could be maintained in these regions if the basement membrane that coats reticular fibers also contributes to retaining fluid within the fiber. This hypothesis is consistent with the current concept that vascular basement membrane contributes to the resistance to fluid and solute movement out of blood vessels (65). The gaps in FRC coverage of the reticular fiber may thus be analogous to gaps in fenestrated endothelium at which basement membrane has been shown to contribute resistance to fluid transfer (66). This is particularly plausible for reticular fibers in which the hydrostatic pressure most likely is quite low.

Molecules within the conduit

We observed a fine linear reticular-pattern staining of keratan sulfate (Fig. 4B) in a patchy distribution within the T cell cortex. Such staining contrasts with the ring staining of tenascin and laminin-α3, and with the broader staining of fibronectin (which may be present both inside and outside the fiber). The simplest interpretation of this fine pattern is that it represents localization to within the very center of the fiber. Keratan sulfate has notable expression in cartilage and cornea, where it is thought to facilitate diffusion of solutes (67). Its presence in the core of the reticular fiber may similarly facilitate hydration and flow within the collagen fiber matrix. We did not find staining of KS in mouse lymph node, however, which may mean that its expression may be species-specific or even dependent on local influences.

Future directions

The lymph node is the macro-anatomic solution to the challenge of how to allow a circulating lymphocyte to make contact with its cognate antigen arising from tissues. Within the node, the labyrinthine cavity appears to be the micro-anatomic extension of this solution. It accomplishes this solution by conducting signals from afferent lymph to HEV for the recruitment of circulating lymphocytes, by forming a matrix-free space in which cells can easily migrate and interact, and by providing paths for migration that direct the cells to productive interactions that result in immune responses. Many important facts have yet to be determined.

For example, we do not understand the connections between the labyrinthine cavity of T cell cortex and B cell follicles, and the deeper medulla—whether there are patent connecting pathways or whether cells must move across cellular barriers between these compartments. Further investigation is warranted, since increased appreciation of lymph node structure will likely lead to increased understanding of normal and abnormal immune responses.

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Abbreviations

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<tr>
<th>Abbreviation</th>
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<tr>
<td>APC</td>
<td>antigen-presenting cell</td>
</tr>
<tr>
<td>ECM</td>
<td>extracellular matrix</td>
</tr>
<tr>
<td>FRC</td>
<td>fibroblastic reticular cell</td>
</tr>
<tr>
<td>HEV</td>
<td>high endothelial venule</td>
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<td>RN</td>
<td>reticular network</td>
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References

T cell cortex is a labyrinthine cavity bounded by FRC


T cell cortex is a labyrinthine cavity bounded by FRC 1253