Human Complement Receptors for C3b (CR1) and C3d (CR2)

DOUGLAS T. FEARON, M.D.

Department of Medicine, Harvard Medical School and Department of Rheumatology and Immunology, Brigham and Women's Hospital, Boston, Massachusetts, U.S.A.

The human C3b receptor (CR1) is a polymorphic glycoprotein comprised of a single polypeptide chain. Of the 4 allotype forms of CR1 that have been described, the 2 most common have M,s of 250,000 and 260,000, and are regulated by alleles having frequencies in a Caucasian population of 81.5% and 18.5%, respectively. CR1 is present on erythrocytes, neutrophils, eosinophils, monocytes, macrophages, B lymphocytes, some T lymphocytes, mast cells, and glomerular podocytes. CR1 number on erythrocytes is genetically regulated, and ranges from less than 100 sites per cell to greater than 1000 sites per cell, the average in the normal population being 500–600 sites per cell. A model accounting for this wide distribution proposes the existence of 2 codominant alleles determining low and high receptor number respectively; CR1 number is not affected by the structural polymorphism, so that the loci for these two phenotypic characteristics are distinct. The function of CR1 on erythrocytes may be to promote the clearance of immune complexes from the circulation. CR1 number on myelomonocytic cells is regulated by chemotactic factors which can rapidly transfer CR1 sites from a latent, presumably intracellular, site to the plasma membrane of these cells, thereby enhancing their ability to interact with opsonized foreign material. The receptor is involved in the endocytic reactions of these cells, and recent findings have demonstrated that this function can be modulated by T cell-derived factors, fibronectin, and phorbol esters. The role of CR1 on lymphocytes remains to be fully explored, although the receptor may enhance the differentiation of B cells into antibody-secreting cells.

Several studies have documented a relative deficiency of CR1 on erythrocytes in patients with systemic lupus erythematosus (SLE). In studies from Boston and Japan, the relatives of patients have been found also to have significantly fewer CR1 sites on erythrocytes than did normal individuals, indicating that genetic factors had a role in the occurrence of the deficiency. In 2 other reports, correlations of low receptor number with indices of disease activity have been found, suggesting that the deficiency may also be secondarily acquired in some individuals.

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Reprint requests to: Douglas T. Fearon, M.D., Department of Rheumatology and Immunology, Brigham and Women's Hospital, 75 Francis Street, Boston, Massachusetts 02115.

Abbreviations:
CR1: C3b receptor
CR2: C3d receptor
EBV: Epstein-Barr virus
EBVR: receptor for Epstein-Barr virus
PMA: phorbol myristate acetate
SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SLE: systemic lupus erythematosus

The human C3d receptor (CR2) is a 145,000 M, protein that is present only on B lymphocytes. Although its normal role in the biology of the B cell is not known, studies employing the CR2-specific monoclonal antibodies, HB5 and anti-B2, CR2 have shown it to be the receptor for the Epstein-Barr virus, a finding that explains the B cell tropism of this virus.

The third component of complement, C3, is the most important protein of the complement system. It is the most abundant of the complement components, being present in plasma at concentrations of 1–2 mg/ml; it is the point at which the classical and alternative pathways converge in the reaction sequences of the complement system; it is capable of forming a covalent linkage with the target of complement activation; and, derived from this protein are 5 cleavage fragments, C3a, C3b, iC3b, C3d, and C3e, that can bind to 5 different receptors that reside on cells which participate in inflammatory and immunologic reactions. In this summary recent findings concerning 2 of these receptors, CR1 (also termed the C3b receptor) and CR2 (C3d receptor) will be described.

STRUCTURAL CHARACTERISTICS OF CR1 (C3b RECEPTOR)

CR1 reversibly binds C3b, iC3b and C4b, with the greatest affinity being for C3b. Multivalent interaction of ligand with this receptor is necessary for effective binding to occur [1], and most activators of complement, e.g., immune complexes and bacteria, covalently bind a multiplicity of C3b molecules. CR1 has been purified to homogeneity from human erythrocytes and specific polyclonal antibody has been prepared [2,3]. CR1 is a large glycoprotein that exists in at least 4 allotypic forms differing by M,, the most common forms having apparent M,s of 250,000 and 260,000 [4-7]. Approximately two-thirds of a Caucasian population have only the lower M, form, one-third have both forms and 2.5% have only the higher M, form [4]. The CR1 allotypes do not differ in their affinity for C3b [4]. Removal of N-linked oligosaccharides from purified CR1 by treatment with endoglycosidase F decreased by 10,000 the M, of both forms of CR1, as assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), but this treatment did not abolish the difference in M, between the CR1 allotypes [4].

CR1 is present on human erythrocytes, neutrophils, eosinophils, monocytes, macrophages, B lymphocytes, a subpopulation of T lymphocytes, and glomerular podocytes. Analysis by SDS-PAGE of immunoprecipitates from erythrocytes, neutrophils monocytes, and B and T lymphocytes also has found no major differences in M, between CR1 of the different cell types [3], although the receptor from neutrophils [8] and T lymphocytes [9] appears to be slightly larger than that from the other cells.

FUNCTIONS OF CR1

Erythrocytes express an average of 500–600 CR1 per cell [1,3,10,11], and a wide range of 100–1000 receptor sites per cell has been found among different normal individuals. Erythrocyte receptor number is a stable phenotypic characteristic. The depiction of erythrocyte CR1 number of normal individuals in Boston as a frequency histogram reveals a bimodal distribution with 94% of the population having 600–1000 receptor sites per
cell and 54% having 350–600 sites per cell. A third, less-distinct group has less than 350 sites per cell and represents 12% of the normal population. Two codominant alleles have been proposed for regulation of the quantitative expression of CR1 of erythrocytes [10]. Persons having high numbers of CR1 (600–1000 per cell) were considered to have the HH phenotype and to be homozygous for an allele determining high receptor number; individuals having fewer than 350 CR1 per cell, the LL phenotype, were homozygous for the low receptor allele; and those having intermediate numbers, 350–600 sites per cell, were heterozygotes exhibiting the HL phenotype. This hypothesis was supported by finding the appropriate patterns of inheritance in 7 families, including 1 normal family in which all 5 members had the relatively rare LL phenotype [10]. No linkage was found between the structural polymorphism of CR1 and its quantitative expression [4]. The finding of a trimodal distribution of this characteristic among normal individuals has been confirmed recently by a study from investigators in Japan indicating this to be an inherited trait that is not limited to a single population.

CR1 on erythrocytes can serve at least 2 functions in the intravascular space where they are the major repository of this receptor. First, as on any cell type, it can serve as a cofactor for the factor I-mediated cleavage of C3b to iC3b [2,12,13] and C3dg [14], fragments that can bind to CR2 on B lymphocytes and CR3 on myelomonocytic cells. Second, the receptor endows erythrocytes with the capacity for cyclical uptake and release [15] of C3b- and iC3b-bearing immune complexes, an activity that may allow erythrocytes to clear immune complexes from the circulation. In support of this are studies examining the fate of model immune complexes in primates that demonstrated the delivery of erythrocyte-bound complexes to the liver, via the portal circulation, where the complexes were transferred to hepatic cells, presumably Kupfer cells [16].

Circulating neutrophils and monocytes express an average of only 5000 CR1 per cell but stimulation of these cells with chemotropic agents, such as C5a, formyl-methionyl-leucyl-phenylalanine or leukotriene B4, causes rapid up-regulation of receptors so that as many as 50,000 CR1 per cell become available for binding of ligand [17]. Increased CR1 expression on neutrophils also has been observed in vivo in patients who are experiencing complement activation secondary to hemodialysis with dialysis membranes that activate the alternative pathway [18]. The latent receptors are presumed to be intracellular, but the precise site has not been identified. This mechanism for increasing the expression of CR1 could prepare chemotactically migrating cells for recognition of material that has been opsonized with C3b.

The primary function of CR1 on neutrophils and monocytes is to mediate or enhance the endocytosis of soluble complexes and particles to which C3b has bound covalently. Although internalization of soluble, multimeric ligand by CR1 on these cells can occur through clathrin-coated pits and vesicles followed by delivery to lysosomal structures [19,20], phagocytosis of particles by CR1 had generally been considered not to occur, most early studies having emphasized the role of CR1 in synergistically enhancing phagocytosis mediated by Fe receptors [21,22]. More recently, murine peritoneal macrophages that had been incubated with a supernatant of stimulated T cells were shown to have acquired a capacity for phagocytosis of sheep erythrocytes bearing C3b [23]. A similar change in the functional capacity of CR1 has been reported for human monocytes that had been cultured for 1 week [24], stimulated with phorbol esters [25] or incubated with fibronectin [26,27], and for neutrophils exposed to phorbol esters [26] or with chemotropic peptides and fibrinogen [28]. A recent study has demonstrated that phorbol myristate acetate (PMA) caused 2 sequential changes in the cellular distribution of CR1 [29]. Incubation of neutrophils with PMA first led to the translocation of latent CR1 to the plasma membrane and this reaction was followed within minutes by the internalization of the CR1. Thus, presumptive activation of protein kinase C, a principal biological effect of PMA, induced the 2 reactions of CR1 previously shown to occur with chemotropic peptides and multivalent ligands, respectively.

Analyses of the possible association of CR1 with the cytoskeleton of neutrophils were stimulated by experiments measuring the lateral mobility of CR1 in the plane of the plasma membrane of neutrophils. Whereas there was relatively unrestricted mobility of CR1 on cells supported by a lipid monolayer [30], adherence of cells to quartz coverslips caused restricted movement of CR1 [31]. Recently, it has been shown that cross-linking CR1 on neutrophils at 0°C with F(ab')2 antibody caused the receptor to become insoluble in nonionic detergents [32]. In addition, cross-linked receptors rapidly redistributed at 20°C from punctate clusters into large patches and caps in a reaction that was accompanied by the subplasmalemmal accumulation of actin at the site of caps [33]. This distribution of the receptor was inhibitable by cytochalasin D and chlorpromazine. In approximately half of the neutrophils which had capped their CR1, there was a corresponding redistribution of Fe receptors. The capping of CR1 and Fe receptors also occurred reciprocally so that unoccupied CR1 were found to redistribute with Fe receptors being capped with aggregated IgG [33]. Thus, there is a cytoskeletal-dependent cooperative interaction between CR1 and Fe receptors induced by multivalent ligands that may augment the phagocytosis by these cells of particles bearing both C3b and IgG.

Essentially all mature human B lymphocytes from spleen or peripheral blood express CR1 [34]. In comparison to unstimulated neutrophils and monocytes, the circulating B cell has available for binding of ligand a relatively large number of receptors, 20,000–40,000 CR1 per cell [17]. This number is not increased by chemotropic peptides, lipopolysaccharide or culture supernatants of mitogen-stimulated lymphocytes. CR1 appears early in B cell ontogeny, being present on 15% of large pre-B cells, 35–50% of small pre-B cells, and 60–80% of immature cells [34], and is lost when B cells differentiate into plasma cells. The presence of CR1 on B cells had led to attempts to demonstrate a functional role for this membrane glycoprotein, but a clear definition is not available. Of great interest in this regard is the recent report that polyclonal rabbit F(ab')2 anti-CR1 enhanced the generation of antibody-secreting cells from peripheral blood mononuclear cells in response to suboptimal doses of pokeweed mitogen [35]. Further studies utilizing natural ligand, lymphocyte growth and differentiation factors, and highly purified populations of B cells, T cells, and monocytes should further define the role of CR1 in B cell differentiation.

The importance of employing highly purified subpopulations of lymphocytes in studying the role of CR1 in lymphocyte function is emphasized by the recent finding that in addition to B cells some T cells express CR1 [9]. An average of 15% of peripheral blood lymphocytes forming rosettes with sheep erythrocytes were stained with fluoresceinated F(ab')2 anti-CR1 and these cells expressed approximately one-tenth the amount of CR1 that was present on B cells. All sheep erythrocyte-rosetting, CR1-positive lymphocytes expressed the T4 antigen, and the T4:T8 ratio among these cells was approximately 4:1. Although greater than 90% of these cells bound aggregated IgG, suggesting the presence of Fe receptors for IgG, they had a typical lymphocyte morphology and were stained with HNK-1, an antibody that recognizes large granular lymphocytes that bear Fe receptors. No functional studies of these CR1-bearing T cells have been performed.

**STRUCTURE AND FUNCTION OF CR2 (C3d RECEPTOR)**

CR2 is present only on B lymphocytes and mediates the binding of particles bearing the C3d fragment of C3. This
patients are relatively deficient in absorbed, conferred on them the specific ability to bind of EBV. These studies may also provide insight into the labelled EBV. Thus, it was concluded that abnormally is geographically widespread and reproducibly demonstrable among various investigative groups [10,11,47-50]. Both functional [10,11,47,49] and antigenic assays of CR1 [10,11,48,50] have been employed with similar results, the mean number among patients being consistently 40-60% that of normals. In one study the number of binding sites for dimeric C3b and for anti-CR1 on erythrocytes from normals and patients was shown to correlate (ρ < 0.005), and the affinity for dimeric C3b of CR1 on cells from patients and normals did not differ [10]. The frequency of the two major polymorphic forms of CR1 was the same among SLE patients and normal persons [6], regardless of CR1 number on erythrocytes [4], except for a possible increased occurrence of a rare variant of 160,000 M, in the patient population [6]. In summary, most patients appeared to have low numbers of normal CR1 on their erythrocytes rather than nonfunctional, structurally altered forms of the protein that were antigenically intact.

Two general mechanisms have been proposed to account for the receptor deficiency in SLE: one postulates that it is inherited [10,11,47] and the other that it is acquired as a consequence of pathologic processes associated with the disease [48,50]. Although having evidence for the occurrence of either process does not exclude the other, establishing the validity of the former is most critical as this would define a genetically regulated trait that, in combination with other inherited and environmental factors, predisposes to the occurrence of SLE. The first study describing the CR1 deficiency in Japanese SLE patients noted that 6 of 24 relatives of patients also had the abnormality, compared to 4 out of 104 normal subjects [47]. In a second study performed in Boston, erythrocytes from 47 relatives of 6 probands were assessed [10]. Cells from the patients had an average CR1 number that was 41% of the normal mean, and the mean CR1 number of the relatives' cells was 63% of normal, both measurements being significantly lower than the normal population. Spouses of 14 patients did not differ from the normal population in this measurement, suggesting that the low number of CR1 among the consanguineous relatives was not acquired through physical contact with patients. This study has now been confirmed by the Japanese investigators who not only found a trimodal distribution of CR1 number on erythrocytes in the normal population, consistent with inheritance of this trait among normals, but also described familial transmission in SLE patients [10]. Sixteen relatives of 5 probands, whose cells averaged 39% of the normal CR1 number, had erythrocytes expressing an average of only 64% of the normal mean CR1 number. Thus, in 2 distinct populations, Japanese and North American, inheritance has a major role in the CR1 deficiency in SLE patients.

A recent study involving patients from Great Britain and North Carolina presented findings that emphasized the role of disease processes in the acquisition of low CR1 number on erythrocytes in SLE [50]. Although a significant correlation was found between erythrocyte CR1 of parents and children in normal families, supporting the model of codominant inheritance, no such correlation was observed in SLE families. Instead, increased amounts of C3dg were found on erythrocytes of SLE patients, and the numbers of C3dg molecules correlated inversely with the number of CR1 molecules per cell. These findings were interpreted as indicating that complement activation may occur on erythrocytes of SLE patients, perhaps by receptor-bound immune complexes, and that this process caused the loss or degradation of CR1 sites by an unknown mechanism. In support of this conclusion were the findings of low CR1 also in patients with autoimmune hemolytic anemia, and in patients with circulating immune complexes associated with infectious diseases. An earlier report [48] that did not perform family studies demonstrated that erythrocyte CR1 number among patients varied inversely with serum immune complex levels and directly with C4 levels, and that CR1 sites increased in 2 of 4 patients during remission of disease activity.

ABNORMALITIES OF CR1 IN SLE

Six studies have reported that erythrocytes from some SLE patients are relatively deficient in CR1, indicating that the
Thus, there is good evidence to indicate that pathologic processes also may secondarily diminish the expression of CR1 by erythrocytes in SLE patients and obscure the detection of genetic regulation of CR1 number which, in this study, was apparent only among normal persons.

Additional studies must be carried out to examine carefully both the cellular biologic mechanisms that determine the number of CR1 on erythrocytes and the pathologic processes involved in secondary diminution of erythrocyte CR1. Further, the nucleated cells that express CR1 should be assessed in SLE patients for possible abnormalities involving this and the other C3 receptors. Studies have already demonstrated the absence of CR1 on glomerular podocytes of patients with proliferative nephritis of SLE, which contrasted with the apparently normal expression of CR1 by these cells in membranous lupus nephritis and in other types of endocapillary proliferative nephritis [51,52]. Finally, the demonstration of diminished CR1 on erythrocytes of patients with other diseases, such as juvenile [53] and adult rheumatoid arthritis [48,49], leukemia [49], autoimmune hemolytic anemia [50], and leprosy [54], and of decreased CR1 function on reticuloendothelial cells of patients with SLE [55] and primary biliary cirrhosis [56] must attempt to discriminate between inherited and acquired abnormalities with the realization that both processes may occur.

Possible mechanisms by which inherited abnormalities of the CR1 might predispose to immunologically-mediated disease have been discussed elsewhere [57], and include impaired clearance and processing of immune complexes. Indeed, even before patients with SLE were recognized to have a relative deficiency of erythrocyte CR1, an association between autoimmune disease and inherited abnormalities of proteins of the classical pathway of complement, C1q, C1r, C1s, C4, C2, and C3, had been appreciated [58]. Because C1, C4, C2, and C3 are the components that are involved in the deposition on antigen-antibody complexes of C3b, the ligand for CR1, it may be that the occurrence of SLE in these and the CR1-deficient individuals involve similar mechanisms.

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