Review

Communication, construction, and fluid control: lymphoid organ fibroblastic reticular cell and conduit networks

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Fibroblastic reticular cells (FRCs) are a crucial part of the stromal cell infrastructure of secondary lymphoid organs (SLOs). Lymphoid organ fibroblasts form specialized niches for immune cell interactions and thereby govern lymphocyte activation and differentiation. Moreover, FRCs produce and ensheath a network of extracellular matrix (ECM) microfibers called the conduit system. FRC-generated conduits contribute to fluid and immune cell control by funneling fluids containing antigens and inflammatory mediators through the SLOs. We review recent progress in FRC biology that has advanced our understanding of immune cell functions and interactions. We discuss the intricate relationships between the cellular FRC and the fibrillar conduit networks, which together form the basis for efficient communication between immune cells and the tissues they survey.

Coordinating communication for immune responses

> The fundamental problem of communication is that of reproducing at one point either exactly or approximately a message selected at another point. … The significant aspect is that the actual message is one selected from a set of possible messages. The system must be designed to operate for each possible selection, not just the one which will actually be chosen since this is unknown at the time of design (C.E. Shannon [1]).

The coordinated interactions of different immune cell populations facilitate the recognition of pathogens or cancer cells to precisely orchestrate the balance between immune activation and regulation. The establishment and maintenance of this balance relies on efficient and accurate transmission of information from inner and outer body surfaces and the communication of this information between diverse immune cell populations. According to Shannon's work on the transmission of radio signals in the 1940s [1], a data communication system is composed of three elements: a source of data, a communication channel, and a receiver. The fundamental problem of 'communication' – as expressed by Shannon – is for the receiver to be able to identify what data were generated by the source, based on the signal received through the channel. At a conceptual level (and paraphrasing Shannon's concept), 'information' can be defined as the resolution of uncertainty. In other words, the analogous loss of data in information transmission systems, also known as Shannon entropy, requires dedicated means and protocols to secure optimal communication. The immune system has – at least partially – resolved the uncertainty in processes underlying the activation and regulation of innate and adaptive immune cells through the establishment of dedicated hubs for immune cell interactions. In this review we highlight recent findings on how the cellular system of lymphoid organ fibroblast and non-cellular **extracellular matrix (ECM**; see Glossary) networks combine to facilitate the transfer of information from tissues and body surfaces

Highlights

FRCs use polarized microtubule networks to direct the secretion of ECM components and construct the mammalian conduit network.

The conduit network functions as a filter that monitors fluid draining peripheral tissues, and as an export system for the systemic dissemination of locally produced molecules including antibodies.

Emerging evidence indicates that there are mechanosensitive mechanisms that control fluid flow through secondary lymphoid organs, and that are important for tissue homeostasis and immune function.

Fluid flow through conduit systems is robust, and the conduit network remains functional throughout the perturbation of lymph node expansion in response to immunogenic challenges.

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to **secondary lymphoid organs (SLOs)**. Moreover, we discuss the current state-of-the-art fibro-blastic **stromal cell** research and how the understanding of lymphoid organ fibroblast and ECM networks can help to further elucidate the essential rules underlying communication and engagement in the mammalian immune system.

Information flow in lymphoid organs

SLOs, namely the **splenic white pulp**, lymph nodes (LNs), and **Peyer's patches**, are perma-nent structures that are generated during embryonic or postnatal development and are present throughout the mammalian body [2]. The basic design principles underlying SLO tissue architec-ture facilitate the coordinated interaction of immune cells in dedicated microenvironments such as an antigen-sampling zone, adjacent **B cell follicles**, a **T cell zon**e, and an immune cell egress zone (Figure 1A, Key figure). Immune cells can congregate in SLOs because they possess spe-cific blood vascular entry channels, which appear, in LNs and Peyer's patches, as specialized postcapillary venules termed **high endothelial venules (HEVs)** [3]. Immune cells recirculating through the tissues can also access SLOs via the antigen-sampling zone, as shown for activated T and B cells entering the LN via the subcapsular sinus [4], or the splenic white pulp via the mar-ginal sinus [5]. Crucial immune cell interactions occur in spatially defined niches such as the prim-ing of naïve T cells by dendritic cells in the T cell zone [6], the instruction of B cells by helper T (Th) cells at the T–B cell zone border, and the affinity maturation of antibodies during the **germinal center reaction** in the B cell zone [7]. Thus, the processing of immunologically relevant informa-tion received from tissues and outer body surfaces, and communication among immune cells, are ensured in different SLOs through standard processes in functionally equivalent environments.

A further principal function of SLOs is to act as a molecular filter and immunological screening sys-tem for extracellular fluids, exemplified by LNs, which form an integral part of the lymphatic system to maintain fluid homeostasis throughout the body [8]. The extracellular liquid generated in all body tissues is collected in initial lymphatic vessels that contain primary flap valves, which permit the collection of interstitial fluid while simultaneously preventing the intralymphatic fluid from leaking out [9]. The initial lymphatics further converge on collecting lymphatic vessels, which rap-idly transport the lymph through intrinsic and extrinsic pumping mechanisms towards the draining LNs (dLNs) [10,11]. The fast access of lymph-borne antigens and inflammatory mediators to congregated immune cells in the LN parenchyma is essential for the immunological surveillance of tissues [12]. Moreover, intestinal immune processes are initiated, sustained, and regulated in gut-associated lymphoid tissues, which include the Peyer's patches in the small intestine [13]. Immune cells congregated in Peyer's patches are covered by a single-cell layer of epithelial cells and have direct access to antigens and immunomodulatory substances derived from vari-ous bacteria, viruses, and fungi in intestinal fluids to stimulate the production of vast amounts of protective immunoglobulin IgA [13]. Antigen-containing fluid is funneled directly from the intes-tinal lumen into the subepithelial dome region of the Peyer's patch by specialized microfold (M) epithelial cells, thereby facilitating direct activation of B cells [14] and continuous liquid flow into the Peyer's patch parenchyma [15]. Extraction and processing of immunologically relevant information from the systemic circulation is coordinated in the splenic white pulp [5]. Furthermore, blood-borne antigens released via ramifications of central arterioles into the marginal sinus are transferred to the white pulp by marginal zone B cells and macrophages [5]. The marginal zone, which segregates the red and white pulp in mice and rats, functions as an antigen-sampling zone and is inhabited by CD169^{+} metallophilic macrophages [16]. Similar myeloid cell subsets line the subcapsular sinuses in LNs or reside in the subepithelial dome of Peyer's patches [17–20]. Lymphocyte trafficking between the splenic white and red pulp is regulated in bridging channels, which provide avenues for naïve lymphocyte entry into the white pulp [21]. In addition, the accumulation and distribution of B cells in the white pulp depends on the presence of the

Key figure

Paradigmatic mammalian secondary lymphoid organ (SLO) structure and the underlying fibroblastic reticular cell (FRC) and conduit networks
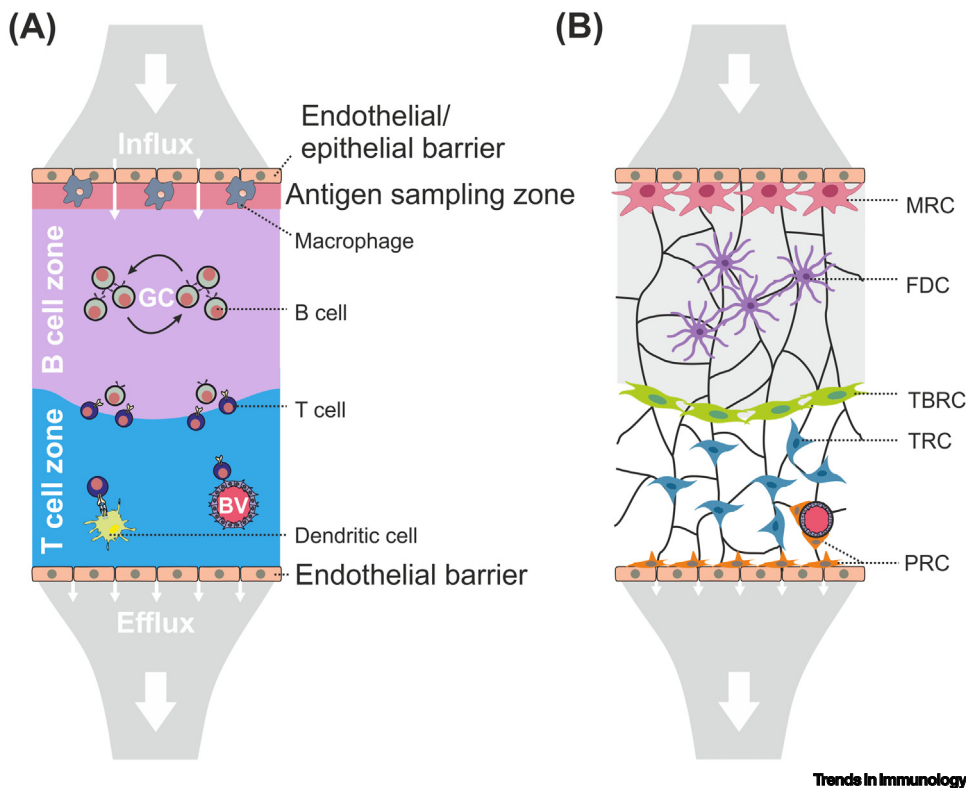

Figure 1. (A) Immune cells enter SLOs through different entry channels and pass through either the endothelial or epithelial cell layers that line the antigen-sampling zone. Macrophages and other myeloid cells closely interact with barrier-forming cells, for example lymphatic endothelial cells lining the floor of the subcapsular sinus in LNs, and capture soluble and particulate antigens from incoming fluids. B cells are activated in the B cell zone, whereas T cell activation occurs upon interaction with dendritic cells in the T cell zone. Specialized blood vessels (e.g., high endothelial venules in LNs and Peyer's patches) facilitate the entry of lymphocytes and myeloid cells from the bloodstream. Immune cells leave SLOs through dedicated exit channels through endothelial barriers. (B) The zonal organization of SLOs is underpinned by specialized FRC subsets. FRCs generate and ensheath extracellular matrix molecules that are arranged in fibrillary strands. The interconnected conduit network links fluid flow between the influx area, blood vessels. and the efflux area [26,97]. Abbreviations: BV, blood vessel; FDC, follicular dendritic cell; MRC, marginal reticular cell; PRC, perivascular reticular cell; TBRC, T–B cell zone reticular cell; TRC, T cell zone reticular cell.

endothelial plasmalemma vesicle-associated protein (PLVAP) [22] – a molecule that forms endothelial diaphragms and regulates liquid homeostasis through the control of serum protein leakage [23], as shown by the reduction of IgM$^+$ B cells in the spleens of mice lacking endothelial expression of PLVAP (*Plvap$^{ECKO-Tie2}$*) relative to wild-type mice [22]. Likewise, lymphatic endothelial PVLAP expression controls the entry of lymphocytes and soluble antigens into the LN parenchyma, as evidenced by lymphocyte immigration to popliteal LNs (pLNs), which was almost threefold higher in PLVAP KO mice (*Plvap$^{-/-}$*) compared with wild-type mice [24]. Thus, communication between SLOs, and the activation of immune cells within SLOs, are closely linked to the regulated flow of liquids through different functional compartments.

The structural scaffold of the distinct microenvironmental niches that harbor different sets of innate and adaptive immune cell populations is formed by specialized lymphoid organ fibroblasts, referred to as **fibroblastic reticular cells (FRCs)** [6,7,25] (Figure 1B). FRCs generate ECM proteins that are uniquely arranged to form a fibrillary mesh known as the **conduit network** [26,27]. FRCs exhibit apicobasal polarity [28] and completely ensheath the conduits. Original tracer studies in rat LNs determined that the conduit system filters and channels liquids from the antigen-sampling zone towards the venous vasculature and the immune cell egress zone (Figure 1B) [29]. Thus, FRCs and FRC-generated conduit networks are at the nexus of immune cell communication and fluid control in SLOs.

FRCs construct the conduit system

Distinct FRC subsets underpin the microenvironmental niches of SLOs to control immune cell positioning and to provide niche factors such as IL-7 [30] and BAFF [31] for immune cell maintenance and differentiation [32]. FRC subsets across all SLOs can be grouped according to the immune cells they interact with and their anatomical location (Figure 1B). For instance, marginal reticular cells (MRCs) underpin the antigen-sampling zone, **follicular dendritic cells (FDCs)** build the germinal center, T–B border reticular cells (TBRCs) form the communication zone of Th and B cells, **T cell zone reticular cells (TRCs)** facilitate DC–T cell interactions, and **perivascular reticular cells (PRCs)** connect the FRC network to the blood vascular system [32]. The development and molecular definition of FRC subsets, as well as their interaction with immune cells, have been recently discussed [6,7,25] and are briefly summarized (see Figure I in Box 1). However, what is universal to every FRC subset is the ability of classical fibroblasts to generate and organize the ECM [32,33]. We focus here on ECM generation, the construction and maintenance of the conduit network, and how the FRC-generated conduit network contributes to liquid flow through the SLO parenchyma, as well as how it impacts on immune cell communication under homeostatic conditions and inflammation.

The ECM is present throughout all developing and adult tissues, providing not only structure and scaffolding but also biochemical signals and mechanical cues to direct the behavior of residing cells [34]. There is broad heterogeneity in matrix composition and arrangement between tissues [35]. The ECM structures within SLOs are atypical, however, compared to other organs where fibroblasts reside surrounded by matrix structures; in SLOs the secreting fibroblasts unilaterally deposit and bundle matrix basolaterally into the conduit network they ensheath [28]. Moreover, the polarized secretion of the ECM is orchestrated by the tethering of microtubules adjacent to focal adhesions between the FRC and the basement membrane [28]. Silencing of pleckstrin homology-like domain family B member 2 (LL5β) and cytoplasmic linker protein (CLIP)-associated protein (CLASP) – which tether microtubules to the plasma membrane – significantly reduces ECM secretion by murine FRCs *in vitro* relative to controls [28], suggesting that FRCs use the same polarity mechanisms as epithelial cells to direct ECM secretion [36]. FRC–FRC and FRC–matrix adhesions to basement membrane proteins such as laminin 8 and 10, which contribute to barrier function in the vascular endothelium of the blood–brain barrier [37], provide a physical cellular barrier between the conduit ECM and trafficking immune cells, thereby sealing the conduit system [38]. Furthermore, microarray analyses [33] and single-cell RNA sequencing (scRNAseq) analyses [32] have identified cadherin-11 as a favored candidate to facilitate homotypic FRC–FRC interactions for FRC network formation, but the mechanisms facilitating FRC network formation and ECM wrapping are still not understood.

The conduit system was first detected in mice as a dispersed reticular structure within LNs [26,39,40]. Researchers observed a network of ECM fibers connected to both the **fibrous capsule** and sinusoid medulla of the LN [39,41,42]. Electron microscopy showed a tight

Box 1. Paradigmatic FRC subsets as defined by single-cell transcriptomics

The term reticular cells was first used in 1968 when a network of thin filamentous processes traversing the B cell follicle of the LN [98] and the spleen [99] was described. Fibroblasts showing these particular topological properties were also found in Peyer's patches [100]. The most common description for FRCs is still based on the expression of podoplanin (PDPN) and absence of the endothelial marker CD31 [30]. Genetic targeting of FRCs in mouse models, for example, based on their signature chemokine expression (*Ccl19*–cre) [101,102], permits the elaboration of the developmental origin, differentiation dynamics, and immunological functions of FRCs *in vivo*. In addition, single-cell transcriptomic analyses have provided high-resolution maps of FRC heterogeneity and differentiation trajectories [32,91,92,103–106] (Figure IA). The major FRC subsets can be assigned to distinct niches within SLOs that correspond to the antigen-sampling regions (MRCs), B cell follicles and germinal centers (FDCs), T–B borders (TBRCs), T cell zones (TRCs), and the perivascular space (PRC). The LN medulla, an anatomical compartment that is unique for this SLO, harbors an additional, largely perivascular FRC subset termed medullary reticular cells (MedRCs) [103,107]. FRC subsets across all SLOs exhibit partially overlapping gene signatures and chemokine gradients that reflect the particular properties of the different microenvironmental niches (Figure IB). MRCs in the antigen-sampling regions express high amounts of *Madcam1*, *Tnfsf11* (encoding RANKL), and *Cxcl13*, whereas FDCs are equipped with complement and Fc receptors to capture and present immune complexes to B cells, for example *Cr2* and *Fcer2a*. TBRCs express low amounts of *Cxcl13*, *Cxcl12*, *Ccl19*, and *Ccl21a*, whereas TRCs maintain the highest expression of *Ccl19*, *Ccl21a*, and *Il7* [32]. PRCs surrounding blood vessels express *Cd34*, *Ly6a*, and *Pdgfrb* – markers that have been associated with stemness and precursor potential [91,93]. The genes encoding ECM proteins forming conduits, for example *Col1a1*, *Col4a1*, and *Dcn*, are expressed by all FRC subsets [32]. Thus, FRCs underpin immune cell communication in SLOs by elaborating migration pathways, providing growth and differentiation factors to immune cells in specific niches, and regulating flow and antigen display [6].
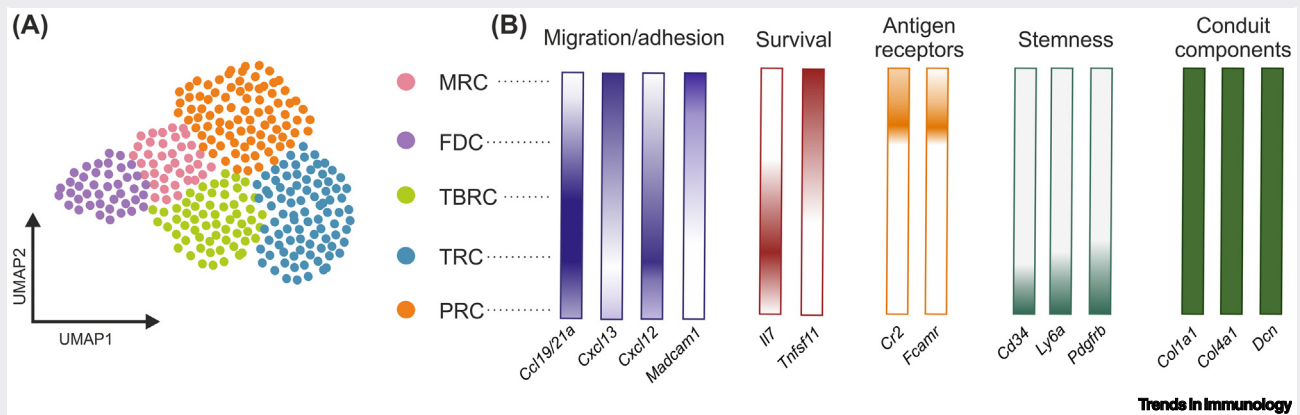

Figure I. Representative diagram of murine single-cell transcriptomics. (A) The FRC landscape in SLO, as revealed by uniform manifold approximation and projection (UMAP), with major FRC subsets as indicated. (B) Gene expression gradients for prototypical genes involved in key FRC functions.

association of fibroblast-like cells with these fibers, which suggested a pipe-like system spanning the LN parenchyma [43]. Using horseradish peroxidase injectable tracers and fluorescein isothiocyanate (FITC)-conjugated antigens in rats, draining lymph was proven to filter through these structures – first termed 'cortical pathways of migration of circulating lymphocytes' [29] – which did not diffuse through the LN tissue [44]. Later mouse studies showed that conduit networks are also present in the thymus and other SLOs, including the spleen and Peyer's patches [15,45,46].

The cable-like structure of the conduit ECM ranges between 0.3 and 2 μm in thickness (average 1.1 μm) in murine resting LNs [27]. The conduit includes all three core **matrisome** categories – collagens, glycoproteins, proteoglycans, plus ECM crosslinkers such as collagen XIV and LOX [33], as well as modifying enzymes, including matrix metalloproteases MMP9 and MMP14 [33]. Both fibrillar and non-fibrillar ECM proteins are bundled together in distinct layers that were first identified in early immunostaining studies of both mouse and human tissues [47]. The center of the conduit lumen, a fibrillar core, is composed of collagen I, collagen III, and collagen VI [38,47,48], together with other non-fibrillar components, including collagen XIV, decorin, biglycan, and fibromodulin [33], which are thought to control the stability and thickness of the fibrils [49,50]. Electron microscopy of mouse tissues revealed around 100 collagen fibers in cross-section, each fibril measuring approximately 13.29 ± 2.43 nm in diameter [28,51]. The

outer layer of the conduit, that is in direct contact with the FRCs, is a basement membrane which contains the characteristic proteins collagen IV, perlecan, laminins 5, 8, and 10, fibronectin, entactin, heparan sulfate, vitronectin, and tenascin [33,38,47,48,52]. Laminins 8 and 10 are crucial for the barrier function of the blood endothelium [37,53] and may perform a similar role in sealing the conduits, although this remains to be further investigated. Between the basement membrane and the fibrillar core is a third layer, termed the microfibrillar zone, that is composed of a protein recognized by the antibody ER-TR7, or proteins such as fibrillin 1 and fibrillin 2, that bridge the two other layers [33,38].

Conduit systems filter fluid in LNs, spleen, and Peyer's patches. Similarly to LNs, splenic conduits in mice are demarcated by a protein recognized by ER-TR7, and are ensheathed by FRCs [46]. However, in the absence of afferent lymphatic vessels, the spleen samples blood-borne antigens [5]. Differential expression of ECM components in mice demarcates microniches in the spleen that support immune cell compartmentalization; for example, endothelial cell-specific deletion of laminin α5 (*Lama5*$^{-/-}$) reduced the numbers of CD23^{low}/CD21^{high} marginal zone B cells in that niche relative to wild-type mice [54]. Conduits in Peyer's patches extend from the subepithelial dome and project into both the B cell follicle and interfollicular regions, carrying absorbed fluid from the intestinal lumen through the Peyer's patches [15]. Piezo1 plays a fundamental role in FRCs to support HEV function in Peyer's patches, but is not required in LNs; this raises the question of whether LNs might use an alternative mechanism of mechanosensing [15]. Of relevance, prolonged disruption of fluid flow in the Peyer's patch conduit system in mice through genetic deletion of *Piezo1* in *Ccl19*-expressing stroma (*Ccl19*-cre; *Piezo1*$^{fl/fl}$) impaired homeostatic lymphocyte migration and reduced mucosal antibody responses compared to control mice [15]; this further highlighted the functional connection between immune regulation and fluid balance in SLOs. Thus, the tissue architecture places FRCs as key stromal cells linking immune function to fluid homeostasis.

Filtering and access of inflammatory mediators to the SLO parenchyma

The conduit system encompasses a fast-track communication system for immune cells to directly sense inflammation and infection in draining tissues. Soluble tracers can reach the conduit in a few minutes, suggesting that this occurs by bulk flow of fluid rather than by diffusion [29,44]. Attempts to measure the flow rate through the LN conduit system (from the subcapsular sinus and follicular conduits into the cortical network) in mice have estimated ~35 μm/minute [55]. Entry of molecules into the conduit system from the afferent lymph is tightly controlled, with the molecular radius as a physical cutoff [26,38]. Large particles (>5 nm) may enter the LN subcapsular sinus via the afferent lymph, but are physically excluded from entering the conduit [44,56]. Soluble molecules larger than 70 kDa are also predominantly excluded from the conduit [56]. Lymphatic endothelial cells (LECs) lining the subcapsular sinus floor express PLVAP, a type 2 transmembrane glycoprotein that forms homodimers and generates diaphragm structures that flank 60 nm diameter transendothelial channels [24]. These diaphragm structures with radial fibrils at the rim make a pore of ~5.46 nm, and were first observed in endothelial cells by electron microscopy [57]. In constitutive PLVAP knockout mice (*Plvap*$^{-/-}$) generated from hybrid intercrosses containing a mixture of BALB/c, C57Bl/6N, and NMRI backgrounds, both large and small antigens enter the conduits, unlike wild-type mice, confirming that PLVAP acts as a physical barrier that regulates the entry of soluble antigens to the conduit system [24]. Moreover, size exclusion is maintained during LN expansion [28], despite extensive conduit remodeling. However, additional questions have since been raised because researchers detected low titers of intact vaccinia virions (virion diameter 250 nm) within the conduits of pLNs following footpad injection in mice [58]. The low viral dose that was permitted entry through the conduit was hypothesized to allow direct and rapid T cell activation; thus, this study made it clear that there is still
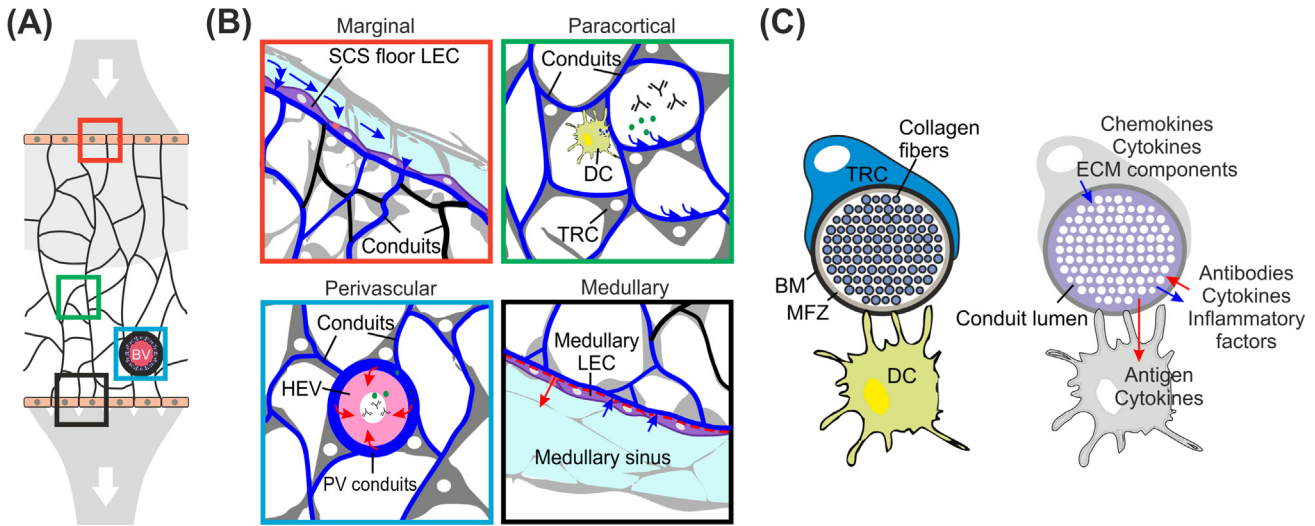
much to be discovered about both the physical structure of the conduit and its direct immune activation functions.

Resident antigen-presenting cells are predominantly associated with the conduit system in the T cell zone of LNs, where they likely adhere to the conduit basement membrane proteins laminin 10 and fibronectin [38,59]. Antigen-presenting cells can sample and process antigens directly from the conduit [60,61]. In B cells areas, FDCs support the conduit system and facilitate the direct access of B cells to lymph-borne antigens, which are essential for naïve B cell activation [62,63]; this was shown in mice in which fluorescent wheat germ agglutinin (WGA) was readily captured from conduits by FDCs [62,63]. However, it has been technically challenging to experimentally address the relative contributions of direct antigen sampling versus cross-presentation of antigens by migratory antigen-presenting cells to adaptive immune cells, and this is an issue that remains to be further addressed.

The conduit also transports immunomodulatory molecules. Although large molecules are predominantly excluded from entering the conduits from the afferent lymph, they can be transported within the conduit and exported from the tissue when locally produced within the lymphoid tissue [55]. Locally produced IgM has been measured within conduits in reactive LNs in mice, and exits the organ via HEVs and medullary sinuses [55]. This export system can ensure the rapid distribution of protective antibodies to halt pathogen dissemination [55]. Chemokines originating in the periphery, or produced locally within the lymphoid tissue, mediate the recruitment and spatial organization of immune cell populations. Furthermore, tumor-derived inflammatory factors can prime or suppress immune responses in dLNs via the conduit [64]. For instance, clinically negative sentinel LNs from breast cancer patients display hampered activation of resident DCs, and increase regulatory T cell (Treg) numbers and T cell anergy relative to controls, as measured by flow cytometry of resected LNs [64]. Thus, the specialized architecture of the conduit system in SLOs directly links peripheral tissues to immune cell populations, thereby allowing rapid exchange of immunologically relevant information. Going forward, understanding the interplay between fluid homeostasis and immune cell function can provide a more complete picture of SLO functions.

Conduits manage fluid control

Although the primary role of the blood circulatory system is to deliver oxygen and nutrients to organs and tissues, the lymphatic vascular system returns fluid collected from the interstitium to the venous circulation to maintain body fluid balance [8,65]. The arterio-venous blood circulation meets the lymphatic system in LNs and Peyer's patches (Figure 2A). Filtering fluid through the conduit network provides an opportunity for immune cells to detect pathogens before fluid is returned to the blood. Afferent lymph is distributed through LNs via both the subcapsular sinus, that engulfs the parenchyma, and the conduit system that pervades the different immune cell-containing compartments (Figure 2B) [26,40]. Computational modeling of LN fluid flow has revealed that 90% of the incoming lymph is carried through the subcapsular and medullary sinuses, whereas only 10% is sequestered through the conduit system [66]. The conduit network emerges from the subcapsular sinus, transports fluid through the B cell follicles, and increases in density in the T cell zone [27]. Conduits exchange fluid at the perivascular interface with HEVs (Figure 2B). Lastly, fluid is carried to the cortical and medullary sinuses of LNs leading the lymph flow out of the organ through the efferent lymphatic vessels and back into systemic circulation [40]. Fluid is reabsorbed via blood capillaries and HEVs in the LNs, resulting in higher total concentrations of soluble proteins in the efferent lymph than in the incoming afferent lymph, as measured by cannulation studies in rats [67–69]. However, specific proteins such as albumin, which control **oncotic pressure**, are efficiently phagocytosed within the tissue, and are reduced in concentration in efferent lymph to 2–20% of their pre-nodal concentrations, thereby

Figure 2. Fluid flow through the mammalian lymph node (LN) conduit system and transport of immunological effector molecules. (A) The conduit network pervades all microenvironmental niches of secondary lymphoid organs (SLOs). Boxes highlight SLO regions (magnified examples in B) that are essential for flow dynamics and cellular communication. (B) Marginal zone (red box): afferent lymph flows through the supcapsular sinus (SCS) and pervades the LN tissue via the conduits after filtering through valves at the floor of the lymphatic endothelial layer. Paracortical zone (green box): the content of the conduits changes while flowing through the cortex and paracortex because antigens and small molecules are sampled by cells that have direct access to the conduit lumen (e.g., dendritic cells, DCs). Molecules produced by lymphocytes such as antibodies can be transported via the conduits. Perivascular zone (blue box): the fibrillary conduit system extends to the perivascular (PV) conduit system around high endothelial venules, where the fluid is partially resorbed. Medullary zone (black box): the conduit system is connected to the LN medulla through a fenestrated medullary lymphatic endothelium. (C) The basic conduit structure comprises a basement membrane (BM) that ensheaths a microfibrillar zone (MFZ) and bundles of collagen fibers. Conduits support information transfer between immune cells in SLOs through the transport and distribution of antigens and inflammatory mediators. DCs can extract immunologically relevant information from conduits through direct access to the luminal conduit compartment [62]. Abbreviations: ECM, extracellular matrix; HEV, high endothelial venule; LEC, lymphatic endothelial cell; TRC, T cell zone reticular cell.

maintaining directional osmotic pressure to prevent tissue edema [69]. Quantitative proteomic analysis of nodal clearance of afferent versus efferent lymph in rats shows that proteins derived from draining peripheral tissues must be significantly cleared after passing through the LN, independently of molecular weight [69]. Fluid absorption by blood vessels is driven mainly by oncotic pressure differences between lymph and blood, corroborating the presence of perivascular sleeves where the conduits exchange fluid with the blood vasculature [27,70]. Moreover, directional drainage of fluid is also maintained through changes in interstitial pressure in inflamed tissues, effectively preventing lymphedema [71]. Following recent data highlighting the requirement for the mechanosensor Piezo1 in controlling fluid homeostasis in Peyer's patches [15], it is tempting to speculate that additional mechanosensitive mechanisms are also necessary in LNs and spleen to respond to changes in fluid flow and to adapt the conduit structure to maintain fluid homeostasis. However, this possibility warrants thorough investigation.

FRC and conduit network remodeling during inflammation

LNs expand to 3–5 times their original size with every immune response, and this requires extensive remodeling of the tissue [72–74]. Moreover, the interactions between FRCs and DCs in mice can initiate LN remodeling [73–75], but many questions remain regarding how the non-cellular structures of the conduit are altered, rebuilt, and extended during LN expansion. Mouse studies have shown that peripheral tissue-resident DCs upregulate the expression of the C-type lectin CLEC-2 as they mature and migrate into LNs [76]. *In vitro* and *in vivo* experiments in mouse models show that CLEC-2 plays a dual role: first, to promote the migration and protrusive activity of DCs [76], and second, to downregulate actomyosin contractility in FRCs via engagement of podoplanin (PDPN) [73,74]. As a consequence, the arrival of migratory antigen-presenting DCs initiates the

relaxation of the FRC network, thus permitting FRCs to elongate and make space for expanding lymphocyte populations (Figure 2C) [73,74]. Indeed, mimicking this interaction using recombinant CLEC-2-Fc protein can augment such LN expansion [73]. During this process of expansion, the FRCs partially decouple from the conduit, thus reducing adhesion, temporarily losing apicobasal polarity, and halting ECM secretion [28]. The conduit structure, although practically disrupted through early LN expansion, remains functional, and the flow of small fluorescent dextran tracers can still be observed throughout the LN parenchyma [28]. Furthermore, resident myeloid cells can still sample lymph-borne antigens in this scenario [28]; therefore the changes accompanying LN expansion highlight the robustness of the conduit system through tissue remodeling. However, the opposite appears to be true during chronic inflammation, and there are many clinical reports showing that conduits lose structure and function as lymphoid tissue becomes progressively fibrotic [65,77]. Likewise, the conduit system in the spleen is maintained during acute microbial infection; by contrast, severe immunopathology-induced impairment of fluid flow through the conduits can be observed during chronic infection with lymphocytic choriomeningitis virus (clone 13) in mice [78]. Thus, understanding how tightly regulated ECM production maintains homeostatic architecture through cycles of tissue expansion, and how controlled ECM production is lost in chronic pathologies, may potentially yield opportunities for investigating treatment options in conditions such as fibrosis; it may be possible to discover druggable targets that can prevent the overproduction of ECM and, ideally, restore the immune function of affected tissues.

High-resolution microscopy in mice has enabled topological mapping of the FRC network [79,80]. Moreover, graph theory-based systems-biology approaches have determined that the FRC network exhibits small-world topology, which is sufficiently robust to tolerate loss of up to 50% cellularity and maintain function [27,80]. The topology of the associated conduit system has also been quantified at both small and large scales and was shown to have a similar structure as the FRC network, albeit being more susceptible to perturbation in mathematical modeling experiments [27,70]. The robust maintenance of the conduit function through tissue remodeling is supported by the large number of alternative routes of fluid drainage to multiple blood vessels. A recent study quantified the entire LN vasculature in mouse LNs to study transmural flow patterns and calculate variations in vessel lengths and diameters, as well as pressure differences [81]. Accordingly, the authors demonstrated that the blood vasculature was non-uniformly distributed, and 75% of it consisted of capillaries distributed in the periphery of the LN. Computational modeling showed that transmural fluid flow was also concentrated in the LN periphery [81]. In addition, as LN expansion progresses, it is surmised that ECM structures might serve as a morphological template for FRC proliferation – a phenomenon which has been recently shown in skin using a combination of *in vivo* clonal lineage tracing in mice, proliferation kinetics, single-cell transcriptomics, and *in vitro* micropattern experiments [82]. It will be interesting to determine whether ECM structures thus direct and align newly divided FRCs to maintain network integrity.

Concluding remarks

The primary and original function of fibroblasts within SLOs is to construct a physical 3D niche to house immune cells. The later development of these fibroblasts into immune-specialized FRCs has led to studies of their roles in supporting immune cell survival and activation, controlling inflammation, and also in immune dysfunction. We now understand that FRCs function at the interface of immunity and fluid homeostasis where they provide the physical and chemical cues to link fluid flow, immunosurveillance, and adaptive immune responses.

There is still much to understand about the mechanics of fluid collection and recirculation through tissues and body cavities; furthermore, there is a need to determine how mechanical cues from fluid flow impact on the structure and function of SLOs (see Outstanding questions). It is clear that

Outstanding questions

Piezo1 has been identified as a key mechanosensor in murine Peyer's patches that impacts on fluid flow, tissue homeostasis, and local immune function. What mechanosensitive mechanisms are triggered by tissue remodeling or fluid flow to influence the structure and function of other SLOs?

Conduit-like structures have been observed in TLS. Do these conduit networks function to direct and filter fluid similarly to SLOs, and, if so, do they contribute to antigen sensing?

FRCs have acquired a specialized phenotype to support immune function, and recent work has shown that FRC subtypes are further specialized in their specific niche within the tissue. What are the signals that direct the maintenance and specification of FRC subsets, and which signals are necessary to regenerate FRCs following perturbations?

The functions of FRCs are broadly similar between different SLOs, but tissue-specific differences in FRC phenotypes have been observed. The field should investigate the progenitor–progeny relationships of FRC ontogeny and clarify the relationship between FRCs and the apparent ubiquitous perivascular fibroblast precursors.

FRC-like cells can be induced through the development of TLS, and existing FRCs in SLOs can lose their immune-specific phenotypes, thus contributing to fibrosis of lymphoid tissue and loss of immune function. How stable is the phenotype of an FRC, and what signals are key drivers of FRC plasticity?

ECM structures generated and maintained by FRCs are key to the filtering function of SLOs; however, fluid flow and hydrostatic pressure vary greatly between LNs, spleen, and Peyer's patches [8]. In LNs, afferent lymph flows at low pressure, and the direction of the flow is likely maintained by selective uptake and degradation of lymph proteins to maintain oncotic pressure, although this remains to be examined. Conversely, the spleen filters fluid directly from the blood where arterial pressure is high, meaning that fluid flow through the spleen is likely to be driven or pushed out by a pressure gradient, whereas Peyer's patches absorb and filter fluid directly from the gut into the efferent lymphatics, and sense the forces of fluid flow through the mechanosensor Piezo1 [15]. Because fluid flow and hydrostatic pressures are specific to each tissue, presumably there are also tissue- and FRC-specific mechanisms that control and respond to fluid flow, but these are not yet understood.

Functional conduits have also been observed in **tertiary lymphoid structures (TLS)**, which can develop during inflammation [83]. Unlike SLOs, these ectopic lymphoid structures might form in the middle of a tissue, or in several sites within a solid tumor [84]. It therefore seems unlikely that TLS play any functional or essential role in fluid homeostasis. However, it remains to be determined whether the conduits of TLS play a role in antigen presentation. By contrast, non-classical lymphoid organs found directly under the mesothelial layer of interstitial areas lining serous body cavities (i.e. peritoneal, pleural, and pericardial) still embody the major principles of linked fluid control and immune surveillance, despite their more rudimentary structure. In the **omentum**, fat-associated lymphoid clusters (FALCs), known as milky spots, appear as dispersed immune cell aggregates underpinned by CCL19$^+$ and CXCL13$^+$ FRCs, myeloid cells, and B cells [85–87]. Similarly to SLOs, mouse studies have shown that FALCs are perfectly situated to sample and filter the flow of fluid from serous cavities, and therefore may also have an important filtering function, provide early warning signals to immune cells, and block the spread of infections [88]. However, despite interesting initial studies [89], further research will be necessary to elaborate the fluid connection of FALCs with the lymphatic system and to determine how FALC FRCs steer immune cell homeostasis and activation during inflammatory diseases of major internal organs.

Another important area for further investigation includes determining the mechanisms that control the maintenance and regeneration of FRC and conduit networks. An important step towards achieving a better understanding of these processes is the identification of progenitor–progeny relationships during FRC ontogeny because FRCs can originate from multiple sources, and this question remains unresolved [90–92]. Moreover, it will be important to clarify the relationship between FRCs within different SLOs in which ubiquitous fibroblastic progenitors appear to reside within the perivascular compartment [93,94]; this may allow us to understand the unique properties of FRCs and how these may differ between tissue sites. Such studies can also contribute to our improved knowledge of the processes that underlie transformative events of FRCs into ECM-producing fibroblasts within fibrotic LNs, as has been observed in various models of fibrotic mice (including organ transplantation) [95,96].

In sum, despite significant progress during recent years in the research arena of stromal–immune cell interactions, future investigations of FRCs and conduit networks can provide pivotal insight into the inter-immune cell communication within lymphoid tissues and the processing of immunologically relevant information received by immune cells from various tissues and organs.

Declaration of interests

The authors declare no conflicts of interest.

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