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Complement and Humoral Immunity

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Abstract

The complement system was discovered almost a century ago as an important effector in antibody-dependent killing of microorganisms. Since this early period much was learned about the biochemistry and structure of complement proteins and their function in mediating inflammation. More recently, a prominent role for complement was identified in *linkage of innate and adaptive immunity*. In this review, I will discuss our current understanding of the importance of complement in enhancing the humoral immune response to both model antigens and pathogens. As discussed below, it is evident that the complement system participates in marking of “foreign” pathogens and “presenting” them to B cells in a manner that enhances both antibody production and long term memory. In this special issue of *Vaccine*, we see examples of how complement is critical in the immune response to bacterial and viral pathogens. Moreover, the finding that most organisms have co-evolved proteins to evade complement detection underscores its importance in host protection.

Keywords

humoral immunity; innate immunity; complement receptors

1. Introduction

The awarding of the Nobel Prize in Physiology or Medicine to Jules Bordet in 1919 for identifying the bacteriocidal component of serum called “complement” recognized the importance of this family of proteins in host protection against infection [1]. Over the past near century, we have gained a much fuller appreciation for the biology of complement and how it is involved in immunity. In this review, I will focus on our understanding of how complement participates in humoral immunity especially from the perspective of vaccine development.

The complement system represents a family of over 25 serum proteins and cell surface receptors that act in a cascade manner leading to innate functions such as inflammation and

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enhancement of adaptive immunity [2]. Three general pathways, i.e. classical, lectin and alternative, activate the complement system. The pathways are not distinct but overlap. For example, the alternative pathway amplifies both the classical and lectin pathways. Interestingly, much of our understanding of the complement system in humoral immunity is based on activation via the classical pathway; therefore this review focuses on models in which complement is activated via immunoglobulin.

After the early studies of Bordet, there was a lengthy gap before it became apparent that complement not only served an effector role but also participated in development of a humoral response. A role for complement in B cell differentiation came first with the identification of receptors for the third component C3 [3]. The finding that humoral immunity was reduced in mice transiently depleted of serum C3 further supported a role for complement in B cell regulation [4]. Further studies of humoral immunity in humans [5] and experimental animals [6-9] bearing genetic deficiencies in early complement components C3 or C4 supported the growing idea that complement was important in development of antibody responses to both thymus-dependent and -independent antigens.

One major path in which complement participates in adaptive immunity became apparent with the identification of the CD21/CD19/CD81 co-receptor expressed on B cells [10]. Carter and Fearon made the striking observation that cross-linking of the co-receptor and the B cell antigen receptor (BCR) together resulted in a significantly stronger response than BCR alone. This observation combined with the earlier work by Alex Law [11], David Isenman [12] and Brian Tack [13] that activated C3 and C4 bind co-valently to the antigen, provides a link between innate and adaptive immunity. Thus, activation of C3 and C4 exposes an internal thioester resulting in either an amide or ester bond with structures on the microbial surface. This step is a critical event in the complement cascade as it not only anchors the enzymatic convertase for cleaving additional complement but provides a molecular tag on the foreign antigen (Fig 1). The importance of this event in terms of host protection is becoming increasingly clear and represents a major topic discussed in this issue of *Vaccine*. As is evident from the articles in this issue, many pathogenic microbes and viruses have evolved mechanisms to evade the active C4b and C3b complexes bound on their surface. For example, expression of homologues of the mammalian complement regulatory proteins (CRP) such as C4b binding protein and Factor H provide a mechanism for the pathogen to limit deposition of complement on the surface and initiation of inflammation. However, as discussed below, the inactive forms of C3b, also provide a ligand for complement receptors important in instruction of the adaptive immune response.

2. Results

2.1 Coupling of C3d alters fate of antigen

The tagging of microbial pathogens or toxins with the cleavage products of C3, i.e. C3b, iC3b, C3d,g or C3d, facilitates their uptake and clearance by specific receptors such as CR3 (CD11b) expressed on most myeloid cells, CR-IG expressed on macrophages and kupfer cells in the liver [14] and Crry expressed on red cells and platelets [15]. In addition, the C3 tag targets foreign antigens to the CD21 (CR2) and CD35 (CR1) receptors which are co-expressed on B cells and follicular dendritic cells (FDC) in mice [16-17] (Fig. 2). In humans

the latter two receptors are more widely expressed including red cells (CD35 only) [18]. In mice both receptors are encoded by the *Cr2* locus and CD21 represents a splice product of the longer CD35 transcript which includes 21 short complement repeats (SCR), i.e. domains of approximately 60 amino acids held together by two-disulphide bonds [19]. Thus, CD21 includes 15 of the overall 21 complement repeat domains observed in CD35 but is missing the six N-terminal domains that includes the binding sites for C3b and C4b as well as the complement regulatory protein function.

2.2 Complement regulates B cell differentiation

Upon activation, mature B lymphocytes undergo various stages of differentiation depending on the strength of signal and the presence of T cell help (Fig. 3). Under optimal stimulation, B cells differentiate into effector cells (B effector) that secrete antibody and long-lived memory cells (B memory). Differentiation occurs primarily within a specialized environment within the B cell follicles referred to as germinal centers (GC) [20]. Effective formation of GC requires presence of both T cell help and a source of antigen which is retained on the surface of FDC via CD21 and CD35 (CR) and FcR (discussed more below). On activation by threshold signal B cells migrate into the follicles and initiate an GC and undergo rapid cell division. During this stage B cells isotype switch (class switch recombination-CSR), i.e. IgM to IgG, their surface receptor and acquire mutations (somatic hypermutation-SHM) that lead to a higher affinity interaction with antigen. Competition for antigen results in “clonal selection” a process that enriches for survival of the higher affinity clones.

Characterization of mice bearing a deficiency in complement C3, C4 or CD21/CD35, demonstrate the overall importance of complement in humoral immunity [21]. Thus, the deficient mice fail to make an efficient humoral response to model T-independent [22-24] and T-dependent antigens [25-29] as well as viral [30-32] and bacterial [33] pathogens. Dissection of the separate role of complement receptors on B cells [26] and FDC [34-35] indicates that both are essential to an efficient humoral response.

Using an influenza model and addressing the question of protective immunity, Gonzalez et al compared the survival of C3^{-/-} and Cr2^{-/-} mice with WT controls following vaccination and challenge at varying time points. Their results demonstrate that complement is essential for survival of a lethal challenge dose of influenza at 8 weeks post-vaccination (see article by Gonzalez et al in this issue). Importantly, survival correlated closely with anti-viral IgG titers prior to challenge with infectious virus. Thus, the anti-flu IgG titer was significantly reduced at 8 weeks relative to WT controls.

2.2 a. B cell co-receptor—Both CD21 and CD35 receptors form a co-receptor on the B cell surface along with CD19 and CD81 [36-37]. As mentioned above the interaction between CR with CD19 is essential for co-receptor activity. Although the CD21 short cytoplasmic domain becomes phosphorylated following cross linking with C3d, the major signaling effect on B cells is mediated by the CD19 moiety.

Cross linking of the B cell antigen receptor (BCR) and the CR/CD19/CD81 co-receptor by engagement of antigen coupled to C3d, enhances B cell immunity at several stages in B cell

differentiation. Naïve B cells in general bear a low affinity receptor for antigen and this limits their accidental activation by self-antigen. However, coupling of C3d to the foreign antigen “instructs” the B cell by activation of the co-receptor [38]. Using the hen egg lysozyme (HEL) transgenic model in which B cells bear a receptor bearing specificity for HEL, Fearon and colleagues demonstrated that coupling of C3d to the model antigen (HEL) reduced the amount of antigen required for an optimal secondary humoral response by as much as 10,000 fold [39]. Thus, the combined effect of co-receptor signaling at various stages of B cell development has a dramatic outcome on the B cell memory response.

To address the importance of co-receptor signaling in lowering the threshold for activation of naïve B cells, Barrington et al examined the primary humoral response to antigens with varying affinity [40]. Notably, using ligands that bind at low affinity, B cells not only failed to respond but underwent cell death in a caspase-dependent pathway. By contrast, coupling C3d to the low affinity antigen resulted in B cell activation and production of antibody. Thus, these results not only support the hypothesis that the co-receptor lowers the threshold for activation but rescues low affinity B cells from cell death on encounter with antigen.

A second stage in which co-receptor signaling enhances B cell immunity is in the GC response (Fig. 3). In a direct comparison of Cr2+ and CR2-/- B cells, Fischer et al demonstrated that antigen specific B cells lacking CR failed to survive within an ongoing GC in competition with WT B cells [41]. Thus, adoptive transfer of Cr2+ or Cr2-/- HEL Tg B cells into an ongoing GC response to lysozyme, the former co-receptor sufficient B cells competed efficiently and underwent proliferation. By contrast the Cr2-/- HEL + B cells failed to proliferate and did not survive. The interpretation of the results is that co-receptor signaling enhances BCR strength of signal and the B cells are clonally selected. In the absence of co-receptor, the B cells fail to compete and undergo cell death.

2.1 b. Follicular Dendritic Cells—A fundamental role for FDC is to trap and retain antigen on their cell surface for presentation to both naïve and GC B cells. As noted above CD21 and CD35 are important in retention of C3d-tagged immune complexes. To test the importance *in vivo* of retention of antigen via CR on FDC, Barrington et al used a chimeric approach in which Cr2-/- mice were reconstituted with bone marrow (BM) prepared from CR sufficient (or deficient as controls) mice [34]. Since FDC are derived from stromal cells; whereas B cells are BM in origin this model provides a novel approach to distinguish the role of CR on the two cell types. The chimeric mice were immunized with T -dependent antigen and their humoral response evaluated. As observed previously [26], the initial IgM and IgG responses were similar in both sets of chimeric mice suggesting that sufficient antigen is available for the initial priming of B cells in absence of CR expression on FDC. However, specific IgG titers declined significantly in the Cr2-/- relative to Cr2+ chimeras. To test for B cell memory, the mice were challenged several months later and the IgG response evaluated. Notably, the B memory response was significantly impaired relative to the control mice. Thus, in the absence of efficient retention of antigen on FDC via CD21/CD35 both long-term antibody production and memory are significantly reduced.

Expression of CR on FDC is also relevant to the host response to viral infection as BM chimeric mice prepared in a similar manner as discussed above also fail to maintain a

durable or long term IgG response to HSV infection [42] (see article in this issue by Knipe et al).

2.3 Trafficking of B cell antigens to lymphoid compartment

How antigens are acquired by FDC is a long-standing question. Studies in mice deficient in C3 or CD21/CD35 demonstrate the importance of CR in FDC retention of C3d-tagged antigen, but how the antigen complex is transported to the B cell follicles is only now becoming clear. Results from early experiments tracking T-independent and T-dependent antigen administered intravenously, demonstrated that immune complexes activate complement and that C3-coupled antigen is taken-up efficiently by marginal zone (MZ) B cells [22-23,43]. This specialized sub-set of B cells which localize within the marginal zone, bear high levels of CD21/CD35. Following uptake of C3-tagged IC they migrate into the splenic follicles where the complex is transferred to FDC, although the mechanism is not clear. More recently, a two-photon intravital imaging approach was used to track uptake of IC by MZ B cells. Interestingly, they found that MZ B cells continuously shuttle between the marginal zone and follicle in a chemokine dependent manner [44]. The frequent shuttling provides an efficient pathway to transport C3-tagged IC from the MZ to the B cell follicles.

Several recent reports have shed light on the trafficking of lymph-borne B cell antigens within the lymphoid compartment (Fig. 4). Using B cell TG models, two groups reported that cognate (antigen specific) B cells in the underlying follicular zone sample lymph-borne antigens from sub-capsular sinus (SCS) macrophages [45-46]. This sub-set of macrophages is characterized by expression of CD169+, CD11b+ and CD11c^{lo}. Since the frequency of antigen specific B cells is relatively low in normal mice and antigen is only retained transiently, it is not clear how relevant this pathway is as a major source of antigen.

Characterization of the transport of lymph-borne antigen in passively immune mice also identified initial uptake of immune complexes by the SCS macrophages [47]. Subsequently, complement-tagged IC are transferred to follicular B cells within the underlying lymphoid follicles. Importantly, the uptake of IC is complement dependent (as found for MZ B cells) as Cr2^{-/-} B cells were significantly less efficient in binding and transport. Based on these results, it was proposed that binding of antigen by IgG in the lymph resulted in tagging of antigen with C3d. Following uptake of C3d-IC, B cells migrate through the follicles and transfer the complexes to FDC in a undefined mechanism. It will be important in future experiments to understand how the C3d-tagged IC are transferred from the B cell to the FDC.

2.4 A novel pathway for B cell access to small antigens

Recent studies by Pape et al observed rapid entrance of small antigen (HEL) into the follicles following subcutaneous injection [48]. They focused on uptake of the labeled antigen by cognate B cells adoptively transferred into the recipients prior to immunization. Transport of the small antigen via SCS macrophages or dendritic cells was ruled out using various approaches; they concluded that small antigen entered the follicles via gaps in the sub-capsular sinus floor.

An alternative pathway for entrance of small lymph-borne antigen into the follicles is via conduits. In the process of tracking various antigens into the lymph node and their uptake on FDC, we found that the size of the antigen dictated its path [49]. In immune mice injected subcutaneously with large protein antigen, i.e. greater than 70 kDa, IC were bound by SCS and taken-up by follicular B cells over a 24 hr period and eventually retained on FDC as reported by Phan et al [47]. By contrast, antigens less than 70 kDa appeared to enter the follicles within minutes of subcutaneous injection as observed by Pape et al [48]. Thus, in the presence of antibody and complement the small antigen (TEL, turkey egg lysozyme) was identified on FDC in the draining LN within minutes of its injection in the footpad. Although complement was not required for entrance into the follicles, it was required for retention on FDC as found for large antigens.

Tracking of labeled TEL (14 kDa) into the draining lymph node and follicle using two-photon intravital microscopy, we observed that the antigen rapidly filled the sub-capsular sinus and drained into the follicular region via defined structures resembling fibroblast reticular fibers previously identified in the T cell (paracortical zone) [50-51]. One advantage of two-photon imaging is that collagen structures are detected through second harmonic emission and therefore conduit structures (as well as the collagen lining of the sub-capsular sinus) are visible without specific labeling [52]. Additional analysis of LNs using confocal microscopy in combination with various antibodies and electron microscopy revealed that the conduit structures within the follicles closely resembled those defined for the T cell zone [49]. Moreover, the collagen rich conduit fibers were wrapped by gp38+ fibroblast reticular cells (FRC) as described for analogous structures in the cortical region.

Thus, the current view is that lymph borne antigens gain entry into the B cell follicles via several pathways dependent on their size. Irrespective of the pathway, however, it is clear that retention on FDC is complement dependent. Further understanding of how pathogens are encountered within the draining lymph node will be important in aiding the design of vaccines.

3. Summary

Since the initial observation that B cells bear complement receptors, we have gained significant insight into how the complement system participates in adaptive immunity. Although this review has focused on humoral immunity, there is a growing understanding that complement also enhances T cell immunity. With respect to humoral immunity, complement is important in at least three different roles. A key event in humoral immunity is recognition of foreign antigen leading to covalent binding of C3d. This event “marks” the antigen and leads to enhancement of B cell effector and memory function by: (i) lowering the threshold for activation and maintenance of B cell survival within germinal centers; (ii) retention of antigen on FDC; (iii) and transport of immune complexes to the FDC by B cell via complement receptors.

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Figure 1.

Co-valent binding of complement C3 and C4 “mark” antigens as foreign. Recognition of foreign antigen by antibody (classical pathway) or lectin binding protein (lectin pathway) leads to covalent attachment of C4b and C3b to the antigen. C4b provides an anchor for the C3 convertase which is composed of a heterodimer of C2aC4b. One enzyme complex will activate up to 1000 C3 molecules. In addition to C4b, C3b and its cleavage fragments are ligands for complement receptors, in particular CD21 and CD35. Thus, the initial covalent attachment of C4 and C3 to foreign antigen not only provides an anchor for continuation of the cascade but ligands for complement receptors important in “instructing the humoral response”.

Figure 2.

Complement receptors CD21 and CD35 link innate and adaptive immunity via B cell co-receptor and retention of antigen on FDC. Both genes are encoded at the *Cr2* locus in the mouse and CD21 is a splice product of CD35. Recognition of split products of C3 (and C4) on a foreign surface provides ligand for complement receptors CD21 (CR2) and CD35 (CR1). In mice the receptors co-localize on B cells and follicular dendritic cells (FDC). On B cells, they form a functional co-receptor with CD19 and CD81. Engagement of the B cell receptor (BCR) with antigen coupled to C3d results in co-ligation of the co-receptor and BCR enhancing downstream signaling and lowers the threshold for B cell activation. In addition, foreign antigen uptake on FDC via C3d tag is essential for clonal selection of B cells within the germinal center and efficient maintenance of long term antibody responses and memory.

Figure 3.

Recognition of C3-tagged antigen via CD21 and CD35 enhances B cell differentiation at 3 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.

Transport of immune complexes by B cells into the LN follicles is CR dependent. Recognition of foreign antigens entering the host following vaccination (or infection) by antibody or lectin proteins results in activation and binding of complement. Immune complexes are transiently retained by subcapsular sinus (SCS) macrophages and subsequently shuttled to follicular B cells in the underlying B cell zone. In the presence of C3d tag, complexes are taken-up by B cells via CD21/CD35 and transported into the follicles where they are transferred to FDC. An alternative pathway of delivery to FDC is via fibroblast reticular cell (FRC) conduits. Small antigens (less than 70 kDa) actively drain from the SCS into the conduits which terminate near FDC. Thus, complement tagged antigens are rapidly delivered to FDC independent of B cell transport. Gaps in the conduits also provide an opportunity for cognate B cells to sample small antigens draining from the SCS.