Immunity
Review

Regulation of Humoral Immunity by Complement

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The complement system of innate immunity is important in regulating humoral immunity largely through the complement receptor CR2, which forms a coreceptor on B cells during antigen-induced activation. However, CR2 also retains antigens on follicular dendritic cells (FDCs). Display of antigen on FDCs is critical for clonal selection and affinity maturation of activated B cells. This review will discuss the role of complement in adaptive immunity in general with a focus on the interplay between CR2-associated antigen on B cells with CR2 expressed on FDCs. This latter interaction provides an opportunity for memory B cells to sample antigen over prolonged periods. The cocrystal structure of CR2 with its ligand C3d provides insight into how the complement system regulates access of antigen by B cells with implications for therapeutic manipulations to modulate aberrant B cell responses in the case of autoimmunity.

Introduction

The paradigm first suggested by Janeway over two decades ago, that innate immunity was essential for regulating adaptive responses, focused attention on how pathogens were recognized and taken up by dendritic cells for presentation to T cells (Janeway, 1989). Later, Fearon and Locksley (1996) proposed that B cells were equally reliant on innate recognition for acquisition of cognate antigens and that the complement system played a critical role in signaling B cell activation. More recently, in vivo imaging studies tracking B cell capture of lymph-borne antigens in real time have again provided a major shift in our understanding of how the innate system regulates humoral immunity. For example, specialized macrophages lining the subcapsular sinus of peripheral lymph nodes (pLNs) have been shown to capture both particulate (Carrasco and Batista, 2007; Junt et al., 2007) and soluble immune complexes via complement receptors (Phan et al., 2007) and display them to cognate B cells in the underlying B cell follicles. Alternatively, small protein antigens have been found to drain directly from the subcapsular sinus into the follicles through collagen-rich conduits enveloped by fibroblast reticular cells (FRCs) (Bajénoff and Germain, 2009; Roozendaal et al., 2009). Although these pathways provide insight into how memory B cells can directly sample antigen within lymph nodes, they leave open the question of how B cell antigens are retained and sampled over longer periods. An important clue to answering this question has come from the recent report by Cyster and colleagues as they made the striking observation that naive B cells are highly efficient at taking up and transporting immune complexes bound to complement receptor (CR2) to B cell follicles within pLNs, where they are transferred to follicular dendritic cells (FDCs) for long-term retention (Phan et al., 2009). These exciting new findings combined with the recent solving of the cocrystal structure of CR2 in complex with the complement fragment C3d (van den Elsen and Isenman, 2011) suggest a new perspective in how the complement system regulates access of antigen by B cells. In this review, we will discuss these recent findings and how they might lead to possible therapies to "tune-down" aberrant B cell responses in the case of autoimmunity.

Complement Receptor CR2 in Humoral Immunity

Starting from an initial observation ~40 years ago that depleting complement component C3 from the serum of an experimental animal results in an impaired antibody response to T cell-dependent antigen (Pepys, 1972), there has been a substantial body of research directed at understanding the molecular basis for this complement link to the regulation of the adaptive humoral immune response. The various milestones in this quest have been documented in several past reviews (Carroll, 2000; Carroll, 2004; Nielsen and Leslie, 2002; Rickert, 2005; Roozendaal and Carroll, 2007). It is now clear that at the heart of complement's effect on adaptive humoral immunity is the interaction between complement receptor 2 (CR2, CD21), which is primarily present on B cells and follicular dendritic cells (FDCs) in humans and mice, with an antigen-bound fragment of C3 in which the C3d moiety is fully accessible (Figure 1).

Before dealing with the models that have emerged for the role of the CR2:C3d interaction in adaptive immunity, it is important to appreciate how the antigen gets "tagged" with the appropriate C3 split product via the classical or lectin complement pathways. These processes are dependent upon antigen recognition by an activator of the classical or lectin pathways, such as natural repertoire IgM antibody (Boes et al., 1998) (Fischer et al., 1996), C-type lectins such as SIGN-R1 (Kang et al., 2006), or mannan binding lectin (Degn et al., 2007; Takahashi et al., 2008). When associated with antigen, IgM and SIGN-R1 engage the C1q subcomponent of the proenzyme form of C1 and this results in C1 activation. The now activated form of the C1s subcomponent of C1 cleaves the next component of the classical pathway, C4, into C4a and C4b, with a portion of the C4b becoming covalently bound to the antigen (see below). There it binds C2, which in turn is also cleaved by activated C1s into the active serine protease fragment C2a. The resulting antigen-attached enzyme, C4b2a, is the classical pathway C3 convertase and it cleaves many molecules of C3

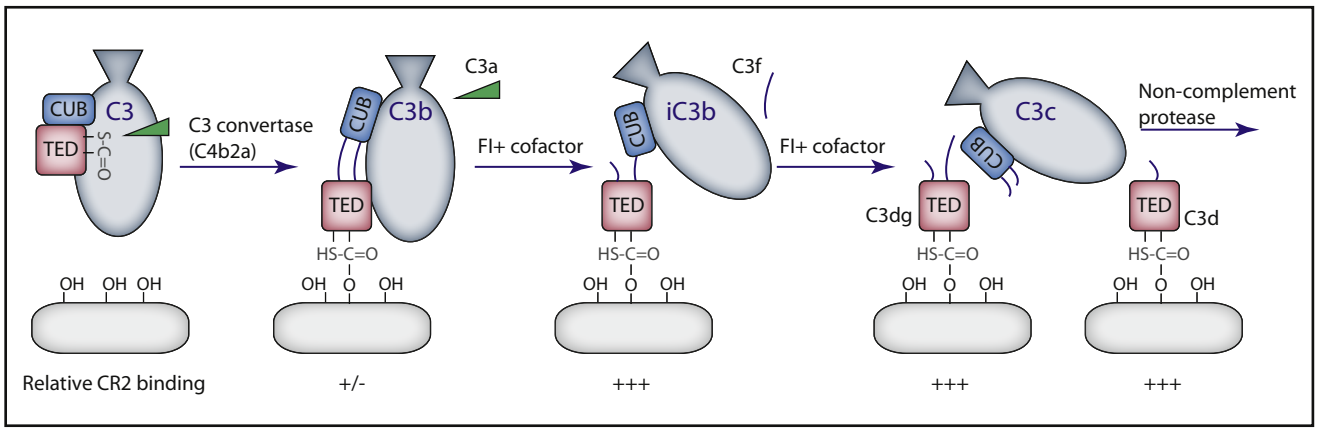

Figure 1. Structure-Based Cartoon Depiction of the Processes Involved in the Covalent Tagging of Antigen with C3b and Its Subsequent Degradation to to the Equivalent CR2 Ligands iC3b, C3dg, and C3d

In native C3, the intramolecular thioester bond of the TED is buried at an interface and is thus denoted in gray. After proteolytic removal of the C3a activation fragment by C3 convertase, which would be present on the antigen surface, a massive conformational change ensues in the C3b fragment. The activated thioester carbonyl becomes exposed and can react with a hydroxyl group on the antigen surface (gray, shaded box), thereby forming a covalent ester linkage. Whereas the CR2 binding surface of the C3d moiety is largely obstructed in C3b, it becomes accessible as a result of the conformational changes accompanying removal of the small C3f peptide of the CUB (for complement C1r and C1s, Uegf, and bone morphogenic protein 1) domain by factor I (in concert with cofactor FH) that generates iC3b. An additional cleavage of the CUB domain by FI (this time requiring CR1 [CD35] as the cofactor) releases C3c and leaves C3dg still covalently bound to the antigen. Further proteolytic trimming of C3dg can yield the C3d limit fragment. These cartoons, although greatly simplified to depict only the most relevant domains as individual entities, are based on the crystal structures of native C3 and C3b (Janssen et al., 2006) and EM images of iC3b (Alcorlo et al., 2011; Nishida et al., 2006). The relative binding affinities of the antigen-bound C3 split products are indicated.

into C3a and C3b (Figure 1). Like C4b, a portion of the nascent C3b molecules become covalently bound to the antigen. In the case of antigen recognition by mannan binding lectin (MBL), the MBL-associated serine proteases (MASPs) become activated, and like C1s, activated MASP2 can cleave C4 and C2, thus yielding an antigen-bound C3 convertase identical to the one formed by via C1 activation.

As alluded to above, the transfer of the homologous proteins C3 and C4 from the fluid phase to a target surface involves a covalent binding reaction. In this transacylation reaction, a surface nucleophile (hydroxyl group in the case of C3, hydroxyl and amino groups in the case of human C4) attacks an activated intramolecular thioester bond formed between the side chains of cysteine and glutamine residues in the sequence CGEQ located within the C3d and C4d subfragments of these proteins (Figure 1). These fragments correspond closely to the respective structurally defined thioester domains (TEDs). Subsequent degradation of the antigen-bound C3b to iC3b and C3dg by the complement regulatory enzyme factor I, and further protease trimming of C3dg to C3d by a noncomplement protease, will leave these C3 secondary and tertiary degradation fragments still covalently attached to the antigen (Figure 1). It is known from structural work that relative to native C3, the TED is in a completely different conformational environment in the C3b fragment, and then again in iC3b (Alcorlo et al., 2011; Janssen et al., 2006; Nishida et al., 2006). The equivalence of the affinities for CR2 of the C3 split products iC3b, C3dg, and C3d (Clemenza and Isenman, 2000; Kalli et al., 1991; Nagar et al., 1998) not only indicates that C3d contains the CR2 binding site, but also that the site is equally accessible to CR2 in the precursor degradation fragments iC3b and C3dg.

There are three distinct modalities that have emerged through which the interaction of CR2 with antigen opsonized with C3d affects antibody-mediated adaptive immunity. The first is depicted in Figure 2A and occurs at the surface of a B cell. It involves the coligation of the B cell antigen receptor (BCR) to CR2, during which both the primary receptor, i.e., the BCR, and the coreceptor, i.e., CR2, are each individually associated with cell signaling platforms. Coligation results in augmented signaling as a result of the redistribution of the entire receptor-coreceptor complex to lipid rafts (Cherukuri et al., 2001). In the presence of large doses of antigen, there is sufficient crosslinking of the BCR complex so that the signaling provided via the associated Igα;Igβ platform is sufficient to induce activation and clonal expansion of the antigen-specific B cell, without the need for complement-mediated coligation of the CR2-CD19-CD81 coreceptor complex. However, under conditions of limiting antigen, as would likely be the case that upon initial encounter with a microbial pathogen, the augmented signaling occurring as a result of CR2-coreceptor ligation effectively lowers the threshold dose of antigen required to trigger B cell clonal expansion.

The second role for the CR2:C3d interaction, as depicted in Figure 2B, involves FDCs within germinal centers in which, as a result of trapping C3d-opsonized antigen via CR2, they can present the antigen to naive or previously antigen-engaged B cells during the processes of affinity maturation, isotype switching, and generation of effector and memory B cells (Fang et al., 1998). These CR2-mediated effects at the surface of B cells and FDCs are collectively referred to as the "molecular adjuvant" effects of complement and have been shown in various experimental systems in which antigen is fused to multiple copies of C3d to lower the threshold dose of antigen required for an antibody response in the absence of other "traditional" adjuvants from two to four orders of magnitude (Barrault et al., 2005; Dempsey et al., 1996; Wang et al.,

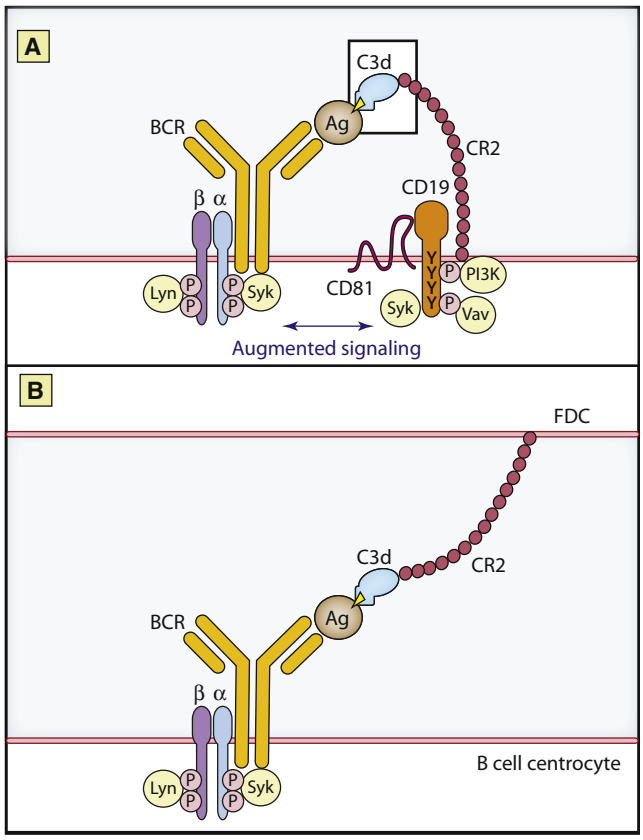

Figure 2. Complement as a Bridge Linking the Innate and Adaptive Immune Systems: The Molecular Adjuvant Role of Antigen-Linked C3d

(A) Coligation of the BCR with the CR2-CD19-CD81 complex leads to augmented signaling when naive B cells first encounter antigen and initiate the process leading to their clonal expansion. CR2 is composed of 15 CCP domains, denoted by circles. The white boxed area indicates the key binding interaction between CR2(CCP1-2) with a C3d TED that is covalently bound (yellow triangle) to the antigen recognized by the BCR of this particular B cell. (B) CR2 present on FDCs may also capture C3d-opsonized antigen and present this antigen to previously primed B cell centrocytes in the germinal center of the lymph node.

2004a; Wang et al., 2004b). However, these same CR2:C3d-mediated molecular adjuvant effects, which are beneficial in the host's immune response to foreign antigen, can also amplify an antibody-mediated autoimmune response in the case of individuals having B cells reactive against self-antigens. This possibility is discussed in more detail below.

The third modality through which complement interfaces with adaptive humoral immunity involves the delivery of antigen to FDCs and this topic will be dealt with separately in a subsequent section.

Complement C3 in T Cell Immunity

Although the focus of this review is primarily on B cell immunity, complement also participates in activation of T cells and several excellent reviews have reported on the topic (Kemper and Atkinson, 2007b; Dunkelberger and Song, 2010; Sacks and Zhou, 2012). Using a murine model of influenza infection, Kopf and colleagues first identified an essential role for complement C3 in the T cell response to the virus. Mice deficient in C3 failed to clear the virus efficiently relative to WT controls and their adaptive response was characterized by an impairment in both the B cell and T cell compartment. Examination of the $CD4^+$ and $CD8^+$ T cell response at early time points showed a marked decrease in T cell proliferation and release of cytokines such as interferon gamma. Later studies confirmed the sensitivity of C3-deficient mice to influenza infection, showing a high incidence of mortality that correlates with impaired B cell memory (Fernandez Gonzalez et al., 2008). Interestingly, mice deficient in CD21 and CD35 ($Cr2^{-/-}$) appear to respond to infectious virus similar to WT controls, suggesting the T cell defect is not mediated through this pathway (Kopf et al., 2002). The results are in agreement with earlier studies by Birgitta Heyman et al., who found that blockade of CD21 receptors leads to an impaired antibody response without limiting the T helper (Th) cells (Gustavsson et al., 1995). A possible explanation for the impaired $CD4^+$ Th1 cell response to influenza response is suggested by a recent study by Pekkarinen et al. (2011). They found that the $CD4^+$ Th1 cell response of C3-deficient mice is reduced relative to WT controls after immunization with ovalbumin (OVA) in Freund's adjuvant and this correlates with a decrease in expression of interleukin-12 (IL-12) and the Th1 cell lineage transcription factor T-bet, together with a deviation to Th2 cell response. They proposed that the defect could be due to either the cytokine environment or effects of antigen presentation by dendritic cells (DCs). For example, DCs can produce complement components locally, which may modulate their cytokine secretion (Sacks and Zhou, 2012). One relevant pathway is the C5a receptor (C5aR), which is known to affect Toll-like receptor (TLR) signaling, especially IL-12, and is linked to Th17 cell and regulatory T (Treg) cell differentiation (Kemper and Atkinson, 2007b; Weaver et al., 2010). In human T cells, the complement receptor CD46 may be a major mediator of the effects of C3 (Kemper and Atkinson, 2007a). Earlier studies have shown that crosslinking of CD3 and CD46 on T cells induces a regulatory T cell phenotype (Kemper et al., 2003). In their more recent report, the authors show that activated $CD4^+$ T cells produce ligands for CD46, i.e., C3b and C4b, that result in IL-10 production and induction of a regulatory phenotype (Cardone et al., 2010). Thus, the CD46 receptor that was first identified as a regulator of C3b and C4b to protect host cells from activated complement has potent effects on the cytokine production of T cells and a strong influence on the overall T helper cell phenotype.

The CR2:C3d Binding Interface

As can be seen in the cartoon depiction in Figure 2, depending on an alternative splice site used, the extracellular region of CR2 consists of 15 or 16 complement control protein (CCP) domains, but the C3d binding site is entirely contained within the two N-terminal-most CCP domains (Kalli et al., 1991). Moreover, both of these domains must be present to observe C3d binding (Carel et al., 1990; Lowell et al., 1989). When the structure of C3d became available (Nagar et al., 1998), it was noted that one of its prominent features was an acidic residue-lined depression that was remote from a more convex face of the molecule harboring the covalent attachment site. On the basis of its accessible location, the known ionic strength sensitivity of the CR2:C3d interaction, and the potential for charge

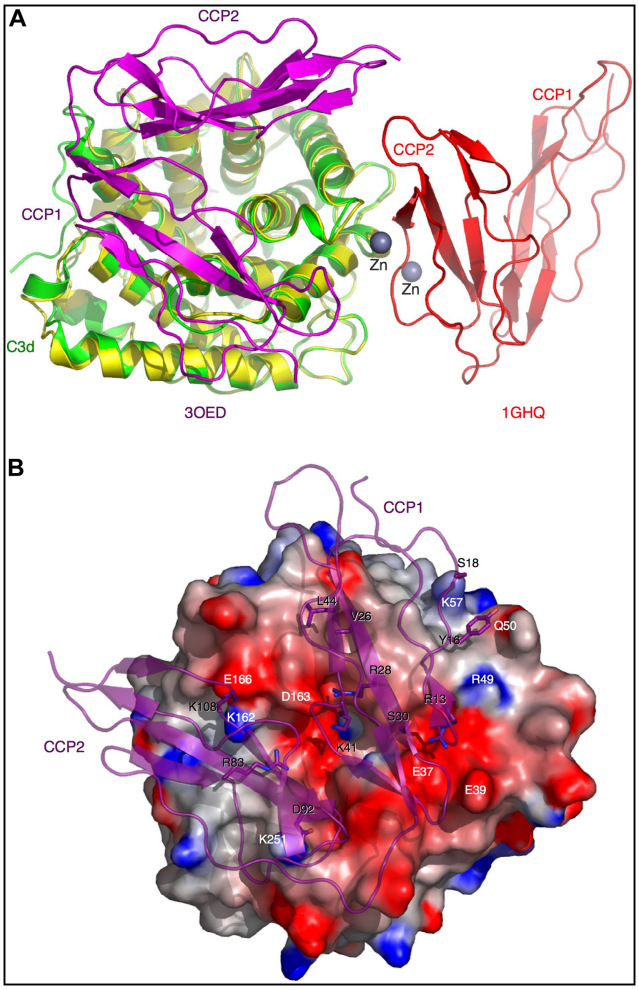

Figure 3. The Structure of the CR2(CCP1-2):C3d Complex

(A) Comparison of the CR2:C3d binding interfaces in the 2001 (PDB 1GHQ, Szakonyi et al., 2001) and 2011 (PDB 3OED, van den Elsen and Isenman, 2011) CR2(CCP1-2):C3d cocrystal structures. Ribbon representations of the essentially identical C3d molecules of the 2001 structure (yellow) and the 2011 structure (green) were superimposed in an orientation where one is looking into the acidic pocket of the C3d molecule. The respective placements of the CR2 (CCP1-2) molecules in the 2001 and 2011 cocrystal structures are denoted by the red and magenta ribbon diagrams, respectively. The gray spheres indicate the respective positions of the two zinc atoms at the interface visualized in the 2001 structure.

(B) Depiction of the charge complementarity at the interface between the positively charged side chains of CR2(CCP1-2) that point down toward the acidic pocket on the concave surface of C3d. C3d is shown as a molecular surface representation colored for electrostatic potential; red, negative; blue, positive; and gray, neutral. The ribbon rendering of the CR2 domains is semitransparent (adapted from van den Elsen and Isenman, 2011).

complementarity in binding to prominent positively charged patches on one face of the CR2(CCP1-2) molecule, as inferred at that time from a homology model of these domains, the acidic residue-lined depression of C3d was proposed to be a major site of interaction with CR2(CCP1-2) (Nagar et al., 1998). Subsequently, an alanine scan mutational analysis of fully exposed residues within the candidate site was published (Clemenza and Isenman, 2000). This study not only provided support for the hypothesis, but further identified two distinct subclusters of residues located at opposite ends of the acidic pocket whose mutation lead to profound defects in CR2 binding. Shortly thereafter, an alternative model for CR2 binding was suggested by a cocrystal structure of CR2(CCP1-2):C3d that, as depicted by the red CR2(CCP1-2) molecule in Figure 3A, shows no interaction within the mutationally defined contact areas on the concave surface but rather shows CR2 binding via CCP2 only to a side face of C3d (Szakonyi et al., 2001). From the outset, this structure was discordant with biochemical data on several fronts, including the strong indication that both CCP1 and CCP2 would mediate the contact (Carel et al., 1990; Lowell et al., 1989). This structure has not been supported by subsequent studies, including one showing that the mutation of several basic residues in CCP1, which made no contact with C3d in the 2001 cocrystal structure, had a major effect on binding (Hannan et al., 2005). Experiments showing that the concentration of zinc ion used in the crystallization of the complex actually abolishes CR2:C3d binding in solution (Isenman et al., 2010) have made it likely that the 2001 structure represented a nonphysiologic interaction promoted by the zinc in the crystallization buffer.

The recent characterization of secreted *Staphylococcus aureus* virulence proteins extracellular fibrinogen-binding protein (Efb) and *Staphylococcal* binder of immunoglobulin (Sbi), which have constituent domains Efb-C and Sbi-IV, respectively, that, like CR2, bind weakly to C3b but strongly to iC3b and C3dg (Burman et al., 2008; Hammel et al., 2007) refocused attention on the acidic residue-lined depression of C3d as being a major contact site for CR2(CCP1-2). Both Sbi-IV (Burman et al., 2008) and Efb-C (Ricklin et al., 2008) were shown to compete with CR2 for binding to C3dg. Moreover, a cocrystal structure of the Efb-C:C3d complex showed the three-helix bundle Efb-C molecule binding on the concave face of C3d, and with several of its helix 2 residues having side chains making specific contact with residues in the acidic residue-lined depression of C3d. These results prompted not only the use of the collection of concave surface C3d mutants to map the interaction site of Sbi-IV, which by this point had also been determined to have a three-helix bundle fold (Upadhyay et al., 2008), but also reexamination using biophysical methodology, as compared to the rosette assays of binding used in the 2000 study (Clemenza and Isenman, 2000), of the CR2(CCP1-2) binding capacity of an expanded collection of C3dg mutants. The results of these studies reconfirmed the original findings regarding the importance of residues in the C3d acidic pocket for CR2 binding, as well as showing that like Efb-C, this region of was also utilized by Sbi-IV for its binding (Isenman et al., 2010).

Cocrystal Structure of the CR2(CCP1-2):C3d Complex

In crystallization conditions that avoid the use of nonphysiologic salts, new diffracting cocrystals of the CR2(CCP1-2):C3d complex have been obtained and yield the model depicted in Figure 3A, in which the magenta-colored CR2(CCP1-2) is the C3d-binding entity (van den Elsen and Isenman, 2011). The V-shaped arrangement of the constituent domains of CR2(CCP1-2) interact with the acidic pocket on the concave surface of C3d, and there is excellent shape complementarity of the surfaces. In the electrostatic surface potential rendering of the C3d molecule shown in Figure 3B, it can be seen how there are several basic side chains of lysine and arginine residues of

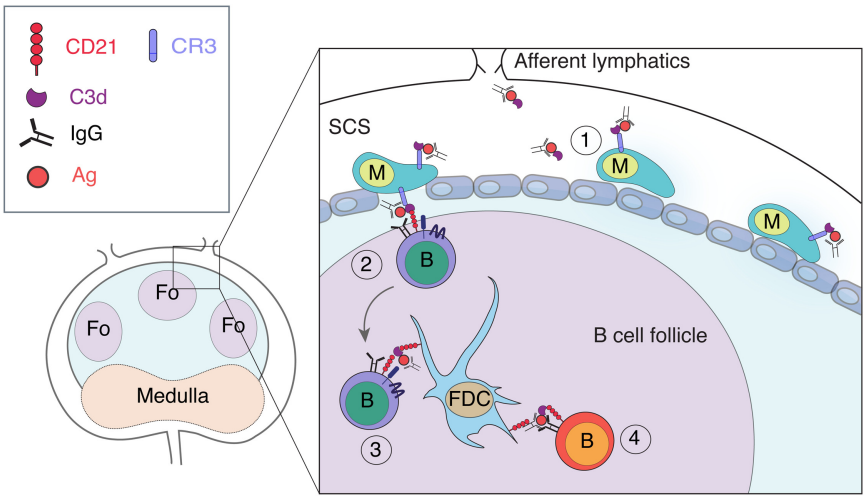

Figure 4. Pathways for the Recognition of B Cell Antigen in the Lymph Node
Processes depicted in the figure are as follows: (1) Immune complexes (ICs), formed by the deposition of complement proteins (in this illustration, C3d) and IgG on the surface of antigen, bind to complement receptor 3 (CR3) on the surface of subcapsular sinus macrophages (M). (2) Naive B cells transport complement-coated ICs from the subcapsular sinus to FDCs. (3) The ICs are transferred in a complement receptor 2 (CR2)-mediated mechanism from the surface of the B cell to the FDCs. (4) Cognate B cells capture small antigen directly from the surface of FDCs, associated with CR2 receptors (adapted from Gonzalez et al., 2011).

CR2(CCP1-2) that protrude down into the negatively charged acidic pocket in a manner that produces the charge complementarity expected from the long-known, ionic strength sensitivity of this binding interaction. Many of the interactions observed at the interface, be they charged, polar, or apolar, conformed to mutagenesis data obtained previously (Clemenza and Isenman, 2000; Hannan et al., 2005; Isenman et al., 2010) or to mutagenesis experiments prompted by the new structure (van den Elsen and Isenman, 2011). Finally, the substantially overlapping contact areas on C3d observed among CR2(CCP1-2), Efb-C, and Sbi-IV in their respective cocrystal structures (Clark et al., 2011; Hammel et al., 2007; van den Elsen and Isenman, 2011) readily explains the competition of the *Staphylococcal* immune evasion molecules for CR2 binding to C3d. Because of the nanomolar range affinity of Efb for C3d, compared with the μM range affinity of Sbi for C3d (Hammel et al., 2007; Upadhyay et al., 2008), the interaction of Efb with any C3d molecules that become covalently attached to the *Staphylococcus aureus* microbe may indeed be a physiologically relevant element through which this human pathogen evades antibody-mediated adaptive immunity (Ricklin et al., 2008).

Delivery of Antigen to Follicular Dendritic Cells

The findings that naive B cells are a major transporter of complement-coated immune complexes in the B cell follicles and that both uptake and transfer of the complexes to FDCs are dependent for the most part on CR2 and C3d provide a third, important pathway by which the complement system regulates humoral immunity (Figure 4). Using multiphoton intravital imaging in anesthetized mice, Phan et al. (2009) have found that soluble immune complexes that form in the afferent lymphatics and activate complement are captured by sinus-lining macrophages, possibly via FcγR and complement CR3 (CD11b; CD18), and are shuttled to naive B cells in the underlying follicles. Complement-coated complexes are efficiently transferred to follicular B cells in a CR2-dependent manner. Although the model system used by Phan et al. relied on rabbit IgG-phycoerythrin immune complexes to activate complement, in a more physiologic setting of vaccination where preexisting antibody is likely to be absent, it seems probable that complement is activated within the lymphatics by other innate recognition proteins such as those noted above, i.e., natural IgM, lectin proteins, or pentraxins like C-reactive protein. The finding that the cell surface C-type lectin SIGN-R1, expressed by sinus-lining macrophages, activates C1q and the classical pathway of complement on binding of *Streptococcus pneumonia* (Kang et al., 2006) provides another mechanism for formation of C3d-tagged complexes. Whether this pathway leads to B cell uptake of C3d-tagged bacteria has not been determined in their model. Given that LN resident dendritic cells also express SIGN-R1, and they have been reported to bind inactivated influenza via SIGN-R1 and transport virus into the follicles (Gonzalez et al., 2010), it is possible that they also transport *S. pneumonia* into the B cell compartment. It will be interesting to learn whether the resident DCs transfer C3-coated particles, such as influenza or bacteria, to naive follicular B cells for the actual delivery to FDCs or whether they deliver the complexes directly.

The actual transfer of C3d-coated immune complexes from naive B cells to FDCs has not been observed in vivo, but CR2-deficient mice show impaired uptake of antigens (Roozendaal and Carroll, 2007). Although FDCs express FcγRII receptors, they are not thought to be constitutively expressed and only appear on activated FDCs (El Shikh et al., 2006). By contrast, CR1 and CR2 are constitutively expressed on FDCs (Roozendaal and Carroll, 2007). Thus, the initial transfer of C3-coated complexes from naive B cells may induce activation of FDCs, leading to expression of FcγRIIb that may subsequently modulate FDC activity (more discussion of FDC activation below).

FDCs Are a Source of B Cell Antigen

The clonal selection hypothesis proposes antigen is required for positive selection and affinity maturation of B cells undergoing somatic hypermutation within germinal centers (Rajewsky, 1996). Studies by Goodnow and colleagues have established the importance of antigen in maintaining germinal centers because blockade of antigen receptors led to ablation of the germinal center response (Goodnow et al., 1995). Notably,

blockade of the B cell coreceptor (CD21;CD19;CD81) with a soluble form of the CR2 receptor also leads to a rapid loss of germinal centers (Fischer et al., 1998), suggesting that the overall strength of signal of the B cell receptor is critical for B cell survival. Early histology studies suggest that the major source of antigen for germinal center responses is FDCs. Studies of the LN and spleen of immunized mice traced labeled antigens to dendritic-like cells that were later identified as FDCs (Hanna and Szakal, 1968; Nossal et al., 1968). Surprisingly, it has been observed that antigen is retained for long periods and in some cases up to 1 year (Mandel et al., 1980). Thus, unlike macrophages and dendritic cells that degrade antigen after relatively short periods, FDCs appear to retain antigens for extended periods.

How these enigmatic stromal cells retain antigen for such extensive periods but make it readily available to B cells is unknown. Ultrastructural studies identify antigen retained as membrane-coated bodies, also known as ICCOSOMES, that are released and taken up by B cells within germinal center reactions (Szakal et al., 1988; Szakal et al., 1989). Alternatively, it is reported that FDCs retain electron dense antigens on their surface as dense deposits that can take a filiform shape.

Despite the strong correlation between FDC retention of antigen and efficient germinal center responses, actual observation of B cell acquisition of antigen from FDC in vivo has been elusive. In a recent elegant study using intravital microscopy, Suzuki et al. (2009) has observed direct uptake of antigen from FDCs by cognate B cells. In their murine model, they adoptively transferred immunoglobulin transgenic B cells specific for hen egg lysozyme (Hel) into mice immunized with Hel conjugated to phycoerythrin up to 9 days earlier. Interestingly, the cognate B cells acquire not only specific antigen but also FDC membrane proteins CR2 and BP-3 (CD157, BST-1). It is known that B cells can take up membrane antigens along with antigen from target cells by a process referred to as trogocytosis. Although the study didn't report whether complement C3d and antibody were also taken up, it is possible that the original C3d-coated immune complexes are retained intact via CR2 on the FDC surface. These findings are important because they establish that B cell antigens are retained by FDCs and can be directly acquired from the cell surface. These elegant results further suggest that FDCs are a likely source of antigen for a primary B cell response.

FDCs Maintain Germinal Centers

In addition to their role in retention of antigen, FDCs are a major source of the B cell chemoattractant CXCL-13, which is required for migration of B cells into the follicles (Cyster et al., 2000). FDCs differentiate from ubiquitous perivascular precursors (preFDCs) that have been recently identified in an elegant report by Aguzzi and colleagues (Krautler et al., 2012). Maturation of the precursors requires signaling by members of the tumor necrosis factor and lymphotoxin α-β family and presumably additional factors within lymphoid tissues (Gonzalez et al., 1998; Katakai et al., 2008; Koni et al., 1997). However, preFDCs can also differentiate within nonlymphoid tissues and are often present in sites of chronic inflammation as found in autoimmune disease and infections.

In the presence of adjuvants and/or immune complexes, FDCs become activated and increase release of B cell chemokines, but also secrete the B cell-activating factor BAFF and proinflammatory cytokines such as IL-6 and IL-10 and upregulate integrins ICAM-I and VCAM-1 (Garin et al., 2010; Wu et al., 2009). Whether activation alters or promotes display of B cell antigen on their surface is not known, but given the importance of antigen for the duration of the germinal center response (~3 weeks) the latter seems most likely.

Earlier studies have demonstrated that treatment of mice with a soluble form of the lymphotoxin receptor leads to transient loss of mature FDCs and disruption of humoral responses (Gommerman et al., 2002). In a definitive study, Wang et al. (2011) have used a genetic approach to selectively ablate FDCs in chimeric mice, demonstrating that FDCs are required for maintenance of B cell follicles and germinal centers. Their model system took advantage of CD21-specific Cre recombinase transgenic mice crossed with a conditional diphtheria toxin receptor (DTR) strain. Through construction of bone marrow chimeras with WT bone marrow to reconstitute irradiated CD21-DTR mice, DTR expression is limited primarily to the $CD21^+$ stromal population. Treatment of immunized chimeric mice with diphtheria toxin leads to ablation of FDCs within lymphoid tissues as expected. Characterization of the mice within 48 hr of treatment reveals loss of germinal centers and a disruption of the architecture of the B cell follicles. A limitation of the model is that only transient responses could be studied because the mice die within several days of treatment with the toxin from an apparent involvement of the central nervous system. An earlier study by Wyss-Coray has identified expression of CD21 mRNA in the nervous system in reporter mice, although the timing of expression is not known (Moriyama et al., 2011). In the future, it will be important to determine the kinetics of CD21 expression within the developing nervous system and identify potential participation in peripheral immunity.

FDC and Autoimmunity

Not all antigens acquired by FDCs lead to immune responses. Using an intricate maternal self-antigen transgenic model in which ovalbumin (OVA) expression is limited to the placentae, McCloskey et al. (2011) has found that self-antigen released at parturition accumulates on FDCs located in draining LNs and its uptake is enhanced by classical pathway complement. To test for T cell response to the self-antigen retained by FDCs, they adoptively transferred carboxyfluorescein diacetate succinimidyl ester (CFSE)- labeled $CD8^+$ OVA-specific T cells (OT-I) several weeks after parturition, at a time when it was predicted that antigen would be cleared from the host with the exception of antigen retained by FDCs. Analysis of harvested LNs at the optimal period has determined that antigen-specific $CD8^+$ T cells were not activated and appeared to be tolerized by resident DC sampling antigen from the FDCs. For a control, McCloskey et al. demonstrated that when OT-1 cells were adoptively transferred before immunization with OVA immune complexes and adjuvant, there was a robust response; by contrast, adoptive transfer several weeks later results in tolerance. Their results are important for several reasons: First, they suggest that resident DCs periodically sample both self- and foreign antigen displayed by FDCs. Second, DC sampling of antigen from FDCs results in a tolerogenic rather than

activation signal for $CD8^+$ T cells under the conditions defined in this study.

Cr2:C3d As a Target for Autoimmunity

Like development of a conventional B cell memory response after vaccination with foreign antigen, autoreactive B cells may undergo a similar pathway of differentiation into isotype-switched memory and effector cells. There is growing evidence that spontaneous germinal center formation may contribute to autoimmunity in mice and humans (Cappione et al., 2005; Vinuesa et al., 2009). Moreover, ectopic germinal centers, i.e., germinal centers formed in nonlymphoid tissues, are a hallmark of chronic autoimmunity such as in arthritis (Grammer et al., 2003). Therefore, it seems plausible that differentiating autoreactive B cells may have a similar requirement for coreceptor engagement of C3d along with B cell receptor binding of self-antigen. Thus, blockade of the CR2 coreceptor could provide a transient therapy to eliminate autoimmune germinal centers without imposing immune suppression to the naive B cell repertoire.

The details of the interface revealed from the cocrystal structure of CR2:C3d may allow one to design a compound that would, for example, interfere with the trapping of C3d-tagged self-antigens by CR2 on FDCs, thereby potentially downmodulating or "tune down" antibody-mediated autoimmunity. It is known from mutagenesis data that removing even a part of the contact interface can lead to a major binding defect and so with therapeutic target surfaces available on both sides of the interface, sometimes involving contiguous stretches of amino acid sequence, a peptide-based mimetic approach to the design of such an antagonist should be within the realm of feasibility. Therefore, it will be important in future experiments to determine whether autoreactive B cells have a similar requirement as conventional B cells for CR2 signal in survival within germinal centers.

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