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Complement receptors and regulatory proteins: immune adherence revisited and abuse by micro-organisms

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Introduction

Phagocytosis is the primary host defence mechanism against most bacteria as well as for many viruses and protozoa. To facilitate ingestion, the microbial membrane is coated with complement and antibody to make it more 'edible' (opsonization). Subsequently, host cell receptors for these proteins bind and immobilize the microbe (immune adherence). C3 and Ig, the two most critical opsonic systems of human blood, are products of independent but cooperative parts of the immune system [1]. The major goal of the complement system is to deposit clusters of C3b on a target. This targeting is mediated by IgM or IgG, in the case of the classical pathway, and by the absence of convertase regulatory proteins, in the case of the alternative pathway. Regulation of C3 activation and destruction of C3b coated particles are therefore salient issues in understanding the complement system. Predominant features of CR1 and MCP are presented in Tables 1 and 2. In this discussion we present recent results relative to the C3b/C4b binding sites of CR1, the structure of 'CR1' on primates and the role of MCP as a receptor for measles virus and group A beta haemolytic streptococci.

Binding sites on CR1 for C3b/C4b

This laboratory has chosen CR1 as a model to analyse SCR/ligand interactions. CR1, a member of regulators of the complement activation (RCA) gene/protein cluster, has two distinct ligand binding sites. The most common variant's extramembraneous portion is composed of 30 SCRs and has one copy of SITE 1 and two copies of SITE 2 [2]. SITE 1, with SCRs 1-4, binds mainly C4b, while SITE 2, with SCRs 8-11 and duplicated in SCRs 15-18, is implicated predominantly in C3b binding [2-6].

SCR-1 and SCR-2 are ~60% homologous to SCR-8 and SCR-9, respectively, whereas SCR-3 and SCR-4 are identical, with the exception of one amino acid, to SCR-10 and SCR-11, respectively. Therefore binding specificity must be determined by amino acids that are *different* in the homologous positions of SCR-1 and SCR-2 versus SCR-8 and SCR-9. To identify aa important for binding, we have prepared two constructs, each carrying only one site, and then employed a strategy of substitution mutagenesis at homologous positions to identify amino acids important for functional activity [3,5]. These studies have led to the following conclusions: (1) SITE 2 binds C3b and C4b. Its C4b binding is more efficient than that of SITE 1, (2) cofactor activity of SITE 1 is much lower than that of SITE 2; cofactor activity of SITE 2 for C3b is significantly higher than for C4b, (3) in each site several amino acids important for ligand binding and cofactor activity were identified. No amino acids were necessary for cofactor activity other than those required for binding, (4) within SITE 2, amino acids important for *both* ligands were identified, (5) in several cases binding/cofactor activity of each site could be increased by transfer of one amino acid from the other site. Interestingly, some of the substitutions conferred C3b specificity on SITE 1.

The generation of sites with increased binding and cofactor activity is an important step toward the generation of more effective inhibitors of complement activation. The characterization of the active sites in CR1 should facilitate the understanding of the evolution and activity of the entire RCA family, and the results should be applicable to other SCR-containing proteins.

Primate immune adherence receptors

Utilizing Western blotting in combination with cell surface labeling and affinity chromatography with homologous C3b and C4b or immunoprecipitation, we have identified distinct size forms of CR1 on erythrocytes (E) of 10 primates [7-9]. Their mol. wts. fell into three classes: ~50-75 kDa, 120 to 150 kDa and ~200 kDa, similar to human CR1. These smaller forms are

predominantly expressed on E while the 200 kDa form is preferentially expressed on peripheral blood leukocytes. On E some species express only the 50 to 75 kDa proteins, others only the 120 to 150 kDa and one (human) only the 200 kDa. The copy number of smaller receptors on primate E was considerably higher than of 200 kDa human CR1 and varied from 1,800 to 15,000. Five species express a combination of the 200 kDa and either the 120 to 150 kDa or the 50 to 75 kDa species [9]. Sequencing the putative 70 kDa 'short form' and full-length chimpanzee CR1 demonstrated that the chimpanzee 200 kDa form is 98.8% identical to the human CR1 and that the short form is an alternatively spliced form of chimpanzee CR1 [10]. It consists of SCRs 1-6, 28, 29, 30 and the transmembrane and cytoplasmic tail. A number of interesting questions are raised by these data including issues of structure/function relationships and evolution of CR1 in primates. For example, how does chimpanzee CR1 on E bind C3b bearing immune complexes if it only apparently contains a C4b binding site and what is the biologic advantage of expressing 15,000 copies/E of a short form of CR1?

MCP - Its abuse by measles virus and Streptococcus pyogenes

MCP recently has been reported to be the cellular receptor for measles virus (MV) [11-13]. Polyclonal and certain monoclonal Ab to MCP block MV binding. Rodent cells, such as CHO and murine fibroblast cell lines, normally resistant to MV, transfected with MCP become permissive to MV infection. The expression of all four commonly expressed isoforms of MCP in CHO cells leads to the appearance of viral proteins within the cell and on the cell surface, to syncytium formation, and to infectious centres [13]. The last point indicates that these cells can transmit virus to uninfected cells. In unpublished observations in collaboration with Manchester and Oldstone at the Scripps Clinic and Lublin at Washington University, we have further evaluated the MV-MCP interaction. As expected, the C3b/C4b binding and MV binding domains are within the SCRs but, surprisingly, are quite distinct. MCP expressing the glycolipid anchor of DAF is permissive of infection by measles viruses. The finding that MCP serves as a (the) MV receptor presents many new and exciting avenues to explore relative to the pathogenesis of infections by measles and related viruses.

The pathogenic Gram-positive bacterium *Streptococcus pyogenes* (group A streptococcus) is the causative agent of numerous suppurative diseases of human skin. The M protein of *S. pyogenes* mediates the adherence of the bacterium to keratinocytes [14]. Through analysis of deletion mutants, Caparon and colleagues at our institution have identified the region of the M protein responsible for cell recognition and found that the SCR domains of MCP are an attachment site for streptococcal adherence to keratinocytes [14].

The binding of factor H to streptococci pyogenes has been previously reported by Horstmann *et al.* [15]. Factor H competes with MCP for binding to keratinocytes [14]. We hypothesize that this organism abuses in an ingenious fashion two complement regulatory proteins. It utilizes a single domain (C3b-like?) to adhere to MCP and thereby gain a foothold in the epidermis and then binds via the same region to factor H in order to protect itself from the alternative pathway as it moves through tissue planes. DAF recently has been demonstrated to be a receptor for *E. coli* [16]. In addition, the Epstein-Barr virus uses CR2 to gain entry into human cells as do several organisms (Babesia, Leishmania, Legionella and Mycobacterum) in the case of CR1 (see Review by Cooper [17]).

Undoubtedly, many other remarkable examples of interactions between complement regulatory proteins and microbes will be defined.

Table 1. Summary of selected features of CR1

Immune Adherence Receptor: C3b/C4b Receptor
Complement Receptor Type One (CR1): CD35

Function	Binds C3b/C4b bearing antigens and immune complexes; ligand and CR1 are both in clusters; adherence>phagocytosis; processing of immune complexes.
Structure	Type I ~200 kDa membrane glycoprotein with N-linked CHO; four alleles with mol. wts. and gene frequencies of 190,000 (.05), 220,000 (.81), 250,000 (.14), 280,000 (.01).
Expression	E, Granulocytes, B-lymph, Mono-Mx, T-lymph (15%), FDC; Cis-acting regulatory element on E; Unusual locations: kidney, nerve, oocyte.
Molecular	Extramembranous portion consists of 23, 30, 37, or 44 SCRs; internal duplicationswith 7 SCRs as the basic unit; multiple binding sites - one specific for C4b plus 1,2,3 or 4 for C3b and C4b; 150 kb gene in RCA gene cluster at 1q32; partial duplicate described.
Blood Group	Knops-McCoy, Swain-Langley, York.
Deficiency	SLE and other syndromes with immune complexes.
Microbial Pathogenesis	Several species of microbes use CR1 to penetrate cells.

Table 2. Summary of selected features of MCP

Membrane Cofactor Protein: gp45-70: CD46

Function	Binds C3b/C4b deposited on self tissue and serves as cofactor for their cleavage by factor I; acts intrinsically.
Structure	Type I membrane glycoprotein with N and O-linked CHO: four commonly expressed isoforms arise by alternative splicing; three patterns on SDS-PAGE upper band, 70% lower band, 5% equal bands, 25%.
Expression	Wide tissue distribution (epithelial, endothelial, blood cells, etc). Absent on E. Unusual locations: inner acrosomal membrane of spermatozoa, trophoblast, oocyte.
Molecular	Four SCRs followed by a heavily O-glycosylatedregion; alternative splicing within O-CHO region and of cytoplasmic tails gives rise to four isoforms; gene is 40 to 50 kb and located in RCA gene cluster; partial duplicate described.
Deficiency	None described.
Microbial Pathogenesis	Measles virus receptor. Streptococcus pyogenes receptor.

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CD21 and CD19: how B cells sense antigen

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Signal transduction by antigen receptors of B lymphocytes, relative to other growth and differentiation receptors, face three unique challenges: the need to recognize an almost infinite number of mutable antigens, to sense low concentrations of these antigens, and to respond in a differential way to self and foreign antigens. The first problem has been resolved by a genetic process that creates antigen receptors from different segments of the immunoglobulin (Ig) genes that encode the antigen binding regions. This leads to extraordinary recombinatorial diversity, but not to high affinity antigen receptors so that the second challenge must be resolved by other receptors on the B cell that do not interact with antigen, but which can amplify signalling when few mIg have been ligated. The third problem also requires the function of membrane proteins others than mIg, but is not related to complement.

The first clue to the identity of the accessory membrane protein that promotes activation of B cells through mIg came from the studies of Pepys in the 1970s which demonstrated that mice depleted of C3 had diminished antibody responses to low doses of antigen, but normal responses to higher amounts. These studies were confirmed when guinea-pigs and humans deficient in the components of the classical pathway of complement that activate C3 were shown to have similar defects in their immune responses. One interpretation of these findings was that the complement system interacted with B cells in some way that augmented their response to antigen. This possibility received support from findings that administering monoclonal antibodies to murine CR2, or a recombinant soluble form of human CR2 (CD21), to mice at the time of immunisation suppressed the production of specific antibodies. Thus, CD21 mediated the capacity of the complement system to enhance the immune response.

The molecular explanation for the *in vivo* effects of CD21 began with the demonstration that coligating CD21 to mIgM, as might occur with antigen that has activated complement and become coated with C3d, greatly amplified the release of intracellular calcium induced by mIgM, while not significantly releasing intracellular calcium by itself. From this observation was formed the concept that the complement system modulated the immune system in an antigen-specific manner by coupling its effects to the antigen receptor. The further suggestion that, because of the short cytoplasmic tail of CD21 (34 amino acids), CD21 mediated its effect through an association with another protein was supported by the coimmunoprecipitation of CD19 with CD21 in digitonin lysates of B cells. The focus of these studies the shifted to this B cell-specific membrane protein of the Ig-super family.

Coligating mIgM with CD19 on human B cells lowered by two orders of magnitude the number of mIgM that needed to be ligated for a proliferative response The mechanism by which CD19 serves as a coreceptor for mIg involves its large cytoplasmic region of 243 amino acids which contains nine tyrosines that are targets of the protein tyrosine kinases activated by the mIg. Two of the tyrosines, Y484 and Y515, that are phosphorylated bind and activate phosphatidylinositol 3-kinase (PI3-kinase), an intracellular enzyme that mediates, by means not yet understood, some of the growth and differentiation functions of receptor tyrosine kinases of other cell types. The roles of other phosphotyrosines of CD19 are not known. Most recently a direct interaction between CD19 and mIg, initially considered because of the unidirectional cocapping of these membrane protein complexes, has been suggested by their communoprecipitation from detergent lysates of B cells activated by ligating mIg.

These *in vitro* and *in vivo* studies provide some molecular basis for the ability of the B lymphocyte to sense low concentrations of antigen before antigen receptors have had an opportunity to hypermutate to higher affinity states. The full importance of CD21 and CD19 will be more completely understood after an opportunity to analyse mice lacking this membrane protein because of targeted interruption of the CD19 gene.

The genetic defect of PNH

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Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hemolytic anemia characterized by intravascular, complement-mediated hemolysis. In patients with PNH, abnormal red cells that are deficient in the surface expressions of DAF and CD59 appear. PNH is a haematopoietic stem cell disorder because DAF- and CD59-deficient neutrophils, monocytes, platelets and lymphocytes also appear. In addition to DAF and CD59, other proteins such as acetylcholinesterase, alkaline phosphatase, CD14, CD16, CD24, CD48, CD52, CD58, CD67, CD73 and CD87 are deficient in various hematopoietic cell lineages.