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COMPLEMENT-DEPENDENT TRANSPORT OF ANTIGEN INTO B CELL FOLLICLES

Santiago F. Gonzalez[1], **Veronika Lukacs-Kornek**[2], **Michael P. Kuligowski**[1], **Lisa A. Pitcher**[1], **Søren E. Degn**[1,3], **Shannon J. Turley**[2], and **Michael C. Carroll**[1]

[1]The Immune Disease Institute and Program in Cellular and Molecular Medicine, Children's Hospital and Departments of Pediatrics & Pathology, Harvard Medical School, Boston, MA, USA

[2]Department of Cancer Immunology and AIDS, Dana Farber Cancer Institute and Department of Pathology, Harvard Medical School, Boston, MA, USA

[3]Department of Medical Microbiology and Immunology, Aarhus University, Århus, Denmark

Abstract

Since the original proposal by Fearon that the Complement System linked innate and adaptive immunity (1), there has been a rapid expansion of studies on this topic. With the advance of intravital imaging, a number of recent papers have revealed an additional novel pathway in which complement C3 and its receptors enhance humoral immunity through delivery of antigen to the B cell compartment. In this review, we will discuss this pathway and highlight several novel exceptions recently found with a model influenza vaccine such as: (a) MBL opsonization of influenza and uptake by macrophages; (b) and capture of virus by dendritic cells residing in the medullary compartment of peripheral lymph nodes.

INTRODUCTION

Peripheral lymph nodes (LN) along with the spleen make-up the secondary lymphoid organ tissue which provide a specialized environment for circulating B and T lymphocytes to interact and encounter cognate antigen(2). While T cells home to the paracortical region of LNs, B cells traffick to the follicles in search of antigen. 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 LN reveal a directed migration along stromal "highways" (3, 4). Fibroblast reticular cells (FRC) not only secrete the collagen-rich fibers that form the network within the paracortical region but also secrete T cell chemokines CCL19 and CCL21. B cell migration within the follicles is dependent on both FDC dendritic processes as well as a less dense network of FRC fibers. Although the reticular network within LNs was characterized over 3 decades ago (5) , it is only more recent that it became apparent that they act as conduits for the delivery of cytokines, chemokines and small protein antigens to both the T (6–9)and B cell areas (10, 11). 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 FRC secrete CXCL-13, whereas, paracortical FRC secrete CCL-19 & 21. Although the outer diameter of conduits is approximately 1–2 μm they are tightly packed with collagen fibers with a spacing of 5–8 nm that acts as molecular sieve (Figure 1). Thus, only proteins less than approximately 60 kDa enter into the conduits. Whether conduit structures are altered to accommodate larger antigens during infection is not clear.

Trafficking of lymph-borne antigens into B cell follicles

Small protein antigens gain direct access into the B cell follicles via either gaps in the sub-capsular sinus floor (12) or through the FRC conduits (10, 11) (Figure 2a). The latter pathway provides a directed flow of small antigen to the FDC for either transient retention or in the presence of antibody and complement long term binding via specific receptors. While, cognate B cells can access antigen draining via the conduits (10), their principal role is more likely directing the antigen to FDC for stable retention. While these initial experiments involved model antigens such as lysozyme (10) or OVA (11), in the natural setting it seems likely that a major source of antigen is degraded products of pathogens that drain from tissues via the lymphatics as suggested by Jenkins and colleagues (13).

Lymph-borne particulate antigens such as vesicular stomatitis virus (VSV) (14) and protein coated beads (15) are rapidly taken-up by macrophages that line the sub-capsular sinus (SSM) (16). Interestingly, the particulate antigens are shuttled to the underlying surface where they are made available to cognate B cells. Similarly, large protein antigens injected sub-cutaneously (s.q.) into passively immunized mice also appear to bind rapidly to SSM. However, in the later example, capture by SSM is complement dependent. Thus, formation of immune complexes (IC) activates complement resulting in formation of C3-coated immune complexes (C3-IC) that enhance uptake via CR3 (Mac-1) and FcRIIb on the SSM (17). Subsequently, C3-IC are relayed to the underlying B cell compartment where they are transferred to naïve B cells (18) (Figure 2a). FcRIIb is known to recycle to the surface following internalization and not go through a lysosomal compartment (19). So it is possible that C3-IC are partially protected by this cycling process. How C3-IC are actually transferred to B cells is not clear; however, uptake on the naïve B cells is CD21/35 - dependent and highly efficient. Strikingly, over 25% of naïve B cells within the LN follicles take-up C3-IC within 8 hrs of s.q. injection of antigen into immune mice (17) (10). In naïve mice where antibody is not preexisting, it is not clear how large protein antigens 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, MBL, Ficolins and pentraxin. Alternatively, protein antigens might be directly taken-up by scavenger or lectin receptors expressed on the sinus-lining macrophages. MM which are more similar to marginal zone macrophages of the spleen express SIGN-R1 and mannose receptor in addition to CR3. However, SSM are more similar to the metalophilic macrophages of the marginal zone and express MOMA-1 (18).

B cell transport of C3-IC is CD21/35 dependent

In mice, complement receptors CD21 and CD35 are encoded at the *Cr2* locus (20, 21). CD21 represents a splice product of the CD35 mRNA; whereas, in humans they are encoded at separate loci (22, 23). Murine CD21 and CD35 (CD21/35) are co-expressed on B cells, FDC and a subset of T cells. CD35 binds activated C3b and C4b and like CD21 binds the cleavage products iC3b, C3d,g and C3d covalently attached to antigens(20, 24–26) . They play a critical role in humoral immunity as blocking with antibodies(27, 28),a soluble receptor (29) or deletion of the receptors (30) (31)leads to impaired humoral immunity to model protein antigens, bacteria(32), and viruses (33, 34) (Figure 3). On B cells, CD21/35 form a co-receptor with CD19 and CD81 and coligation with the BCR lowers the threshold of B cell activation(35–37).

A second major function of CD21/35 is retention of antigen on FDC. They represent the major antigen receptors on FDC(38–40). Antigen is also retained for long periods via FcRIIb; however, its expression is not constitutive but upregulated on activated FDC(41). FDC are thought to be the major source of antigen required for clonal selection of B cells during the germinal center (GC) reaction (42)and possibly for maintenance of memory (43–

45). They not only retain antigen but secrete B cell growth factors and the B cell attractant CXCL-13 as mentioned above (46). Mice deficient in C3 or CD21/35 have fewer and smaller GC and B cells fail to survive(47, 48).

There is growing evidence that B cells acquire antigen and are most efficiently activated when it is attached to membrane(49–51). Therefore, retention of antigen and C3d on the FDC surface could enhance formation of the B cell synapse. Support for a role for FDC as an important source of antigen comes from the recent studies on Cyster et al as they demonstrate direct capture of antigen from FDC surface by cognate B cells using multi-photon intravital microscopy (MP-IVM) (52). Importantly they also identify a role for CD21/35 in efficient uptake of medium affinity antigens. In their model system, MD4 B cells deficient in CD21/35 acquired less antigen from FDC. One explanation of their results is that the co-receptor 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-IC into the B cell follicles and transfer to FDC. As discussed above, naïve B cells acquire C3-IC from SSM lining the sub-capsular sinus via their CD21/35 receptors. Whether uptake induces a direct signal via CD21/35 or involves the co-receptor CD19 and CD81 is not clear. In a recent study using mice in which CD21/35 are uncoupled from CD19 ($Cr2^{\Delta/\Delta}$) naïve B cells in the mutant line appeared to take-up C3-coated immune complexes normally and deliver them to FDC(43). CD21/35 receptors include a cytoplasmic domain so it is possible that uptake of C3-IC induces a signal by CD21 independent of CD19 (53) and triggers B cell migration to the FDC. An alternative possibility, is that B cells within the follicles migrate constitutively to FDC based on a chemokine gradient in a manner similar to that described for marginal zone (MZ) B cells (54); therefore direct signaling by C3-IC may not be not essential. Thus, as B cells enter the peripheral LNs via HEV, they cycle to the sub-capsular sinus area which is rich in MRC (marginal reticular cells) that secrete B cell chemokine (55)and subsequently to FDC which are also a source of chemokine(46).

Earlier studies identified a role for C3 and CD21/35 in capture of IC by MZ B cells and transport to FDC within B cell follicles (56–58). Using intravital imaging, Cyster and colleagues identified a similar pathway for capture of C3-IC in the spleen as reported for the LN (54). They found that MZ B cells continuously shuttled between the splenic marginal zone and the B cell follicles by a CXCR5-chemokine-sphingosine 1- phosphate receptor - dependent mechanism. Thus, naïve MZ B cells capture C3-coated antigens within the marginal zone sinus and transport them into the follicles where they are transferred to FDC. How C3-IC are handed-off to FDC is not clear; however the uptake on the FDC is dependent on CD21/35 and FcRIIb. Since the latter receptors (FcRIIb) are not constitutively expressed but upregulated following FDC activation, complement receptors are critical for the initial capture and retention of C3-IC (41).

Influenza virus is captured via a novel pathway

Influenza infection represents a major pathogen of humans; in general it is controlled by annual vaccination and induction of a protective humoral response (59) (60, 61). Complement C3 and CD21/35 are a important component in humoral immunity to influenza based on earlier studies (62) (63) (34). While much of the focus on the enhancing role of complement has been on the B cell co-receptor, the C3-CD21/35 pathway is also important for transport and retention of antigen on FDC as discussed above.

Recent studies have identified a novel pathway by which viral antigen is captured and possibly transported to the FDC. Gonzalez et al used an UV (ultra-violet light) inactivated form of influenza virus (strain PR8) as a model vaccine. Using a fluorescent-tagged form of

UV-PR8 injected in the footpad (s.q.) and imaging by MP-IVM they identified rapid filling of the sinus and capture by macrophages in both the sub-capsular (SSM) and medullary (MM) regions. As discussed earlier, macrophages lining these regions represent distinct populations as distinguished by functional maturity and cell surface markers (18). For example, MM which express SIGN-R1, F4/80 and mannose receptor appear more mature than SSM based endocytosis and degradation of immune complexes.

The study by Gonzalez reported that labeled-UV-PR8 virus was rapidly bound by both the SSM and MM similar to that reported for VSV (14). In contrast to the earlier studies, UV-PR8 was not retained on the surface but was internalized. Binding and phagocytosis was dependent on MBL as SSM in MBL−/− mice failed to bind the virus. Opsonization by MBL is in agreement with earlier studies by Ezekowitz and colleagues who reported that guinea pig mannan-binding lectin bound and neutralized influenza (64). Interestingly, blocking of UV-PR8 uptake by either SSM alone or both SSM and MM did not impair humoral immunity. This was somewhat surprising as earlier reports suggested that capture of particulate antigens by SSM was important for activation of cognate B cells. However, capture of UV-PR8 by sinus-lining macrophages was important in limiting spread of the virus to downstream LNs and the spleen. Thus, the role of macrophages lining the sinus of pLNs is important in efficient capture and spread of virus but not important for humoral immunity to inactive influenza.

How MBL- opsonized UV-PR8 is phagocytosed by SSM is not clear. Since a known receptor for murine MBL is not clear (65) it is possible that uptake is via a complement ligand since the lectin pathway activates both C2 and C4 or C3 directly via a by-pass pathway as described in human serum (Figure 2b)(66).

Medullary dendritic cells bind influenza

In addition to capture of virus by sinus lining macrophages, Gonzalez et al reported that a resident population of dendritic cells (DC) also bound virus in the medullary region. Using flow cytometric assays, they identified $CD11c^{+}$ $CD11b^{hi}$, $SIGN\text{-}R1^{+}$ population that bound a significantly high amount of virus. Interestingly about 50% of the PR8 binding DC expressed a receptor for the cysteine rich (CR) domain of the mannose receptor (MR) based on binding with an Ig Fc fusion protein with the CR domain, i.e. CR-Fc (67). 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 antigen (68).

Previous studies by Park and colleagues identified murine SIGN-R1 as the major receptor in uptake of *S. pneumoniae* by marginal zone macrophages (69). SIGN-R1 (specific intracellular adhesion molecule-grabbing nonintegrin 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 (70, 71). It is the major receptor for *S. pneumoniae* as pretreatment of mice with a monoclonal (clone 22D1) that down-regulates macrophage expression of SIGN-R1 led to an impaired humoral response and increased mortality (69). Notably, binding of bacteria or purified polysaccharide by SIGN-R1 induces receptor aggregation and activation of C1q and C3 deposition (69). Mice deficient in C3 have impaired immunity to *S. pneumoniae* and this is associated with impaired uptake of bacteria on FDC. Thus, one interpretation of the findings is that opsonization of bacteria with C3 via SIGN-R1 is critical for uptake on FDC and access by follicular B cells.

SIGN-R1 is required for local humoral immunity to influenza

Using a similar approach as Park et al (69), Gonzalez et al showed treatment of mice with a monoclonal antibody to SIGN-R1 that down regulates receptor expression significantly reduced uptake of UV-PR8 by resident DC(72).

Moreover, blocking of UV-viral uptake via SIGN-R1 in MBL−/− mice not only substantially reduced the capture of UV-PR8 in the draining LN; but the local B cell response was reduced by three-fold. However, in these experiments the systemic response wasn't 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 DC in viral capture and humoral immunity was determined by elimination of DC systemically in CD11c-DTR bone marrow chimeric mice (73). In this model, CD11c ^{hi} cells express the monkey diptheria toxin receptor (DTR) and are sensitive to ablation on treatment with diptheria 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. Since the primary IgM response to inactive influenza under the conditions used in the study was largely independent of CD4 T cells (74) (M.C.C, unpublished), the results support a requirement for DC in transport of antigen to the B cell compartment. It is noted that under different conditions in which mice are infected with live influenza virus that the primary response is partly T cell-dependent (75).

Additional support for a role for resident DC in transport of UV-PR8 into the B cell compartment came from MP-IVM analysis of CD11c-EYFP mice injected with labeled influenza. Tracking of CD11c ^{hi} cells both before and after injection of labeled virus revealed that only those cells that bound virus moved in a non-random manner and that their net displacement and velocity was significantly increased relative to neighboring DC that did not bind virus. Moreover, calculation of the net vector of directionality, i.e. net overall direction of migration, indicates that DC that bind UV-PR8 migrate towards the B cell follicles (72).

It is tempting to speculate that capture of influenza by SIGN-R1 on 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 if LN resident DC deliver C3d-coupled inactive virus to the FDC much like that identified for naïve B cells (Figure 4).

Conclusions

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

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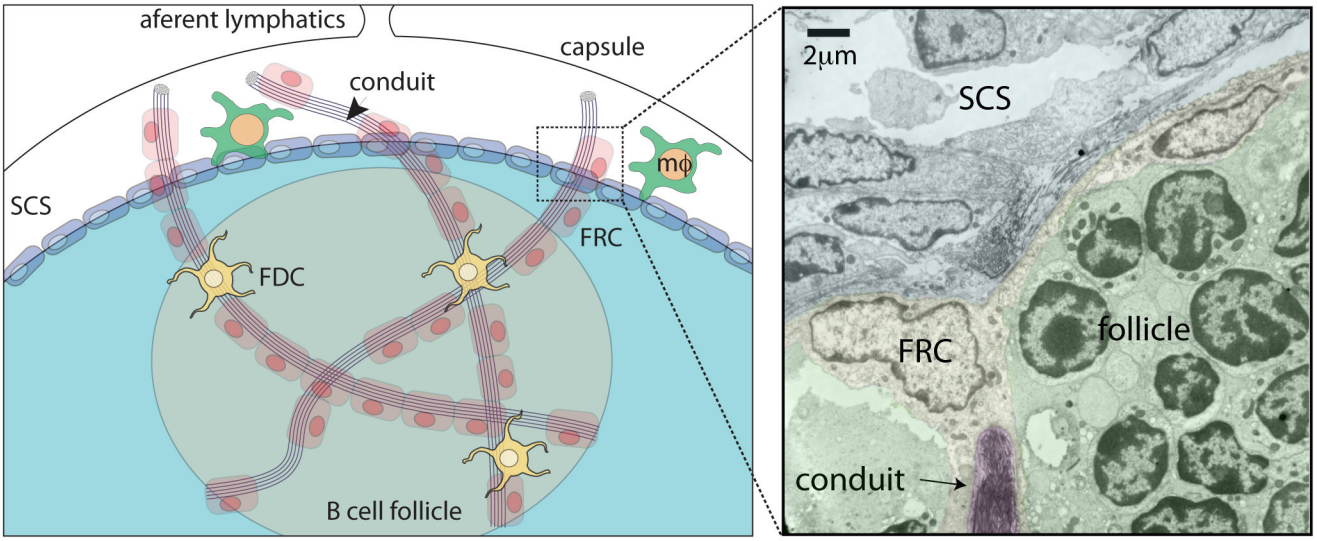

Figure 1.

The conduit network, formed by collagen fibers, is secreted by the fibroblastic reticular cells (FRC) and drains small antigens from the subcapsular sinus (SCS) area of the lymph node to the B cell follicle. Follicular dendritic cells (FDC) present in the follicle are closely associated with the collagen fibers. MΦ; indicates subcapsular sinus macrophages. Arrows indicate conduit opening into the sinus in electron micrograph on right panel. EM is from S. Gonzalez, unpublished.

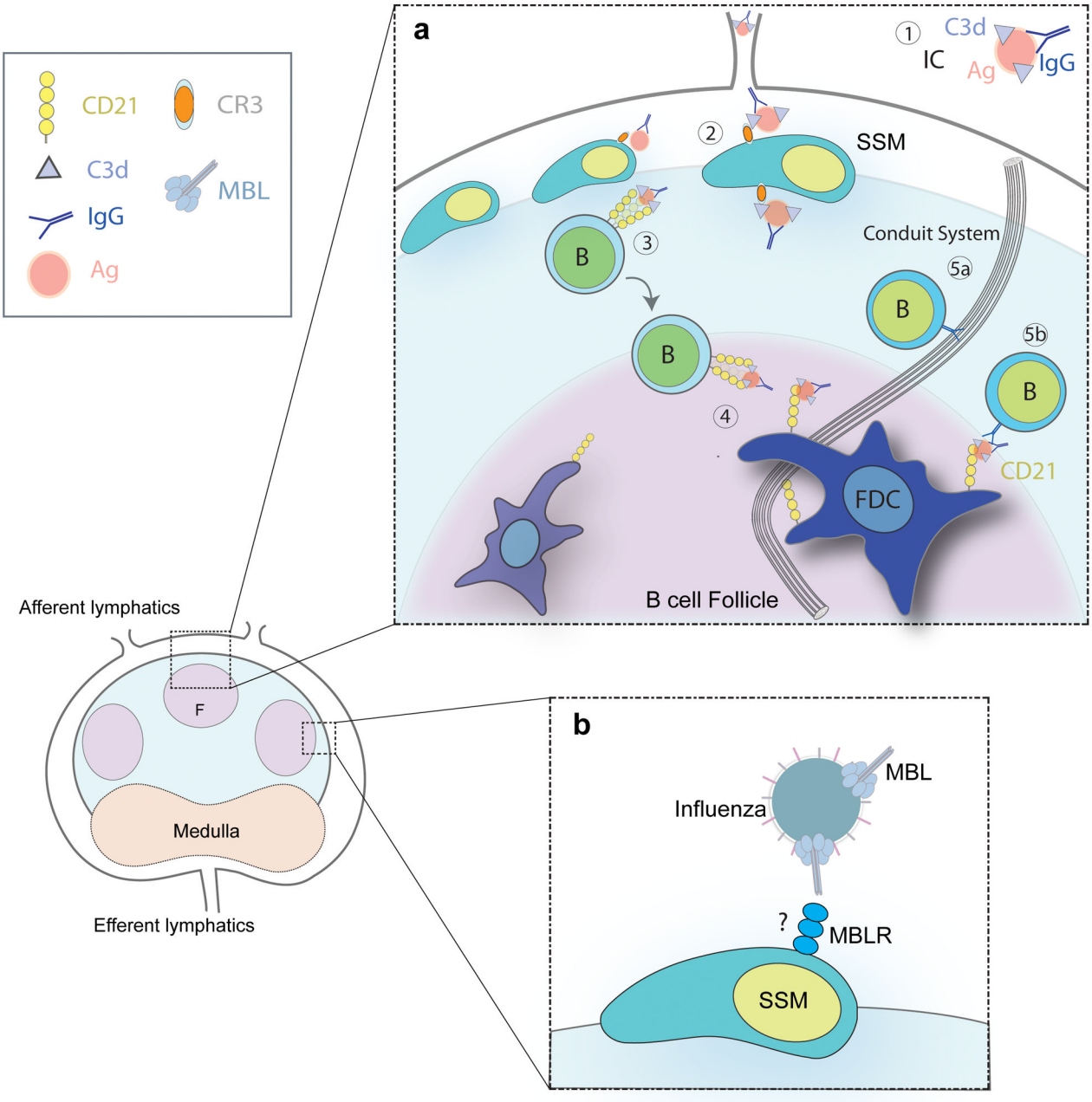

Figure 2.

(a) Pathways for the circulation of antigen (Ag) in the LN. (1) Complement C3 opsonizes antigen in presence of antibody. C3-coated Immune complexes (C3-IC) are formed by the deposition of complement proteins and IgG on the surface of the antigen. (2) The retention of C3-IC on the surface of the subcapsular sinus macrophages (SSM) is CR3 and FcRIIb dependent. (3) B cells transport C3-IC from the surface of the SSM to the FDC in a CD21 dependent manner. (4) C3-IC are transferred to FDC. (5) Cognate B cells acquire small Ag drained via FRC-conduits directly or from FDC surface. (b) Influenza virus uptake by SSM is MBL dependent.

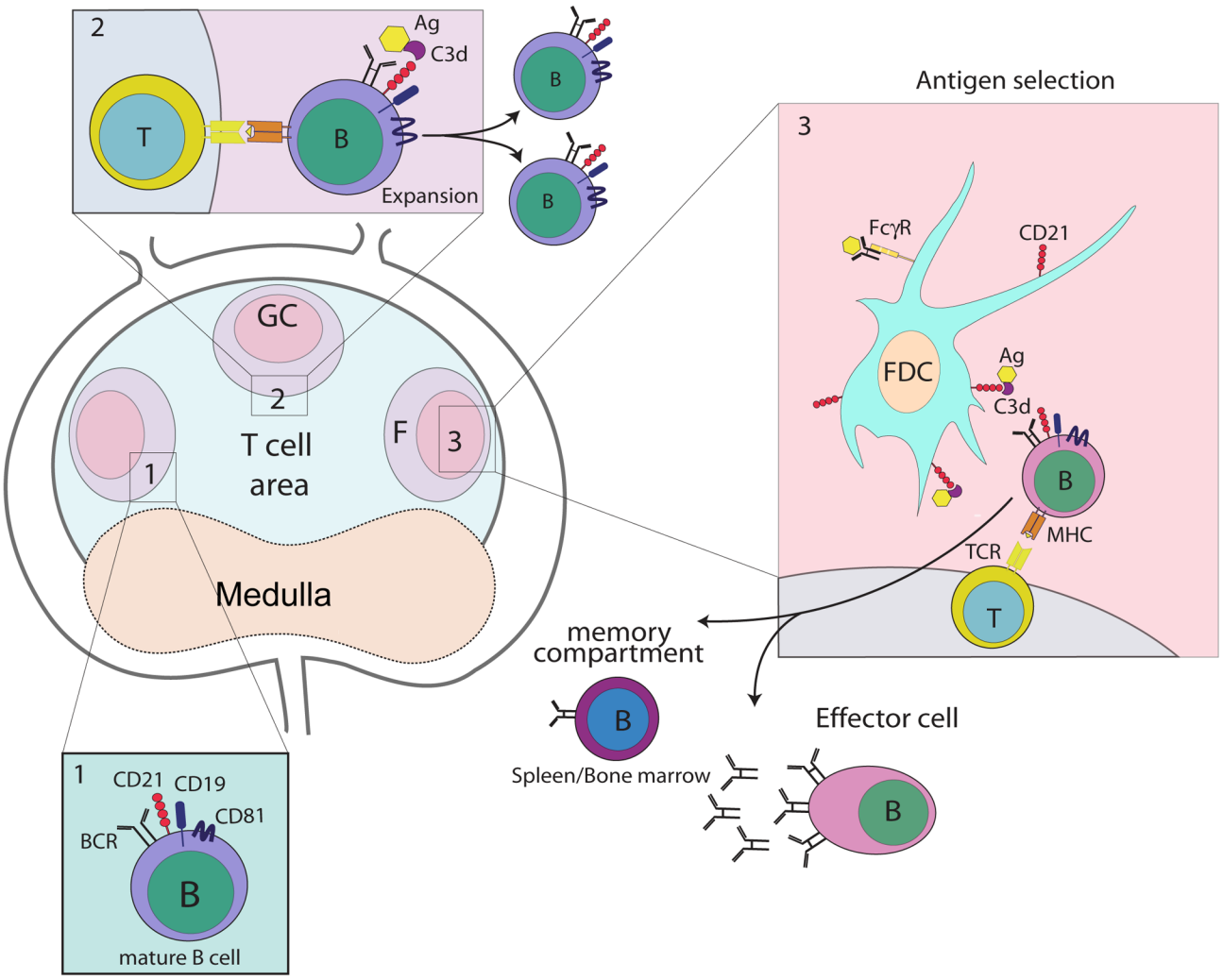

Figure 3.

Recognition of C3-tagged antigen via CD21 and CD35 enhances B cell differentiation at three major stages. Complement receptors CD21 and CD35 play an important role in at least three stages of B cell differentiation. Stage 1: co-ligation of C3d-antigen 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 co-stimulation via CD40. Stage 2: activated B cells enter a germinal center where they begin further differentiation including rapid cell division, somatic cell hypermutation (SHM) and class switch recombination (CSR). Stage 3: following clonal selection (binding of antigen 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 antigen on FDC.

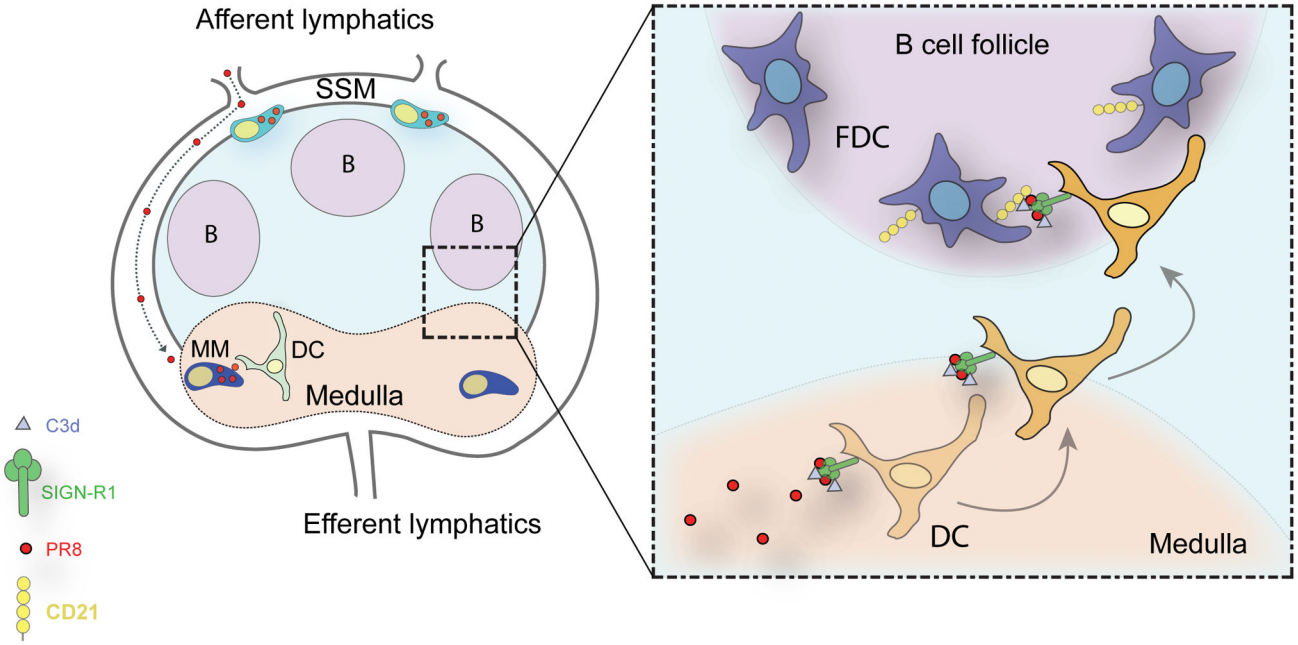

Figure 4.

Lymph-borne UV-inactive influenza (strainPR8) fills the subcapsular (SCS) of the LN, where it is partially taken up by SSM. Binding of virus to SSM is MBL-dependent. Virions are channeled to the LN medulla where medullary macrophages (MM) and resident dendritic cells (DC) capture them in a SIGN-R1-dependent manner. Model speculates that C1q is activated by SIGN-R1 leading to C3 activation and deposition on PR8. Subsequently, resident DC transport C3-coated virus complex to the follicles where it is transferred to FDC.