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Complement-Dependent Transport of Antigen into B Cell Follicles


Since the original proposal by Fearon and Locksley (Fearon and Locksley. 1996. Science 272: 50–53) that the complement system linked innate and adaptive immunity, there has been a rapid expansion of studies on this topic. With the advance of intravital imaging, a number of recent papers revealed an additional novel pathway in which complement C3 and its receptors enhance humoral immunity through delivery of Ag to the B cell compartment. In this review, we discuss this pathway and highlight several novel exceptions recently found with a model influenza vaccine, such as mannose-binding lectin opsonization of influenza and uptake by macrophages, and the capture of virus by dendritic cells residing in the medullary compartment of peripheral lymph nodes. The Journal of Immunology, 2010, 185: 2659–2664.

Peripheral lymph nodes (LNs) and the spleen make up the secondary lymphoid organ tissue that provide a specialized environment for circulating B and T lymphocytes to interact and encounter cognate Ag (1). Although T cells home to the paracortical region of LNs, B cells traffic to the follicles in search of Ag. This directed migration is dependent on chemokines produced by stromal cells in the respective compartments. Recent elegant intravital imaging of T and B cell trafficking within the peripheral LNs revealed a directed migration along stromal “highways” (2, 3). Fibroblast reticular cells (FRCs) secrete the collagen-rich fibers that form the network within the paracortical region, and they secrete T cell chemokines CCL19 and CCL21. B cell migration within the follicles is dependent on follicular dendritic cells (FDCs) dendritic processes and a less-dense network of FRC fibers. Although the reticular network within LNs was characterized more than three decades ago (4), only more recently did it become apparent that it acts as a conduit for the delivery of cytokines, chemokines, and small-protein Ags to the T (5–8) and B cell areas (9, 10). B cell conduits are structurally and immunochemically similar to those in the T cell area. They differ primarily by specificity of the chemokine secreted (i.e., follicular FRCs secrete CXCL-13, whereas paracortical FRCs secrete CCL19 and CCL21). Although the outer diameter of conduits is ∼1–2 μm, they are tightly packed with collagen fibers with a spacing of 5–8 nm, which acts as a molecular sieve (Fig. 1). Thus, only proteins less than ∼60 kDa enter the conduits. Whether conduit structures are altered to accommodate larger Ags during infection is not clear.

Trafficking of lymph-borne Ag into B cell follicles

Small protein Ags gain direct access into the B cell follicles via gaps in the subcapsular sinus (SCS) floor (11) or through the FRC conduits (Fig. 2A) (9). The latter pathway provides a directed flow of small Ags to the FDCs for transient retention, or in the presence of Ab and complement, long-term binding via specific receptors. Although cognate B cells can access Ag draining via the conduits (9), their principal role is more likely directing the Ag to FDCs for stable retention. Although these initial experiments involved model Ags, such as lysozyme (9), or OVA (10), in the natural setting it seems likely that a major source of Ag is degraded products of pathogens that drain from tissues via the lymphatics, as suggested by Jenkins and colleagues (12).

Lymph-borne particulate Ags, such as vesicular stomatitis virus (13), and protein-coated beads (14) are rapidly taken up by macrophages that line the SCS (SSMs) (15). Interestingly, the particulate Ags are shuttled to the underlying surface where they are made available to cognate B cells. Similarly, large protein Ags injected s.c. into passively immunized mice also seem to bind rapidly to SSMS. However, in the latter example, capture by SSMS is complement dependent. Thus, formation of immune complexes (ICs) activates complement, resulting in formation of C3-coated ICs (C3-ICs) that enhance uptake via CR3 (Mac-1) and FcRIIb on the SSMs (16). Subsequently, C3-ICs are relayed to the underlying B cell compartment where they are transferred to naive B cells (Fig. 2A) (17). FcRIIb is known to recycle to the surface following internalization and

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not go through a lysosomal compartment (18). Therefore, it is possible that C3-ICs are partially protected by this cycling process. It is not clear how C3-ICs are actually transferred to B cells; however, uptake on the naive B cells is CD21/35 dependent and highly efficient. Strikingly, >25% of naive B cells within the LN follicles take up C3-ICs within 8 h of s.c. injection of Ag into immune mice (9, 16).

In naive mice in which Ab does not pre-exist, it is not clear how large protein Ags within the lymph are captured by sinus-lining macrophages. The lymph, in general, is thought to include a similar repertoire of recognition proteins that activate complement as found in blood, such as mannose-binding lectins (MBL), ficolins, and pentraxin. Alternatively, protein Ags might be directly taken up by scavenger or lectin receptors expressed on the sinus-lining macrophages. Medullary macrophages (MMs), which are more similar to marginal zone (MZ) macrophages of the spleen, express specific intracellular adhesion molecule-grabbing nonintegrin R1 (SIGN-R1) and mannose receptor (MR), in addition to CR3. However, SSMs are more similar to the metallophilic macrophages of the MZ and express metallophilic macrophages Ab-1 (17).

**B cell transport of C3-ICs is CD21/35 dependent**

In mice, complement receptors CD21 and CD35 are encoded at the Cr2 locus (19, 20). CD21 represents a splice product of the CD35 mRNA, whereas in humans they are encoded at separate loci (21, 22). Murine CD21 and CD35 (CD21/35) are coexpressed on B cells, FDCs, and a subset of T cells. CD35 binds activated C3b and C4b and, like CD21, it binds the cleavage products iC3b, C3d,g, and C3d covalently.
attached to Ags (19, 23–25). They play a critical role in humoral immunity because blocking with Abs (26, 27), a soluble receptor (28), or deletion of the receptors (29, 30) leads to impaired humoral immunity to model protein Ags, bacteria (31), and viruses (32, 33) (Fig. 3). On B cells, CD21/35 forms a coreceptor with CD19 and CD81, and coligation with the BCR lowers the threshold of B cell activation (34–36).

A second major function of CD21/35 is retention of Ag on FDCs. They represent the major Ag receptors on FDCs (37–39). Ag is also retained for long periods via FcRIIb; however, its expression is not constitutive but upregulated on activated FDCs (40). FDCs are thought to be the major source of Ag required for clonal selection of B cells during the germinal center (GC) reaction (41) and possibly for maintenance of memory (42–44). They retain Ag and secrete B cell growth factors and the B cell attractant CXCL-13, as mentioned above (45). Mice deficient in C3 or CD21/35 have fewer and smaller GCs, and B cells fail to survive (46, 47).

There is growing evidence that B cells acquire Ag and are activated most efficiently when it is attached to membrane (48–50). Therefore, retention of Ag and C3d on the FDC surface could enhance formation of the B cell synapse. Support for a role for FDCs as an important source of Ag comes from the recent studies of Cyster et al. (51); they demonstrated direct capture of Ag from FDC surface by cognate B cells using multiphoton intravital microscopy (MP-IVM). Importantly, they also identified a role for CD21/35 in efficient uptake of medium-affinity Ags. In their model system, MD4 B cells deficient in CD21/35 acquired less Ag from FDCs. One explanation for their results is that the coreceptor acts to enhance BCR signaling in the synapse between the BCR and FDC.

A third novel role for CD21/35 is transport of lymph-borne C3-ICs into the B cell follicles and transfer to FDCs. As discussed above, naive B cells acquire C3-ICs from SSMs lining the SCS via their CD21/35 receptors. Whether uptake induces a direct signal via CD21/35 or involves the coreceptor CD19 and CD81 is not clear. In a recent study using mice in which CD21/35 are uncoupled from CD19 (Cr2ΔD), naive B cells in the mutant line seemed to take up C3-ICs normally and deliver them to FDCs (42). CD21/35 receptors include a cytoplasmic domain so it is possible that uptake of C3-ICs induces a signal by CD21, independent of CD19 (52), and triggers B cell migration to the FDCs. An alternative possibility is that B cells within the follicles migrate constitutively to FDCs based on a chemokine gradient in a manner similar to that described for MZ B cells (53); therefore, direct signaling by C3-ICs may not be essential. Thus, as B cells enter the peripheral LNs via high endothelial cells, they cycle to the SCS area, which is rich in marginal reticular cells that secrete B cell chemokines (54), and subsequently to FDCs, which are also a source of chemokines (45).

Earlier studies identified a role for C3 and CD21/35 in the capture of ICs by MZ B cells and transport to FDCs within B cell follicles (55–57). Using intravital imaging, Cyster and colleagues (53) identified a similar pathway for capture of C3-ICs in the spleen as reported for the LNs. They found that MZ B cells continuously shuttled between the splenic MZ and the B cell follicles by a CXCR5-chemokine-sphingosine 1-phosphate receptor-dependent mechanism. Thus, naive MZ B cells capture C3-coated Ags within the MZ sinus and transport them into the follicles where they are transferred to FDCs. How C3-ICs are handed off to FDCs is not clear; however, uptake on the FDCs is dependent on CD21/35 and FcRIIb. Because the latter receptors (FcRIIb) are not constitutively expressed but are upregulated following FDC activation, complement receptors are critical for the initial capture and retention of C3-ICs (40).

**FIGURE 3.** Complement receptors CD21 and CD35 play an important role in at least three stages of B cell differentiation. Stage 1: Coligation of C3d-Ag with BCR lowers threshold of B cell activation, leading to migration of the activated B cell to the T cell–B cell boundary, where cognate interaction occurs and B cells receive costimulation via CD40. Stage 2: Activated B cells enter a GC where they begin further differentiation, including rapid cell division, somatic cell hypermutation, and class switch recombination. Stage 3: Following clonal selection (binding of Ag on FDC), the GC B cell differentiates into an effector cell (plasma cell) or memory B cell. Maintenance of B effector and memory cells is dependent on presence of Ag on FDC.
Influenza infection represents a major pathogen of humans; in general it is controlled by annual vaccination and induction of a protective humoral response (58–60). Complement C3 and CD21/35 are important components in humoral immunity to influenza, based on earlier studies (33, 61, 62). Although much of the focus on the enhancing role of complement has been on the B cell coreceptor, the C3-CD21/35 pathway is also important for transport and retention of Ag on FDCs, as discussed above.

Recent studies identified a novel pathway by which viral Ag is captured and possibly transported to the FDCs. Gonzalez et al. (63) used a UV-inactivated form of influenza virus (strain PR8) as a model vaccine. Using a fluorescent-tagged form of UV-PR8 injected in the footpad (s.c.) and imaging by MP-IVM, they identified rapid filling of the sinus and capture by SSMs and MMs. As discussed earlier, macrophages lining these regions represent distinct populations, as distinguished by functional maturity and cell surface markers (17).

The study by Gonzalez et al. (63) reported that labeled UV-PR8 virus was rapidly bound by the SSMs and MMs, similar to that reported for vesicular stomatitis virus (13). In contrast to the earlier studies, UV-PR8 was not retained on the surface but was internalized. Binding and phagocytosis was dependent on MBL, because SSMs in MBL−/− mice failed to bind the virus. Opsonization by MBL is in agreement with earlier studies by Ezekowitz and colleagues (64), who reported that guinea pig mannose-binding lectin bound and neutralized influenza. Interestingly, blocking of UV-PR8 uptake by SSMs alone or SSMs and MMs did not impair humoral immunity. This was somewhat surprising because earlier reports suggested that capture of particulate Ags by SSMs was important for activation of cognate B cells. However, capture of UV-PR8 by sinus-lining macrophages was important in limiting the spread of the virus to downstream LNs and the spleen. Thus, the role of macrophages lining the sinus of peripheral LNs is important in the efficient capture and spread of virus, but it is not important for humoral immunity to inactive influenza.

It is not clear how MBL-opsonized UV-PR8 is phagocytosed by SSMs. Because a known receptor for murine MBL is not clear it is possible that uptake is via a complement ligand, because the lectin pathway activates C2 and C4 or C3 directly via a bypass pathway, as described in human serum (Fig. 2B) (65).

**Medullary dendritic cells bind influenza**

In addition to capture of virus by sinus-lining macrophages, Gonzalez et al. (63) reported that a resident population of dendritic cells (DCs) bound virus in the medullary region. Using flow-cytometric assays, they identified a CD11c− CD11b+ SIGN-R1+ population that bound a significantly large amount of virus. Interestingly, ~50% of the PR8-binding DCs expressed a receptor for the cysteine-rich (CR) domain of the MR based on binding with an Ig Fc fusion protein with the CR domain (i.e., CR-Fc) (66). The receptor for the CR domain of MR is associated with dendritic-like cells that accumulate within the B cell follicles following immunization and possibly represent cells transporting Ag (67).

Studies by Park and colleagues (68) identified murine SIGN-R1 as the major receptor in the uptake of Streptococcus pneumoniae by MZ macrophages. SIGN-R1 is structurally similar to human DC-SIGN and is a C-type lectin that binds glycans rich in mannose, such as dextran and capsular polysaccharides of pneumococcus (69, 70). It is the major receptor for *S. pneumoniae*, because pretreatment of mice with a monoclonal Ab (clone 22D1) that downregulates macrophage expression of SIGN-R1 led to an impaired humoral response and increased mortality (68). Notably, binding of bacteria or purified polysaccharide by SIGN-R1 induces receptor aggregation, activation of C1q, and C3 deposition (68). Mice deficient in C3 have impaired immunity to *S. pneumoniae* and this is associated with impaired uptake of bacteria on FDCs. Thus, one interpretation of the findings is that opsonization of bacteria with C3 via SIGN-R1 is critical for uptake on FDCs and access by follicular B cells.

**SIGN-R1 is required for local humoral immunity to influenza**

Using a similar approach as Park and colleagues (68), Gonzalez et al. (63) showed that treatment of mice with a mAb to SIGN-R1 that downregulates receptor expression significantly reduced the uptake of UV-PR8 by resident DCs. Moreover, blocking of UV-viral uptake via SIGN-R1 in MBL−/− mice substantially reduced the capture of UV-PR8 in the draining LN, and the local B cell response was reduced by 3-fold. However, in these experiments, the systemic response was not affected, suggesting that blocking of viral capture locally led to efficient spreading of the virus and response in downstream LNs and spleen. Further support for the importance of the resident DCs in viral capture and humoral immunity was determined by the elimination of DCs systemically in CD11c-DTR bone marrow chimeric mice (71). In this model, CD11chi cells express the monkey diphtheria toxin receptor and are sensitive to ablation on treatment with diphtheria toxin. Thus, the CD11c DC-ablated chimeric mice had a significantly
reduced CD4 T cell-dependent (IgG) and -independent (IgM) day-10 humoral response to UV-PR8. Because the primary IgM response to inactive influenza under the conditions used in the study was largely independent of CD4 T cells (72) (M.C. Carroll, unpublished observations), the results support a requirement for DCs in the transport of Ag to the B cell compartment. It is noted that under different conditions, in which mice are infected with live influenza virus, the primary response is partly T cell dependent (73).

Additional support for a role for resident DCs in the transport of UV-PR8 into the B cell compartment came from MP-IVM analysis of CD11c-EYFP mice injected with labeled virus. Tracking of CD11c+EYFP cells before and after injection of labeled virus revealed that only those cells that bound virus moved in a nonrandom manner and that their net displacement and velocity were significantly increased relative to neighboring DCs that did not bind virus. Moreover, calculation of the net vector of directionality (i.e., net overall direction of migration) indicates that DCs that bind UV-PR8 migrate toward the B cell follicles (63).

It is tempting to speculate that capture of influenza by SIGN-R1 on the DC surface leads to activation of C1q and deposition of C3 on the inactive virus, as reported with S. pneumonia. Thus, it will be important in future studies to learn whether LN-resident DCs deliver C3d-coupled inactive virus to the FDCs, much like that identified for naive B cells (Fig. 4).

Conclusions

In this review, we discussed a novel role for complement in the transport of soluble Ags, ICs, and viral Ags to the B cell compartment. The finding that complement C3 and its receptors (i.e., CD21/35 and CR3 [CD11b/18]) participate in the capture and transport of lymph-borne Ags to the FDCs provides another important mechanism by which complement enhances adaptive immunity. Moreover, the identification of a network of conduits that directly funnel small Ags to the FDCs further elucidates how Ags are made accessible to naive B cells. We also highlight a new pathway in which capture of inactive influenza via the SIGN-R1 receptor signals the resident DCs to migrate toward the B cell compartment. It will be important, in future experiments, to determine whether C3 is activated and deposited on the virus following binding by SIGN-R1 and to track resident DCs with bound PR8 into the B cell follicles and determine whether they hand-off viral Ag directly to FDCs (Fig. 4).

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