

Cords, channels, corridors and conduits: critical architectural elements facilitating cell interactions in the lymph node cortex

Authors' addresses

J. Elizabeth Gretz¹, Arthur O. Anderson², Stephen Shaw¹,
¹The Human Immunology Section, Experimental Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda Maryland, USA.

²Department of Clinical Pathology, Diagnostics Systems Division, US Army Medical Research Institute on Infectious Diseases, Fort Detrick, Frederick, Maryland, USA.

Correspondence to:

Stephen Shaw
Chief, Human Immunology Section
EIB/NCI/NIH Bldg 10/4B17
10 Center Drive MSC1360
Bethesda MD 20892-1360
USA
Fax: 301-496-0887
e-mail: sshaw@nih.gov

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Summary: The lymph node cortex is a critical site for encounter between recirculating T cells and their specific antigens. Due to its extreme plasticity, little is understood of the underlying functional unit of the lymph node cortex, the paracortical cord. The idealized paracortical cord (approximately 100 μm by 1000 μm) stretches from a medullary cord to the base of a B-cell follicle. In cross-section, a cord can be visualized as a set of nested cylinders consisting of spaces bounded by cells. The spaces are: i) the lumen of the high endothelial venule (HEV), ii) perivenular channels – narrow potential spaces (0.1 μm) tightly encircling the HEV, iii) corridors – broad spaces (10–15 μm) constituting the majority of the parenchyma, and iv) the cortical sinus. In addition to these spaces for cell traffic, the conduit (fifth space) is a special delivery system for the transit of soluble factors to the HEV and emigrating lymphocytes. The cellular barriers between these spaces are high endothelium, fibroblastic reticular cells, or sinus-lining cells. This review describes the spaces of the paracortical cord and their cellular boundaries, outlines the movement of cells and fluids through these spaces, and discusses how this anatomy affects the efficiency of surveillance by T cells.

Glossary of terms relevant to lymph node cortex (4 terms that are most critical to this review are highlighted).

channel	see perivenular channel
cortical lobule (also called lobule)	major repeating unit within lymph node
cortex	one of two major areas in lymph node (the other being the medulla) the region closest to the capsule, subdivided into the superficial cortex and a deeper region called the paracortex
cortical sinus (also known as intermediary sinus)	a plexus of sinuses which penetrates the cortex, bounds the outer surface of the paracortical cords, and connects the subcapsular sinus to the medullary sinuses
corridor (space)s	spaces between the fibroblastic reticular cell network branches which constitute most of the volume of the cortical parenchyma: an aqueous compartment distinct from the sinus which is the primary site of T-lymphocyte interactions with APCs; each corridor is typically 10–25 microns in diameter
conduit (space)s	a distinct compartment for fluid transport within the cortex (and paracortical cords), almost completely enclosed within cell processes of fibroblastic reticular cells and thus running within the fibroblastic reticular cell network; tracers administered intralymphatically delineate the fibroblastic reticular cell network, suggesting fluid moves in the spaces between the fibers of the fiber bundle

interfollicular region	the T-cell-dependent area of the superficial cortex which lies between follicles
fibroblastic reticular cell (FRC)	predominant stromal cell in the cortex (also known as a myofibroblast) i) FRCs envelop the reticular fibers to create the conduit space ii) in our view, FRC (somewhat specialized) is the pericyte which surrounds the HEV iii) FRCs envelop the fibers which cross the sinuses iv) FRCs may be the sinus-lining cells
fibroblastic reticular cell network (FRCN)	assembly of FRCs and reticular network in the cortex
follicle	B-cell-dependent region of superficial cortex
intermediary sinus	see cortical sinus
paracortex	deeper region of cortex (see cortex)
paracortical cords	the fundamental repeating functional unit of the cortex, consisting of concentric spaces: HEV lumen, perivenular channels, corridors, finally surrounded by cortical sinuses; elucidating this structure is the primary objective of this review
pericyte	specialized cell type which wraps the HEV (see fibroblastic reticular cell)
perivenular channel (PVC)	a potential space which lies between the abluminal side of the endothelium and the surrounding pericytes and is therefore part of the HEV; lymphocytes migrate from the HEV outwards along this space to the corridors and conduit fluid moves in the opposite direction through PVCs into the HEV lumen
reticular fibers	bundles of collagen fibers that make up the lymph node infrastructure: in the cortex they are enclosed within fibroblastic reticular cells and are therefore in the conduit (space)
reticulum or reticular network	assembly of reticular fibers
sinus	fluid-filled compartment through which lymph flows; the cortex is bounded by the subcapsular sinus; many cortical sinuses run through the cortex and coalesce into medullary sinuses
sinus-lining cell (SLC)	cell which provides boundary between corridors and sinuses; cell type unknown (endothelial cell or FRC)
septum	fibrous division between cortical lobules; unlike trabecula, the capsule accompanies the division
sleeve	(see perivenular channel) assembly of FRCs and PVCs surrounding the HEV
superficial cortex	the more superficial part of the cortex which includes the follicles/germinal centers and the interfollicular region
T-cell-dependent area	consists of the interfollicular regions and the paracortex
trabecula	fibrous divisions between cortical lobules; unlike septae, the capsule does NOT accompany the division

Introduction

Lymphocytes circulate in blood and enter tissue to find their relevant antigen, as required by acquired immune responses. However, virtually all primary immune responses are initiated in lymph nodes (LNs) and other secondary lymphoid tissue. This evolutionary strategy succeeds because the immune system has also evolved efficient systems by which relevant antigens are transported to LNs by antigen-presenting cells (APCs)

(1–3). The benefits are dramatic: instead of a lymphocyte having to recirculate throughout the entire body, it needs only recirculate through secondary lymphoid tissue. Thus the LN is a critical crossroad for encounter between APCs and soluble factors in incoming lymph and lymphocytes recruited into LN from the blood.

There is a great advantage to efficient interactions within the LN cortex. As we understand it, the LN cortex:

- i) fosters entry of APCs from lymph, and T lymphocytes from blood;
- ii) makes possible high frequency encounter between T lymphocytes and APCs;
- iii) provides a site for expansion of antigen-stimulated T lymphocytes;
- iv) facilitates the migration of B lymphocytes and certain T lymphocytes into the B-lymphocyte-dependent area;
- v) optimizes activation of naive B lymphocytes.

“Paracortical areas (of lymph node) are zones of variable histological appearance whose functional anatomy is poorly understood” (4). An understanding of lymphoid structure is essential in order to incorporate the ever-increasing fund of molecular information into our knowledge base of host immune responses *in vivo*.

LN architecture in mammals defies easy explanation for multiple reasons. First, it is a complex three-dimensional structure and its apparent organization differs between species (4–6). Second, it undergoes dramatic acute changes in response to antigenic challenge (7–10), inflammatory stimuli (11–13), and even neural modulation (14–17). Third, it undergoes marked chronic changes with normal aging and in many disease processes (18). Therefore, if we are to understand it, we need to be able to define constant elements and understand how changes in them account for the overall changes in the LN.

This review attempts a function-oriented summary of our understanding of the architecture of the T-cell-dependent area of the LN. We explore the conceptual implications largely as they apply to the binary interactions of T cells with APCs, rather than the more complex interactions inferred to involve T-cell help for other cell types such as cytotoxic T cells or B cells. Our intent in this review is not to summarize past work systematically, but to present a working hypothesis regarding the microanatomy of the T-cell-dependent area and the rules governing movement of cells and fluids within that area. These hypotheses are both incremental and radical. They are incremental because they build from concepts which began to emerge decades ago. They are radical because they propose an architecture which is far more elegant than has been generally accepted.

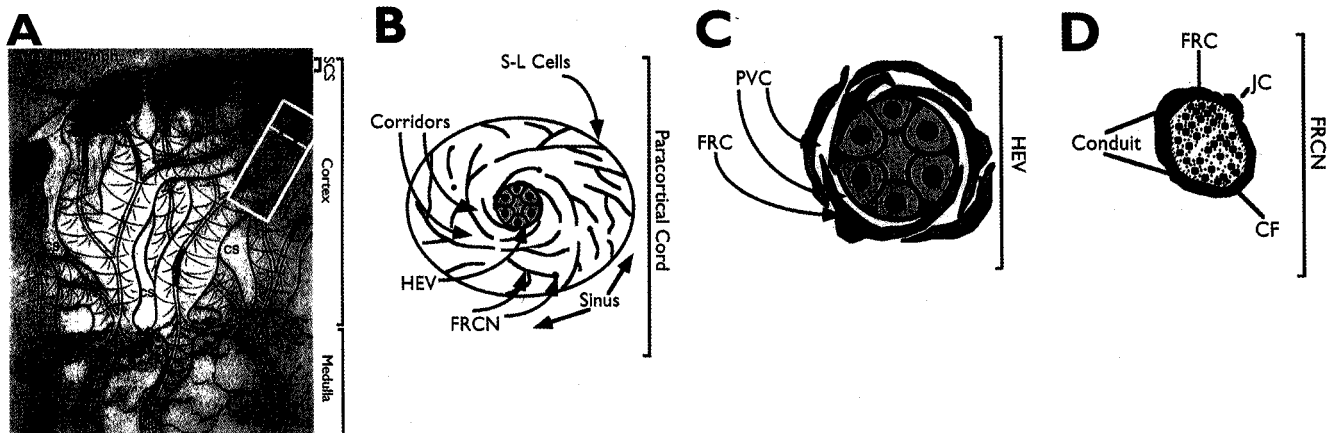


Fig. 1. Paracortical cords as the fundamental structural unit of lymph node paracortex.

A. An anatomical drawing of a partial lobule in the rabbit lymph node shows the paracortical cords with their basketry of reticular fibers surrounding each post-capillary venule/HEV (representative cord in white box). Paracortical cords can either begin near the base of follicles or in the interfollicular regions (not shown) then coalesce into medullary cords at the cortico-medullary boundary. For simplicity, the arterial side of the vasculature is not shown and starts with the basketry of capillaries and post-capillary venules around the follicles. The spaces around the paracortical cords are cortical sinuses.

[Reproduced with permission from Kelly RH. Functional anatomy of lymph nodes. I. The paracortical cords. *Int Arch Allergy Appl Immunol* 1975;48(6):836–849, Fig. 6, p. 844.]

B. Idealized illustration of a cord cross-section at the level of the white dotted line in A. Note the relationship of the corridors bounded by the FRC network to the centralized HEV. The main conduits from the subcapsular sinus run parallel to the HEV; one such conduit appears here in cross-section and interconnects to the HEV via the FRCN.

C. Enlarged illustration of an HEV in cross-section. The overlapping pericyte-like FRCs surrounding the high endothelium are the barriers of the PVCs.

D. Enlarged illustration of FRCN in cross-section. The conduit is made up of Collagen I and III fibers with associated proteoglycans. Note that the junctional complex of the FRC is shown to emphasize the relationship of the FRC enwrapping the conduit.

(CF, collagen fibers; CS, cortical sinus; F, follicle; FRC, fibroblastic reticular cell; FRCN, FRC network; HEV, high endothelial venule; JC, junctional complex; PVCs, perivenular channels; SCS, subcapsular sinus; S-L cells, sinus-lining cells.)

We view the LN cortex as a highly structured set of spaces and boundaries that makes the LN an optimal environment for efficient regulated cell interaction. We have chosen terminology, schematics and figures which we hope will make it easier to understand the complex anatomy. Most of our terminology derives from that used previously. However, we take the liberty of coining new terminology where no suitable terminology exists; in those cases the new concepts/structures have been assigned unambiguous descriptive terms. As we understand it, the fundamental structure in the cortical area is the paracortical cord (4). This review outlines our understanding of its elegant design which facilitates orchestrated movement of cells and soluble factors.

larger animals lobules are separated by fibrous radial bands called trabeculae. The most obvious structure within a lobule is the follicle/germinal center, the B-lymphocyte-dependent area, which will only be discussed in passing. Within the rest of the cortex, high endothelial venules (HEV) and cortical sinuses are easily discernible. Although not readily apparent, there is a repeating structural unit in the cortex, the paracortical cord, which is centered on the HEV and bordered by the cortical sinus. These units are packed together in a more or less parallel fashion to constitute the T-cell-dependent area of the cortex. The term paracortical cord was coined by Kelly to describe the anatomy of the paracortex of rabbit lymph node (4) (Fig. 1A).

To simplify the description, we start with a model paracortical cord and subsequently comment on the reality, which is always somewhat more complicated. An idealized paracortical cord in 3 dimensions would look like a set of nested cylinders. In cross-section, it would appear as a set of concentric rings (Fig. 1B). The rings are barrier-type cells and the spaces between the rings are filled with lymphocytes and other cells (Fig. 2). The center is the lumen of the HEV, the outside of the

Lymph node architecture

Paracortical cord

The LN cortex is subdivided into lobules. Although mouse LNs typically consist of a single cortical lobule, LNs of larger animals may have 10 or more. In small animals such as the rat, lobules are separated by open communicating sinuses, whereas in

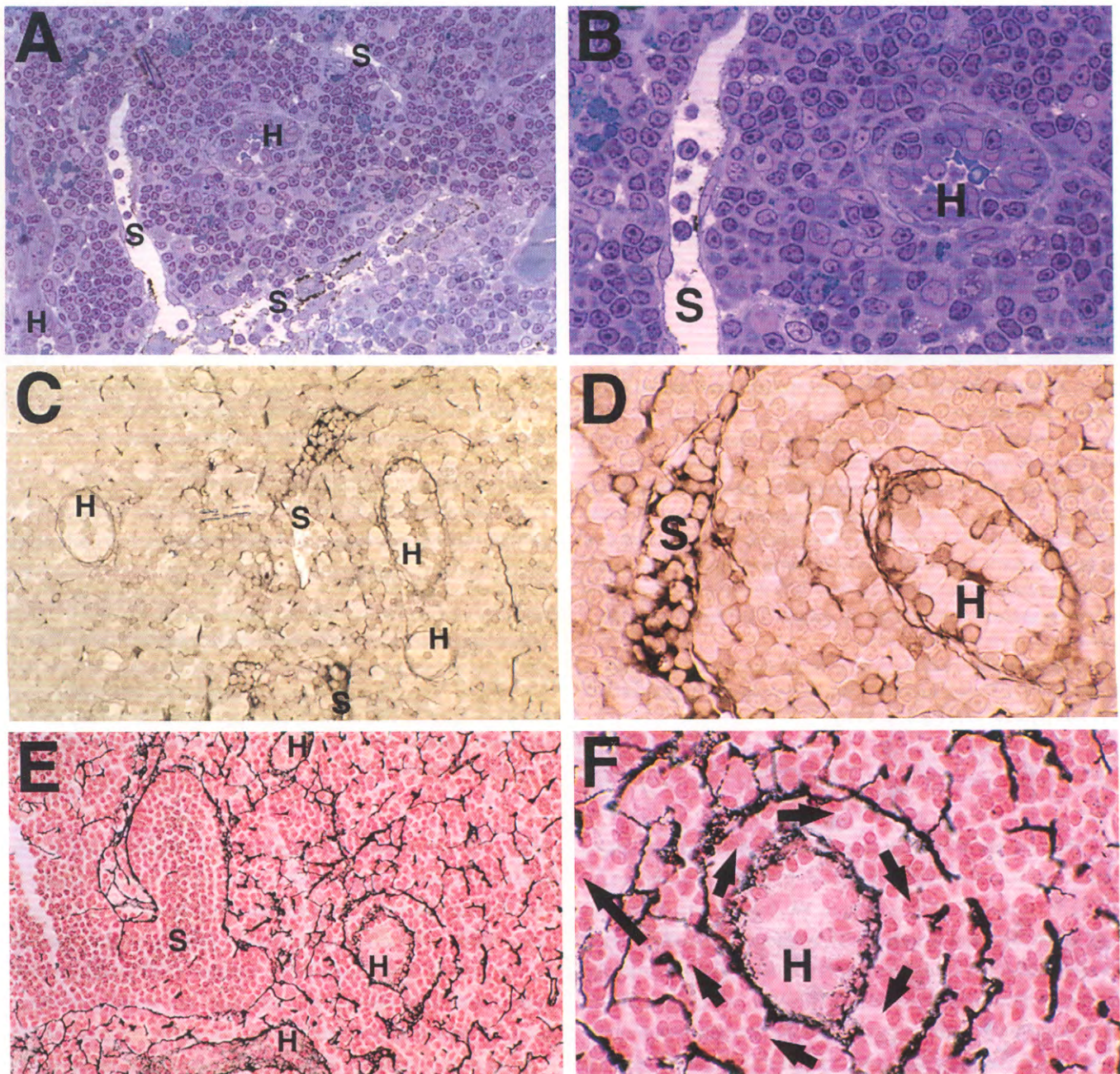


Fig. 2. Transverse sections of paracortical cords in the rat lymph node. Light micrographs of LN paracortex, centered on paracortical cords in cross-section, emphasize the concentric rings of cell-filled spaces and their cellular boundaries (low (A, C, E) and high (B, D, F) magnification). **A, B.** The HEV in the center of the micrograph (A) is surrounded by cortical sinuses – showing a paracortical cord in cross-section. All cells are stained with toluidine blue. Carbon (administered intralymphatically) decorates the lumen of the cortical sinuses.

C, D. The cortical sinus, the conduit and perivenular channels around the HEV are traced by horse radish peroxidase administered intralymphatically. The conduit appears discontinuous due to its interweaving in and out of the thin section shown. Cells appear light brown due to osmium tissue processing.

E, F. The pinwheel-shaped pathway of the corridor wrapping around the HEV is highlighted by silver stained reticular fibers (Gomorri) (as well as arrows in (F)). Cells are counter-stained with nuclear fast red stain. (H, HEV; S, cortical sinus.)

final ring is the cortical sinus space. The intervening structures and spaces form the environment through which lymphocytes must transit as they move through the LN. A representative radius of the cord is 50–100 μm , or roughly 10 lymphocyte diameters. We discuss this structure as we imagine the lymphocyte encounters and moves through it – from the inside out.

The boundary of the lumen of the HEV is the high endothelium. Lymphocytes initiate entry into the LN by binding to the luminal surface of the endothelium. This process has been the subject of much recent interest and is understood to involve a cascade of adhesion events (19–22). It is widely appreciated that the vessel wall poses a barrier to lymphocyte migration, thus requiring special transmigratory mechanisms. There are two potential routes for transmigration – between or through endothelial cells. There is evidence for both, and distinguishing between them is not essential to the current discussion (22–27). Two conceptual issues are worth stressing, however. First, the penetration of the HEV wall appears to be a-traumatic (i.e. not requiring disruption or enzymatic degradation of barriers) since tissue integrity remains intact despite incessant transit of a large number of lymphocytes. This is consistent with passage via interendothelial channels through gaps created by spot welds (macular junctional complexes) between endothelial cells. Second, if lymphocytes do transit through endothelial cells, they are never exposed to endothelial cytoplasm, but travel in an extracellular space transiently surrounded by membranes of one or more endothelial cells (23, 26, 28).

Perivenular channels

It is essential to distinguish at least 3 compartments within the cord: channels, corridors and conduits – each critical to the function of paracortex (Fig. 1B–D). As the lymphocytes cross the high endothelium, the space into which they emerge is a narrow compartment we call the perivenular channel (PVC). This compartment is actually a potential space which lies between the abluminal side of the endothelium and the surrounding pericytes and is part of the HEV (14, 29). This area has been previously referred to as the sleeve (30) or sheath (13, 14, 31, 32). However, sleeve and sheath connote boundaries rather than spaces, therefore we have adapted the perivascular channel terminology used by Sainte-Marie and co-workers (33, 34). The term perivenular channel reflects the reality that this is a space beginning immediately adjacent to the high endothelium of the HEV. We choose the more precise term perivenular in preference to perivascular especially because we believe these structures are important in the characteristic perivenular inflammatory responses seen in non-lymphoid tissues.

The perivenular channels are bounded by pericytic cells which we consider to be fibroblastic reticular cells (FRCs). FRCs are highly pleiomorphic stromal cells which can adopt flattened rectangular morphology (adjacent to HEV or sinus) or take on a tube-like shape (important in the conduit described below). We refer to this assembly of FRCs and embedded PVCs as the perivenular sleeve. FRCs circumscribe the HEV with at least 2 layers of overlapping cells. Unlike the regular ring-like structure of vascular smooth muscle, the FRCs cover the abluminal surface of the HEV in a plate-like fashion much like a shingled roof (with FRCs often centering over intercellular junctions). This arrangement creates an inner and at least 1 outer PVC which are in series and thus continuous with each other. The first PVC lies between the basement membrane of the HEV and the adjacent FRC and is an extremely narrow space, approximately 0.1 μm across in the absence of any lymphocytes. The outer PVC is bounded on both sides by FRCs. PVCs can be discerned by light microscopy when highlighted by appropriate tracer, but only incompletely (Fig. 2C, D). By electron microscopy (EM) they appear as a thin layer of collagen fibers and ECM until distended by a lymphocyte (Fig. 3A, B). Entry into the PVC appears to be rate-limiting since lymphocytes often accumulate in the abluminal endothelial space. The purpose of this delay is unclear. The lymphocyte flattens dramatically in its migration into the PVC as it expands the PVC from potential space into a channel several micrometers wide (Fig. 3). Interestingly, movement through the PVC does not appear to require crossing a basement membrane but rather movement along a basement membrane that is shared by an FRC process and a high endothelial cell (29, 35).

Lymphocytes must migrate out of the PVC into the more open space of the surrounding cord. We believe that they follow the spiral of FRCs outwards. In the inner PVC lymphocytes appear to be flattened, probably reflecting the resistance of the sleeve to distention, in contrast to lymphocytes in the outer PVCs which appear round. In normal and activated LNs, there are many lymphocytes present in the PVCs along this postulated route. Evidence indicates that lymphocytes do not linger in the PVCs, but rather migrate along them, then exit into the cord. This conclusion is supported by our findings (and those of others) that the PVCs rapidly become depleted (in 1–2 h) of lymphocytes following heparin infusion, which inhibits lymphocyte migration into LN (36–38).

Lymphocytes spend 10–100 min in the rich extracellular matrix (ECM) environment of the PVCs. Components of this ECM, particularly fibronectin and laminin, serve as excellent substrates for lymphocyte migration (39–42). In addition, other distinctive ECM components may be localized here, such

anatomic compartment which, when filled with lymphocytes, constitutes most of the parenchyma of the LN cortex. Like the perivenular channels, the corridors are bounded by FRC and some of the same ECM components. However, unlike the PVCs, which are narrow potential spaces, the corridors are typically 10–25 μm in diameter so that they readily accommodate within themselves 2 lymphocytes side by side. The corridors do not have a regular geometric arrangement; the walls are incomplete so that corridors join with and split from each other at frequent intervals (see discussion of walls below). Based on our studies of a variety of ECM components (Kaldjian et al. unpublished), as well as those of others, ECM decorates the corridor walls but is absent in the corridor space (39–42, 45, 46). This has profound implications for lymphocyte migration. First, there is no structural barrier to migration in the corridors. Second, since lymphocytes do not swim, their progress through corridors is likely to occur by active crawling – making regulated adhesive contacts with the walls of the corridors and with other cells in the corridors.

The corridor walls and the conduit

The walls of the corridors are the FRC network. This structure consists of collagen-containing reticular fibers that are enclosed by FRCs. Scanning EMs of LN stroma, after cellular material has been removed by alkali maceration, provide dramatic visualization of this reticular network as a network of collagen fibers (Fig. 4) (47). Fibers, each a bundle of perhaps 10 to 100 parallel collagen fibrils, course through the cortex, subdividing it into individual corridors. This is completely different from the tight random weave of fibers of a collagen gel generated in a

test tube. Fibers stretch from the capsule, across the subcapsular sinus, into the cortex. Some of these fibers run from sinus to HEV, others from sinus to sinus, with finer fibers interweaving among them. Those fibers which intertwine with the HEV basement membrane spiral out from the HEV much like a pinwheel. Histochemically, these fibers can be visualized in LN sections using silver-based reticulin stains which highlight collagen bundles with associated sulfated proteoglycans (Fig. 2E, F) (48).

Meshworks of collagen fibers are prominent in most tissues. However, in the LN cortex they assume a special role because of their distinctive relationship with FRCs. The collagen fibers in the LN cortex are isolated; they are partitioned into their own tiny extracellular space by the surrounding cell processes of an FRC. A given collagen fiber will be ensheathed along its length by a single layer of FRCs (Fig. 5A, B). This novel extracellular compartment of collagen fibers in the paracortex we have termed the conduit (22); this term refers to the ECM-filled space itself, not to the surrounding FRCs.

It is generally agreed that the fibers are largely enclosed by a cellular barrier (of FRCs) in the cortex, and largely unenclosed in the medulla. There is controversy about the integrity of this cellular barrier between the corridors and the conduit (that is, in the walls of the corridors). Fibers that cross the sinuses are completely enclosed by FRC-like sinus-lining cells (49–53). Disagreements arise as to how many perforations there are in the FRC coverage of the fibers in the cortex. Since we are convinced on functional grounds that the conduit space operates as a largely enclosed compartment (see below), we infer that the holes in the cellular barrier visualized in some EM

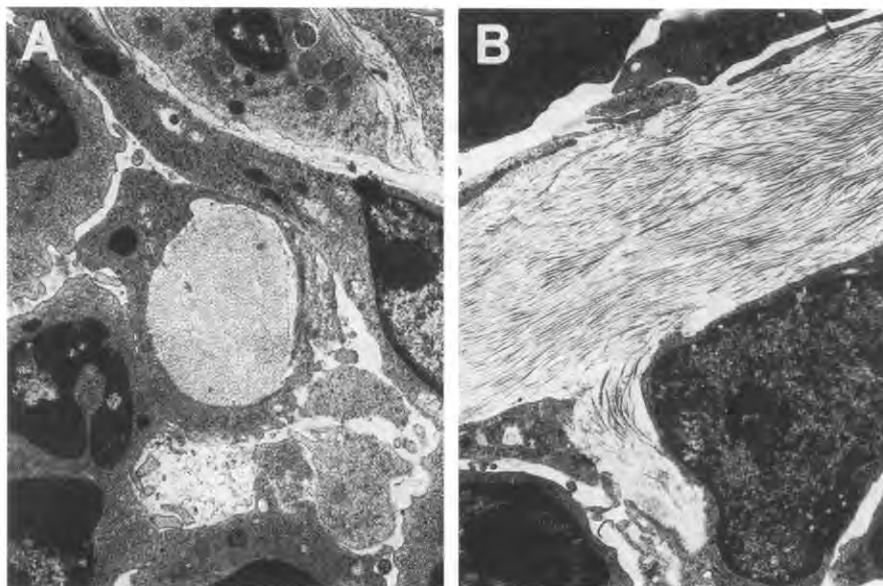


Fig. 5. An FRC-enclosed conduit in the rat lymph node cortex in transverse (A) and longitudinal (B) sections. The complete enclosure of the fiber by FRCs and other cells suggests a separate and distinct pathway for soluble material. Close contacts are evident between lymphocytes and the enclosing FRCs.

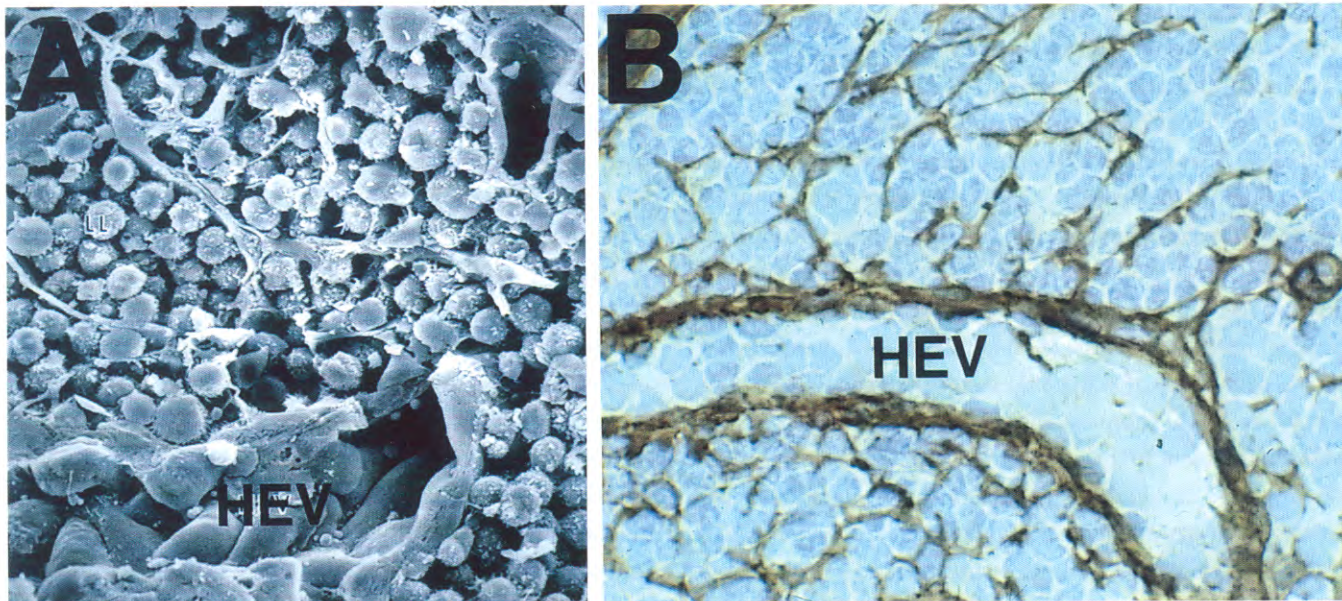


Fig. 6. Lymphocytes fill the corridors surrounding the HEV. Those in contact with the corridor wall appear to reach out and hold the FRC network.

A. Scanning electron micrograph of rat lymph node. [Micrograph reproduced with permission from Dr. Y. He. *Arch Histol Jap* 1985;48:1–15.]

B. Immunohistochemistry shows that fibronectin, an efficient ligand for lymphocyte adhesion, decorates the perivascular channels and FRC network in human lymph node. The intervening space constitutes the corridors which are devoid of fibronectin (courtesy of Dr. E.P. Kaldjian).

studies are transient in duration and are usually covered by cells (either FRCs or other resident cells, e.g. IDCs, macrophages or lymphocytes) (47, 54).

The FRC network is rich in ECM (39–42, 45, 46). However, for most of the ECM components, it has not been demonstrated whether they are exposed to the corridor space or sequestered within the conduit. Operationally, however, it is clear that the FRC network provides traction for lymphocyte migration, as illustrated by lymphocyte contact with the outer surface of the FRC network (Figs. 5 and 6). We infer that this traction is provided both by integral membrane proteins of the FRC and by ECM components produced by the FRC (Fig. 6B).

Cell-cell encounter in the corridor space

The corridors are optimally designed to foster efficient cell-cell interaction between sessile APCs and migratory T lymphocytes. Interdigitating dendritic cells (IDCs) are (understood to be) the preponderant APC in the cortex (1, 2, 55). They derive from tissue dendritic cells (such as Langerhans' cells) which migrate via lymph into the corridor space where they become immobilized on the FRC network (see discussion below). Although they constitute a small fraction of the cellular elements in the LN (perhaps 2%), they provide a very large surface area of contact due to their many long cellular processes.

We envision that T-lymphocyte encounters with IDCs occur much like the receiving line at a wedding; lymphocytes file down the corridors past the stationary IDCs “shaking hands” with each one they contact. During the handshake, when cellular processes of the T cell and IDC interact, immune surveillance occurs. At this time, the T-cell receptor (TCR) on the T cell encounters antigen presented in the form of MHC-peptide complexes on the IDC. Since the IDCs are extremely efficient APCs, rich in co-stimulatory molecules and cytokines, T-cell activation will occur if the TCR to MHC-peptide complex contact is a good fit. This process is spatially distributed throughout the entire corridor space. Judging by the time elapsed between injection of lymphocytes into the blood and their emergence in lymph, lymphocytes typically spend 3–6 h in the corridor space. During this time, we infer that they are more or less continuously migrating along the corridors to interact with multiple IDCs. Conversely, if a T cell meets its antigen, T-cell proliferation begins within the corridors.

Boundary between corridors and the sinus space

There is an outer boundary of the paracortical cord which separates it from the surrounding paracortical sinus. This boundary is made up of sinus-lining cells which are plate-like in morphology. There is no general agreement as to the identity of the

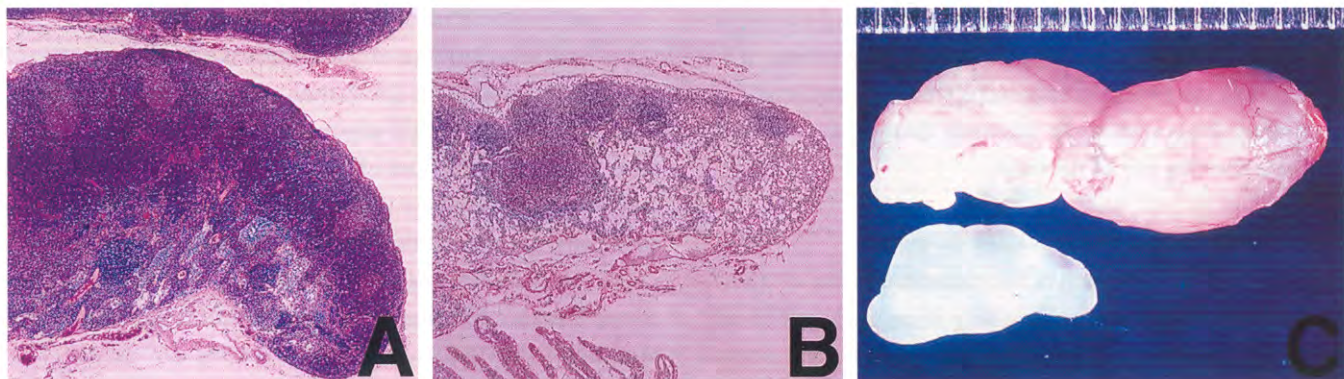


Fig. 7. The plasticity of the lymph node cortex.

Large differences in size of the LN cortex due to changes in lymphocyte population can occur in relatively short periods of time.

A. Post-denervation hypersensitivity results in a greatly enlarged lymph node with an extensive cortex – Hematoxylin and eosin stain.

B. Lymphocyte-depleted paracortex after 14 days of thoracic duct lymph (TDL) drainage.

C. Normal rat lymph node (bottom) versus rat lymph node with post-denervation hypersensitivity (7 days) (top, scale with 1 mm notches).

sinus-lining cells, endothelial or FRC in nature, or on how intact the boundary of sinus-lining cells is between cortical sinuses and the corridor spaces (29, 49, 51, 56–60). However, based on functional evidence, we believe that the sinus-lining cells form a formidable functional barrier between sinus and corridor spaces. When a variety of tracers are introduced into lymph, they do not gain free access to the corridor space. For example, when carbon particles (about 0.05 μm in diameter) are introduced into lymphatics they freely enter the cortical sinus space but are virtually excluded from the corridor space (14, 56). Furthermore, EMs which image lymphocytes moving across the sinus-lining barrier suggest that this transmigration is an active process; unlike the round morphology of lymphocytes in the corridor space, the transmigrating cells have a distorted hourglass morphology of cells working their way through a restricted space (5, 61). Thus, the corridor space and sinus space are spatially distinct compartments with regulated cellular movement between them. T lymphocytes end their journey through the cord by migrating between the sinus-lining cells into the sinus.

outwards, lymphocytes also move circumferentially around the cord through the PVC and the corridors as they spiral around the cord (Figs. 2E, F and 3).

The physical form of an idealized cord is a cylinder 100 μm in diameter by 800–1500 μm long. In addition to inwards/outwards and circumferential co-ordinates, there is an additional co-ordinate system along the long axis of the cylinder. An idealized paracortical cord typically starts either near the subcapsular sinus in the interfollicular area or at the base of a B-cell follicle. It courses towards the hilum, becoming a medullary cord as it enters the medulla. We refer to this axis as capsad/hilad because other terminologies seem misleading or cumbersome. Bloodborne lymphocytes enter the cord through HEV which begin capsad in the cortex and run hilad towards the medulla. Although endothelial transmigration occurs along the full length of the HEV, the highest frequency of transmigration occurs towards the middle segments of HEV, deep in the paracortex. This may be a function of the number of endothelial cells in the circumference of the HEV cross-section (23).

Is there oriented migration within the cortex? The overall pattern of lymphocyte migration is from HEV to sinus; however, we believe that lymphocytes entering the cords can migrate capsad or hilad, inwards or outwards, and circumferentially. Immunohistochemical stains show localized accumulations of B cells in cord-like distribution underlying individual follicles (Fig. 4 in (18)). This suggests to us that there may be increased recruitment of B cells in individual cords and that B cells migrate capsad within the cords. Conversely, T cells migrate towards the hilal end of the cord where the outer reticulin investment of the cord is incomplete (4, 62). Regulation of directional migration within the cord is not yet understood. We can think of three possible mechanisms that would impart

Overall directionality within the cord

In the foregoing discussion we have taken a largely two-dimensional view of the cord, as though lymphocytes move along a transverse cut section of the cord in a single plane. This may often occur and the concepts presented so far already provide a powerful understanding of the basic dynamics of lymphocyte traffic through the cortex. Lymphocytes move from the inside (HEV lumen) to the outside (sinuses) via 2 intervening spaces (perivenular channels and corridors). To do so, they transmute across 3 cellular boundaries: the HEV endothelium, the PVC FRC cell, and the sinus-lining cell. In their journey radially

directionality to lymphocyte traffic: i) gradients of soluble factors giving rise to chemotaxis, ii) immobilized gradients of factors/ligands giving rise to haptotaxis, and iii) directional fluid flow along the corridors. At this time, there is no compelling evidence favoring any of the three.

Cord variations from an idealized cylinder

If the foregoing description of the paracortical cord is substantially correct, why has its existence been such a well-kept secret? Probably because of its complex geometry and extreme plasticity. For ease of description we have described it as a straight uniform cylinder. In reality it is seldom either symmetrical (e.g. Fig. 2) or straight. The distance between an HEV and a sinus (i.e. the width of the corridor space) can be a few cell diameters or several hundred micrometers. Furthermore, it changes dramatically during immune responses. At some stages during such responses, lymphocytes accumulate in the corridors, resulting in marked distention of the cords. In addition, reactive LNs undergo elongation of paracortical cords (Fig. 7), together with proliferation of the HEV endothelial cells (7, 8, 63–65). Conversely, under circumstances involving depletion of lymphocytes from lymph nodes, the cord diameter shrinks as a result of fewer lymphocytes in the corridors and therefore decreased separation between individual strands of the FRC network (see Figs. 22 and 23 in (31)).

There was some early foreshadowing of the concept of paracortical cords in studies by Soderstrom (32), who described the perivenular orbits of parenchyma around the HEV. However, the complex three-dimensional structure precludes simple assessment of precisely how much of the paracortex consists of paracortical cords. Kelly's serendipitous studies with euchrysin revealed that paracortical cords are the global structure in the rabbit and, by extension, we believe, in the human. Euchrysin (or some contaminating agent in the preparation) causes sinus distention; the enlarged sinuses outline the free surfaces of the cords, allowing the cords to be traced from the medulla up through the cortex (4).

Lymph flow through the lymph node

Afferent lymph carries many elements into the LN: i) lymphocytes from tissue or upstream LNs, ii) APCs from tissue, iii) cytokines, iv) soluble antigen, and v) particulates. In general, lymph has long been thought to percolate throughout the lymph node. Exactly how does this flow intersect with the compartments of the paracortical cord?

Lymph in the sinuses

Carbon particles introduced intralymphatically trace the path of lymph flow through the LN. Carbon is carried from the afferent lymph vessel into the LN subcapsular sinus. Thereafter it follows openings in the floor of the subcapsular sinus into cortical sinuses which penetrate the cortex to connect to underlying medullary sinuses. Little carbon penetrates into the parenchyma; instead it remains in the sinuses. Operationally, this means there is a functional barrier to lymph-borne material (at least larger than 0.05 μm) between sinuses and parenchyma. Two explanations seem plausible. First, there may be no constitutive pores in the floor of the subcapsular sinus; in that case macrophages and other cells transit through the floor making their own pathways (57). Alternatively there may be constitutive pores (59) with a mechanism (e.g. efficient phagocytosis by resident phagocytic cells or perhaps positive fluid pressure in the parenchyma) by which particle penetration, but not cell movement, is impeded.

Delivery of soluble mediators through the conduit

Particulate tracers do not represent all of the material present in lymph. Lymph also carries soluble materials from peripheral microenvironments which may be timely information, important for efficient immune surveillance by lymphocytes. For example, factors in lymph are responsible for maintaining the distinctive morphology and recruiting capacity of the high endothelium (66–68). Furthermore, lymph-borne soluble factors can increase lymphocyte recruitment by the HEV within minutes (69). However, the foregoing discussion emphasizes that there are multiple boundaries between sinus and HEV. Therefore, if soluble material does cross into the LN parenchyma the conventional mechanisms of percolation and diffusion would allow only inefficient access of lymph-borne materials to the abluminal surface of the HEV.

We have previously proposed and discussed the conduit as a highly specialized system to move lymph-borne soluble information from the sinuses directly to the abluminal surface of the HEV (22, 23, 30, 31), in effect, tiny pipes. This concept of a miniaturized flow system may arouse some skepticism since it is unlike any other we know of in mammals. However, its reality is suggested by two distinct lines of reasoning and is confirmed by direct investigation. First, details of the architecture are highly suggestive; the unique enclosure of reticular fibers by FRC gave rise to informed speculation in the heyday of electron microscopy (51). Second, the teleological need for such a mechanism is strong. It is highly paradoxical that there is an elaborate anatomical isolation of lymphatics from HEV but nevertheless clear functional communication exists between

them. Third, proof of the existence of a functional conduit system comes from tracer studies (14, 23, 31 (see Figs. 11–16 for detailed structure), 70).

Molecular tracers, when administered intralymphatically, rapidly highlight the reticular network in the cortex of the LN. For example, horseradish peroxidase (HRP) administered intralymphatically traces the reticular network from the sinus to the HEV within 1 min (14). This distance averages 100 μm and is therefore unlikely to be diffusion-driven. Rather, we think that the rapid appearance of tracer within the FRC-enclosed reticular fiber suggests a conduit through which the rapid transit of selected material moves via bulk flow of fluid. In addition, directed transit of material is reflected in the advancement of the tracers, as well as changes in tracer density, from the sinuses to the HEV through the reticular network over time (14, 70).

What determines the directionality of movement within the conduit system from the sinuses to the HEV? We believe that the explanation is deceptively simple: there is almost complete enclosure of the conduit everywhere but at the abluminal surface of the HEV (23). Once fluid enters the conduit, its principal exit would be at the HEV. This raises the question of how fluid gets into the conduit from the sinus and whether there are special mechanisms to allow fluid to move from the conduit system into the HEV.

We hypothesize that some of the fluid that gets into the conduit is derived by regulated transport across sinus-lining cells (transcytosis). Indeed, the most striking microanatomical feature of the sinus-lining cells and the FRCs covering the fibers in the sinus are “the extraordinary number of simple and compound uncoated invaginations of the plasmalemma” (49). Compton and co-workers commented that the simplest interpretation for these observations was transport vesicles, but minimized that possibility since they saw no need for transport across this barrier. In contrast, transport vesicles are perfectly consistent with our model.

Fluid may also enter the conduit system as capillary transudate. Intriguingly, some fibers that course through the superficial cortex perpendicularly from the subcapsular sinus to the HEV intersect with capillaries (14). This could provide an additional source of fluid to carry material through the conduit. We envision that the enclosed conduit space conducts soluble materials much like a molecular sizing column, passing the material between the loosely packed collagen fibers. Because of the very small void volume in this conduit column, molecular transport would occur rapidly, despite low flow rates.

Conduit-delivered material at the HEV

– possible implications

HRP, administered intralymphatically, can not only be found in the sinuses and the conduit system but also in the PVCs, between high endothelial cells and in the lumen of the HEV with minimal leakage of material into the parenchyma. Conversely, after peripheral intravenous injection, there is no leakage of HRP from the HEV lumen between high endothelial cells into the PVC except when it accompanies emigrating lymphocytes (14, 31). These studies suggest a one-way passage of material through the conduit as well as the possibility that the conduit system connects directly with the interendothelial spaces of the HEV via the PVCs. The architecture of the intersection of the conduit with the HEV may explain how the tracer reaches the interendothelial junctions. The conduit is completely enclosed by FRCs all the way to the HEV. At the HEV, the fibers of the conduit intertwine with the fibers in the PVCs, and the PVCs spiral to the interendothelial junctions. This intersection remains enclosed because the last FRC ensheathing the conduit fans out like a trumpet bell and flattens out, becoming the outermost FRC plate around the HEV (23, 31). These studies are the basis of the conduit concept and have remarkable implications: i) material in the conduit system (which includes the PVCs) is completely isolated from the parenchyma so that the system is a privileged information delivery system to the HEV lumen; ii) if material moves through the conduit via bulk flow, as suggested by the capillary input, fluid pressure in the conduit exceeds that in the HEV and changes in venous pressure would then result in changes in conduit delivery; and iii) there is less resistance to flow along the PVCs and between endothelial cells than in inter-FRC junctions or other leaks in the system.

Situated at the intersection of incoming lymphocytes and conduit-delivered material, the PVC is a space in which sophisticated mixing of cells and fluid in transit is facilitated at 3 entry/exit points: i) at the interendothelial junction and the PVC, lymphocytes move into the PVC while fluid moves out into the HEV lumen; ii) at the conduit junction and the PVC, conduit fluid enters but cells generally do not move into the conduit; and iii) at the FRC junctions and the corridor space, lymphocytes exit into the corridors and presumably some conduit fluid may accompany them. What is remarkable is that sufficient fluid pressure is maintained in the PVCs with the steady lymphocyte traffic.

More than 50 years ago, Dabelow noted unique properties of the interendothelial junctions in HEVs and observed that lymphocytes cross between high endothelial cells “like ships in canal locks” (71). The design of the junction is that of a flap valve

allowing fluid movement in one direction and cell movement in the opposing direction (22). We now extend that concept, and propose that the PVCs are part of an extended closure system to allow movement of cells and fluid in opposite directions.

A corollary of these concepts is that the PVC is a specialized microenvironment which influences lymphocytes immediately following their entry via the HEV. In addition to the ECM-rich environment in the PVCs, the PVCs may be suffused with conduit fluid on its way to the HEV lumen. Chemokines, presumably delivered to the HEV via the conduit, are displayed on the surface of the high endothelium (72, 73). In addition, functional and immunohistochemical studies suggest that chemokines are present in conduit fluid (Kaldjian et al. unpublished, (69)). We predict that this combined rich environment will influence the lymphocyte migration and even differentiation.

The issue of how information gets from the lymph to the HEV and to the lymphocytes emigrating from the HEVs has received little attention. The assumption is that lymph factors percolate through the parenchyma. We think of this as the trickle-down model in which the soluble materials reach the core of the paracortical cord last. In contrast, we refer to our model of fluid movement through the conduit as the special delivery model. The two models have many operational differences. First, the special-delivery model would allow selective uptake and delivery of particular molecules, while in the trickle-down model there is no regulation of information delivery. Second, the special-delivery model allows rapid transport, whereas the trickle-down model would result in much slower delivery, especially given the boundaries between compartments which have been outlined above. Third, the special-delivery model endows the PVCs with special properties. They are the focal point for rapid delivery of factors where the emi-

grating lymphocytes are immediately exposed to factors coming down the conduit. Factors which regulate lymphocyte motility, such as chemokines, could therefore influence lymphocytes very early in their transit through the LN. Furthermore, we recognize the possibility that certain forms of antigen may be delivered via conduit to the PVC, thereby allowing extremely early antigen exposure to recirculating B cells.

Caveats

The model formulated in this review provides a conceptual framework which has been enormously helpful to us in understanding events in the LN cortex. Several of our conclusions are based on inference rather than direct demonstration, however, and remain to be confirmed experimentally. Furthermore, there are some reported structures which we have not identified and cannot yet integrate into our model, such as labyrinths (53) and the deep cortical unit (74–78).

Conclusions

Form dictates function. We believe that the functional T-cell unit of the LN is much simpler in some ways than generally understood – a simple repeating unit (the cortical cord) with geometrically simple design (concentric rings). Yet in other ways, it is much more complex – more compartments with regulated movement of fluids and cells between them. We consider that the elegant form of the paracortex envisioned in our model dictates, in large part, the marvelous efficiency of secondary lymphoid tissue in facilitating and regulating immune responses.

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