The conduit system of the lymph node

Ramon Roozendaal, Reina E. Mebius and Georg Kraal

Department of Molecular Cell Biology and Immunology, Vrije University (VU) Medical Center, PO Box 7057, 1007MB Amsterdam, The Netherlands

Keywords: fibroblast reticular cells, lymphoid compartment, subcapsular sinus

Abstract

The lymphoid compartment of lymph nodes is impermeable to many molecules that are delivered via afferent lymphatic vessels. In the lymphoid compartment, fibroblast reticular cells form an interconnected network—the conduit system. This network has a structural function supporting tightly packed lymphocytes and antigen-presenting cells; however, it also has an important function as a molecular sieve, since it contains tubules that are the only entry point for fluid and allow only small molecules and particles (including antigens) to flow along the network. This size exclusion may prevent pathogens entering the blood from lymph. Dendritic cells can sample antigens from the conduit system and present them to nearby lymphocytes; this may be particularly important in initiating immune responses. The importance of larger antigen transport via macrophages or other cells is unclear. Lymphocytes and antigen-presenting dendritic cells actively move and interact along the conduit system, perhaps in response to chemokines or cytokines transported by the conduit system; these molecules may also be transported to high endothelial venules and regulate the attraction of blood leukocytes to the lymph nodes. The conduit system is also important for fluid distribution between afferent lymphatics and blood, but the mechanisms are not yet established.

Introduction

Lymph nodes are essential for the initiation of immune responses by creating an environment in which lymphocytes and antigen-presenting cells can optimally interact. Antigen and antigen-presenting cells (as well as some lymphocytes) arrive in the lymph node with the afferent lymph, whereas the majority of lymphocytes enter the node from the bloodstream via specialized venules (high endothelial venules). The so-formed counter flow of lymphocytes and antigen-presenting dendritic cells enables rapid selection of antigen-specific lymphocytes from the vast number of cells passing through the node. Specific activation of the lymphocyte may lead to clonal expansion and further differentiation, whereas cells that do not encounter specific antigen will leave the lymph node with the efferent lymph and may try their luck in another lymph node.

With the recent advances in intravital microscopy, it has been possible to actually show these encounters of dendritic cells and lymphocytes, and this has led to better insight into the dynamics of these fascinating and important processes (1, 2). An intriguing question has been how antigen reaches the lymphoid compartment, considering that because of the anatomy of the lymph node only very small-sized particles and proteins can enter this compartment through a highly specialized conduit system. Here we describe the structure of the conduit system and discuss recent insights into its function with regard to the physiology of the lymph node.

The structure of the lymphoid compartment

To fully grasp the contribution of the conduit system for the functioning of the lymph node, it is important to realize that it is in essence part of the structural basis of the lymphoid compartment of the node, with a highly specialized function in fluid transport. Via the afferent lymphatic vessels, lymph enters the lymph node into a large subcapsular sinus leading to a slowing down and percolating of the lymph flow through this area (Fig. 1). The bottom of the subcapsular sinus, being the roof of the lymphoid compartment, is formed by sinus-lining cells. They form a basement membrane that can be actively crossed by cells that enter the sinus with the afferent lymph but is otherwise impermeable for fluids. This way a closed lymphoid compartment is created that is shielded from afferent and efferent lymph.

This lymphoid compartment is composed of a three-dimensional network of fibroblasts, creating spaces that completely filled with lymphocytes, with different niches for the T and B cells; for example, B cells are localized in follicles in the outer cortex, whereas the paracortex is rich in...
Phages can deliver antigens to underlying lymphocytes or engender lymph for pathogens. Because of their position, macrophages, which lymphocytes can leave the lymphoid compartment, at protrusions. This may serve to enhance the surface area from compartments forms a more labyrinth-like structure with many leakages. Explaining the large amounts of macrophages found in sections, the paracortex, to allow for active immigration of lymphocytes from the blood into this compartment. This means that most of the fluid that enters the lymph node via the subcapsular sinus is shunted to the medullary region of the node, simply bypassing the lymphoid compartment, and subsequently leaves the lymph node through effenter lymph vessels.

Incoming afferent lymphatic vessels distribute the lymph (plus dendritic cells, macrophages and some lymphocytes) into the subcapsular sinus. Most of the fluid passes around much of the lymphoid compartment to leave through the effenter lymphatic vessels. During this passage, it is filtered by macrophages that line the outer borders of the lymphoid compartment. Part of the lymph fluid enters the conduit system inside the lymphoid compartment and leaves the lymph via the blood circulation at high endothelial venules, which are also the main site of entry of lymphocytes to the lymph node.

The lymph node is vascularized from the lower, hilus part of the lymph node, in which parts of the venous branches are adapted, as high endothelial venules in the paracortex, to allow for active immigration of lymphocytes from the blood into this compartment. This means that most of the fluid that enters the lymph node via the subcapsular sinus is shunted to the medullary region of the node, simply bypassing the lymphoid compartment, and subsequently leaves the lymph node through effenter lymph vessels.

Particles and larger molecules are removed from the incoming lymph by a large population of macrophages in the subcapsular sinus that are positioned on top, in between and underneath the sinus-lining cells. This close association of macrophages with sinus-lining cells is probably found all along the outer boundaries of the lymphoid compartment, explaining the large amounts of macrophages found in sections of the medullary regions of the lymph nodes where the compartment forms a more labyrinth-like structure with many protrusions. This may serve to enhance the surface area from which lymphocytes can leave the lymphoid compartment, at the same time enabling more macrophages to scan the passing lymph for pathogens. Because of their position, macrophages can deliver antigens to underlying lymphocytes or antigen-presenting cells by passing the antigen over to these cells rather than by moving into the T or B cell areas (3, 4).

The structure of the conduit system

As a result of the initial, groundbreaking studies of Anderson, Shaw, Gretz and colleagues (5–8), it is now well accepted that the lymphoid compartment is a shielded unit, and although cells are able to pass across the layer of sinus-lining cells by active movement, fluid can only enter via small tubules originating between the sinus-lining cells, starting at the bottom of the subcapsular sinus and filtering out any molecule >70 kDa or with a hydrodynamic radius >4 nm. This conduit system is composed of a three-dimensional network of collagen fibers that starts at the sinus-lining cells in the upper part of the node and can be found throughout the paracortex and also in the B cell follicles from the medullary sinuses.

The fibers are produced by fibroblasts, the basic cell type that forms all the connective tissue components of our body. But, in contrast to most connective tissues where the extracellular components that are produced by the fibroblast surround the cells, in lymphoid organs a special adaptation leads to a situation where the fibroblasts called fibroblast reticular cells (FRC) surround the extracellular matrix. In addition, the FRC are connected to each other forming a three-dimensional reticulum, much like a sponge, and lymphocytes fill up the spaces of this network (Fig. 2). In addition, the extracellular matrix is highly organized in a collagen fiber network, consisting of 20–200 parallel bundles, up to 1 μ in diameter, of mostly type I and type III collagen fibers.

As a result of careful analyses by Sixt and coworkers, we now know that several stabilizing and cross-linking molecules are associated with the collagen fibers, including fibromodulin, decorin, and lumican, and that between the bundles of collagen a meshwork of fibrils can be found (9, 10). Microfibrils, consisting of fibrillins, are located in particular around the outer collagen bundles and form the connection between the bundles and a surrounding basement membrane. The complete circular ensheathment of the collagen bundles by a basement membrane forms a tube, which is the most important feature of the conduit system, with its molecular sieve probably based on the meshwork formed by collagen and connecting fibrils and associated glycosaminoglycans.

All the collagen and basement membrane are made by the FRC and completely surrounded by them, involving intracellular cell–cell contacts by each individual cell, but the FRC are also connected along the longitudinal axis by junctional complexes (7). This way most of the conduit is shielded from the lymphoid and myeloid cells that are in the spaces associated with the reticulum. This means that lymphocytes are not in contact with the constituents of the basement membrane and will have to move directly over the cell membrane of the FRC. This is facilitated by the presence of collagen IV on the outer cell membrane of the FRC (11). Collagen IV can bind and present several chemokines including CCL21 and CCL13 and may thereby play an important role in the guidance of lymphocytes across the reticulum (12). Gaps that exist between the FRC are covered by...
port of entry for lymphocytes, the high endothelial venule. Cytokines from the draining region of the lymph node to the system to transport signal molecules like chemokines and suggested that the conduit system could act as a messenger for sustaining the response it was necessary that dense dendritic cells, which gain access to the contents of the conduit by extending processes through the basement membrane (13). The interesting consequence of this structure is that in the spaces between the FRC network, there is little or no fluid present and the densely packed lymphocytes are more or less sliding along each others' cell membranes. It also means that inside the lymphoid compartment, there is no real flow, except for the flow in the inner core of the conduit, and that all the movements of the lymphocytes are based on active migration, guided by the FRC (2, 14).

The function of the conduit system

The original tracer studies (5, 6, 15, 16) that led to the discovery of the conduit system not only showed the limited accessibility of the system but also made it clear that smaller molecules could be rapidly transported from the subcapsular sinus to the lumen of the high endothelial venules, where most of the tubules of the conduit system end. This suggested that the conduit system could act as a messenger system to transport signal molecules like chemokines and cytokines from the draining region of the lymph node to the port of entry for lymphocytes, the high endothelial venule. Chemokines produced at a site of inflammation would reach the lymph node with the afferent lymph and quickly be transported to the high endothelial venules via the conduit system. The conduit system ends in a perivenular region surrounding the venule and it is assumed that the chemokines can be transported to the lumen of the vessel by active transcytosis and presented there to attract blood-borne cells, such as lymphocytes, monocytes or even granulocytes, to enter the node depending on the types of transported chemokine.

This model, known as the remote-control function of the conduit system, suggests that the immune system can sense the start of an inflammation in a remote region drained by the lymph node and is able to direct appropriate cells types to the lymph node where the response is to be initiated. This remote-control function was convincingly demonstrated in experiments in which minutes after subcutaneous administration of chemokines, these were demonstrated in the lumen of the high endothelial venules of the draining lymph node and able to attract leukocytes (17, 18).

The FRC not only transport chemokines through their conduit but also actively produce the homeostatic chemokines C-C motif chemokine ligand 19 (CCL19) and CCL21, which are ligands for C-C motif chemokine receptor 7 that is present on naive T cells (19). Remarkably, most of these chemokines seem to be associated with the basement membrane of the conduit, not accessible for the T cells. It is unclear whether this is a matter of misleading experimental detection or whether the FRC actively shuttle chemokines from their basal side to the apical side of the cells. It is clear that these chemokines function to keep the T cells in an active migratory state, whereby they continuously move along the FRC and associated dendritic cells. CCL19 is also involved in the homeostasis of T lymphocytes, together with the survival factor IL-7, which is also produced by the FRC (20).

But what about antigens? It is obvious, on the basis of the anatomy of the lymphoid compartment, that antigens >70 kDa cannot enter, and only rather small antigens or part of antigens enter the conduit system to be transported to the T and B cell areas. With regard to T cells, in the T cell zone lymphocytes have no access to the antigen because it is shielded from them inside the basement membrane of the conduit. Only resident dendritic cells, associated with the conduit, can gain access to the contents of the conduit by crossing the basement membrane with their cell extensions. This way they can pick up antigens and will be able to present them to the T cells that pass by.

That this type of presentation is actually happening was demonstrated by Itano et al. (21) using an elegant system to discriminate between unprocessed and MHC-presented antigen. However, these authors also clearly showed that resident dendritic cells can initiate a first T cell activation but that for sustaining the response it was necessary that dendritic cells from the site of antigen entered the node. This need for a second wave of antigen presentation suggests a threshold system by which the rapid sensing of antigens and chemokines of the draining region via the conduit leads to an immediate alertness of the lymph node that can contribute to a more efficient immune response when, hours later, activated antigen-presenting cells will eventually arrive.
in the lymph nodes. If this does not occur, the alertness subsides and T cell activation seems to wane.

Although it is less elaborate than in the T cell areas, the B cell compartments of the lymph node (the follicles) also contain an FRC network with a conduit system. It is as yet unclear whether this follicular conduit system has a role in the transport of small antigens to B cells. Gaps (0.1–1.0 μ) have been detected in the subcapsular sinus floor by means of electron microscopy (22, 23) showing that antigen may passively diffuse into lymph node follicles. However, the size of these gaps appears to be inconsistent with the fact that a similar size restriction exists in antigens that have entered either the follicles or the T cell area conduit (8). Higher molecular weight (>70 kDa) antigens were shown to translocate across the subcapsular sinus floor on the surface of subcapsular sinus macrophages (3, 4), but antigen-specific B cells acquire small soluble antigens at a substantially higher rate (24). Thus, it is likely that the nature of the antigen at least in part determines the mode of transport. It remains to be seen whether there are qualitative differences between different modes of antigen transport into B cell follicles and whether conduits play a role.

An important function of the conduit system seems to lie in fluid distribution since the interstitial lymph that enters the conduit from the afferent lymphatics is directly drained from the conduit system into the venules of the lymph node. It is not clear how the fluid distributes itself into the conduit. An active pumping of the interstitial fluid transported by the afferent lymphatics into the conduit has been suggested with active pumping of the interstitial fluid transported by the afferent lymphatics into the conduit has been suggested with a likely role for the sinus-lining cells, perhaps through high transcytotic activity. However, as pointed out by Lämmerman and Sixt (9), it is unclear how such a pressure can be maintained through a complex and interconnected tubular network. These authors, assuming that the conduit is the only open space where fluid can enter, propose that all significant fluid movements are occurring inside this system. The driving force for the movement is the continuous filtration in the capillaries in the draining interstitial tissues that is based on the differences in blood pressure and interstitial pressure, whereby suction of the lymphatic vessels maintains a continuous flow into the lymph nodes.

Since it has been well documented that the afferent lymph contains lower amounts of proteins than the efferent lymph, this concentrating effect may result from the rapid translocation of part of the lymph fluid directly into the bloodstream via the conduit, thereby excluding larger, and probably the majority of, proteins. Alternatively, it has been suggested that water transport from the conduit into the venules of the lymph node is mediated by pumps, aquaporin channels, that are able to actively transport water across sinus-lining cells and endothelial cells from the lymph node, where these channels are highly expressed (25). It remains to be seen whether the activity of these channels is sufficient to achieve the amount of water transport that is associated with the normal physiology of the lymph node.

Apart from lymph nodes, conduit systems have also been described for spleen and thymus (26, 27). In both organs, the conduit system gets its fluid directly from the blood and the direction of the conduit also suggests an ending into capillaries or venules. It is obvious that its function in these organs is therefore much more important as a transport system for signaling molecules like chemokines and cytokines and small antigens, than as a system for fluid homeostasis.

An important issue of the conduit system is the size exclusion, which is remarkably set at ~70 kDa. Considering the fact that a sizeable part of the afferent lymph is swiftly led into the blood by the conduit, its size exclusion effectively prevents pathogens like bacteria and viruses from entering the bloodstream. Instead, any large particle arriving with the lymph is bound to be caught by one of the many potent macrophages in the subcapsular sinus and medullary region. In addition, dendritic cells in the sinus can actively ingest and process larger antigens. Without the size exclusion, the filter function of the lymph node would severely be impaired.

**Concluding remarks**

The unique organization of the connective tissue of the lymphoid compartment of the lymph node creates a relatively fluid-free environment in which densely packed lymphocytes can interact with dendritic cells, moving along the network of FRC. The only fluid movement in this compartment is in the conduit system that transports part of the incoming lymph from the subcapsular sinus directly into the blood system. Because of the intriguing size exclusion of the conduit system, no pathogens can directly enter the bloodstream from a draining region via the conduit of the lymph node. The constant transport of fluid from tissue into the blood may play a role in the homeostasis of tissue fluids. In addition, an important role for the conduit is in the ability to transport chemokines and antigen fragments from a draining tissue. The antigens can be picked up by dendritic cells associated with the conduit, whereas the chemokines, being exposed on the luminal side of the high endothelial venule, can help to attract cells from the bloodstream into the node. This remote sensing of the conditions in areas drained by the lymph node helps to prepare the lymph node for upcoming infections and can lead to a rapid and efficient immune response, tailored to the occasion.

**Abbreviations**

CCL19 C-C motif chemokine ligand 19
FRC fibroblast reticular cells

**References**


