Complement Receptors

Secondary article

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Complement receptors are membrane proteins expressed on the surface of immune cells. They interact specifically with complement factors leading to the removal of antigen from the circulation.

Article Contents

Introduction

In addition to promoting microorganism destruction by lytic attack, the complement system exerts a wide-ranging influence on the activities of cells involved in the immune response. Thus, complement is instrumental in the direction of blood leucocytes to a site of inflammation; the induction of granule release and synthesis of cytotoxic oxygen- and nitrogen-containing compounds by leucocytes of the myeloid lineage; the promotion of particle phagocytosis by these cells; the clearance of soluble immune complexes (ICs) from the circulation; and the induction of a primary B-cell response to antigen. These effects are exerted through interaction of the activation products of complement factors C1, C3 or C5 with specific receptors on the responding cells. The receptors can be divided into three categories: (1) those recognizing the anaphylotoxic polypeptides, C3a, C5a and C5a-desarg, (2) those binding the active C3 fragment, C3b, and its degradation products, iC3b and C3dg, and (3) receptors for C1q and related collagenous lectins.

The general characteristics of the complement receptors are summarized in **Table 1**. Attention will be primarily focused on the human complement receptors, although mention is also made of the receptors in other species; especially where these diverge structurally or functionally from their human counterparts.

The Anaphylatoxin Receptors

The C5a receptor (C5aR, CD88)

C5aR is a membrane glycoprotein of about 42 kDa displaying high affinity for the C5 fragments C5a and C5a-desarg.

Structure

C5aR belongs to the superfamily of G protein-coupled receptors, which contain seven transmembrane segments distributed throughout the whole protein. Other members of this superfamily include the receptors for C3a, formyl MetLeuPhe (fMLP), interleukin 8 (IL-8) and platelet-activating factor (PAF) as well as the tachykinin, adrenergic and opsin receptors. The C5aR gene is located in q13.3–13.4 of human chromosome 19, together with the fMLPR gene with which it displays 34% identity. The C5aR genes in mice, rats and dogs have also been sequenced and show considerably greater interspecies diversity (68% conservation between humans, mice, rats, canines and bovidae) than do the other members of the G protein-coupled receptor family (85–98% conservation); especially in the putative extracellular segments. This diversity is presumed to reflect interspecies divergence of the ligand involved.

Table 1 The complement receptors

Receptor	Ligand	CD number	Protein superfamily	Function
C5aR	C5a; C5a-desarg	CD88	G protein-coupled receptors	Leucocyte chemoattraction, degranulation
C3aR	C3a	–	G protein-coupled receptors	NO synthesis
CR1	C3b	CD35	Regulators of complement activation	Promotion of phagocytosis, immune complex (IC) clearance, processing of IC-bound C3b
CR2	C3dg (iC3b)	CD21	Regulators of complement activation	B-cell proliferation, alternative pathway activation
CR3	iC3b	CD11b/CD18	β_2 integrin	ROM/NO synthesis, degranulation
CR4	iC3b	CD11c/CD18	β_2 integrin	Phagocytosis, leucocyte migration
cC1qR	C1q, MBL, SPA	–	–	Chemotaxis, promotion of phagocytosis, ROM
C1qRp	C1q, MBL, SPA	–	–	Synthesis, platelet aggregation, leucocyte migration
gC1qR	C1q	–	–	Regulation of B-cell activity

ROM, reactive oxygen metabolites; NO, nitric oxide; MBL, mannan-binding lectin; SPA, lung surfactant protein A

Cellular distribution

C5aR is expressed on neutrophils, eosinophils, basophils and monocytes, as well as on some nonmyeloid cells, including bronchial and alveolar epithelial cells, vascular endothelial cells, Kupffer cells, stellate cells and, to a lesser extent, sinusoidal epithelial cells in the liver, as well as astrocytes and microglial cells in the brain (**Tables 2** and **3**). The C5aR on eosinophils is somewhat larger than that on neutrophils (50–55 kDa versus 42 kDa) and this difference is presumed to arise either from alternative splicing of the C5aR gene or from variable posttranslational modification. Expression of C5aR on monocytic cell lines can be upregulated upon stimulation of the cells with interferon γ (IFNγ) and phorbol myristate acetate (PMA).

Function

The 42-kDa form of C5aR binds C5a with high affinity ($K_d \sim 1$ nmol L^{-1}) and C5a-desarg with an approximately 10-fold lower affinity. On neutrophils, the receptor is expressed as a functionally uniform population at a level of 200 000 copies per cell, whereas on eosinophils, two distinct populations are discerned; one (15 000 sites per cell) with a K_d of 31 pmol L^{-1} and the other (375 000 sites per cell) with a K_d of 100 nmol L^{-1}. Current evidence suggests that a dual binding motif is involved in the interaction with C5a; a recognition site for the N-terminal end and disulfide-linked core of C5a, located in the N-terminal extracellular domain of the receptor, and a secondary site, involving Glu199 and Arg206 of C5aR in binding Lys58 and the terminal arginyl carboxylate group of C5a.

Activities initiated in granulocytes and monocytes via C5aR include cytoskeletal remodelling, shedding of L-selectin and upregulating of adhesion molecules/complement receptors (CR1, CR3 and CR4), chemotaxis, granule release and synthesis of cytotoxic reactive oxygen metabolites (ROM). Stimulation of endothelial cells via C5aR results in upregulation of the adhesion molecules P-selectin, E-selectin and intercellular adhesion molecule 1 (ICAM-1), involved in trapping leucocytes, while C5aR triggering in the liver initiates the production of acute-phase proteins.

Signalling

Signalling via C5aR, in which ligand engagement of Arg206 is believed to play a crucial role (Raffetseder *et al*., 1996), is mediated by receptor-associated, heterotrimeric (αβγ) GTP-binding proteins, containing the α subunits, $G_{i\alpha 2}$ and $G_{i\alpha 3}$, which are pertussis toxin (PTX)-sensitive, and/or the PTX-insensitive $G_{\alpha 16}$. GTP-induced release of the βγ subunit from α leads to activation, by these subcomponents, of phosphatidylinositol (PI)-specific phospholipase C-β2 and A_2 as well as phospholipase D. Their activation leads, in turn, to the generation of phosphatidic acid and diacylglycerol, calcium mobilization, activation of protein tyrosine kinases, protein kinase C (PKC) and phosphatidylinositol 3-kinase (PI3 kinase) and, ultimately, initiation of the mitogen-activated protein kinase (MAPK) cascade, which is involved in nuclear signalling.

In the course of activation, the C5a receptor is transiently phosphorylated, by a G protein-independent, staurosporin-sensitive mechanism, at serine residues in its cytoplasmic tail. In its phosphorylated form, C5aR is rapidly internalized into endosomes that cluster near the nucleus after 10 minutes (Naik *et al*., 1997). The internalized C5aR may subsequently be recycled to the surface membrane following dephosphorylation.

Table 2 Distribution of complement receptors on blood cells in humans

Cell type	C5aR	C3aR	CR1	CR2	CR3/4	cC1qR	C1qRp	gC1qR
Monocytes (macrophages)	+	+	+	–	+	+	+	–
Neutrophils	+	+	+	–	+	+	+	+
Eosinophils	+	+	+	–	+	–	–	+
Basophils (mast cells)	+	+	+	–	+	+	+	+
Natural killer cells	–	–	+	–	+	–	–	–
B cells	–	–[a]	+	+	–	+	–	+
T cells	–	–	(+)[b]	(+)[b]	–	–	–	–
Erythrocytes	–	–	+[c]	–	–	–	–	–
Platelets	–	–[d]	–[e]	–	+	–	+	+

[a] C3aR has been detected on tonsillar B cells.
[b] Present on a minor T-cell subpopulation.
[c] On human and primate cells only.
[d] C3aR is expressed on guinea-pig platelets.
[e] CR1 is expressed on murine and rabbit platelets.

Table 3 Distribution of complement receptors in human tissues

Tissue and cell type	C5aR	C3aR	CR1	CR2	CR3/4	cC1qR	C1qRp	gC1qR
Lymphoid organs								
Follicular dendritic cells (FDCs)	–	–	+	+	+[a]	–	–	–
Liver								
Kupffer cells	+	–	+	–	+	–	–	–
Stellate cells	+	–	–	–	–	–	–	–
Brain								
Microglia	+	–	–	–	+	–	–[b]	–
Astrocytes	+	–	–	+	–	–	–	–
Other tissues								
Vascular endothelial cells	+	–	–	–	–	+	+	–[e]
Epithelial cells	+[c]	–	+[d]	–	–	+	–	–
Gingival fibroblasts	–	–	–	–	–	+	–	–

[a] CR3 only.
[b] Present on rat microglial cells.
[c] On bronchial and alveolar cells.
[d] On glomerular podocytes.
[e] Found in association with mitochondria.

The C3a receptor (C3aR)

Structure

Human C3aR resembles the C5aR in that it contains seven transmembrane segments within its 482 amino acid sequence and shows 37% sequence identity with C5aR (Ames *et al*., 1996). However, distinctive for C3aR is a 175 amino acid insert in the second extracellular loop, between the fourth and fifth transmembrane segments (Ames *et al*., 1996; Crass *et al*., 1996). Murine C3aR shows 65% overall sequence identity with its human counterpart and contains a 165 amino acid insert.

Like C5aR, C3aR displays diversity in size; being found as two species of 54–61 kDa and 86–107 kDa on the human mast cell line HMC-1, and in up to three forms on guinea-pig platelets (83–114 kDa).

Cellular distribution

C3aR is expressed on monocytes/macrophages, neutrophils, eosinophils and basophils, but rarely on peripheral lymphocytes (**Table 2**). A single report has described the receptor's presence on human tonsillar B cells. The receptor is also found on a human hepatoma and some leukaemia cell lines and has been identified in murine testis, brain, liver, lung, muscle and kidney tissue as well as on guinea-pig platelets.

Function

C3aR exists as two functional variants displaying different affinities for its sole ligand, C3a. The high-affinity form (K_d = 2–5 nmol L^{-1}) is expressed in relatively low copy numbers (200, 63 000 and 30 000–60 000 per cell on guinea-pig platelets and macrophages and the HMC-1 cell line, respectively) while the low-affinity receptor (K_d = 30–150 nmol L^{-1}) is more prevalent (500, 120 000 and 5–23×10^5 per cell, respectively, on the same cells). On human neutrophils, the receptor is found as a single low-affinity species at a level of 40 000 copies per cell. Expression of C3aR on human myeloblastic cell lines is enhanced by treatment with IFNγ.

The C3aR mediates the chemotaxis of eosinophils and mast cells, but not neutrophils. It also induces histamine release and leucotriene synthesis by mast cells/basophils, ROM synthesis by eosinophils, neutrophils and macrophages and degranulation of eosinophils and neutrophils. In the case of human basophils, the response is greatly increased upon costimulation with either IL-3 or granulocyte–macrophage colony-stimulating factor (GM-CSF). The C3aR has also been reported to suppress human tonsillar B-cell responses to polyclonal activation.

Signalling

Ligand engagement of C3aR causes a rise in intracellular free Ca^{2+} which is inhibited by PTX, indicating G protein involvement. In the HMC-1 cell line, internal Ca^{2+} stores are mobilized, whereas the Ca^{2+} flux in neutrophils is derived externally. This is in contrast with neutrophil stimulation via the C5aR, where both intra- and extracellular sources may be tapped, depending on ligand concentration. In keeping with this finding, no activation

of PI3 kinase is observed upon C3aR stimulation, while inositol trisphosphate (IP_3) generation is detectable. C3aR-mediated activation is also staurosporine sensitive, although not to the degree seen with PMA stimulation, indicating that a protein kinase other than PKC may be involved.

The Complement Receptors for C3b and its Derivatives

Complement receptor type 1 (CR1)

Complement receptor type 1 (CR1, CD35) is a 210–290 kDa membrane glycoprotein with specificity for the complement products C3b, C4b and, with lower affinity, iC3b.

Structure

CR1 shares structural similarities with a group of proteins, including factor H, C4-binding protein (C4bp), decay-accelerating factor (DAF), membrane cofactor protein (MCP), CR2, C1r, IL-2 receptor, β_2-glycoprotein 1, haptoglobin α chain and factor XIIIb, many of which are involved in the control of complement activity. These proteins are known as the regulators of complement activation (RCA) family. The extracellular domain of CR1 consists of an array of 30 or more homologous units (short consensus repeats, SCRs) comprising of 60–65 amino acids, of which 10–15 are highly conserved in all members of the family; including four half-cystines linking in a first–third, second–fourth arrangement to create a triple-loop structure within each SCR (**Figure 1a**). Distinctive for CR1 is the arrangement of the SCRs in three to six long homologous repeats (LHR) comprising seven SCR units and displaying 70–95% identity between each LHR. The transmembrane region of CR1 consists of 25 hydrophobic amino acids followed by four positively charged residues and the C-terminal cytoplasmic domain consists of 39 amino acids.

The LHRs have probably arisen from duplication of gene segments encoding the constituent SCRs of an LHR, while the variability in the size of CR1 reflects allotypic polymorphism involving different numbers of LHR. Thus, the two most prevalent CR1 alleles among Caucasians (F and S, gene frequency = 80% and 20%, respectively) are composed of four and five LHRs, while two rarer allotypes contain three and six LHRs, respectively. In humans, the CR1 gene is located on the long arm of chromosome 1, band q32, in close association with the genes encoding CR2, DAF, factor H and C4bp.

Murine CR1 differs from its human counterpart in that it is expressed as part of a composite receptor consisting of a six SCR N-terminal segment with CR1 activity and a 15 SCR core corresponding to murine CR2 (see below). The CR1/2 composite receptor and CR2 arise from alternative splicing of a gene which is located on murine chromosome 1.

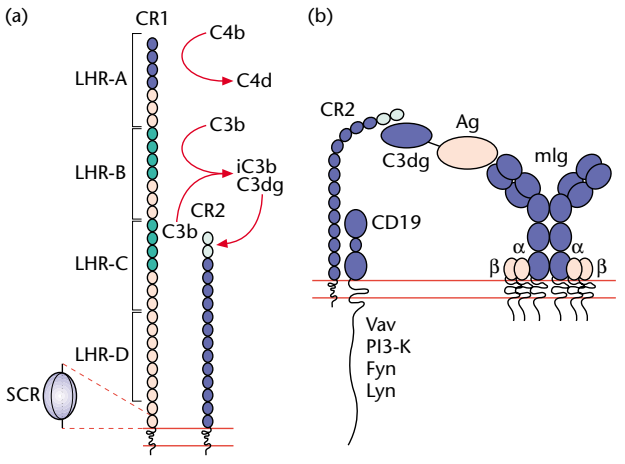

Figure 1 Structure and function of CR1 and CR2. (a) The most prevalent allele of CR1 is comprised of 30 short consensus repeats (SCRs) arranged in four long homologous regions (LHRs), where the ligand-binding sites are contained in the four N-terminal SCRs of each of the first three LHRs, while the ligand-binding site of the 15 (or 16) SCR CR2 is located in SCR1/2. On B cells, CR1 and CR2 are found in noncovalent association with each other. As a cofactor for factor I, CR1 promotes degradation of its ligand C3b, to iC3b and then C3dg, thus providing CR2 with its ligand. (b) The association of CR2 with CD19 ensures recruitment of the latter to the B cell receptor (BCR) complex upon BCR/CR2 crosslinking by opsonized antigen. By binding and activating the protein tyrosine kinases Lyn and Fyn, and PI3 kinase, CD19 supplements the signalling transduced through BCR upon antigen engagement. (Figure 1b is adapted from O'Rourke L, Tooze R and Fearon DT (1997) Co-receptors of B lymphocytes. *Current Opinion in Immunology* **9**: 324–329.)

Cellular distribution

CR1 is expressed on monocytes/macrophages, neutrophils, eosinophils, basophils, natural killer (NK) cells, B cells and some T cells, as well as follicular dendritic cells (FDC), glomerular podocytes, Kupffer cells and, in the case of most primates, erythrocytes (**Tables 2** and **3**). Although the number of CR1 molecules expressed on the erythrocytes (about 250 per cell) is 25- to 50-fold lower than that on blood leucocytes, the preponderance of erythrocytes in whole blood ensures that 85–90% of the cell-bound CR1 pool resides on these cells. The surface expression of CR1 on neutrophils is highly susceptible to modulation, being rapidly upregulated 5- to 10-fold by the chemotactic peptides fMLP and C5a-desarg, by GM-CSF and even by changes in temperature, and downregulated by 60–70% by PMA. The internal pool of CR1, responsible for the rapid upregulation, is located in smooth-surfaced vesicles distinct from the granulocyte's primary and secondary granules. A soluble form of CR1 (sCR1), present in low concentration (about 30 ng mL^{-1})

in normal plasma, is thought to arise from cleavage of membrane CR1 from blood leucocytes.

Function

CR1 binds to the active complement fragment, C3b, with an association constant (K_a), for dimer, of 2–$5 \times 10^7\,L\,mol^{-1}$, to C4b with similar affinity, and to iC3b with about 100-fold lower affinity. Each CR1 contains multiple C3b-binding sites located within the first four SCRs of each LHR and a single, high-affinity C4b-binding site in the same region of the first LHR. In addition to acting as a receptor, CR1 regulates complement activation by promoting factor I-mediated degradation of C3b to iC3b and, subsequently, C3c and C3dg, and of C4b to C4c and C4d. CR1 also binds C1q with a K_a of $2.6 \times 10^8\,L\,mol^{-1}$ in isotonic saline.

CR1 triggering of activities such as phagocytosis generally requires other forms of priming or enhancing stimuli (e.g. phorbol esters, fMLP, fibronectin, C1q, mannan-binding lectin or the pulmonary surfactant protein SPA). However, induction of lactoferrin release from granulocytes and IL-1 production, nuclear translocation of nuclear factor-κB (NF-κB) and prostaglandin synthesis by monocytes occurs upon stimulation via CR1 alone. CR1 has been reported to promote differentiation of activated B cells to immunoglobulin-secreting cells following antigenic stimulation. However, an equally important role for CR1 on B cells may be the conversion of antigen-bound C3b to C3dg, thereby facilitating attachment of the opsonized antigen to CR2 (**Figure 1a**).

In humans and primates, erythrocyte-borne CR1 plays a critical role in the clearance of soluble immune complexes (ICs) from the circulation. By transporting C3b-bearing complexes to the liver for disposal by Kupffer cells, erythrocytes ensure that IC deposition in sensitive sites such as the capillaries or kidney glomeruli, and consequent tissue destruction by inflammatory responses, is prevented. Furthermore, by competing directly with the blood leucocytes in binding the ICs, erythrocytes hinder inappropriate activation of these cells by the ICs. In this connection, erythrocyte CR1 performs a dual function by (1) competing with leucocyte CR1 in the binding of C3b-bearing IC and (2) degrading IC-bound C3b to C3dg, rather than to the intermediate product, iC3b; thereby changing the IC from an inflammatory stimulus, acting via neutrophil or monocyte CR3, to a potentially B cell-regulating stimulus via CR2 (Nielsen *et al*., 1997). The importance of this clearance mechanism is illustrated in the so-called immune complex-associated diseases, such as systemic lupus erythematosus (SLE), where failure of the erythrocytes to cope with the circulating IC load results in capillary and kidney damage leading to renal failure. In nonprimates, the clearance of complexes may involve platelets bearing C3b-binding receptors.

Signalling

There is no evidence that CR1, in its nonphosphorylated, resting state, is capable of inducing signal transduction. Phosphorylation of CR1 occurs in neutrophils and monocytes (but not B cells), following activation of PKC with platelet-activating factor or PMA, and converts the receptor to an activated state, in which it is capable of triggering such activities as phagocytosis. However, the signal transduction mechanisms employed by activated CR1 have not yet been characterized.

Complement receptor type 2 (CR2)

Complement receptor type 2 (CR2, CD21) is a 145-kDa glycoprotein with specificity for the complement fragments iC3b and C3dg. It is also the site of attachment for Epstein–Barr virus (EBV), the causative agent of infectious mononucleosis.

Structure

Like CR1, CR2 is a member of the RCA family. CR2 is expressed as two alternatively spliced gene products encoding 15 or 16 SCRs; where the additional SCR in the longer version lies between SCR10 and SCR11 of the shorter CR2. C-terminal to the SCR lies a 24 amino acid transmembrane segment and 34 amino acid cytoplasmic tail. Electron microscopy of CR2 has revealed that the extracellular portion of the receptor is highly flexible. Allelic polymorphism (at least three alleles in humans) has been reported for both versions.

Cellular distribution

CR2 is expressed on mature B cells (about 8000 per cell), some T cells, FDCs, pharyngeal epithelial cells, astrocytes and platelets (in humans) (**Tables 2** and **3**). The receptor is not expressed on pre-B or immature B cells and disappears upon differentiation of activated B cells to antibody-secreting plasma cells.

Function

CR2 binds iC3b, C3dg (with high affinity), C3b, and hydrolysed C3 (C3i) (with low affinity), as well as gp350/220 of EBV via a site, or sites, located in the first two SCRs of the molecule. However, SCR3 and SCR4 may also be required for binding to C3b and C3i. CR2 can also activate the alternative pathway (AP) of complement by a mechanism that involves the formation of a C3iBbP complex (i.e. the AP convertase) at the ligand-binding site. *In vitro*, the activation, on normal B cells, results in the deposition of predominantly C3dg fragments in an approximately 5-fold excess over the number of expressed CR2, while the naturally occurring deposition *in vivo* is about 10-fold lower.

A central role for CR2 in primary antibody responses against T cell-dependent antigen has been demonstrated in

mice by blocking CR2 function with monoclonal antibody that recognizes the binding site, or by infusion of soluble CR2, and by developing CR2 knockout mice. In all cases, major impairment of the humoral immune response was observed. Conversely, immunization with antigen covalently linked to two or more C3dg fragments was found to enhance the antibody response by up to 10 000-fold, compared to immunization with antigen alone (Dempsey *et al*., 1996). Studies in mice, which were manipulated to express CR2 only on their B cells or their FDC, have shown that CR2 on B cells plays an important role in initiating a humoral response while FDC CR2 is essential for the maintenance of this response

CR2 forms noncovalent binary complexes with three other membrane glycoproteins, CR1, CD19 and CD23 (the 'low-affinity immunoglobulin E' receptor), present either on the B cell itself or on other interacting cells. Association with B-cell CR1 is presumed to enhance the effectiveness of the transfer of opsonized antigen to CR2, following CR1 processing of antigen-bound C3b (see above), while association with the signalling molecule, CD19, ensures recruitment of the latter to the B cell receptor (BCR) signal complex (**Figure 1b**). Two roles have been proposed for CR2 complex formation with CD23; the regulation of immunoglobulin E production, and the rescue of activated B cells from apoptosis by CD23-bearing FDCs in the germinal centres.

Signalling

CR2 possesses only a short cytoplasmic tail and is unlikely to act directly as a signal transducer. However, by virtue of its association with the trimolecular glycoprotein complex CD19/CD81(TAPA-1)/Leu 13, CR2 plays a pivotal role in augmenting the B-cell response to opsonized antigen by bringing CD19 into close proximity with the BCR. Coligation of CD19 with BCR reduces the threshold for stimulation via BCR by at least two orders of magnitude. CD19 becomes tyrosine phosphorylated upon ligation with BCR and associates with the protein tyrosine kinases (PTK), Lyn and Fyn, PI3 kinase and the Rac guanine nucleotide exchange factor Vav, which is responsible for activating the MAPK cascade (**Figure 1b**). The enhancement of BCR signal transduction by CD19 is thought to involve at least two elements: (1) phosphorylation, by CD19-bound Lyn, of potential substrates in the BCR complex and (2) Ca^{2+} mobilization by a PI3 kinase-dependent mechanism (Buhl *et al*., 1997), distinct from the phospholipase Cγ-mediated mobilization initiated by BCR.

Complement receptors types 3 and 4 (CR3 and CR4)

Complement receptor type 3 (CR3, Mac-1, CD11b/CD18) and complement receptor type 4 (CR4, p150/95, CD11c/CD18) are heterodimeric glycoproteins with a shared β chain (CD18). Both receptors show specificity for the iC3b fragment.

Structure

CR3 and CR4 are members of the integrin superfamily of adhesion proteins. They are termed β2 integrins because, in common with leucocyte function-associated molecule 1 (LFA-1, CD11a/CD18), they contain the same β subunit; a 95-kDa transmembrane glycoprotein, encoded by a gene in the q22 region of human chromosome 21 and in chromosome 10 in mice. The human β2 is comprised of a 678 amino acid extracellular domain containing 57 Cys residues, of which 24 occur in three repeating units each with eight residues, a 23 amino acid transmembrane segment and a 46 amino acid cytoplasmic tail. The subunit contains a putative metal ion-dependent adhesion site (MIDAS) involving the residues Asp134, Ser136, Asp232 and Glu235, which contributes to the ligand-binding activity of the intact receptor. The α subunit of CR3 is a 155-kDa glycoprotein, comprising a 1092 amino acid extracellular domain, a 26 amino acid transmembrane segment and a 19 amino acid cytoplasmic tail. The CR3 α subunit shows 63% sequence identity with that of CR4, a 150-kDa glycoprotein, in which the extracellular domain, transmembrane segment and cytoplasmic tail consist of 1081, 26 and 29 amino acids, respectively. Both proteins are encoded by genes located between the bands p11 and p13.1 on human chromosome 16. The striking common features of these two proteins are their highly (87%) homologous divalent cation-binding sites, contained in three tandem repeats of approximately 60 amino acids, and a domain of about 200 amino acids, lying N-terminal to the cation-binding sites. This extra insert or 'I' domain displays homology with complement factors B and C2. The crystal structure of the I domain shows that it adopts a classic α/β 'Rossman' fold and displays a MIDAS-type Mg^{2+}-coordination site at its surface.

Cellular distribution

CR3 and CR4 are both present on neutrophils, eosinophils, basophils, monocytes/macrophages, NK cells, Kupffer cells, microglial cells and platelets, while CR3 is also expressed on FDCs (**Tables 2** and **3**). Like CR1, CR3 on the neutrophils is rapidly upregulated by a range of stimulants, including GM-CSF, fMLP, C5a, PMA, ionophore A23187 and IL-4, and it has been suggested that the intracellular pool of CR3 is located in the same exocytic vesicle compartment as CR1.

Function

CR3 and CR4 both bind iC3b, in a Mg^{2+}-dependent fashion, with an affinity for monomeric ligand of about 2×10^6 L mol^{-1}, in the case of CR3. CR3 also functions as a receptor for fibrinogen, ICAM-1 (CD54), bacterial

lipopolysaccharide (LPS) and neutrophil inhibition factor (NIF) from the hookworm *Ancylostoma caninum*; all of which react with closely related sites in the I domain of the α subunit. CR3 also binds zymosan (Z) via a β-glucan-binding lectin site remote from the I domain (Thornton *et al*., 1996).

Stimulation of neutrophils and monocytes via CR3 or CR4 results in phagocytosis, production of ROM and, in the case of neutrophils, release of the specific granules. CR3 is also involved in the IgE antibody-dependent cytotoxic response of eosinophils towards parasites and their elaboration of ROM, and in the induction of nitric oxide synthesis by monocytes/macrophages. Both CR3 and CR4, along with LFA-1, are intimately involved in leucocyte adhesion to endothelial cells and their migration to sites of inflammation.

Signalling

The extent to which CR3 and CR4 act as independent signal transducers is uncertain. Current evidence suggests that prior PKC-dependent phosphorylation of the β_2 subunit is required and that many of the functions initiated by CR3 involve cooperation with other receptors such as the glycosylphosphatidylinositol (GPI) anchored proteins, urokinase plasminogen activator receptor (CD87) and FcγRIII (CD16). Stimulation of CR3, in its primed state, results in tyrosine phosphorylation of several endogenous proteins, activation of phospholipases A2 and D and NADPH oxidase. Calcium ions are also mobilized in a biphasic fashion, with an initial release of Ca^{2+} from stores, located just under the plasma membrane, followed by global mobilization driven by influx of extracellular Ca^{2+} (Pettit and Hallet, 1998). Both the initial Ca^{2+} burst, which is localized to the sites of CR3 engagement, and the global mobilization are accompanied by changes in cell morphology, indicating an intimate connection between signalling via CR3 and reorganization of the cytoskeleton. The role of CR3 in cytoskeleton rearrangement is further emphasized by the fact that the cytoskeletal protein paxillin becomes phosphorylated upon stimulation via CR3.

The C1q Receptors

Three cell-associated proteins with affinity for C1q have been described: cC1qR, a 56-kDa protein closely resembling calreticulin which binds the collagen-like tail of C1q; C1qRp, a phagocytosis-promoting receptor of 126 kDa that has similar ligand specificity, and gC1qR, a 33-kDa protein, which recognizes the globular head regions of C1q.

Structure

cC1qR is a relatively hydrophobic protein consisting of 42% hydrophobic and 12.6% hydrophilic residues, with a pI of 5.5–6.0. The amino acid sequence of cC1qR displays near identity with the Ca^{2+}-binding intracellular protein, calreticulin (CRT); a 475 amino acid 52-kDa polypeptide containing three zinc-finger motifs, a leucine zipper and a ret finger protein (rfp)-like domain. While CRT is anchored to the endoplasmic reticulum via its C-terminal KDEL sequence, the means of attachment of cC1qR to the cell membrane remains unclear.

The primary C1qRp gene product is a 641 amino acid protein, consisting of a 21 amino acid leader sequence, a 156 amino acid segment resembling a C-type carbohydrate-recognition domain, five EGF-like domains, a 25 amino acid transmembrane segment and a short (47 amino acid) C-terminal cytoplasmic tail.

gC1qR is an acid protein (pI = 4.5) of 33 kDa at the cell membrane and 88–90 kDa in neutrophil intracellular granules. The gene for gC1qR encodes a 282 amino acid protein containing a 13 amino acid putative leader sequence followed by a relatively hydrophobic 60 amino acid segment which is not present in the mature protein. Of the 209 amino acids in mature gC1qR, 73 are charged residues, with an acid:base ratio of 2:1, while no putative transmembrane sequence is discernible.

Cellular distribution

cC1qR is expressed on monocytes, neutrophils, B cells, murine mast cells, endothelial and epithelial cells, human gingival fibroblasts (**Tables 2** and **3**), as well as on myeloid, pre-B and B-cell lines. The level of expression varies widely between cell types, from 8000 per cell on the B-cell line Raji to 1.6́106 per cell on U937 cells and 3́106 per cell on gingival fibroblasts. Expression of cC1qR on neutrophils is down-regulated by receptor shedding upon activation with PMA. cC1qR on gingival fibroblasts, on the other hand, is upregulated by 30–60% upon activation with IL-1b or transforming growth factor b (TGFb), while the calcium ionophore, A23187, increases expression of the receptor on U937 cells.

C1qRp is also expressed on myeloid leucocytes, endothelial cells, platelets (Nepomuceno and Tenner, 1998) and rat microglial cells, but not on lymphoid or epithelial cells (**Tables 2** and **3**). There are conflicting reports regarding the capacity of fMLP and PMA to upregulate this receptor on neutrophils, while the expression on rat microglial cells may be upregulated by IFNg.

gC1qR is found on neutrophils, eosinophils, mast cells and B cells, as well as on platelets, following adhesion to collagen, fibrinogen or fibronectin (**Table 2**). Although gC1qR is not expressed on the surface of endothelial cells, it is found in association with their mitochondria, as it is in fetal mouse tissue (Dedio et al., 1998).

There are approximately 4105 receptors for C1q on monocytes and neutrophils, although the individual contribution of each receptor to this binding remains unclear.

Function

cC1qR binds C1q, mannan-binding lectin (MBL), lung surfactant protein A (SPA), collectin CL43 and bovine conglutinin, via clusters of charged amino acids in the collagenous tails of the ligands. Its affinity for C1q in isotonic buffer is relatively low, lying in the micromolar range. The binding occurs through a site in the S region (N160–283) of the receptor (Stuart *et al*., 1997). C1qRp is thought to display similar specificity, at least in regard to C1q, MBL and SPA. gC1qR, on the other hand, reacts specifically with C1q.

The activities triggered by C1q include the induction of chemotaxis and chemokinesis in mast cells and neutrophils, ROM production (neutrophils, eosinophils and monocytes), promotion of FcR- and CR1-mediated phagocytosis (monocytes), increased expression of E-selectin, VCAM-1 and ICAM-1 by endothelial cells, platelet aggregation and fibroblast adhesion, as well as inducing an antiproliferative response, depressed IL-1 production and modulation of immunoglobulin production by B cells. There still exists some confusion as to which receptors are involved in these processes, although cC1qR and gC1qR have been implicated in neutrophil chemotaxis and ROM production, fibroblast adhesion and platelet aggregation, while the involvement of C1qRp in phagocytosis has been clearly demonstrated. However, it is likely that the various receptors cooperate with each other in triggering the above-mentioned activities.

In addition to triggering cellular activities cC1qR is also thought to regulate classical pathway activation of complement by preventing formation of the active C1 complex (C1q-$C1r_2$-$C1s_2$).

Signalling

The data available on signal transduction by the C1qR are extremely scant. Neutrophil chemotaxis mediated by the collagenous tail of C1q is inhibitable by PTX, wortmannin and LY294002, indicating involvement of a G protein and PI3 kinase in the signalling processes initiated by cC1qR and/or C1qRp. Stimulation via cC1qR has also been shown to induce changes in K^+ conductance and Ca^{2+} influx in mouse fibroblasts.

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