MINIREVIEW

Molecular Structure and Expression of Anthropic, Ovine, and Murine Forms of Complement Receptor Type 2\(^7\)

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Complement receptor type 2 (CR2), also called CD21, is a cell surface glycoprotein expressed on the surfaces of mature human B lymphocytes, follicular dendritic cells (FDCs), a few T cells, and some striated epithelia (1). Two splice variants of CR2 have been detected in human B cells. The extracellular region of the prototype “short” form (145 kDa) contains 15 tandem short consensus repeat (SCR) domains (1), while the “long” form of CR2, containing 16 SCR domains, has been detected at the transcript level (58). Human CR2 associates with other molecules on the B-cell surface to form two distinct complexes. The most commonly expressed form of CR2 consists of a noncovalently associated complex formed between CR2 and CD19/CD81/Leu13 (104), although CR2 can also form complexes with CR1 (CD35) (110). The two complement receptors that are expressed by B lymphocytes both bind fragments of C3 that become covalently attached to activators of complement, providing the B cell with a nonclonal means for interacting with antigen that has been identified by this innate immune system. Therefore, CR2 rather than CR1 was the more likely candidate for the receptor that communicated innate recognition of antigen to the B lymphocyte (29).

COMPLEMENT IS A KEY COMPONENT OF INNATE IMMUNITY

The complement system is comprised of a number of serum and membrane-bound proteins that play an important role in the elimination of foreign microorganisms while protecting the host organism from complement-related damage. It has three critical physiologic activities. (i) It defends against microbial infections by triggering the generation of a membranolytic complex (C5b9 complex) at the surface of the pathogen and complement fragments (named opsonins, i.e., C1q, C3b, and iC3b) which interact with complement cell surface receptors (CR1, CR3, and CR4) to promote phagocytosis. Soluble complement anaphylatoxins (C4a, C3a, and C5a) greatly control the local proinflammatory response through the chemotaxis and activation of leukocytes. (ii) It bridges innate immunity and adaptive immunity (essentially through CR2, expressed by B cells). (iii) It disposes of immune complexes and the products of inflammatory injury (i.e., other danger signals, such as toxic cell debris and apoptotic corpses) to ensure the protection and healing of the host. The regulatory mechanisms of complement are finely balanced so that on the one hand, the deposition of complement is focused on the surface of invading microorganisms, and on the other hand, the deposition of complement on normal cells is limited by several key complement inhibitors (e.g., CD46, CD55, and CD59) (40).

Complement can be activated essentially by three distinct routes, the classical pathway (CP), the alternative pathway (AP), and the lectin pathway (Fig. 1). The initiation of the AP (involving C3, factor B, factor D, and properdin) does not depend upon the presence of immune complexes but is initiated following interactions with carbohydrate-rich particles lacking sialic acid (for a review, see reference 28). The AP is activated, for instance, by a diverse set of “natural” substances, including yeast walls, bacterial cell walls, and cobra venom factor. This will lead to the deposition of C3 fragments on the target cells. The CP (involving C1q, C1r, C1s, C4, C2, and C3 components) is activated primarily by the interaction of C1q with immune complexes (antibody-antigen), but activation can also be achieved after interaction of C1q with nonimmune molecules such as polyanions (bacterial lipopolysaccharides, DNA, and RNA), certain small polysaccharides, viral membranes, complement-reactive protein (CRP), serum amyloid P component (SAP), and, more importantly, some bacterial, fungal, and virus membrane components that are yet to be fully characterized (41). CRP in mammals is an acute-phase protein which can interact directly with microorganisms in a calcium-dependent manner to bring about complement activation through the CP but which, allegedly, does not initiate an efficient terminal pathway with the formation of C5a and the membranolytic complex C5b9 (62). Along with SAP, CRP is a member of the pentraxin family of proteins, which are unrelated to other known proteins but are themselves stably conserved in vertebrate and invertebrate (e.g., Limulus polyphemus [horseshoe crab]) (3, 69, 105). In vertebrates, CRP binds to a plethora of microbial polysaccharides, while SAP interacts with carbohydrate moieties. Pentraxins are also known to bind to cell components (e.g., fibronectin and chromatin) in damaged tissues to aid in their removal through interactions with the opsonic complement system as well as to stimulate macrophages through direct binding to Fc receptors (types I and II) (8).

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and is homologous in structure to C1q. MBL is probably the most remarkable pattern recognition molecule of the innate immune system owing to its selective binding to arrays of terminal mannose groups on a variety of bacteria (59, 84). MBL activates complement by interacting with two serine proteases called MBL-associated serine protease 1 (MASP1) and MASP2. MASP2 cleaves and activates C4 and C2, and MASP1 may cleave C3 directly. The lectin pathway is initiated by the binding of MBL or serum ficolins to repeating carbohydrate moieties found primarily on the surfaces of microbial pathogens (38, 75, 94). In addition, though, the protein cytokeratin, when it is exposed on ischemic endothelial cells (12), also activates this pathway, as can antibodies bearing a specific form of agalactosyl carbohydrate designated G0 (73). Of interest, although the best evidence for the mechanism by which MBL binding leads to C3 activation is that this pathway proceeds through the initial cleavage of C4 and C2, several lines of research suggest that C3 may be directly activated by the lectin pathway without utilizing C4 or C2 (38, 100). These components of the complement system are part of the lectin pathway (17, 99, 101) (Fig. 1). Each of these activating pathways converges at the step of C3, which leads to the covalent attachment of C3b to the activating substance. C3b fragments generated from C3 cleavage are covalently attached to the activating substrate and serve as ligands, along with C4b-bound antigens, for CR1 (CD35). Inactivation of C3b with iC3b and C3d generates the specific ligands for CR2 (CD21) and serves as a means of targeting antigen or immune complexes to cells expressing CR2, linking complement activation to B-cell biology and tolerance (9).

The family of complement receptors. Four complement receptors have been described: CR1 (CD35), CR2 (CD21), CR3 (CD11b/CD18), and CR4 (CD11c/CD18). CR1 and CR2 are expressed mainly on B cells and FDCs, while CR3 and CR4 are integrins expressed on macrophages and dendritic cells (85).

CR1 is a principal regulator of the activation of the complement system of plasma proteins. It is a membrane-bound, single-chain glycoprotein, ranging in size from 210 to >300 kDa (21–23, 114), and is expressed on erythrocytes, monocytes, neutrophils, B cells, some T cells, FDCs, and glomerular podocytes (31). It is capable of binding C4b and C3b fragments of complement (30), functioning on phagocytic cells to facilitate the ingestion by these cells of particles that have activated complement (39).

The complement system through CR2 plays immunoregulatory roles such as enhancing humoral immunity to T-cell-dependent and T-cell-independent foreign antigens (2, 13, 37, 47, 77) and regulating T-cell immunity to self and nonself antigens (26, 60, 88). Recently, an additional complement receptor, designated CR1g for circulating C3b-bound antigens, has been found on Kupffer cells and likely plays the major role in clearance of pathogens from the blood system (50).

In addition to immunoregulatory functions, other studies have focused attention on the roles that the complement system plays in recognition and effector functions during self tissue injury (54, 106) and in shaping the development of the natural antibody repertoire by influencing the development of reactivity with certain self antigens (14, 35, 55, 95, 120, 121). C3b interacts with CR1, which promotes its proteolytic processing eventually to C3dg, which is the ligand for CR2. Therefore, CR2 rather than CR1 was the more likely candidate for the receptor that communicated innate recognition of antigen to the B lymphocyte (24, 29). Below we focus our attention on the available data from CR2, as it has been shown to be the main receptor of C3d.

CR2 (CD21): the link between the innate and acquired immune responses. CR2 (CD21) is an important receptor in the innate and acquired immune responses. It is now established that complement-derived fragments play an important role in shaping the antibody response of acquired immunity. This was shown first by Pepys, who demonstrated that the formation of antibodies against T-cell-dependent antigens was reduced in animals in which C3 had been depleted (83). On the plasma membrane of B cells is a complex of two proteins: CD19, which...
is a component of the acquired immune system, and CD21 (CR2), a receptor for C3d. It has been proposed that activation against a newly encountered antigen occurs via “natural” immunoglobulin M (IgM), CRP, collectins, or alternative pathway and leads to C3 and C3d opsonization at the surface of the pathogen. The proposed paradigm is that binding of the antigen-C3d complex to the membrane Ig and CR2 and signaling through CD19 induces a sustained activation of B cells. Indeed, when an antigen was coupled to C3dg molecules, much less antigen was required to evoke a given level of antibody than was the case for the native antigen (20). C1q also acts to bridge the innate and adaptive immune systems following binding to natural IgM complexed to antigens, including microbial antigens and certain autoantigens (51). A novel interface between complement and B-cell activation has been unveiled with the report that the alpha chain of C4b binding protein activates B cells through the CD40 receptor (10).

Complement activation is an essential component of the early response to infection. Rapidly generated complement cleavage products recruit and activate effector cells and promote the lysis or phagocytosis of microbes. In addition, the complement cleavage products C3d and C3dg (collectively referred to as C3d (g)) enhance adaptive immunity via binding to CR2 (CD21) on B cells and FDCs, resulting in enhanced antigen-specific antibody production. Expression of CR2 on FDCs promotes retention of opsonized antigens and immune complexes for presentation to B cells, whereas CR2 expression on B cells enables coaggregation with the B-cell antigen receptor (BCR) and augmented B-cell activation through recruitment of the B-cell-restricted CD19 molecule. For these reasons, C3d (g) possesses potent adjuvant activity (20, 46, 98), making its generation a key regulatory step linking the innate and adaptive immune responses (97).

The exact mechanisms by which CR2 plays a role in B-cell tolerance have also not been delineated. Since coligation of CR2 with surface Ig (sIg) is known to lower the threshold for B-cell activation, one hypothesis is that coligation of CR2 with sIg also lowers the threshold for B-cell tolerance to autoantigens. If CR2 expression or function is impaired, then autoactive B cells may be able to escape from tolerance. Altered CR2 expression or function may also diminish the levels of complement-coated autoantigen that are targeted to FDCs in secondary lymphoid organs and remove a reservoir of self antigen that may be critical in maintenance of self-tolerance. Expression of CR2 on both B cells and FDCs is important in the generation of normal immune responses (9, 27, 92), and ligand binding to both cell types may also be important in the regulation of B-cell tolerance.

Differences in CR2 gene expression in mice and humans. Not everything about what determines the transcriptional control mechanisms dictating CR2 expression in different species is yet clear, but some general conclusions about CR2 gene expression in mice and humans have been drawn and will be discussed here. In addition, structural differences between human, sheep, and mouse CR2 will be summarized below.

The human CR2 gene encodes a single RNA transcript that generates a 145-kDa type I transmembrane glycoprotein that contains 15 or 16 SCRs, a 24-amino-acid transmembrane domain, and a short (34-amino-acid) cytoplasmic tail. The murine CR2 gene encodes two alternatively spliced RNA transcripts that generate two distinct glycoproteins: a 145-kDa direct homologue of human CD21 (CR2) and a 190-kDa isoform produced by the inclusion of an additional five exons encoding six N-terminal SCRs (CR1, CD35) (66, 78). Thus, the two mouse CR2 proteins share identical sequences throughout except for the inclusion of the additional six SCRs in the larger form (119). All native murine cells that express the CR2 gene produce both the CR2/CD21 and CR1/CD35 products, although the exact mechanisms used for this alternative splicing control are not known. No activation signal that induces the alternative splicing of one form over the other has been discovered.

The human CR2 gene is expressed by B lymphocytes and transformed B-cell lines, FDCs (68, 96), thymocytes, and some T-cell lines (33, 109). Conflicting reports have been published regarding CR2 gene expression by a small fraction of human peripheral T lymphocytes (1, 36). The physiological relevance of CR2 expression on T cells remains unclear, since these cells do not acquire and present antigen, nor do they express the accessory molecule CD19. However, since we have demonstrated that T cells can express CR2 following appropriate activation (118), there may be benefits of CR2 expression on T cells that differ from those realized by B cells, but these have yet to be determined.

CR2 gene expression in mice, producing both the CR2/CD21 and CR1/CD35 products, appears to be more restricted than that in humans, reportedly occurring only in B lymphocytes and FDCs (63, 74). CR2 is generally considered a developmental and cell-specific marker for both murine and human B cells, which initiate CR2 expression as they mature and express IgM and terminate CR2 expression when they are activated and switch Ig isotypes (119). This developmental expression pattern corresponds well with its biological functions in the B cell, which can use C3-bound immune complexes for antigen presentation as well as antigen-specific cellular activation following surface IgM/CR2 cross-linking. After isotype switching and affinity maturation, the requirement for such signals diminishes. In contrast, FDCs, which spend their entire lifetime holding antigen complexes on the cell surface, constitutively express CR2.

Molecular structure and expression of human CR2

Human CR2 (CD21) is a 145-kDa type I transmembrane glycoprotein that binds the surface-fixed cleavage fragments of C3, iC3b, C3dg, and C3d and serves as the receptor for Epstein-Barr virus (EBV) on B lymphocytes (35, 52, 113). CR2 is a member of the structural family of C3/C4 receptor and regulatory proteins known as the regulators of complement activation (RCA) family. Members of this family are characterized by the presence of repeating modules known as SCRs, also called complement control protein or sushi domains (48).

Molecular structure of human CR2. The molecular structure of CR2 is well known and comprises a 15- or 16-SCR extracellular domain, a 24-amino-acid transmembrane domain, and a short (34-amino-acid) intracellular tail. Each SCR unit is about 60 to 70 amino acid residues in length, and variable linker regions of three to eight amino acid residues separate neighboring units. All SCRs contain a number of conserved residues, including four cysteine residues and an invariant tryp-
The conserved cysteine residues form a pattern of disulfide bridges connecting Cys1-Cys3 and Cys2-Cys4 (48). The X-ray crystal structure of human CR2 SCR1-2 in its complex with C3d has been elucidated at a 2.0-Å resolution (48); this is the first such ligand-receptor pair in the RCA family for which a cocrystal has been generated (102). Crystal structures of human forms of free CR2 SCR1-2 and C3d have also been determined (91, 109). The structures of CR2 SCR1-2 in both the C3d-bound and unbound states reveal that both SCR domains are in the compact β-barrel configuration characteristic of these proteins and that both domains are packed against each other in a tight V shape (102). The interface region between the two SCRs appears to be lined with nonpolar and hydrophobic residues (48, 102). However, recent analytical ultracentrifugation studies and X-ray scattering experiments, in conjunction with previous electron microscopy data, appear to dispel the ready conclusion that SCR1 and SCR2 exhibit the same side-side packing in solution and instead indicate that CR2 SCR1-2 in solution is in fact in a much more extended conformation than the two crystal structures suggest (42, 45, 80, 81). It has also been suggested that the presence of a large N-glycan moiety attached to N107 of SCR2 would sterically inhibit the close packing of SCR1 and SCR2 (48, 91); notably, both the bound and free forms of CR2 were deglycosylated prior to crystallization (91).

The complex contains a V-shaped CR2 receptor binding to a globular C3d ligand (Fig. 2). The V-shaped CR2 molecule has a span of 42.6 Å at the base, and the height of the V structure is 38.5 Å. The C3d ligand, which has a dome-shaped helical structure, interacts with the receptor using one portion of the edge of the dome. Because the CR2 contact edge of C3d is located nearly opposite the amine (NH₂) and carboxyl (COOH) termini, binding to CR2 will likely orient the rest of the iC3b form of C3, a larger molecule that contains the C3d domain and also binds CR2, away from the interaction site. The site of the ester bond linkage to antigens (Q20) is approximately halfway between the receptor contact edge and the NH₂ and COOH termini of C3d (102).

Expression of human CR2. Human CR2 is expressed by B lymphocytes and B lymphoblastoid cell lines, human thymocytes (109), a fraction of human T lymphocytes (36), certain leukemia T-cell lines, the pharyngeal and cervical epithelia, and FDCs. The level of expression of CR2 is developmentally regulated; it is highest on mature B lymphocytes and on a subpopulation of immature blastic thymocytes. An additional ligand for CR2 is CD23, a type II transmembrane protein expressed on a variety of hemopoietic cell types that serves as the low-affinity receptor for IgE. Cleavage of the membrane receptor generates soluble forms of CD23 that are endowed with “cytokine-like” activity (19). Fluorescent liposomes carrying CD23 were shown to interact specifically with CR2 on B cells, some T cells, and FDCs and with hamster kidney cells transfected with CR2 cDNA (5, 86). Binding of CD23 involves

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FIG. 2. Structure of the CR2-C3d complex. (A) Overall view of the structure of CR2 binding to C3d. (B) Part of CR2 electron density, showing clear features of amino acid side chains. (Adapted from reference 102 with permission of the publisher.)
Molecular Structure and Expression of Sheep CR2

The primary structure of sheep CR2 is similar to those of known mammalian homologues, but the higher-order structure is unusual. Two distinct CR2 isoforms occur, one of which is ubiquitinated in the cytoplasmic domain, and the two molecular forms are expressed at the cell surface as noncovalently associated dimers. The percentage of sheep B cells that express CR2 changes during development and varies between different body compartments (116).

Molecular structure of sheep CR2. Lemmon et al. have recently characterized the structure of sheep CR2 at the protein and transcript levels (67). Two distinct glycoproteins, with apparent molecular masses of 150 kDa and 190 kDa, were immunoprecipitated from B-cell lysates by a panel of eight monoclonal antibodies (MAbs). NH2-terminal sequencing of affinity-purified target proteins showed that both were the sheep homologue of CR2. Surprisingly, the 190-kDa protein contained ubiquitin as well as CR2 in a 1:1 molar ratio. This result was confirmed by Western blotting experiments using antisera specific for an NH2-terminal peptide of sheep CR2 or native ubiquitin. There was no difference between the 150-kDa and 190-kDa glycoproteins in terms of the degree of N-linked glycosylation; both contained about 30 kDa of carbohydrates. These results demonstrated that mature sheep B cells express two distinct isoforms of CR2, one that is similar to the human prototype (150-kDa isoform, designated CR2no) and another that has undergone ubiquitination (190-kDa isoform, designated CR2ub).

Lemmon et al. (67) and MacKenzie et al. (70) then used a variety of PCR approaches to generate a series of overlapping cDNA clones that encoded a full-length transcript of sheep CR2. The mature 4.0-kb transcript had a conserved structure with 69 and 66% overall amino acid identity to the human and mouse proteins, respectively. The extracellular region of the sheep CR2 transcript contained 15 SCR domains and lacked SCR11, similar to the “short” form of human CR2. Compared with other regions of the molecule, the primary structure of the transmembrane region of sheep CR2 was relatively poorly conserved, which is unusual for membrane-anchored cell surface proteins. Closer inspection of the transmembrane region identified a stretch of sequence (LIXxxxxGVxxxxxxGVxIT) with significant similarity to a rare seven-amino-acid motif (LIXxG VxxGVxxT) known to mediate the dimerization of the glycoporphin receptor on human red blood cells (67, 70). The seven critical amino acid residues forming the motif are conserved in sheep CR2, albeit with different spacing, although the predicted secondary structure aligns them along the face of an α-helix where they could stabilize adjacent transmembrane helices. The cytoplasmic region of sheep CR2 contained two conserved lysine residues that provide potential sites for ubiquitination and contained conserved tyrosine, threonine, and serine residues, which offer potential phosphorylation sites.

During immunoprecipitation experiments, Hein et al. found no evidence that CR2 associated with other molecules in the molecular weight range expected for CD19/CD81 or CR1, irrespective of the detergents or sodium dodecyl sulfate-polyacrylamide gel electrophoresis conditions used (49). We suspected that there might be something unusual about the higher-order structure of sheep CR2, since one of the eight MAbs (Du2-128) selectively immunoprecipitated only one isoform, CR2ub, but always produced straight-line fluorescence-activated cell sorter (FACS) profiles when used for two-color FACS analysis in combination with any of the remaining seven MAbs that identified both isoforms. This consistent result indicated that the target epitopes for each MAb were expressed in a fixed ratio on the B-cell surface. A series of experiments were then done using different chemical cross-linkers to show that the two isoforms of sheep CR2 were expressed as noncovalently associated complexes. These experiments established CR2no-CR2ub dimers as a minimum stoichiometry, although the possible occurrence of other forms, such as homodimers or higher multimers, was not excluded.

In summary, sheep CR2 has several interesting structural properties. Two distinct isoforms are expressed on the surface of B cells and one of these has undergone posttranslational ubiquitination of the cytoplasmic region. This is not unusual by itself, since a growing number of cell surface molecules are known to become ubiquitinated, but it is the first occasion that this protein modification has been demonstrated on CR2 in any species. The transmembrane region of sheep CR2 contains a dimerization-like motif, and the two isoforms are expressed as dimers on the cell surface. The external topography of the two isoforms is distinctive in some respects, since one MAb selectively binds CR2ub. The occurrence of a unique epitope on a splice variant of sheep CR2, coupled with selective ubiquitination of that isoform, would provide a plausible molecular basis for the observations noted above, but so far there is no evidence for alternative splicing of sheep CR2 (49).

Expression of sheep CR2. As for other species, immunohistochemistry and FACS analysis show that sheep CR2 is expressed on B cells, FDCs, a small proportion of T cells, and some striated epithelia. The expression of CR2 on B cells changes in defined ways at different stages of development and also varies between different lymphoid compartments. Virtually all the immature B cells in the ileal Peyer’s patch express CR2ub, but these cells have a significantly lower level of expression of the CR2ub isoform than do peripheral B cells. In the periphery, the expression of CR2 changes in an age-related manner. In late-term fetuses, and in lambs for the first 4 to 5 weeks after birth, virtually all B cells in blood express both the CR2 isoforms. From relatively early postnatally, CR2 expression therefore defines two distinct subsets of peripheral sheep B cells, CR2+ and CR2−. These two subsets of B cells differ in a number of other phenotypic properties that have been defined in studies done independently by three groups (15, 43, 117). However, a
subset of CR2− B cells that do not express either isoform then becomes detectable in blood. The size of this subset increases steadily with advancing age, stabilizing at about 10 to 12 months, when it accounts for around half of all blood-borne B cells. B cells in solid lymphoid organs such as lymph nodes and spleen have a more variable pattern of CR2 expression, ranging from levels higher than those found in blood to none (116).

At this time it is difficult to know what functional significance should be attached to the CR2− and CR2+ subsets of sheep B cells. Several of their phenotypic properties suggest that they could be sheep counterparts of the so-called B1 and B2 subsets, respectively, characterized previously in mice and humans (15). Although uncertainty remains about the functional significance of these two subsets of B cells, they can now be identified using a number of phenotypic criteria, and experiments in the next few years, particularly with disease models, should provide new and interesting insights into their biological roles (116).

**MOLECULAR STRUCTURE, EXPRESSION, AND MODULATION OF MURINE CR1/2**

CR2 is a surface glycoprotein located almost exclusively on B cells and FDCs in mice, with a somewhat broader cell distribution in humans. The level of CR2 expressed on cells is controlled by regulatory elements in the proximal promoter (93, 112, 115), and cell and lineage specificity is controlled by an intrinsic silencing mechanism (56, 71, 72, 119). In mice, both CR1 and CR2 are transcribed from a single gene by alternative mRNA splicing (78), while in humans these proteins are derived from two distinct but closely linked genes on chromosome 1. Both human and mouse CR2 bind the C3 activation products iC3b, C3dg, and C3d (57, 113); human CR2 also binds EBV (35), CD23 (5), and alpha interferon (18). CR2 is composed of 15 repeating 60- to 70-amino-acid extracellular subunits termed SCRs, a transmembrane domain, and a short cytoplasmic tail. In humans, a 16-SCR form of CR2 has been identified that is generated by alternative splicing of a single exon (9, 45). Interaction of human CR2 with its C3d ligand has been shown by X-ray crystallographic studies to occur primarily via SCR2 (102). CR2 has been demonstrated to amplify antigen-induced B-cell activation through sIgM, to rescue peripheral B cells from sIgM-mediated apoptosis, to promote antigen processing and presentation of C3d-bound targets (9), to modulate the expression of costimulatory molecules, to stabilize the BCR in lipid rafts, and to target immune complexes to germinal centers in secondary lymphoid organs. Many of these functions may occur via interactions of CR2 with CD19 and CD81 on the B-cell surface, where these receptors form a multimolecular signal transduction complex (9, 20). Mice deficient in CR2 by homologous recombination have defects in T-cell-dependent and T-cell-independent antibody responses, germinal center formation, generation of memory B cells, and development of a normal natural antibody repertoire (9, 59, 95). The defect in T-cell-dependent antibody responses results from a lack of CR2 on both B cells and FDCs (27).

Murine complement receptors CR1 and CR2 (CD35/CD21) have also been implicated in the maintenance of self-tolerance: self-reactive B cells of animals deficient in the receptor or the complement protein C4 are not energized, leading to deterioration of the immune response. The most likely explanation, proposed by Prechl et al. (90), is that self-antigen recognition by natural antibody initiates the classical pathway of complement activation, and focusing these complexes by C4b-CR1/2 interactions to cells within the bone marrow or secondary lymphoid compartment would enhance contact with, and elimination of, self-reactive immature B cells.

**Molecular structure of murine CR2.** In the last decade, the genes, protein structures, and functions of murine complement receptors CR1 and CR2 (CD35/CD21) have been deciphered (90). Murine CR1 and CR2 are encoded by a single gene, mCr2 at the RCA locus. Alternative splicing gives rise to the two products: murine CR1 comprises 21 SCRs (complement control proteins), while murine CR2 is identical to murine CR1 except that it lacks the first six SCRs (34, 66, 78). Thus, it is these six N-terminal SCRs that endow murine CR1 with activities characteristic of human CR1: factor I cofactor activity and C3b binding (64). Since the rest of the receptor is exactly identical to murine CR2, most MAbs against murine CR2 also recognize murine CR1 (65). This, coupled with the fact that knocking out the Cr2 gene results in mice lacking both CR1 and CR2, is the reason that the two receptors are usually simply referred to as murine CR1/2. Since it is the membrane-proximal, transmembrane, and intracytoplasmic regions that are shared, the two receptors are likely to associate with the same membrane and cytoplasmic proteins. The C3d binding region was located in the two N-terminal SCRs of CR2 (87), while shuffling of the SCRs in recombinant proteins revealed that the first two SCRs in CR1 are responsible for C3b binding (79). Sequence analysis reveals that the intracytoplasmic domain contains a K/RxxE/Dxxxy Tyrphosphorylation motif and a YxxO endosomal targeting motif. Accordingly, tyrosyl phosphorylation of the receptors (111) and delivery of murine CR1/2 ligands to the antigen-processing pathway have been reported (7, 89).

**Expression and modulation of murine CR2.** Murine CR1/2 is expressed mainly on B lymphocytes and FDCs. Tissue-specific expression is controlled by the first intronic sequence in the gene where both positive and negative regulatory elements were identified (56). Later the same group showed that cell-type-specific histone deacetylation served to repress transcription of the Cr2 gene (118). Cells other than B lymphocytes or FDCs, such as activated T cells (25, 61), may also express CR1/2. B cells express CR1/2 when they leave the bone marrow and start their maturation, the stage called transitional B cells. In the spleen, marginal-zone B cells express the highest levels, while follicular B cells have intermediate levels, of these complement receptors (103). Murine CR1/2 is lost again when the B cell differentiates into plasma cell. The level of CR1/2 may also be altered in certain disease states, as was found in MRL/lpr mice (103) and in a recent model of induced autoimmunity (32).

In vitro activation of splenic B cells by lipopolysaccharide, anti-κ-Sepharose, or CD40L reportedly increases expression (6), while exposure to TI-2 antigens was shown to decrease murine CR1/2 on the cell surface (82). In vivo introduction of high doses of anti-CR1/2 MAbs also down modulates murine CR1/2 on B cells (65), an effect that was exploited to study the role of CR1/2 in the immune response (44, 107). On the cellular level, extensive cross-linkage resulted in capping of mu-
rime CR1/2 (108); interestingly, cross-linking the BCR induced cocapping of loaded CR1/2 in the same studies.

CONCLUSION

The essence of innate immunity is the detection of molecules that are unique to infectious organisms and noxious substances in order to induce clearance of the intruders, and it also dictates the conduct of the acquired immune response in vertebrates. Complement is widely accepted to constitute this critical link between the innate immune response involved in the selective recognition and clearance of potentially noxious substances, whether they are derived from the host following injury (apoptotic cells or toxic cell debris) or following an infectious challenge (microbial agents), and the interface with the acquired immune response. Whole-genome sequencing studies and the evidence of experiments involving the genetics of invertebrates indicate that complement is certainly an archetypal component of innate immunity (40). Receptors for C3 activation fragments, especially CR2/CD21, play central roles in linking the B-lymphocyte adaptive immune system to the complement system. CR2/CD21-deficient mice demonstrate pronounced immune defects. These defects are apparent because CR2/CD21 plays an important role in the activation of B lymphocytes, FDCs, and likely T lymphocytes in vivo (53).

In recent years, foundational work on the relationship of complement C3, CD19, CR2/CD21, and CR1/CD35 has resulted in a growing appreciation of the importance of complement for the induction and recall of antibody responses to foreign antigens (97). Manipulations of CR2/CD21 activities by either blocking the binding of ligands to CR2/CD21 or targeting antigens to this receptor in vaccination strategies are currently being explored as options to regulate several human immune responses. The regulation of immune responses through these and other strategies will likely further define the pivotal role that CR2/CD21 plays in connecting innate immunity to acquired immunity (53). Thus, the study of CR2, its biologic roles, and its many ligands continues to provide surprises as well as a unique window into important events during the development and effector functions of the innate and adaptive immune systems (52).

CR2 from many animals has been sequenced, and the CR2-encoding genes are known. The mouse is still the best model for research on the functions of CR2, complement components, and receptors in autoimmune disease. For example, levels of CR1/CR2 were found to be decreased prior to the development of clinically apparent disease in the MRL/lpr mouse model of lupus, suggesting that this decrease may indeed contribute to the initiation or progression of autoimmune disease (9). Based on the current data, ovine and murine models are the better representative mammal research models for the entire animal kingdom.

Although much progress has been made in the characterization of structure/function relationships of individual complement components, there are many more fundamental questions to address. Importantly, we will have to decipher when, where, and with what associated molecules do complement molecules engage to promote either an anti-inflammatory or a full proinflammatory response. Moreover, we will have to decipher the ill-characterized cross talk between the complement and other innate immune signaling pathways (e.g., Toll-like receptors and scavenger receptors) to promote the safe clearance of the intruders. The answers to these questions will greatly expand our understanding of the complex interactions between pathogens and the host immune response (40).

It is expected that future studies will reveal more and more complement-mediated links between innate immunity and adaptive immunity (90). Future challenges include understanding how complement and complement receptors contribute to the prevention or, conversely, exacerbation of autoimmunity in particular contexts. In addition, the molecular interplay of the BCR, CD19, CR2/CD21, and CR1/CD35 remains to be fully resolved. The attractive paradigm that CR2/CD21 signals exclusively through CD19 remains to be experimentally validated in the case of B-cell activation as well as antigen uptake and presentation (97). Much work about the functions of these three sorts of CR1/2 needs to be conducted to determine their structural differences and relation to complement biology. A better understanding of complement function and the antibody response holds great promise for the development of complement (e.g., C3d)-based vaccines as well as modalities for the treatment of B-cell-dependent autoimmune disease.

In summary, complement components and complement receptors have been implicated to play a role in the pathogenesis of autoimmune disease, although the mechanisms for their involvement remain unclear. Promising therapies are being developed to block the end organ damage induced by complement activation (9). It is intriguing to speculate that specific complement components or complement receptors might also be targeted in autoimmune disease in order to regulate B-cell tolerance to autoantigen.

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