Trafficking of B Cell Antigen in Lymph Nodes

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Abstract
The clonal selection theory first proposed by Macfarlane Burnet is a cornerstone of immunology (1). At the time, it revolutionized the thinking of immunologists because it provided a simple explanation for lymphocyte specificity, immunological memory, and elimination of self-reactive clones (2). The experimental demonstration by Nossal & Lederberg (3) that B lymphocytes bear receptors for a single antigen raised the central question of where B lymphocytes encounter antigen. This question has remained mostly unanswered until recently. Advances in techniques such as multiphoton intravital microscopy (4, 5) have provided new insights into the trafficking of B cells and their antigen. In this review, we summarize these advances in the context of our current view of B cell circulation and activation.
ROLE OF ANTIGEN IN B CELL DIFFERENTIATION

From their birth in the bone marrow to when they first express a mature B cell receptor (BCR), B cells’ fate is determined by cognate antigen (6). Immature B cells go through two checkpoints, one in the bone marrow (checkpoint 1) and the second in the spleen (checkpoint 2) prior to maturation and migration to the B cell follicles (7–9). Engagement of antigen in the bone marrow leads to deletion (10, 11), anergy (12), or receptor editing (13–16). Cells surviving negative selection in the bone marrow migrate to the spleen, where encounter with self-antigen leads to anergy and death (checkpoint 2) (9, 17). How self-antigen is presented to immature B cells is unclear, although investigators speculate that they engage antigen held by stromal cells, possibly follicular dendritic cells (FDCs) (18).

Mature B cells circulate though the secondary lymphoid organs (SLOs) regularly (approximately every 24 h) and in the absence of antigen have a half-life of a few days. They leave the vascular system and enter lymph nodes via specialized endothelia termed high endothelial venules (HEV) (see 19 for review). Lymph nodes are organized into discrete compartments: B cells localize within the cortical region near the subcapsular sinus, whereas T cells localize in the paracortical region (Figure 1). Until recently, it was held that migration within the lymph nodes was determined principally by chemokine gradients. B cells are attracted to the follicles by the release of CXCL13 by follicular stromal cells [FDCs, already mentioned, and fibroblastic reticular cells (FRCs)] (20, 21), whereas T cells migrate to a gradient of CCL19 and CCL21 (19). More recent studies using multiphoton intravital microscopy (MP-IVM) have identified migration of T cells and dendritic cells (DCs) along a network of reticular fibers (22, 23). Similarly, B cells were shown to traffic within the follicles.

Figure 1
(a) Lymphocytes enter the lymph node via high endothelial venules (HEV) and are directed to the follicular (Fo) or paracortical (Pc) areas following chemokine gradients on the FRC reticular fibers. (b) Fibroblastic reticular cells (FRCs) are divided into follicular conduits or paracortical conduits according to the different chemokines expressed. Follicular conduits transport CXCL13, a chemoattractant for B cells, and paracortical conduits transport CCL19 and CCL21, which attract T cells and dendritic cells (DCs) to the paracortical area. (c) Electron micrograph of a FRC and a follicular conduit in the subcapsular sinus area (SCS) of the lymph node. The black arrow indicates the lumen of the conduit. [Panel c adapted with permission from Current Opinion in Immunology (Reference 104).]
along the FDC dendritic processes (23). Thus, lymphocytes are guided within the lymph node along a complex of reticular fibers or FDC processes in a nonrandom manner.

LYMPH NODE STROMAL CELL RETICULUM

FRCs and their reticular fibers were identified 30 years ago in studies examining the anatomy of rat lymph nodes (24, 25). The collagen-rich reticular fibers, which are ensheathed by FRCs, not only provide an infrastructure, but also act as conduits for delivery of small proteins within the draining lymph to the HEV within lymph nodes (26–30). For example, chemoattractants such as monocyte chemoattractant protein 1 (MCP-1) (now known as CCL2) draining from a possible infection in the skin are delivered to the HEV of local lymph nodes, where they provide a signal to circulating leukocytes, as proposed by the “remote control hypothesis” (31). Reticular fiber conduits have also been described in the spleen, where they appear to deliver small proteins from the blood to the white pulp areas (32).

As noted above, recent imaging studies indicate that reticular fibers act as highways for migrating T lymphocytes and DCs and that this is enhanced by the localization of T cell chemokines (CCL19 and CCL21) secreted by FRCs. In addition to their secretion of chemokines, FRCs are an apparent source of self-antigen that acts to regulate CD8+ T cells (33, 34).

DC uptake and presentation of peripheral antigens to T cells appear to occur in two time frames. Migratory DCs take up antigen from the tissues and arrive in the lymph node 12–18 h after injection, whereas small antigens gain rapid access to lymph node–resident DCs within minutes after subcutaneous injection (35). FRC conduits provide pathways for passive delivery of small antigens from the draining afferent lymph to resident DCs within the para-cortical region of lymph nodes. Resident DCs localize to the T cell area conduits and sample small lymph-borne antigens for presentation to cognate T cells migrating along the fibers (36, 37). Thus, the conduits provide an efficient network for alerting resident DCs to small foreign antigens, cytokines, or chemokines entering the draining lymph node from a potential site of infection (36, 37).

The FRC conduits, which have an outer diameter of 1–2 μm, are composed of a core of tightly packed type I collagen fibers with spacing of approximately 5–8 nm (37, 38) (Figure 2). This structural arrangement would explain the known size exclusion of proteins over 60–70 kDa. Although the collagen core is enveloped by FRCs, our studies indicate that the contents of the conduits are directly accessible to B cells and FDCs (M.C. Carroll, unpublished results; Reference 38).

The B cell area also includes FRC-like stromal cells and collagen-rich reticular fibers that are structurally and immunohistochemically similar to those in the T cell area (38). However, they are less dense than in the cortical region. Notably, the FRC conduits are interconnected with the FDC network (Figure 2). We discuss the potential importance of this intersection in more detail below. One major difference between FRCs in the T and B cell areas is that the latter (also referred to as marginal reticular cells) are a source of B cell chemokine (CXCL13). Therefore, release of the chemoattractant into the conduits would provide a guide or highway for B cells migrating within the follicles.

The spleen serves as a major component of SLOs and filters antigens from the circulation. Although its architecture is different from that of lymph nodes, the spleen’s overall organization is similar, and FRC reticular fibers appear to play a similar role in guiding T cells within the T cell zone (32, 39) (Figure 2). Therefore, for this review we point out similarities and differences with lymph nodes but do not provide detail.

GUARDIANS OF THE LYMPH NODE: SINUS-LINING MACROPHAGES

The lymph node sinus is lined with CD169+ CD11c+CD11b+ macrophages that act as
Figure 2

(a) Transmission electron micrograph showing a longitudinal section of a follicular conduit (Co). (b) High magnification image showing the space between collagen fibers inside a conduit. (c) Electron micrograph showing a transverse section of a follicular conduit. The double pointed arrow indicates the conduit diameter. To clarify the electron micrograph, the conduits and the FRC in panels a, b, and c are colored in blue and green, respectively. (d) Multiphoton intravital microscopy (MP-IVM) snapshot showing small antigen [turkey egg lysozyme (TEL), 14 kDa, red] within the follicular conduits, although large antigen (PE-TEL, 240 kDa, green) is restricted to the subcapsular sinus (SCS) space. Arrowheads indicate cognate MD4 B cells, which rapidly acquire TEL directly from the conduits. (e) Follicular conduits intersect with FDCs. MP-IVM snapshot showing the intersection of follicular conduits (filled with TEL, green) and FDCs (blue) in the follicles. Arrows indicate colocalization of TEL and FDC. [Panel d adapted with permission from Immunity (Reference 38).]

Guardians to sample and clear microorganisms entering via the afferent lymph (Figure 3). Two major subsets of macrophages, i.e., subcapsular sinus macrophages (SSMs) and medullary macrophages (MMs), have been identified based on location and expression of cell surface markers (40). SSMs line the sinus in the region of the afferent lymph vessels and are characterized by expression of the metallophilic antigen monoclonal antibody-1 (MOMA-1) (41). They are similar to the metallophilic macrophages that line the inner marginal zone sinus in the spleen, and they are dependent on lymphotoxin cytokines (LTα/β) for their localization (42). In contrast, MMs line the medullary sinus, are distinguished by expression of mannose receptor, SIGN-R1, and F4/80, and are more similar to the outer marginal zone macrophages in the spleen (38, 42, 43). In general, SSMs are less endocytic relative to MMs and their lysosomal compartment is less mature (42). This may be an important property in retaining captured lymphborne antigens on their surface, as is discussed in more detail below. In contrast, MMs are more typical of mature macrophages and efficiently take up and clear opsonized particles and antigens from the lymph.

FOLLICULAR DENDRITIC CELLS AS A DEPOT FOR B CELL ANTIGEN

The Ig receptor expressed on B cells readily binds antigen in fluid phase, at least in vitro; however, it seems unlikely that free antigen is
bound efficiently in vivo. A more likely source of B cell antigen is antigen that is concentrated on the surface of cells. Model binding studies in vitro using transgenic B cells and membrane-bound antigen have identified a reorganization of the cell surface proteins on both the B cell and the target cell following antigen engagement (44–46). Thus, the BCR forms a dynamic synapse within the cell surface, which results in membrane spreading that facilitates an increase in signaling and an efficiency in antigen uptake similar to that described for the T cell synapse (47). In model systems in vitro, B cell encounter with membrane antigen results in formation of microclusters that include components of the BCR, i.e., IgM and IgD, CD19, and adhesion molecules ICAM-1 (intercellular adhesion molecule-1) and LFA-1 (lymphocyte function-associated antigen-1) (48, 49). Although initial studies suggested that the CD19/CD21/CD81 coreceptor was not required, more recent studies in vivo support a role for complement receptors in acquisition of membrane antigen; we discuss these studies in more detail below (50).

Early studies tracking radiolabeled protein antigens injected intravenously into immune animals identified rapid uptake within the spleen (51). Closer inspection revealed capture of the labeled immune complexes (ICs) on dendritic-shaped cells that were referred to as FDCs because of their morphology and location within the splenic follicles. Use of cobra venom factor, which transiently depletes C3, demonstrated that complement was important in the localization of ICs to the FDC surface (52). More recent studies have shown that ICs are captured on naive FDCs principally by complement receptors CD21 (CR2) and CD35 (CR1) (53, 54) and by FcRIIb (55). CD21 and CD35 are expressed on naive FDCs, whereas FcRIIb is expressed following activation (Figure 4).

FDCs originate from mesenchymal cells and depend on LTα/β for their mature phenotype (for review, see 56). A primary source of LTα/β for FDC differentiation is derived from circulating naive B cells. For example, reconstitution of RAG-1−/− mice, which are deficient in mature lymphocytes, with B cells restores FDCs and the architecture of lymph nodes. FDCs are a major source of CXCL13, as noted above, and also provide the B cell survival factor B cell–activating factor (BAFF). Thus, B cells and FDCs interact to maintain normal lymph node architecture.

Initial electron microscopy (EM) images of spleen sections from mice injected intravenously with 125I- and 131I-labeled ICs

![Figure 3](image)

(a) A MP-IVM snapshot showing UV-inactivated influenza virus PR8 (red) at 7 min after footpad injection. The virus is already present in the subcapsular sinus (SCS) and in the medulla of the lymph node, and it colocalizes with subcapsular sinus macrophages (SSMs) (MOMA-1+, green) and medullary macrophages (MMs) (F4/80+, blue). (b) Schematic drawing showing trafficking of influenza PR8 in the lymph node. SSMs and MMs capture and internalize influenza virus. (c) Electron micrograph showing influenza virions inside a SSM at 30 min after footpad injection. [Panel a adapted with permission from Nature Immunology (Reference 72).]
Figure 4
Role of complement receptors CD21 and CD35 in B cell differentiation. (a) Mature B cells are located in the follicle and express complement receptors CD21/35 (CD21 in figure), which form a coreceptor with CD19 and CD81. (b) Binding of C3d-coated antigen with the B cell coreceptor lowers the threshold of B cell activation and directs the activated B cells to the T cell–B cell boundary, where they further differentiate and undergo somatic cell hypermutation and class switch recombination. (c) B cells acquire cognate antigen deposited on FDCs within the germinal center (GC) and present it to follicular T helper cells (TFH). Following the encounter of the B cells with antigen and TFH, B cells differentiate into effector and memory B cells. [Model adapted with permission from the Journal of Immunology (Reference 105).]

identified deposits on FDCs within the B cell or follicular zone (57). Subsequent EM histology using ICs composed of horseradish peroxidase and substrate provided further support for uptake of ICs along the FDC reticular processes, and, based on their beaded structure, they were referred to as IC-coated bodies (iccosomes) (58). The beaded structures of approximately 0.25–0.38 µm in diameter appeared to be enveloped by a membrane. Similarly, ex vivo cultures of FDCs loaded with ICs via FcRIIb revealed a distinct periodic array of deposits.
along the dendritic processes similar to that observed in vivo. However, in contrast to the beaded structures reported earlier, the array of ICs was located on the outer membrane surface (59). Recent studies have shown that the FDCs retain antigens and can activate cognate B cells for more than one week after antigen administration (50). Together, these data indicate that FDCs serve as a central depot for long-term antigen retention in the B cell follicle.

**LYMPH NODE GERMINAL CENTERS**

A general feature of a B cell response to T cell–dependent antigen is formation of germinal centers (GCs) within the follicular region of SLOs (6, 60, 61) (Figure 4). These are specialized microenvironments where activated B cells undergo clonal expansion, class switch recombination (CSR), somatic hypermutation (SHM), and affinity maturation (20). They are critical in host immunity as they are the principal site of B cell differentiation into long-term memory and effector cells. On the basis of immunohistochemical studies, investigators have divided GCs into dark and light zones. The light zone is typically more proximal to the subcapsular sinus in lymph nodes and to the marginal zone in spleens. This orientation may enhance entrance of naive B cells and/or antigen. In the traditional GC model (60), the dark zone serves primarily as the region where B cells undergo rapid cellular division, CSR, and SHM. Subsequently, they migrate into the light zone within the FDC network and undergo antigen selection in the presence of follicular helper T cells (TFH) (62) (Figure 4c).

This traditional view of GC function was recently revised based on studies using MP-IVM (63–66). Tracking GC B cells in real time revealed that B cells within the two zones are more similar than previously proposed and that migration between the two regions in both directions is common. Importantly, B cells undergo cellular division and antigen selection in both zones, and it is unlikely that CSR and SHM are limited to the dark zone only. Thus, the real-time imaging provides a view of a dynamic movement in both regions and migration between them. However, certain features distinguish the two regions and favor separate functions consistent with the traditional model. For example, B cells in the dark zone express the chemokine receptor CXCR4 and are attracted to this region based on expression of stromal cell–derived factor (SDF) ligand by stromal cells (67). Downregulation of CXCR4 by dark zone B cells leads to migration into the light zone because the GC B cell is attracted to the FDC network by CXCL13. In addition to the presence of the FDC, the light zone is further distinguished by an enrichment of TFH (CD4+ CXCR5+).

A hallmark of the GC is selection of B cells after undergoing CSR and SHM for antigen reactivity (6, 68). Thus, GC B cells are selected for both BCR recognition of antigen and presentation to TFH cells (62). These events would be expected to occur more efficiently in the light zone that is enriched with antigen retained on the FDC and in the presence of TFH cells. GC B cells that fail to obtain a signal via the BCR and CD40 undergo apoptosis and are cleared by tingible body macrophages.

One striking observation from the real-time imaging studies is that the morphology of the GC B cell appears different from that of naive B cells (64). GC B cells appear more irregular in shape, with extended filopodia apparently probing the surrounding FDCs. In some images, GC B cells were tethered to the FDC by long, thin extensions. This distinct morphology could enhance GC B cell interaction with cognate antigen bound to the surface of FDCs.

**B CELLS ACQUIRE ANTIGEN FROM FOLLICULAR DENDRITIC CELLS**

Direct evidence of B cell acquisition of antigen from the FDC surface in vivo was recently reported (50). To track the uptake of antigen, passively immune mice were injected subcutaneously with labeled lysozyme that binds to MD4 Ig transgenic B cells at
either high, i.e., hen egg lysozyme (HEL) \((K_d \approx 1 \times 10^9)\), or intermediate, i.e., duck egg lysozyme (DEL) \((K_d \approx 1 \times 10^7)\) affinity.

Nine days later the mice were administered fluorescent-labeled antigen-specific (MD4) B cells. MP-IVM imaging of B cells within the follicles identified acquisition of the labeled antigen. In some examples, the antigen was removed along with a fragment of the FDC membrane. Notably, efficient capture of intermediate affinity DEL antigen was dependent on CD21/CD19/CD81 coreceptor, given that MD4 B cells deficient in CD21 acquired significantly less labeled antigen than did \(\text{Cr}^2+\) control B cells. One explanation for the requirement of the coreceptor in antigen uptake is that BCR and coreceptor coligation of C3d-DEL complexes on the FDC surface enhances signaling in microsignalosomes and in turn increases the efficiency of acquisition of membrane antigen.

In summary, FDCs not only provide a critical source of antigen for clonal selection of B cells within the GC, but also provide a potential source of antigen for naive B cells in a primary response.

**LYMPH NODE SINUS MACROPHAGES CAPTURE PARTICULATE ANTIGENS**

The recent use of MP-IVM to track the movement of fluorescent-labeled antigen into draining lymph nodes has provided an insight otherwise unattainable into the uptake and accessibility of antigen by B cells (40). Multiple pathways are apparently used to transport antigen into the follicles. The pathway(s) involved is determined by factors such as antigen size, presence of preexisting antibody, and activation of the complement system, as well as by whether migratory DCs transport antigen into the lymph node (Figure 5).

Particulate antigens draining via afferent lymph such as vesicular stomatitis virus (VSV) (69), protein-coated beads (70), and ICs composed of large proteins and IgG (71) are captured and displayed on the surface of SSMs. Strikingly, cognate B cells within the underlying follicles were observed to acquire antigen directly from the SSM surface and to migrate to the T-B cell border, where antigen is presented to T cells. Elimination of sinus-lining macrophages by pretreatment with clodronate-loaded liposomes (CLLs) reduces the frequency of antigen-specific B cells that acquire virus within the first few hours of immunization (69). MP-IVM imaging identifies antigen-specific B cells in direct contact with antigen bound to the surface of the SSM and shows a reduction in their velocity, suggesting the formation of a synapse (70). In one study, fluorescent-labeled beads draining into the peripheral lymph node were observed to accumulate on FDCs over 24 h. How the beads were transported in the absence of specific B cells was not determined; however, it was proposed that naive B cells or low-affinity B cells participated in their transport (70). Whether SSMs provide a long-term source of antigen was not examined, but it seems unlikely in this dynamic environment given that SSMs are constantly bathed in afferent lymph.

In the Jun et al. (69) study using VSV as the antigen, SSMs not only provided a source of antigen to B cells, but also limited systemic spread of the virus entering lymph nodes via the lymph. Elimination of SSMs and MMs using CLLs led to systemic spread of the virus, confirming the importance of the sinus-lining cells as guardians against microbial infections. How VSV is captured by SSMs was not addressed; however, binding of virus by SSMs and MMs was observed in C3 \(^{-/-}\) mice, suggesting that complement was not required, although direct opsonic effects of C1q, mannann-binding lectin (MBL), and/or ficolins cannot be ruled out.

**OPSONIZATION OF INFLUENZA BY MANNAN-BINDING LECTIN**

Not all lymph-borne particulate antigens are retained on the surface of SSMs for relay to B cells. In a recent study using a fluorescent-labeled UV-inactivated form of influenza A virus (A/Puerto Rico/8/34; PR8; H1N1) injected in the footpad, Gonzalez et al. (72) found...
that the inactivated PR8 virus was rapidly captured and internalized by SSMs and MMs. In contrast to the earlier models, the inactive virus was not transferred to naive B cells but rather was transported by a novel pathway. We discuss this in more detail below.

The envelope of influenza A virus particles bears two surface glycoproteins, the hemagglutinin and the neuraminidase. Both are critical to the ability of the virus to replicate in susceptible target cells, and the oligosaccharides attached to the viral glycoproteins play a number of important biological roles. The mannose-rich glycoproteins provide a PAMP (pathogen-associated molecular pattern) for recognition by C-type lectins, Ca\(^{2+}\)-dependent carbohydrate-binding proteins that share primary and secondary structural homology in their carbohydrate-recognition domains (CRDs) (73). The members of one subgroup of C-type lectins, the collectins, appear to be especially important for host defense against influenza. The collectins contain a collagen-like domain and usually assemble into large oligomeric complexes, allowing high-avidity binding based on multiple low-affinity interactions of their CRDs (74). This enables collectins to discriminate not only specific carbohydrate moieties but also specific patterns of these present on pathogens. Most collectins, including MBL (75), are present in serum and body fluids. In addition to the collectins, a number of cell surface–associated C-type lectins appear to play a role in innate defense toward influenza, such as the macrophage mannose receptor (76), the macrophage galactose-type C-type lectin 1 (MGL1) (77), the human DC-specific intercellular adhesion molecule (DC-SIGN) (78), and the related mouse DC-specific ICAM-3-grabbing nonintegrin homolog, SIGN-related 1 (SIGN-R1) (79). The latter receptor (SIGN-R1) is discussed in more detail below.
Influenza virus strain PR8, which has very little glycosylation on its envelope proteins, is poorly recognized by the C-type lectin activity of either the macrophage mannose receptor or the MGL1 and is only very weakly bound by surfactant protein D (80). Thus, it is taken up poorly by macrophages (81). Notably, binding of PR8 by MBL in a solid-phase binding assay (72) led to lectin pathway activation and C4 and C3 deposition in vitro (S.E. Degn and M.C. Carroll, unpublished results). Importantly, virus was opsonized by MBL in lymph and was rapidly endocytosed by SSMs and MMs. Because activation of the lectin pathway via MBL activates complement C3, binding of the virus is likely mediated by the CR3 receptor, a scenario that is similar to that observed for C3-coated ICs (discussed below). However, MBL is also thought to bind to specific receptors on macrophages, so it will be important to understand the specific pathway.

In the study with UV-inactivated PR8 virus, macrophages were not required for an effective humoral response, but on the contrary seemed to dampen the ensuing response. Indeed, MBL has previously been reported to modify the humoral response of mice toward viral antigens, dependent on their background (82, 83). This effect may mechanistically be explained with the increased clearance and degradation of antigen, as is also indicated above regarding viral infection of macrophages. Although this effect is detrimental in the scenario of an influenza vaccine, it may obviously be crucial in the control of infection with live virus. Presumably, the MBL-mediated opsonization in the blood and subsequent uptake by macrophages serves an important barrier function restricting spread of virus, as has been previously suggested concerning the spread of influenza virus into the bloodstream from the lungs (84).

COMPLEMENT-DEPENDENT CAPTURE OF IMMUNE COMPLEXES

Soluble proteins in the afferent lymph gain entry into the B cell follicles via several routes depending on size, presence of specific antibody, or recognition by innate immunity leading to opsonization or direct binding to macrophage surface receptors. For example, proteins larger than approximately 60 kDa injected into passively immune mice form ICs that activate complement, resulting in covalent attachment of C3 (C3-IC). The C3-coated complexes are captured by SSMs and made available to B cells in the underlying follicles, as observed with particulate antigens (71) (Figure 5). Notably, C3-coated ICs are taken up by SSMs and are relayed in a unidirectional manner to naive B cells (42). Because SSMs express the CR3 receptor, this receptor is likely involved in the binding and shuttling process. Strikingly, C3-ICs are off-loaded onto noncognate (naive) B cells that take up the complexes via CD21 and CD35 and possibly FcRIIb receptors (38, 71). This is an efficient process, given that a relatively high frequency of follicular B cells acquire ICs within a few hours after administration of antigen in preimmune mice. FcRIIb is known to internalize Ig complexes on DCs into a nondegradative compartment and then return ICs to the cell surface for presentation to B cells (85). Whether this pathway occurs in the case of C3-IC uptake and relay is not clear, but this could help explain the relatively high efficiency of transfer of C3-ICs to naive B cells.

In a model in which preexisting antibody is not present in the lymph, it seems likely that innate recognition proteins such as natural IgM; C-type lectins such as MBL and ficolins; pentraxins, including C-reactive protein; and other complement activators could effectively bind the foreign protein and activate complement, resulting in uptake via CR3.

Noncognate B cells loaded with C3-ICs migrate to FDCs, where the complexes are transferred. Again, this process is apparently efficient given that, within a few hours after antigen administration, FDCs bear complexes on their surface (38, 71). The mechanism for transfer of C3-ICs from B cells to FDCs is not clear because both employ CD21 and CD35 receptors in binding. One possible explanation is that the relatively high density of CD21 and
CD35 on the surface of FDCs mediates the directional transfer. However, further studies are needed to understand this important process. A similar transport pathway was predicted almost a decade ago based on studies of the uptake of labeled ICs from circulation by marginal zone B cells in the spleen (86–88). More recent studies demonstrate a constitutive pathway in which marginal zone B cells cycle between the marginal zone and the B cell follicles by a process dependent on CXCR5 for migration to the follicles and sphingosine 1-phosphate receptors (S1P1 and S1P3) for retention within the marginal zone (89). Marginal zone B cells that express relatively high levels of CD21 and CD35 pick up C3-ICs from the sinus and deliver the complexes to FDCs as they migrate through the follicles. As discussed above, the mechanism by which C3-ICs are stripped from the B cell and captured by FDCs is not known.

ENTRY OF SMALL ANTIGENS INTO LYMPH NODES VIA FRC CONDUITS

Small proteins gain direct access to the B cell follicles in a manner similar to that described above for chemokines, cytokines, and small T cell antigens, i.e., via FRC conduits (Figures 2, 5). Although the distribution and density of follicular conduits is different from that of the paracortical area, they provide a passive entry for small molecules to the B cell area. Follicular conduits intersect with FDCs, providing a direct connection for C3-coated antigens to bind to the FDC surface, where they can be taken up via complement and Fc receptors (38, 90). Confirmation that small antigen directly enters the follicles via the conduits was obtained using MP-IVM (Figure 2). In one study, small (turkey egg lysozyme; TEL) and large (TEL-coupled phycoerythrin) antigens were mixed and injected in the footpad of anesthetized mice, and the popliteal lymph node was then imaged in real time. The results showed rapid draining of the small antigen through the conduits and access to the FDC processes; antigen that was too large to enter the conduits remained adjacent to the subcapsular sinus floor (38) (Figure 2). In another intravitral imaging study, Germain and colleagues (90) tracked various small lymph-borne antigens, i.e., wheat germ agglutinin, ovalbumin, and HEL, draining from subcutaneous ear tissue into the B cell follicles of the ear-draining lymph nodes and binding to FDCs. Interestingly, cognate B cells appear to efficiently access small antigens within the FRC conduits as observed by MP-IVM (38). On acquisition of antigen, the movement of the cognate B cells (but not noncognate cells) slows, suggesting that the antigen-specific B cells become activated. High-resolution EM images of the follicles of popliteal lymph nodes identify B cell filopodia, displacing the FRC sheath and directly accessing the conduits (38). Therefore, B cells migrating along the conduits survey the reticular fibers for cognate antigen, although this is more likely only a transient source of antigen because the FDCs would provide a long-term and more efficient site for display of C3-ICs.

The size exclusion of conduits presents somewhat of a dilemma for understanding how antibody complexes composed of small antigens access the follicles. One explanation is that antibodies bind small antigens within the follicles as they exit the conduits and that activation of complement provides a ligand for uptake on CD21 and CD35 receptors. Alternatively, ICs form in the tissues or draining lymph and activate complement, resulting in tagging of the antigen with C3d ligand, which is approximately 30 kDa. Although IgG is too large to enter the conduits, it is transported across the subcapsular sinus by the neonatal Fc receptor (91). An alternative pathway for small antigens to enter the follicles independent of conduits was proposed by Pape et al. (92). Using conventional confocal imaging of cryosections of lymph nodes from mice injected subcutaneously with HEL antigen, they identified HEL antigen bound to cognate B cells throughout the B cell area within minutes of subcutaneous injection. Elimination of sinus-lining macrophages with CLLs prior to injection of antigen did not block acquisition of
antigen by cognate B cells. Their interpretation of the results was that the HEL antigen entered the follicles through gaps in the sinus floor.

**DENDRITIC CELLS TRANSPORT B CELL ANTIGEN**

A third major pathway for entry of foreign antigens into lymph nodes is active transport by migratory DCs. This pathway is best described for delivery of antigens into the T cell compartment (93). For example, during lung infection with influenza, at least two distinct populations of respiratory dendritic cells (RDCs) — CD103⁺ and CD103⁻CD11c⁺CD11b⁻/⁺ — are known to transport influenza into the draining mediastinal lymph nodes (mLNs) (94, 95). Migratory DCs enter lymph nodes via either HEV or lymph vessels. In the example of lung infection with influenza, RDCs enter the draining mLNs via the lymphatics by a pathway that is CCR7 dependent.

In addition to antigen presentation by the migratory DCs, a resident population of CD11cʰCD8α⁺ DCs within the mLN also take up the viral antigen and present it to CD8 T cells (95, 96). This opens the interesting possibility that migratory DCs transfer or hand off antigens to resident populations within the mLN. Whether the antigen is processed before delivery or is transferred intact is not clear. It seems reasonable to speculate that migratory DCs enter the subcapsular sinus via the afferent lymph and migrate along the FRC reticular network, where they encounter resident DCs. A similar pathway was proposed for skin migratory DC transfer of herpes simplex virus to resident CD11c⁺CD8α⁺ DCs (97). This pathway suggests that the viral antigen is transferred from the migratory skin DCs to the resident DCs.

To test whether B cell antigens are also transported via migratory DCs, Germain and colleagues (98) adoptively transferred DCs loaded in vitro with labeled HEL into mice seeded with lysozyme-specific B cells. Using MP-IVM, they tracked labeled DCs into the HEV of draining lymph nodes, where the HEL antigen was acquired by the cognate B cells. This was an important observation because it not only supported a clear role for migratory DCs to transport B cell antigen into local lymph nodes via the circulation, but it also suggested that the antigen is maintained intact. It will be important to learn whether migratory DCs that take up antigen at peripheral sites such as the lung also transport B cell antigen into the local lymph node via the lymphatics and present it directly to B cells or hand off the intact antigen to a resident population of DCs.

**RESIDENT DENDRITIC CELLS CAPTURE LYMPH-BORNE ANTIGENS DIRECTLY**

Sinus-lining macrophages are not the only cell type within the lymph node to filter particulate antigen; DCs residing in the lymph node medulla also sample particulates. Recent studies have identified a novel role for this relatively uncharacterized population of CD11cʰCD11bʰ DCs. Using a UV-inactivated PR8 strain of influenza A as a model vaccine, Gonzalez et al. (72) found that the virus was captured by the lymph node–resident DC population via SIGN-R1 (Figure 6). As noted above, the inactive virus is also bound by both SSMs and MMs, but binding is not required for humoral immunity. Instead, blocking of SIGN-R1 and MBL impairs the local humoral response. Moreover, elimination of CD11cʰ DCs in CD11c-DTR bone marrow chimeric mice treated with diptheria toxin blocks both the T-dependent (as expected) and the T-independent humoral responses to UV influenza (72). Notably, binding of the virus by lymph node–resident DCs induced a significant increase in velocity in a nonrandom manner and a net directional movement toward the FDC region. By contrast, neighboring resident DCs that did not take up the inactive virus failed to increase motility or gain a nonrandom movement. The combined results support a model in which the resident DCs transport the virus to the B cell follicles, where it is handed off either directly to FDCs or to other DCs.
Figure 6

(a) A MP-IVM snapshot showing the capture of influenza (PR8, red) by medullar resident dendritic cells (DCs) (green, white arrows) at 46 min after injection of the virus in the footpad. The lymph node capsule and the medullary conduits are shown in blue. (b) Schematic drawing showing the cells involved in the capture of lymph-borne UV inactive influenza virus in the lymph node (SSM, subcapsular sinus macrophage; MM, medullary macrophage). (c) DCs capture influenza virus in a SIGN-R1-dependent manner. This model speculates that C1q is activated by SIGN-R1 (not illustrated) and leads to C3 deposition on the surface of the virus. After binding to the virus, SIGN-R1 receptors could cluster on the surface of the cell, and the resident DCs could then transport the C3-coated virus to the follicles, where it could be transferred to the FDCs. (Panel a adapted with permission from Nature Immunology (Reference 72).)

Unlike the observations with C3-ICs, B cells did not appear to participate in viral transport. Whether the resident DCs also transport the virus to the paracortical T cell area was not examined, but it seems reasonable that they might also be involved in T cell priming or handing off the antigen to other subsets such as CD11c+CD8α+ DCs.

SIGN-R1 is one of several homologs of human DC-SIGN and is expressed by marginal zone macrophages in the spleen and MMs in the pLNs (79). Although SIGN-R1 shares many ligands with DC-SIGN (99), such as glycans rich in mannan, it contains an altered intracellular domain that signals via SRC family kinases through a JNK-dependent pathway rather than the canonical ERK pathway attributed to DC-SIGN (100). Although much of the literature describes SIGN-R1 as a phagocytic receptor, zymosan binding data have indicated that the receptor may actually be poorly phagocytic in the absence of other phagocytic receptors such as dectin-1 (101). This finding may be important in understanding antigen transfer in the lymph node, as DCs would have to retain antigen on their surface or recycle in a nonlysosomal compartment, as discussed above for FcRIIb (85). Interestingly, DC-SIGN can retain specific antigens without digestion through the use of low-pH, recycling, nonlysosomal compartments (102), suggesting a potential mechanism for SIGN-R1-dependent retention of viral antigens on lymph node–resident DCs.

A novel function of SIGN-R1 is that binding of Streptococcus pneumoniae activates complement C3 via C1q through the classical pathway (103). In their study, Kang et al. (103) found that blocking of SIGN-R1 binding or absence of C3 resulted in impaired humoral immunity to the bacteria. They proposed that C3 deposition was essential for uptake of S. pneumoniae on FDC. However, the mechanism of transport of opsonized bacteria to FDC was not explored.

Based on the observations of Gonzalez et al. (72) with inactive influenza, one might speculate that S. pneumoniae is captured by resident DCs as well as by MMs and transported to the FDC by a mechanism similar to that proposed above for influenza (Figure 6). Thus, one could envision that binding of S. pneumoniae to SIGN-R1 leads to C3 deposition on the pathogen surface that provides a ligand
for the CD21 and CD35 receptors on FDCs. Thus, resident DCs might transport the C3-opsonized bacteria to the B cell follicles and hand off the complex to FDCs (Figure 6).

**SUMMARY**

A long-standing enigma in immunology has been how and where B lymphocytes acquire their cognate antigen. The use of MP-IVM has allowed fresh insight into trafficking of leukocytes and the fate of lymph-borne antigens and their entry into the B cell follicles. In this review, we discussed three major pathways in which B cell antigens are captured and delivered to the B cell compartment and deposited on FDCs. In the first pathway, macrophages lining the lymph node subcapsular sinus are critical not only to limiting the spread of pathogens but also to capturing and relaying particulate antigens and ICs to naive and cognate B cells in the underlying follicles. B cells play a major role in the transport of ICs via complement receptors in this pathway. In a second pathway, small antigens in the afferent lymph drain passively into the follicles through collagen-rich conduits. The conduits, which are secreted by FRCs, intersect with a network of FDCs, providing a direct connection for capture and retention of antigens via complement and Ig Fc receptors. In a third major pathway, DCs residing along the medullary sinus sample lymph-borne antigens either directly or in conjunction with MMs. One model system suggests, for example, that capture of a viral antigen via SIGN-R1 leads to activation of the DC and migration in the direction of the B cell compartment.

Future challenges lie in characterizing the innate receptors and recognition proteins such as C-type lectins that participate in opsonization of various microorganisms and in determining how they alter antigen retention on the macrophage surface. Similarly, we must understand the signals involved in induction of resident DC migration to either the B cell or T cell compartments and whether they deliver antigens directly to FDCs or hand off the antigens to either B cells or other resident DCs.

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