REVIEW

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B cell follicles and antigen encounters of the third kind

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Defining where and in what form lymphocytes encounter antigen is fundamental to understanding how immune responses occur. Although knowledge of the recognition of antigen by CD4+ and CD8+ T cells has advanced greatly, understanding of the dynamics of B cell–antigen encounters has lagged. With the application of advanced imaging approaches, encounters of this third kind are now being brought into focus. Multiple processes facilitate these encounters, from the filtering functions of lymphoid tissues and migration paths of B cells to the antigen-presenting properties of macrophages and follicular dendritic cells. This Review will discuss how these factors work together in the lymph node to ensure efficient and persistent exposure of B cells to diverse forms of antigen and thus effective triggering of the humoral response.

At any one time, the majority of B cells in the mammalian body are situated inside follicles in the lymph nodes, spleen, Peyer's patches and other mucosal lymphoid tissues. B cells enter these tissues from the blood, migrate rapidly into follicles and spend about a day in the tissue before returning to the circulation to travel to another tissue. A striking feature of lymphoid tissue anatomy is that the B cell follicles are situated directly opposite the portals of antigen entry: lymph node follicles are located beneath the subcapsular sinus (SCS) of the lymphatic, splenic follicles are beneath the blood-filled marginal sinus, and mucosal follicles are situated immediately adjacent to antigen-transporting M cells. Early studies demonstrated that opsonized (complement- and/or antibody-coated) antigens become deposited on follicular dendritic cells (FDCs) within hours of immunization, and a model emerged that B cells travel to follicles to survey FDCs for antigen[1]. Moreover, the continued display of antigen on FDCs over periods of days or weeks was suggested to be important in driving the germinal center (GC) response[2]. The mechanisms that promote encounter with nonopsonized antigens were less obvious but were thought to involve fluid-phase exposure. However, through a combination of fluorescence-based fixed-tissue and real-time imaging approaches, a more intricate picture of the dynamics of B cell–antigen encounters in lymph node follicles has emerged[3–5]. To consider these dynamics, it will be helpful to first review how B cells travel to and migrate within lymphoid follicles.

Cues that guide follicular B cell migration: CXCR5 and EBI2

The chemokine–chemokine receptor pair CXCL13-CXCR5 has an essential role in attracting B cells to follicles (**Fig. 1**). CXCL13 is made by follicular stromal cells, including marginal reticular cells (MRCs) in the subcapsular region of the follicle and FDCs in the center[6,7], whereas CXCR5 is expressed by all mature B cells[8]. CXCL13 binds heparin sulfate and collagen[9,10] and is found concentrated in association with collagen fibers and stromal cells[6,11]. In the follicle, B cells move at an average velocity of 6 μm/min with a 'random walk' motion[12] that may be promoted in part by CXCL13 (ref. 13). Whether CXCL13 acts principally in a surface-bound (haptokinetic) way or a soluble (chemokinetic) way remains to be established. B cell movement can be stromally guided[14], although the cells do not always seem to be associated with stroma[15], and if studies of DCs translate to B cells, the cells may move at similar speeds whether on or off stromal support[16]. As B cells 'go walkabout' in the follicle, they contact not only FDCs, MRCs and each other but also cells located around the follicle perimeter, including sinus-associated macrophages and T cell zone–associated DCs (**Fig. 1**). As will be discussed further below, this migration activity facilitates both encounter with cognate antigen by individual B cells and the mass transport of opsonized antigens from the exposed follicle perimeter to the protected center for long-term display. How much of the dwell time in a lymphoid tissue is spent navigating a single follicle versus exploring several B cell areas is not known. Egress from the tissue occurs in a manner dependent on the sphingosine 1-phosphate receptor type 1 via lymphatic sinuses situated at the border of follicles and T cell zone, called 'cortical sinuses' (**Fig. 1**), that connect to medullary sinuses and hence the efferent lymphatics[17–19].

CXCL13 is not the only chemoattractant that controls cell distribution in the follicle, as hinted by the finding that B cells in CXCR5- or CXCL13-deficient mice continue to reside in the outer regions of lymphoid tissues[8,20]. Published work has identified a role for the chemoattractant-type receptor EBI2 (also known as GPR183) in guiding naive and activated B cells to the outer follicle and interfollicular regions of lymph nodes (**Fig. 1**), spleen, Peyer's patches[21,22] and isolated lymphoid follicles in the intestine (L.M. Kelly. and J.G.C., unpublished data). Like CXCR5, this receptor is present in all mature B cells, but unlike CXCR5, its expression increases rapidly after B cell activation. The nature and

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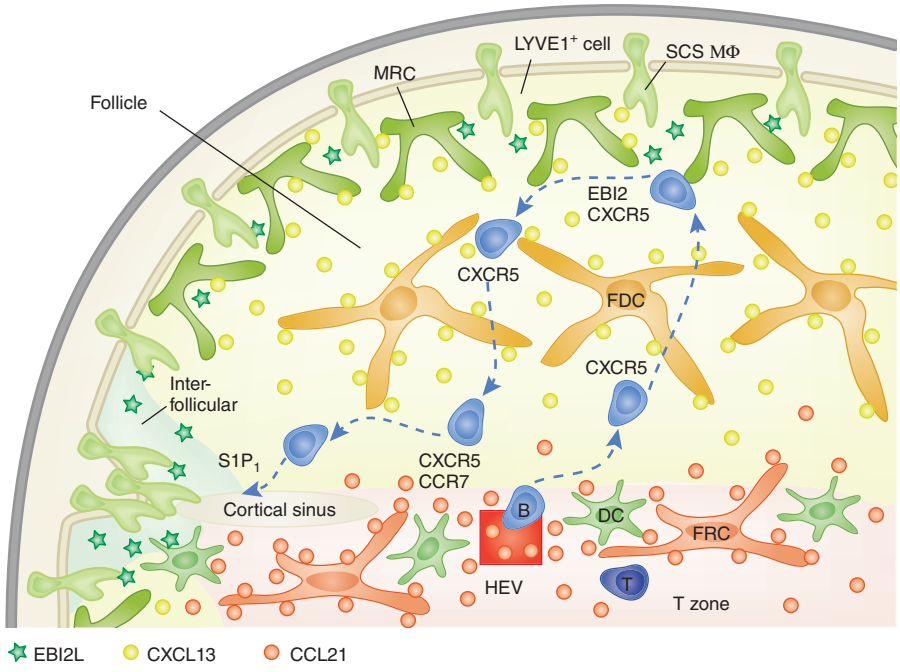

Figure 1 Multiple cues direct B cell migration through the lymph node follicle. A B cell enters from an HEV, migrates into a follicle in a CXCR5-CXCL13–dependent manner and then is attracted to the outer follicle in response to both CXCL13 and EBI2 ligand (EBI2L). After migrating back through the FDC network, the cell travels through the T cell zone (T zone)–proximal follicle using CCR7 to respond to CCL21 (and CCL19; not presented here) and CXCR5 before exiting into a LYVE1$^+$ cortical sinus in an sphingosine 1-phosphate receptor type 1 ($S1P_1$)-dependent manner. CCL21 is produced by fibroblastic reticular cells (FRC) and HEVs; CXCL13 is produced by MRCs and FDCs; and the source of EBI2L is not known, but it is suggested to be more abundant in the interfollicular regions and outer follicle than in the follicle center. The morphology of MRCs are not well defined and there is probably additional heterogeneity in the follicular stroma not presented here. Some of the stromal cells are associated with collagen fibers (not presented here) and the cells form an interconnected network. MΦ, macrophage.

source of the ligand for EBI2 has not yet been reported, but EBI2 overexpression studies suggest that this ligand may be concentrated in the outer follicle and interfollicular regions[21,23]. Consistent with the roles of CXCR5 and EBI2 in promoting antigen encounter, both CXCR5 deficiency and EBI2 deficiency are associated with diminished antibody responses[15,22,24,25].

A third chemokine receptor system that influences B cell distribution in the follicle is CCR7 and its ligands, CCL21 and CCL19 (ref. 26). Mature B cells have low expression of CCR7 and respond to CCR7 ligands[27]. Although CCR7 ligands are expressed principally in the T cell zone, CCL21 extends as a gradient from the T cell zone into the follicle, and access of B cells to the T cell zone–proximal half of the follicle is partially CCR7 dependent[28] (**Fig. 1**). B cells rapidly upregulate CCR7 after encountering cognate antigen, and they use this receptor to navigate to the B cell–T cell boundary in search of T cell help[27,28].

Early antigen traffic to lymph nodes

Small soluble antigens that enter the body across the skin are picked up in the flow of transudated plasma and are carried through flap-like junctions between endothelial cells of the initial lymphatics[29,30]. These vessels join larger collecting lymphatics that later become lymph node afferent lymphatics. Transudated plasma, after entering a lymphatic, becomes lymph fluid, and any material it carries drains through these vessels into the SCS located between the collagenous capsule and the lymphocyte-rich cortex. The passage of entering lymph fluid through the SCS before it reaches the medullary sinus, located on the lymph node surface connecting to the efferent lymphatic, ensures that antigenic particles have the opportunity to encounter SCS macrophages overlying B cell follicles and possibly to gain access to follicles; antigen traveling on to the medullary sinus is taken up and destroyed by highly phagocytic medullary macrophages. Large antigens, complexes too large to freely enter lymphatics, can arrive in the lymph node later, carried by DCs or other myeloid cell types. The size of particles that can freely reach and enter lymphatics varies with the properties of interstitial spaces at the site of injection and with the type of local lymphatics[29,31]. By whole-body imaging, subcutaneously injected fluorescent microspheres 20–200 nm in diameter (a size range that includes many viruses) have been found to gain ready access to lymphatics, whereas particles 500–2,000 nm in diameter (the size of many bacteria) are inefficient in reaching draining lymph nodes unless carried by cells[32]. Other studies have found that particles 1,000 nm in diameter can gain access to skin-draining lymph nodes[33], and particles of this size readily drain from the peritoneum, where stomatal openings in the mesothelium overlying diaphragmatic lymphatics facilitate their passage, although microspheres 5 μm in diameter do not drain[34,35]. The size limits of particle draining may be influenced by the extent of local inflammation and associated increase in interstitial fluid flow[31]. The protozoan pathogen *Toxoplasma gondii* is several micrometers in size and reaches draining lymph nodes within hours of subcutaneous inoculation[36]. Some large pathogens reach draining lymph nodes because of their own migratory activity. For example, *Plasmodium falciparum* sporozoites have been visualized migrating into skin lymphatic vessels (as well as blood vessels) after a bite by an infected mosquito[37]. More generally, many microbes secrete antigenic material or are attacked by proteases and other enzymes, releasing lymphatic-accessible antigens.

SCS macrophages and display of particulate antigens

The floor of the SCS is lined by cells positive for the lymphatic endothelial cell marker LYVE1, lying on a layer of extracellular matrix[38–41]. This endothelium-like lining limits the free access of molecules and fluid to the tissue parenchyma[42]. Although some reports have suggested the existence of pores in the lining, most studies have emphasized the presence of macrophage-type cells that extend across the sinus wall[38,40,43] and that express sialoadhesin (CD169)[44,45] (**Fig. 2**). Early studies considered the macrophages to be in the act of passing through the sinus lining cells, but real-time imaging experiments have shown that they are resident in this 'transcellular' position[41,46,47]. How they orientate themselves in this polarized way is not yet clear, but in experiments in which afferent lymph flow is interrupted, they move rapidly into the follicle and disappear[48,49], which suggests that there are signals in the lymph that promote positioning and homeostasis of the cells. On the follicular side, SCS macrophages are in contact with B cells, and they require B cells

and the B cell–expressed cytokine lymphotoxin-α1β2 for their development and/or maintenance[50]. This B cell–macrophage crosstalk may be a mechanism that links changes in follicle size with the maintenance of SCS macrophage coverage[51]. The development of SCS macrophages is defective in mice lacking colony-stimulating factor 1, consistent with their macrophage designation[52].

Early studies showed that SCS macrophages capture a variety of foreign particulate antigens that enter from the afferent lymphatics, such as colloidal carbon, ferritin, liposomes and opsonized antigens[39,45,53,54]. In contrast to the sinus-situated medullary macrophages that fit the classical macrophage definition of being strongly phagocytic cells that rapidly degrade internalized material[39,45,53], SCS macrophages have a low rate of antigen internalization and degradation[39,50,54]. SCS macrophage have a lower rate of depletion by clodronate-containing liposomes that kill cells after internalization than do medullary macrophages, which is also consistent with their different rates of phagocytosis[45]. Medullary macrophages can be distinguished from SCS macrophages by their expression of the macrophage marker F4/80 and the C-type lectin SIGN-R1 (refs. 45,50,55). A study has used the marker F4/80 to distinguish $CD169^+$ SCS and medullary cells by flow cytometry[50], although the ability to isolate SCS macrophages at higher purity continues to be a challenge, and some of the $CD169^{hi}F4/80^{lo}$ cells identified in cell suspensions seem to be lymphocytes masquerading as macrophages because of acquisition of macrophage membrane blebs (E.E. Gray, T.G. Phan and J.G.C., unpublished data). Whether this membrane transfer[56] occurs *in vivo* or during tissue digestion and cell isolation requires further investigation, but these observations suggest that some lymphocytes have receptors that promote strong interactions with SCS macrophages.

Real-time imaging experiments have visualized SCS macrophages capturing virus-sized (200-nm) beads, opsonized phycoerythrin, vesicular stomatitis virus, vaccinia virus and adenovirus particles within minutes of their subcutaneous injection[41,46,47]. For each of these antigen types, the cells make the captured material accessible to B cells that migrate over their follicular tail processes[41,46,47] (**Fig. 3**). Real-time imaging has shown immune complexes moving unidirectionally from the sinus-accessible macrophage head region to the follicle-associated tail region[50]. The complexes seem to be predominantly surface associated (**Fig. 4**, model 1), but the real-time imaging approaches have been of insufficient resolution to exclude the possibility of movement by transcytosis (**Fig. 4**, model 2). The cells express the complement receptor CR3 (also called Mac1 or CD11b-CD18; **Fig. 2**) and have low expression of CR4 (CD11c-CD18)[47,50,57] as well as FcγRs (Fc receptors for immunoglobulin G (IgG; E.E. Gray and J.G.C., unpublished data). They also have a selective ability to bind the cysteine-rich domain of soluble mannose receptor, in part via CD169 (refs. 58,59). However, the involvement of any single receptor in the capture of opsonized antigens has been difficult to establish[47,50], which perhaps indicates that multiple classes of receptors are often used together. Capture of ultraviolet irradiation–inactivated influenza virus is partially dependent on mannose-binding lectin, an initiator of the complement cascade[60]. This suggests a role for SCS macrophage CR3 or CR4 in capture but might also indicate involvement of receptors for mannose-binding lectin or CD169-mediated binding via mannose receptor.

After encountering cognate antigen displayed by SCS macrophages, migrating B cells decrease their velocity and transiently accumulate in the SCS region[46,47]. This accumulation may reflect close interaction with the SCS macrophage surface, perhaps mediated by the integrin ligands VCAM-1 or ICAM-1 on the macrophage[41,46,47]. In addition, antigen-receptor engagement can deliver migration 'stop' signals. These effects are transient, and within a few hours, encounter with cognate antigen leads to CCR7-dependent migration to the T cell zone[41,46,47].

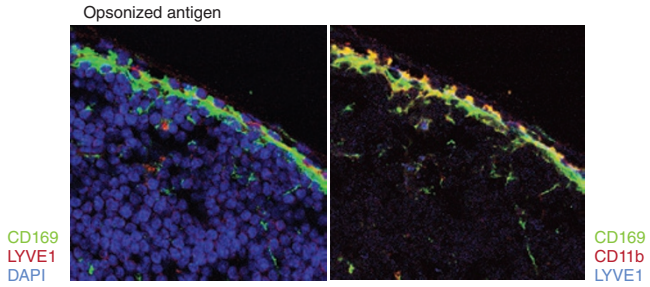

Figure 2 SCS macrophages overlying a lymph node follicle. Confocal microscopy of a peripheral lymph node frozen section stained for various markers (bottom left corners); $CD169^+CD11b^+$ macrophages project through the $LYVE1^+$ SCS-lining cells. Original magnification, ×63. Images by Elizabeth E. Gray.

Although SCS macrophages capture many types of foreign particles, they ignore much of the material that enters the SCS. Lymph contains substantial amounts of self protein, carbohydrate and lipid[61]. Lymph traveling from the intestine is particularly rich in lipids (for example, chylomicrons) and contains other inert dietary components. These self and dietary molecules are thought to have free passage through the node sinuses[29], which highlights the point that the capture and retention of foreign material requires specific receptors.

As well as capturing and displaying antigens, SCS macrophages are an early site of infection by pathogens, including vaccinia, vesicular stomatitis virus, murine cytomegalovirus, cowpox and *Toxoplasma gondii*[35,36,47,62,63]. It seems possible that a lower propensity for antigen degradation may cause these cells to serve as a safe haven for pathogens. Indeed, one theory is that they may be specialized to support infection and presentation or cross-presentation of antigenic material to facilitate rapid mounting of T cell responses[64]. The cells express major histocompatibility complex class I, and when infected by *T. gondii*, they are killed by effector $CD8^+$ cells[65]. However, whether they can prime the activation of $CD8^+$ T cells is less clear, and they might instead deliver antigen to nearby DCs[64]. SCS macrophages are able to take up, process and present lipid antigens in the context of the antigen-presenting molecule CD1d for direct activation of invariant natural killer T cells[66]. Published work has highlighted an important innate role for SCS macrophages: after infection with vesicular stomatitis virus, they produce interferon-α/β, which is necessary to protect nerves situated in the SCS from infection by this neurotropic virus[63]. Marginal zone metallophilic macrophages in the spleen also produce interferon-α/β during viral infection[67]. How infection of SCS macrophage by pathogens affects the B cell response is not yet clear, but locally produced interferon-α/β may directly costimulate antibody production[68,69].

In addition to macrophages, lymph node lymphatic endothelial cells bind and endocytose antigen[40,43,70], including viruses[47]. These cells express mannose receptor[71] and scavenger receptors such as stabilin I (ref. 72). Whether antigen captured by these cells is displayed for B cell recognition has not been determined. Lymph node lymphatic endothelial cells have been found to express a variety of tissue-specific autoantigens[73]. Other stromal cell types in lymph nodes, including cells expressing the transcriptional regulator Aire, which are more abundant near follicles, also express a range of tissue-restricted antigens and help mediate T cell tolerance[74,75]. It will be interesting to discover whether these cells secrete or display autoantigens in a way that affects B cell tolerance.

Despite evidence that SCS macrophages capture and transiently display particulate antigens, macrophage-ablation experiments have so far not led to lower B cell responses[60,63,76]. As these treatments broadly

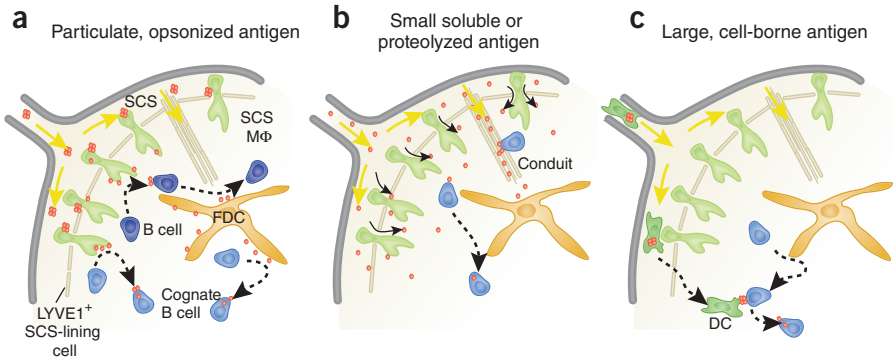

Figure 3 Multiple modes for the encounter of follicular B cells with antigen. The modes by which particulate antigens (such as immune complexes and viral particles; **a**), soluble antigens of small hydrodynamic radius (in some cases released from large antigens by proteolysis; **b**), or antigens too large to enter passively into lymphatic vessels (**c**) encounter B cells. (**a**) Antigen is captured and displayed by SCS macrophages and then is encountered directly by cognate B cells or is transported by noncognate B cells to FDCs for later encounter with cognate B cells. (**b**) Antigen accesses the follicle via gaps in the SCS lining or via conduits and is then encountered by cognate B cells. (**c**) Antigen is carried into the follicle-proximal cortex inside DCs, is recycled to their cell surface and is encountered by cognate B cells.

remove macrophages from the lymph node and the injection site, any decrease in efficiency of antigen delivery to follicles might be compensated for by less antigen clearance and greater availability. Although it has been reported that the SCS is not markedly perturbed by macrophage ablation[47], a more detailed assessment is needed to rule out the possibility that gaps are left at the sites of the ablated macrophages. Future studies with systems that allow more selective ablation or perturbation of SCS macrophages will help to further elucidate the importance of these cells in various types of antibody responses.

Conduits and follicular access of small soluble antigens

In contrast to particulate antigens, small soluble unopsonized antigens bypass SCS macrophages and gain direct access to the follicle (**Fig. 3**). Lymphoid tissues contain a network of collagen-rich fibers ensheathed by fibroblastic reticular cells, called 'conduits'[42]. The fibers are not tightly packed, and conduits contain spaces that allow passage of lymph and molecules with a dynamic radius of less than ~5.5 nm (molecular size, ~70 kilodaltons) through the tissue parenchyma[29,42]. Conduits are most abundant in the T cell zone and interfollicular regions, and tracer studies indicate that the direction of flow in conduits is from the SCS to high endothelial venules (HEVs). These structures may facilitate the delivery of some of the lymph fluid arriving in lymph nodes to venules for return to circulation[29,42]. By passing through conduits, small proteins such as chemokines can travel rapidly from the lymph to HEVs[29,42]. It seems likely that small antigens are delivered in a similar way to HEVs, which would facilitate their encounter by entering B cells, although this has not been tested directly. Tracer molecules are also delivered to cortical sinuses[30,70,77], and conduits may help supply these sinuses with the lymph flow needed to promote the passage of egressing lymphocytes from cortical sinuses to medullary sinuses[18]. In the developing lymph node, conduits are similarly abundant in the nascent follicle and T cell area, but as B cells arrive and the follicle develops, some of the fibroblastic reticular cells and associated conduits are pushed aside and FDCs take their place in the center of the follicle[78]. However, some conduits remain and may have a role in the delivery of small antigens to B cells and the FDC network[78,79] (**Fig. 3**). Other work has suggested that small protein antigens gain direct access to the follicle, moving in a front from the SCS[80], perhaps gaining access via junctions between LYVE1$^+$ SCS-lining cells and SCS macrophages[39]. It seems possible that small antigens access follicles by both of these pathways, but conduits may contain a higher concentration of (possibly matrix-associated) antigen. This might mean that if a low-affinity B cell probes the interior of a conduit, it has a better chance of becoming activated. To definitively establish the role of conduits in antigen delivery it will be necessary to test the effect that disrupting them has on B cell activation.

Although many antigens are greater than 70 kilodaltons in size, large antigens traveling in lymph fluid can become proteolyzed[33]. Protein antigens are cleaved from the surface of beads 1 μm in diameter and gain access to follicular B cells without a requirement for SCS macrophages or DCs or for extensive migration of the follicular B cells, which suggests that the cleaved antigens directly access the follicle[33]. The ability of B cells to present peptides to T cells requires that the B cell and T cell determinants be linked in the same protein rather than simply being present on the same beads. This observation may connect with the finding that T cell help for B cells responding to vaccinia virus is largely intramolecular rather than of the intermolecular type identified in classical studies of responses to influenza virus (80 nm in diameter) and hepatitis B virus (20–40 nm in diameter)[81]. Vaccinia virus is large (360 nm in diameter), and proteolysis may be an important factor in allowing efficient access of B cells to vaccinia virus antigens. These observations might typify events for pathogens that are more than a few hundred nanometers in size.

Antigen-presenting roles for migrating and local DCs

DCs are specialized to internalize antigens at peripheral sites and then travel to draining lymph nodes to display complexes of peptide and major histocompatibility complex to T cells. DCs have lower abundance of lysosomal proteases than do classical macrophages, and they degrade internalized antigens more slowly[82]. This facilitates the prolonged generation of complexes of peptide and major histocompatibility complex but might also allow the cells to regurgitate intact antigen for encounter with B cells (**Fig. 3**). DCs internalizing immune complexes via FcγRIIb can recycle the complexes to the cell surface[83], and the C-type lectin DC-SIGN re-exposes captured viral particles in human DCs[84]. Early studies demonstrated the activity of antigen-bearing DCs in stimulating B cells *in vitro* and after adoptive transfer[85,86]. Subsequently, transferred DCs localized near HEVs have been visualized presenting hen egg lysozyme to entering B cells[87]. B cells migrating through the follicle are also likely to encounter DCs that are densely distributed at the follicle–T cell zone boundary (**Fig. 3**), and the activity of CCR7 in naive B cells may favor such encounters. DCs are also present in the SCS and are occasionally present in follicles[88], possibly providing further opportunities for antigen presentation to B cells. DCs in the medullary or interfollicular regions of lymph nodes may also capture antigen, such as inactivated influenza virus, arriving in lymph and present this to B cells, although interaction between such DCs and B cells has not yet been visualized[60,89]. It remains to be determined whether the ability of B cells to respond to large antigens is augmented by DC-mediated antigen presentation or whether other pathways such as proteolysis are more important mechanisms for achieving B cell exposure to antigens that do not travel passively to the SCS.

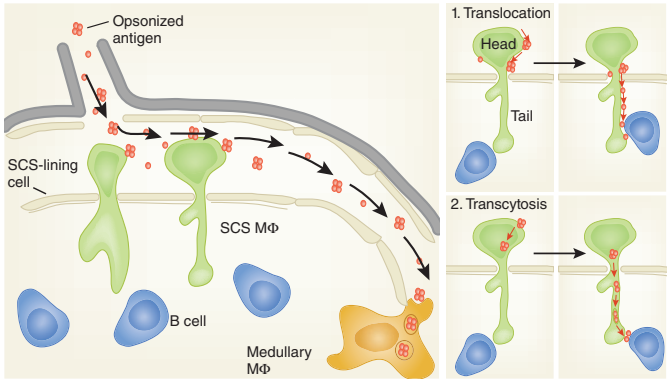

Figure 4 Models for the translocation of opsonized particulate antigen from the sinus to the follicular surface of the SCS macrophage. Particles in the lymph percolate through the SCS and some are captured by unknown receptors on the SCS macrophage, whereas excess material reaches medullary sinuses and is phagocytosed and degraded by medullary macrophages. In model 1 (top right), particulate antigen is retained on the SCS macrophage surface, undergoes fragmentation and moves unidirectionally on the cell surface by unknown mechanisms from the SCS to the follicle, where it is displayed to migrating B cells. In model 2 (bottom right), the particulate antigen is endocytosed into vesicles, transported directionally within the cell and then returned to the cell surface for display. Over a period of hours, antigen that is not removed by migrating B cells is probably degraded by the SCS macrophage.

Antigen delivery to and capture from FDCs

FDCs have a prominent role in the capture and display of opsonized antigens, having the unique property of retaining them on their surface for long periods[2,90]. In primary follicles, FDCs have very high expression of complement receptors 1 and 2 (called 'CR1/2' here); within GCs they also express FcγRIIb[2]. Unlike macrophages and DCs, they express few pattern-recognition receptors and have little ability to capture nonopsonized antigens[2]. The classical pathway of complement activation ensures that once specific IgM or IgG is available, even small soluble antigens can be coated with breakdown products of complement component C3 (C3b, iC3b and C3d)[91]. Many complex antigens can be engaged by the alternative or lectin pathways of complement activation, which allows opsonization before the availability of specific antibody. Polymeric antigens and pathogen surfaces are sometimes bound by pre-existing polyreactive (natural) antibodies. As early as 2–3 days into a primary response to pathogens containing T cell–independent antigens, specific antibody can be induced. Thus, there are many ways that particulate antigens can become opsonized during primary exposure, and for most infections in humans, some form of the pathogen has been encountered before (prior infection or vaccination), allowing opsonization by pre-existing antibody. Thus, FDCs are involved from the outset or quickly become involved in the capture and display of antigen during most immune responses. Although mice selectively deficient in FDCs have not been studied, mice lacking CR1/2 in FDCs are compromised in antibody responses to a variety of antigens[91].

A notable feature of the FDC network is that it is located centrally in the follicle and typically does not extend to the SCS, interfollicular regions or T cell zone[92]. The logic for this separation of FDCs from the sites of earliest antigen capture has not been defined. Perhaps being centered in the follicle and not in substantial contact with macrophages, DCs or circulatory fluids provide a protected environment in which opsonized antigens can be displayed for long periods without being proteolyzed or removed by phagocytic cells. In agreement with this hypothesis, follicular stromal cells have high expression of serpin-a1 (α1-antitrypsin), a protease inhibitor[93,94]. The physical isolation of FDCs in follicles necessitates mechanisms for antigen to travel from the first point of capture to the FDC. Early studies postulated the involvement of B cells[95,96], myeloid cells or FDC precursors[54] in this transport. Imaging studies have shown that noncognate B cells capture immune complexes from SCS macrophages in a CR1/2-dependent way and then migrate through the follicle with complexes attached at their uropods (**Fig. 3**). The delivery of immune complexes to FDCs is less efficient in mice in which B cells lack CR1/2 (ref. 41), and an *in vitro* experiment has shown that B cells loaded with opsonized gold particles give the particles up to FDCs[96]. This may occur as a consequence of the much higher CR1/2 expression by FDCs than by B cells[91].

B cells may also capture immune complexes in the blood and carry them into lymphoid tissues for unloading on FDCs. As well as CR1/2-based capture, B cell can use the low-affinity FcεR CD23 in mice with large amounts of antigen-specific IgE[97]. When B cells lack CR1/2, the movement of IgG-containing immune complexes to FDC networks is less efficient but is not blocked. The continued delivery might reflect a contribution of B cell FcγRIIb or the involvement of additional antigen-transporting cell types. Moreover, the dominant antigen-transport pathway may vary depending on the properties of the antigen, such as their content of ligands for Toll-like receptors. Exposure to lipopolysaccharide causes splenic marginal zone metallophilic macrophages to migrate rapidly into follicles[98], and such movement can be observed in lymph nodes, although the effect is less prominent (T.G. Phan and J.G.C., unpublished data). Slow movement of SCS macrophages into follicles has also been reported after immunization with antigen in adjuvant[58,99]. Thus, under some conditions, SCS macrophages themselves may deliver immune complexes to FDCs.

Evidence that FDC-displayed antigen drives B cell responses is extensive[1,2,92]. Cognate B cells have been visualized capturing phycoerythrin-coupled antigen from the surface of FDCs[15]. This visualization was facilitated by the use of high-affinity B cells, and acquisition occurred rapidly (~6.5 min of contact time). For low-affinity B cells, antigen acquisition was enhanced by CR1/2 expression on the B cell[15]. Consistent with the ability of FDCs to display opsonized antigens for long periods[90], FDC-retained antigen was available for acquisition by naive B cells over a 9-day period[15]. By this late time point, the follicles often showed GC responses, which suggested that naive B cells were able to access antigen displayed on FDCs in GCs, in agreement with evidence that GC light zones are open for the entry of naive B cells[100].

Ongoing immune complex delivery helps drive the GC response

GCs are antigen-driven structures that form in the follicle center, acquiring a light zone centered over the FDC network—orientated toward sites of antigen entry—and a dark zone that extends toward the T cell area. GCs take about a week to form and can last for many weeks[101]. GC B cells migrate within the confines of the GC at speeds similar to those of naive B cells but have a probing, dendritic morphology that gives them a much larger surface area and greater opportunity for antigen encounter[13,100,102]. Although cells have been observed pausing for long periods in the FDC network, for the most part the cells seem to continually move over and through the FDC network. These data might suggest continual binding and ripping off of antigen from FDCs as the cells move, similar to the antigen capture witnessed for naive B cells[15], but actual visualization of antigen capture by GC B cells has not yet been achieved. Naive B cells have been visualized ferrying immune complexes into GCs, and when they lack CR1/2, there is less deposition of immune complexes on light-zone FDCs and less effective selection

of high-affinity B cells[50]. These findings and other data showing that specific antibody can augment the GC response[103] suggest a model in which initial antibody produced in the primary response can opsonize the remaining antigen and facilitate its loading onto FDCs to drive the high-affinity antibody response.

Summary

One goal of gaining greater understanding of how B cells encounter and respond to antigen is the ability to design better vaccines. With this view in mind, it is worthwhile to try to put the knowledge discussed in this review to work and imagine the pathways that ensure B cell encounter with and response to an important vaccine. The hepatitis B vaccine was the first recombinant vaccine and is now being used as a platform for the development of other vaccines[104]. It is generated by expression of hepatitis B surface antigen (HBsAg) in yeast, yielding HBsAg particles 20–22 nm in diameter that are collected and prepared in alum adjuvant for intramuscular injection[105,106]. After primary immunization, particles of this size can be expected to gain efficient access to local lymphatics, and some arrive within minutes to the draining lymph node SCS. Given their yeast origin, the particles probably become opsonized through lectin and alternative complement pathways. SCS macrophages can be expected to capture the particles through a combination of receptors and transport them from the sinus for transient surface display on their follicular processes. Follicular B cells already moving over the macrophage processes immediately begin capturing the particles using CR1/2 and transport them through the follicle, delivering them to FDCs. Some antigen is also proteolyzed and able to travel into the follicle through the sinus floor or via conduits. Within the first hours of immunization, rare cognate B cells encounter the particles on the SCS macrophage processes, but at later times greater amounts are accessible on the FDC network. In the primary response, during which the few available cognate B cells have low-affinity B cell antigen receptors for HBsAg, B cell activation is more effective when intact particles, rather than soluble antigens, are encountered. Membrane-bound arrays of opsonized particles are particularly effective at engaging low-affinity B cells. The 'depot' effect of the adjuvant ensures that particles continue to drain to the lymph node for many hours, perhaps even days, keeping SCS macrophages 'in play' for a sustained period. Cognate B cells that capture antigen go on to produce antibody several days later, and this serves to improve the capture and deposition of the remaining HBsAg particles on the FDCs. By this stage, some B cells have received signals to differentiate into GC B cells, and after downregulating EBI2, they move to the follicle center and migrate in close association with the antigen-bearing FDC network. The continued relay of remaining antigen as IgG-coated immune complexes from the SCS to the GC, in part by follicular B cells, helps drive the affinity maturation of antibody. In a booster immunization, these events are accelerated because of the pre-existence of specific IgG and more efficient opsonization and capture of the viral particles, as well as the presence of a greater frequency of cognate B cells. The outcome of these events is production of high-affinity HBsAg-specific antibody.

Of course, many aspects of this hypothetical response may turn out to be incorrect or oversimplified, and much more work needs to be done before accurate modeling will be possible. One implication of the work cited here is that intentional targeting of antigens to SCS macrophages may be a means to augment antibody responses, and there is some preliminary support for this possibility[107,108]. The studies reviewed here also highlight how the entire primary follicle is organized to coordinate antigen encounter and prolonged antigen display, with follicular B cells doing double duty surveying for cognate antigen while helping to deliver noncognate antigen from the exposed outer follicle to the follicle center. An idea that merits further investigation is that prolonged antigen display by primary follicle FDCs is facilitated not only by specialized properties of these cells but also by the inner follicle's being an 'antigen sanctuary', protected from circulating proteases and migrating phagocytes. Finally, the existence of multiple overlapping mechanisms that facilitate encounters between B cells and antigens may help to ensure that pathogens have difficulty in outmaneuvering this crucial first step of the humoral response.

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