Review

The role of complement in the success of vaccination with conjugated vs unconjugated polysaccharide antigen

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Abstract

The complement system, a well-characterised arm of the innate immune system, significantly influences the adaptive immune response via direct cell-cell interaction and maintenance of lymphoid organ architecture. Development of vaccines is a major advance in modern health care. In this review, we highlight the importance of the marginal zone in response to both, polysaccharide and conjugated vaccines, and discuss the relevance of complement herein, based on findings obtained from animal models with specific deletions of certain complement components and from vaccination reports of complement-deficient individuals. We conclude that both, intactness of the complement system and maturity of expression of its components, are relatively more important to aid in the immune response to polysaccharide vaccine than to conjugated vaccines.

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running title

Complement essential for polysaccharide vaccines

The importance of complement activation in adaptive immunity

The complement system is part of the innate immune defence, because it partakes in first-line, pattern-mediated recognition via its germ-line encoded serum proteins and receptors. It has evolved from a primordial fluid-phase system that "simply" tags and mediates uptake of micro-organisms by cells of early chordates. Large-scale gene duplications, exon shuffling and transposition events are the main evolutionary mechanisms that have generated a wide range of diversified molecules, which we now ascribe to innate and adaptive immune defences [1]. Innate immune defence molecules are of importance in the initial phase of an infection prior to the phase in which an adaptive immune response is mounted in our secondary lymphoid organs, and may lead to a resolution of local inflammation. However, pattern recognition systems in general, such as TLRs, NODs, and scavenger receptors, do have an impact on the generation of the adaptive immune response as well [2-6]. The complement system encompasses both, fluid-phase molecules, and cell surface receptors. Components are widely expressed, including in primary and secondary lymphoid organs, which are a more recent acquisition in vertebrate evolution. The formative role of complement activation for immune complex tagging, immune complex localisation, and B-cell co-receptor triggering has now been clearly defined [7]. At the interface of innate and adaptive immunity, complement activation effects an enhanced antibody response by directing the binding of split products of one of its most abundant proteins, C3, to relevant surfaces and molecules. Most studies have been conducted using T-dependent antigens, therefore, the focus in the bigger body of literature is on follicular B cells; antigen, when coated with C3d, binds to membrane-bound immunoglobulin of follicular B-cells, the B-cell receptor (BCR), and, in addition, to one of the co-receptors, CD21. This results in co-ligation of a receptor complex, composed of BCR, CD21, CD19, and CD81 [8], which decreases a signalling threshold and, in the presence of T-cell help,

leads to expansion of B-cells and increase of secreted immunoglobulin production. C3d-tagged antigen localises to follicular dendritic cells in lymphoid follicles by binding to the receptors CD21 and CD35. This retention of antigen leads to stimulation of follicular B-cells, affinity-driven selection and maintenance of memory B-cells. C3-coated immune complexes are trapped by splenic marginal zone B-cells expressing high levels of CD21 and CD35 and are transported by these cells to the follicles.

The influence of complement on adaptive immunity involves, in particular, survival, stimulation, and maintenance of B-cells. Here, we review the evidence that complement influences differentially the response to unconjugated vs conjugated polysaccharide antigen.

Microanatomic site of splenic immune reaction towards polysaccharide antigens

Total splenectomy or congenital asplenia in man carries a significant risk of infections caused by encapsulated organisms. Complex polysaccharides, as they are found in cell walls of encapsulated bacteria, such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis*, are poorly immunogenic in comparison with peptide antigens owing to some degree to their chemical character (negative charge), which can make phagocytic uptake difficult [9]. However, a group of specialised cells in the marginal zone of the spleen appear to be of predominant importance in dealing with this particular challenge. In the mouse, the marginal zone surrounds the marginal sinus, which are vascular endbuds of the central artery with a leaky endothelium, and harbors marginal zone macrophages, B-cells, dendritic cells and metallophilic macrophages (fig. 1). In human, the splenic morphology differs in that a marginal sinus as described for mice and marginal metallophilic macrophages are absent [13]. Positivity for sialoadhesin, associated with marginal metallophilic macrophages in rodents, is found in macrophages of the human perifollicular zone

(capillary sheaths), the proposed entry of antigen into the human spleen [14]. About 90% of splenic blood throughput is directed into this area of discontinuous arterial terminals and open-ended venous sinuses (slow flow), whereas the remainder passes via direct arteriovenous connections through the red pulp (fast flow) [15].

Unconjugated polysaccharide antigens, due to the multivalent nature of the repetitive epitopes [16] crosslink polyreactive B-cell receptors and lead to their activation. Marginal zone B-cells are distinct from follicular B-cells (of the germinal centers) and function within the innate immunity [17]. Polysaccharide antigens are avidly bound by $CD21^{hi}$ marginal zone B-cells and are, in the mouse, additionally captured by marginal zone macrophages expressing complement receptors CR3 and CR4 [18]. Marginal zone B-cells are a non-activated B-cell subset [19], but on activation, they migrate to the follicular zone where CD21 (CR2) is proteolytically cleaved and antigen transferred to follicular dendritic cells. There is, notably, no T-cell help, because i) there is no efficient processing and presentation of polysaccharides, and ii) solely CR2 mediated B-cell triggering does not upregulate co-stimulatory molecules [20]. Importantly, upon binding of C1q-tagged complexes to the BCR of follicular B-cells, the B-cell provides a surface on which complement activation can occur and C3 ligands that are engendered can bind in strict vicinity of the BCR, to CR1 or CR2 [21].

Antibodies detected after immunisation with T-independent antigens, usually generated simultaneously as IgM and IgG within 3 days, exhibit lesser avidity, opsonophagocytic and bacteriolytic activities, compared to antibodies elicited by immunisation with T-dependent antigens [22-24], reflecting absent affinity maturation of these antibodies, but the polyreactive and persistent stimulation of B-cells by polysaccharide structures can provide long lasting antibody levels. This is consistent with the idea that antigen persistence and continuous stimulation are necessary to maintain memory B-cells [25]. However, to date, polysaccharides were not thought to be

able to elicit a memory B cell response [26]. It seemed difficult to achieve, given the fact that polysaccharide antigens are poorly presented by MHCII, thereby do not recruit T cell help, may induce tolerance or generation of plasma cells by crosslinking BCR and might be poorly retained in the germinal center [27]. Yet, importantly, recent analyses demonstrate that IgM memory B-cells are indeed generated, independent of T-cell help [28].

Coupling of vaccines with attenuated toxins, such as diphtheria or tetanus toxin, enhances the delivery of low immunogenic vaccines, such as polysaccharides, to antigen presenting cells, and recognition by T-cells. In the mouse, this T-dependent immune response involves metallophilic macrophages of the marginal zone, marginal zone B-cells and follicular B-cells of the germinal center in the spleen. Antibodies in circulation, initially of the IgM-, subsequently of the IgG-type, are specific for the protein carrier as well as the polysaccharides [29].

During T-dependent immune response to hen egg lysozyme, using an adoptive transfer model in mice transgenic for anti hen egg lysozyme immunoglobulin, purified marginal zone B-cells were less efficient in migrating to the outer periarteriolar lymphatic sheath and were therefore less available for T-dependent responses than follicular B-cells [30]. In principle, however, follicular B-cells and marginal zone B-cells are able to respond to challenge with both, T-dependent and T-independent antigen [31].

Importantly, after subcutaneous administration of T-independent or T-dependent antigens in mice, it is the spleen that reacts with formation of antibody forming cells before the draining lymphnodes, especially in response to T-independent antigen [32].

Germinal centers are formed during an immune response elicited with a so-called T-independent antigen. This reaction is, however, of short duration. It is possible that the absence of somatic hypermutation of the immunoglobulin V genes is due to both, the curtailed germinal center reaction – as somatic hypermutation is a relatively late event -

and the lack of T-cell help [33]. Of note, marginal zone B-cells are incapable of participating in the process of somatic hypermutation due to the lack of expression of activation-induced cytidine deaminase, an enzyme essential for this process [34].

The involvement of T-cells is not strictly excluded in the antibody response against polysaccharides, as B7 ligand dependent costimulation of B-cells (by T-cells) is needed, however, the interaction in this early response is short and T-cell receptor unspecific [35]. The absence of somatic hypermutation does, however, explain the low affinity binding of anti-polysaccharide antibodies.

The contribution of complement activation towards vaccination success

In the marginal zone, innate and adaptive immunity interact on a cellular level in the first encounter of hematogenously spread antigen with lymphatic tissue. Table 1 lists the source of expression for scavenger receptors and complement receptors in this anatomic area. Complement components assist in the marginal zone in focussing antigenic material to specialised splenic cells, but they clearly shape the germinal center reaction in the follicle of both, spleen (and lymphnodes). Table 2 lists the expression of complement components in the germinal center.

Germinal centers of secondary lymphoid follicles are positive for the deposition of C1, C4b, C4d, C3b, C3d, C5b-9, and C4Bp [45], which is consistent with a model of complement activation on immobilised immune complexes. Relevant to this concept is detection of C1r mRNA in the spleen [46], and of properdin mRNA, precursor of a relevant amplifier of ongoing complement activation [47], which is possibly produced by cells of the dendritic cell lineage [48].

Pneumococcal polysaccharides bind C1q [49] and can trigger alternative pathway-mediated C3 deposition [50]. Pneumococcal polysaccharides and C1q both,

bind to SIGN-R1, a lectin expressed by marginal zone macrophages [51]. They are highly phagocytic, but their antigen presentation potential is low.

In rats and non-immune humans, pneumococcal polysaccharide antigens localise to splenic marginal zone B-cells and follicular dendritic cells of the germinal center, in strict dependence of complement C3d and CR2 [52-54]. In immune humans, pneumococcal polysaccarides co-localise with splenic CR1 [54], which captures immune complexes and participates in enzymatic degradation of C3 and C4. Both, CR1 and CR2 are expressed by marginal zone B-cells. CR2 binding of marginal zone B-cells triggers their migration to follicular dendritic cells, aided by myeloid dendritic cells (secretion of activating signals). CR2 is able to promote alternative pathway-mediated C3 deposition [55], so, on involvement, may provide the necessary ligands for CR3 expressing dendritic cells.

The importance of complement in the generation of an adaptive immune response towards T-dependent antigen is well studied using complement-deficient mice: Expression of CR1 and CR2 on follicular dendritic cells and B-cells are necessary for a specific antibody (IgM and IgG) response and increase of titre after secondary i.v. immunisation with T-dependent antigen [44, 56], as deduced from CR1/2-deficient mice. CR1/2-deficient mice may be impaired in germinal center formation and B1-cell numbers, depending on the type of T-dependent antigen applied [57]. C1q-deficient mice do not localise immune complexes to follicular dendritic cells [58]. C3- and C4-deficient mice show a deficiency in isotype switching and formation of germinal centers in a model of i.v. administration of T-dependent antigen [59]. After i.p. and epicutaneous administration of T-dependent antigen, C3-deficient mice are impaired in both, Th1 and Th2 responses [60]. As the humoral response to T-dependent antigen is normal in Factor B-deficient mice [61], it seems that it is the classical pathway of complement activation that is predominately more relevant than the lectin or alternative pathways for

generation of specific antibody response and maintenance of germinal center architecture.

Complement activity can be depleted *in vivo*, for a limited period, by administration of cobra venom factor (which forms a stable C3 convertase). In this situation, there is absent splenic localisation of pneumococcal polysaccharide (administered i.v.) and impairment of specific antibody response. This is not observed for i.v. administered conjugate vaccine [53]. In splenectomised rats, polysaccharide vaccines are far less efficient than conjugated vaccines [62].

Is complement relatively more important to raise an appropriate antibody response to polysaccharide antigens, compared to proteinaceous, T-dependent antigens? The fact that primary complement deficiency predisposes to infection with micro-organisms with a complex polysaccharide capsule seems to suggest this and casts doubt on the use of polysaccharide-type vaccines in these patients [63].

Complement deficiencies are rare [64], and few studies investigate the impact of these deficiencies on vaccine success. In the largest of these studies, 53 patients with a variety of genetic complement deficiencies (properdin, Factor H, late components) were immunised with the tetravalent meningococcal polysaccharide vaccine ACYW135 [65]. There was wide variation in the specific antibody response between individuals but, importantly, there were no vaccine failures. Of note, these patients on the whole showed not only vaccination-induced, but also infection-induced, protective immunity. Vaccination of three properdin-deficient patients with meningococcal vaccine was protective against meningococcal disease [66, 67]. However, not every properdin-deficient male succumbs to fatal meningococcal disease and there may indeed be compensation of this defect by certain immunoglobulin allotypes that are especially efficient towards polysaccharide antigens [68]. The naturally acquired anti-polysaccharide antibody response of two C1q-deficient patients consisted of elevated

IgM levels compared to controls but lesser IgG and IgA levels [69], showing low class switching ability in these patients. By contrast, three out of four C3-deficient patients showed negligable levels of anti-pneumococcal capsular polysaccharide antibodies and suffered from recurrent pneumococcal sepsis [70]. Hereditary C3-deficiency predisposes to infections with not only encapsulated, but a variety of microorganisms [48], reflecting the central role of C3 and its activation/degradation products in the complement activation cascades.

Maturation of the immune response to polysaccharide vaccines

Immune response, splenic microarchitecture, and complement repertoire mature with age. Neonatal mice have low C3 serum levels, low C3 production of peritoneal macrophages, low follicular dendritic cell numbers, and immature B cell function [71,72]. The splenic marginal zone is the important substructure because it efficiently and quickly captures, presents and relocates to the germinal centers T-dependent and T-independent immunogens and other blood-borne antigens [73-75]. In term-newborns, the spleen shows no evidence of a marginal zone [54]. In infant spleen, the marginal zone shows low abundance of CD21 (CR2) expression, but is positive for CD35 (CR1). Pneumococcal polysaccharides pre-incubated with normal human serum localise to follicular dendritic cells in infants, but to follicular dendritic cells and marginal zone B-cells in adults [54]. Development of the marginal zone is delayed in mouse (up to 3- 4 weeks) and human (up to two years, but it becomes visible as of 4 months) and encompasses maturation and organisation of marginal zone B-cells (expressing CR1, CR2, CD1d) [76] as well as population with memory B-cells [77, 78]. This is the basis for the impairment in the immune response of the young in both, human and mouse, but it can be overcome by conjugation of polysaccharide vaccines [79, 80]. In the elderly, by

contrast, unconjugated polysaccharides and protein-conjugated oligosaccharides prove equally efficacious [81, 82], at least in the absence of functional hyposplenism [83].

Conclusions

Polysaccharides are poorly immunogenic. Marginal zone B-cells are instrumental in generating an immediate anti-polysaccharide immunoglobulin response through their ability to polyvalently bind polysaccharides on the one hand (via BCR) and to bind to C3d-decoration on the other hand (via CR1/CR2). Marginal zone B-cells are found concentrated in the spleen and in mesenteric lymphnodes [84].

Though the pieces of work using experimental mice aimed to elucidate relevant immune mechanisms in human, it is important to remember that there are differences that caution against over-extrapolation. Not only does the mouse spleen harbour hematopoietic activity (signalling the presence of niches distinct form human), it exhibits a different structure of the white pulp as explained above [85]. B1 cells, not or hardly found in humans, can contribute significantly to the murine antibody response to polysaccharide antigen [86]. However, as in humans, splenectomised mice are significantly more susceptible to sepsis with encapsulated organisms [87]. The significance of marginal zone B-cells as crucial effector cells in the response to T-independent and T-dependent antigen lies in the rapidity of their involvement ad the efficiency with which a specific antibody response is initiated [31]. In particular, complement tags and captures polysaccharide antigens on marginal zone B-cells [88]. Cells expressing complement components or complement receptors are involved in the transportation of both, polysaccharide and proteinaceous antigens, to the germinal center. Where antigen processing and presentation occurs, namely in the case of proteinaceous antigens, complement, in addition, aids in maintaining T-cell sustained germinal center development and in establishing an adaptive immune response.

Receptors for the central complement component C3 and its cleavage /degradation products are found on all immune effector cells, macrophages, follicular dendritic cells, B-, T-cells. In the immune response to polysaccharide antigens, germinal centers do develop, but, in the absence of T-cell help, they are transient. Through the ability to retain and focus antigen, complement plays a role in the generation of memory B-cells. These are selected in the spleen during both, T-dependent and T-independent antigen challenges [78]. Mice genetically engineered to be deficient in complement components, which, to date, have only been investigated in their responses to T-dependent antigens [89], are suitable tools to investigate the relative contribution of complement activation and complement-mediated effects on splenic antigen localisation, its maturation, and ensuing antibody responses.

An intact complement system influences differentially the response to unconjugated vs conjugated antigen because of the inability of non-zwitterionic polysaccharide antigens to be presented in the context of MHC class II, causing ineffectiveness of a cognate T-cell response. A defect in the complement system therefore compromises the host especially on exposure to polysaccharide antigens. C3 deficiency is the most serious complement defect because C3 split and degradation products link the adaptive and innate immune response on the surface of the B-cell [90]. In other complement-deficient individuals, adequate immunoglobulin responses to polysaccharide antigens may be found, therefore, in persons with a complement deficiency other than C3 and an inability to mount a protective titre of antibodies to polysaccharide antigens, other functional polymorphisms, immaturity or old age may play a more dominant role. After splenectomy, it is, on the one hand, the annihilation of the anatomic substructure of the marginal zone of the white pulp that leads to a poor immune response to encapsulated microorganisms. On the other hand, the significant reduction of the MPS/RES represented by the red pulp with its repertoire of plasma cells

and C1q-producing macrophages leads to impairment in clearance of these organisms. Hence, autotransplantation or maintenance of residual splenic tissue is now standard practice [91]. Vaccinations against encapsulated organisms are administered to manage these two cohorts showing physical and/or functional impairment of the splenic marginal zone, however, the use of conjugate vaccines (where available) in these cases appears to be more justified than that of polysaccharide antigens.

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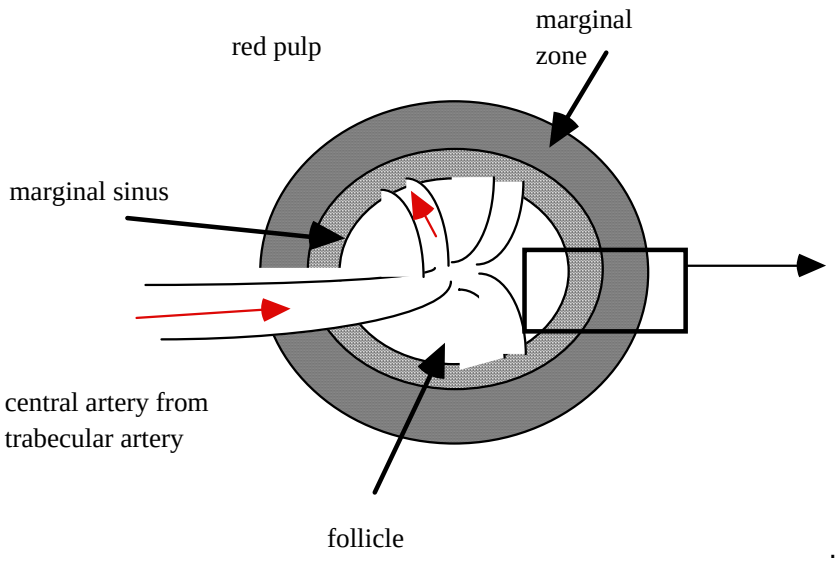
Figure 1: Architecture of the white pulp of the mouse spleen (A) and depiction of the cell types involved at the first interface between blood and lymphatic tissue. Red arrows indicate the flow of the blood from the marginal sinus into the marginal zone, at the right of panel B. Here, depending on the interactions made, antigenic material is either moved on to the red pulp or passed across the marginal sinus to the white pulp. From there, it is transported to the germinal center (black arrows). Complement proteins or described receptors thereof are indicated in green. Splenic T-cells may exhibit surface receptors for complement C3 [13-15].

Salehen N, Stover C: The role of complement in the success of vaccination with conjugated vs unconjugated polysaccharide antigen, Fig.1

A



B

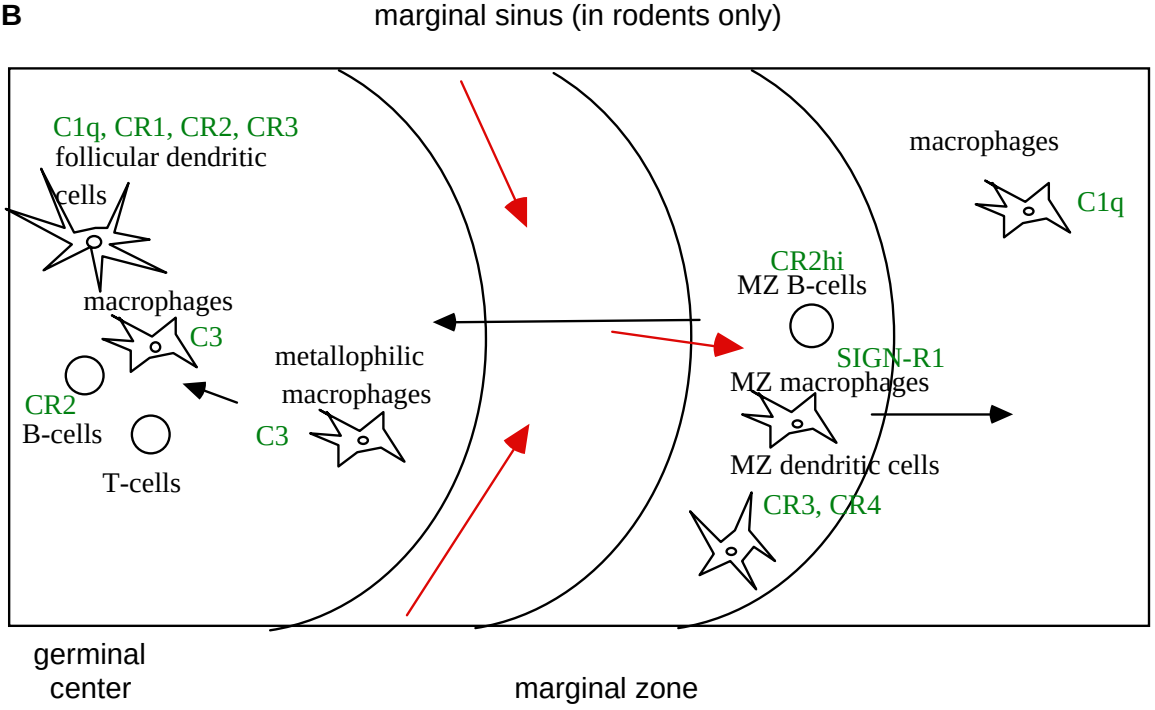


Table 1 Innate immune molecules expressed by cells of the marginal zone in the mouse [36-40]

Type of cell	**molecule**	**Function**
Macrophages of the marginal zone	SIGNR1	Scavenger receptor
	MARCO	Scavenger receptor, retains B-cells in marginal zone
	TLR	Pattern-recognition receptor
B-cells of the marginal zone	CD1	Non-classical MHC molecule, binds non-peptidic antigen
	CR1 (CD35)	Binding of C3b and C4b, binding of immune complexes, decay acceleration (cofactor activity) of C4b2b, C3bBb/C4b2b3b, C3bnBb, binding of iC3b, C3d
	CR2 (CD21)	Binding of iC3b, C3d, C3dg
Dendritic cells of the marginal zone	CR3	Integrin, binding of iC3b, ICAM-1, LPS, phagocytosis, apoptotic cell uptake
	CR4	Integrin, binding of iC3b, apoptotic cell uptake
Marginal zone metallophilic macrophages of the white pulp	C3	Central complement component giving rise, after enzymatic cleavage and degradation, to C3a, C3b, iC3b, C3dg
	sialoadhesin	Binds microbial polysaccharides

Table 2 Complement molecules expressed by cells of the germinal center [41-44]

Type of cell	**Molecule**	**function**
macrophages	C3	Central complement component giving rise, after enzymatic cleavage and degradation, to C3a, C3b, iC3b, C3dg
Follicular dendritic cells	C1q	Binding of immune complexes bound to FcγRII, initiation of classical pathway of complement activation
	C1 Inhibitor	Regulation of classical pathway of complement activation
	CR1 (CD35)	Binding of C3b and C4b, binding of immune complexes, decay acceleration (cofactor activity) of C4b2b, C3bBb/C4b2b3b, C3bnBb
	CR2 (CD21)	Binding of iC3b, C3d, C3dg
	CR3	Integrin, binding of iC3b, ICAM-1, LPS, phagocytosis
B-cells	CR2 (CD21)	Binding of iC3b, C3d, C3dg