The Human C3b Receptor: Function and Role in Human Diseases

Francisco Tausk, M.D., and Irma Gigli, M.D.
Division of Dermatology, University of California, San Diego, School of Medicine, San Diego, California, U.S.A.

The human C3b receptor (CR1) is a polymorphic glycoprotein which functions regulating the complement system by inhibiting the activation of C3 and C5, through its effect on their convertases, and serving as cofactor for factor I in mediating the degradation of C3b to its inactive fragment C3bi and further to C3d-g. The latter are then ligands for their respective receptors on leukocytes, CR3 and CR2. Additionally, CR1 on erythrocytes endows these cells with the capacity to deliver immune complexes (IC) to the reticuloendothelial system, resulting in their clearance from the circulation. On phagocytes, this receptor participates in the process of endocytosis of foreign particles.

There is a wide inherited variation of CR1 expression on erythrocytes (CR1/E) of different individuals. Patients with diseases which feature elevated levels of IC, such as systemic lupus erythematosus, leprosy, and AIDS, have a marked decrease of CR1/E, which may result in an altered clearance. This reduction appears to be related to disease activity, and the most probable site for CR1/E loss is during the transfer of IC to macrophages.

Healthy neutrophils increase tenfold their expression of CR1 in response to the effect of chemotactic peptides. Neutrophils from patients with AIDS display an altered response to stimulation. This defect may be of relevance in the process of endocytosis. J Invest Dermatol 94:141S–145S, 1990

This work was supported by a grant from the National Institutes of Health (AI 20067).
Reprint requests to: Dr. Francisco Tausk, Division of Dermatology (H-811J), UCSD Medical Center, 225 Dickinson Street, San Diego, CA 92103.

Abbreviations:
AIDS: acquired immunodeficiency syndrome
B: factor B
CIC: circulating immune complexes
CR1: C3b receptor
CR2: C3d receptor
CR3: C3bi receptor
CR1/E: erythrocyte CR1
DAF: decay accelerating factor
DLE: discoid lupus erythematosus
E: erythrocytes
FMLP: F-Met-Leu-Phe
HIV: human immunodeficiency virus
IC: immune complexes
MCP: membrane cofactor protein
RFLP: restriction fragment length polymorphism
SLE: systemic lupus erythematosus

FUNCTIONS OF CR1

The best characterized functions of this receptor are the regulation of complement activation, the clearance of immune complexes, and its participation in the process of phagocytosis.

Regulation of Complement Activation Following the activation of complement, enzymatic complexes are assembled which act on C3 and C5. The C3 convertases of the classical (C4b2a) or alternative (C3bBb) pathways cleave C3, releasing the 9000-kD peptide C3a (anaphylotoxin). An internal thioester is transiently exposed allowing for the covalent binding of the resulting C3b fragment to carboxy or amino groups of activating particulate surfaces. Subsequently the C5 convertases of the classical (C4b2a3b) or alternative (C3bBb3b) pathways cleave C5 to C5b, depositing the latter, and mediating the release of the C5a anaphylotoxin. This is followed by the “passive” deposition of C6, C7, C8, and C9, which do not require enzymatic activation. Thus the regulation of the C3/C5 convertases becomes critical to the outcome of complement activation and deposition of the membrane attack complex (C5b-9) on cellular surfaces.

The C3 and C5 convertases are substrates for the action of CR1 [5]. This receptor regulates the classical pathway activation [6–8] by accelerating the natural degradation (decay function) of C2a from the C4b2a enzyme and impairing the uptake of C2 by C4b. Similarly, it affects the alternative pathway convertases [5,7] by accelerating the decay of C3bBb and impairing the uptake of B by C3b. CR1 participates in restricting complement activation. This receptor functions as a cofactor for the proteolytic action of the serine protease factor I (F1) in the cleavage of C3b. This reaction generates the inactive fragment C3bi, which becomes the ligand for the specific receptor CR3, or it can be further degraded resulting in the release of the C3d-g fragment. C3d-g can no longer bind to CR1 or CR3 and becomes the ligand for the C3d receptor (CR2) on B lymphocytes [9–12].

These functional properties of CR1 and structural characteristics are partly shared with other complement regulatory serum and
membrane proteins, such as the decay accelerating factor (DAF), C4 binding protein, factor H, and the membrane cofactor protein (MCP) [13].

Clearance of Immune Complexes  During the last decade, numerous studies have examined the role of CR1 in the clearance of immune complexes. This receptor, has variable C3b binding sites depending on particular phenotypes (see W. Wong, this issue). The monomeric form of C3b has very low affinity for CR1 [14], and increases progressively as C3b clusters in multimers as seen in immune complexes (IC). This results in the lack of competition between IC and fluid phase C3b for CR1 on erythrocytes (E).

Because erythrocytes are found in large relative numbers in the circulation, their membranes are the repository of the major pool of circulating CR1, which endows these cells with the capacity of clearing circulating immune complexes (CIC). Indeed, when soluble IC that had activated complement were incubated with a mixture of blood cells, they were found preferentially on erythrocytes [7]. The presence of FI mediates the quick dissociation of these cell-bound complexes, although erythrocytes (E) retain the capacity to rebind fresh IC, whereas the dissociated IC progressively lose the capacity to attach to E. This suggests that CR1 in the presence of FI may render them unable to continue activating the complement system [15].

Cornaff et al [16] studied the CIC clearing function of E-associated CR1 by injecting IC formed in vitro into the aorta of primates. These complexes rapidly bound to E, which after traversing the liver were found to exit this organ devoid of IC, which remained trapped in the hepatic tissue. These experiments suggested that the complexes are transferred from the E surface to local macrophages for their final processing. Following C3 depletion by cobra venom factor, the IC did not bind to E, and were partially deposited in organs which could suffer immune complex–mediated damage [17]. In support of this role of CR1 in vivo are experiments [18] showing that soluble complement fixing tetanus–anti-tetanus complexes bound more to E of individuals with high expression of CR1. Similar results were observed utilizing cells from SLE patients and in vitro formed IC. Erythrocytes of such patients also had a decreased binding of IC and the latter had an increased pathologic clearance by peripheral tissue (reviewed in [19]).

In this manner, CR1 on erythrocytes plays a role in the trafficking of IC, delivering them to the reticuloendothelial system, thus preventing their deposition in organs such as kidney, lung, and brain. As discussed below, this may be of importance in the pathogenesis of autoimmune diseases.

Endocytosis  CR1 has been found to be present in variable numbers on the surface of phagocytic cells and its expression can be modulated by temperature and pharmacologic agents [20–22]. Neutrophils and monocytes isolated at 4°C express relatively few receptors which can be increased by incubation at 37°C by inducing the translocation of intercellular pools of CR1 to the cell membrane. The presence of C5a or the synthetic chemotacticant F-Met-Leu-Phe, among others, mediates a marked increase of the surface expression of CR1. Therefore, during inflammation chemotactants generate an increase of CR1 to facilitate phagocytosis. This process most likely requires phosphorylation of the receptor [23].

Following the binding of cross-linked C3b to the membrane of neutrophils, CR1 clusters, permitting a multipoint attachment. During this process, a cytoskeletal rearrangement results in co-capping of CR1 and Fc receptors. The binding of particle-bound C3b to phagocytes results in the endocytosis of C3b ligand [24,25] creating the necessary environment to recruit Fc receptors and cooperate with Fc-mediated phagocytosis. The clearance of immune complex and endocytosis functions are summarized in Fig 1.

ALTERATIONS OF CR1 IN DISEASE

The number of CR1 molecules on erythrocytes (CR1/E) surfaces varies widely from individual to individual; we have observed differences ranging from 100 to 1200 receptors per cell. These numbers are genetically determined, as demonstrated by Wilson et al [26]. Normal subjects can be segregated phenotypically into three groups: those homozygotes for high or low expression of CR1, and those heterozygotes for this receptor. In support of this notion Wilson and co-workers reported [27] a 6.9-kb or a 7.4-kb restriction fragment length polymorphism (RFLP) that correlates with the number of CR1 per erythrocyte. Subjects having the 7.4-kb fragment had high CR1/E, whereas those homozygotes for the smaller fragment had low expression. In contrast, the CR1 expression on other cell types does not vary among individuals.

Several reports coincide in the finding of a marked decrease of CR1 on erythrocytes of patients with systemic lupus erythematosus (SLE) [28–31,26] as well as on glomerular podocytes [32] and leucocytes [33,34]. Various studies have shown that the reduction in CR1/E in patients with SLE is inherited (reviewed in [35]). These patients had a decrease in CR1 expression that was approximately 50% of that seen in the normal population and this defect was shared by blood relatives. Long-term follow-up studies of these patients revealed that the number of CR1 did not vary in patients as well as in their relatives and the control group; however, the patient population consisted of relatively quiescent cases. Further numbers of patients with SLE were homozygotes for the alleles identified by the 7.4-kb RFLP when compared to normal controls, but they did not have an increased frequency of homozygosity for the low CR1/E-associated 6.9-kb fragment [27]. Similar studies by Moldenhauer et al [36] showed no difference between the gene frequency for the alleles correlating with high and low expression of CR1 between normal subjects and SLE patients. Additionally, analysis of the frequency of the molecular weight polymorphic variants of CR1 has shown a significantly increased presence of the 160-kD receptor among patients with SLE [37].

It was also intriguing that when groups with the same allelic expression were compared, SLE patients still had lower CR1/E [27,37], suggesting that other mechanisms could be involved in determining the defect. The notion that CR1/E is genetically determined but that its reduction in SLE is associated to disease activity is sustained by various studies. Walport et al [38] have reported that relatives of SLE patients have normal expression of receptors, and patients with low numbers have been found in families where the healthy probands were homozygotes for high CR1/E expression. Ross et al [39] examined the relationship between CR1/E and disease activity in patients with SLE as well as other autoimmune...
conditions such as autoimmune hemolytic anemia, cold agglutinin disease, and Sjögren's syndrome. They observed that although during remission these patients had decreased CR1/E, there was a loss of receptors during exacerbations. They observed an inverse correlation between CR1/E and the deposition of the C3d-g fragment of C3 on the surface of erythrocytes, suggesting that CR1 could be related to the regulation of complement activation in vivo. It is of interest that transfused erythrocytes in patients with SLE and hemolytic anemia showed a progressive loss of CR1 molecules from the donor cells, concomitant with the deposition of C3 fragments on their surface [40]. This in vivo study strongly supports the acquired nature of CR1 deficiency in SLE.

In addition to the nature of this defect, the mechanism by which CR1 molecules are reduced remains in question. It has been reported that the receptor on erythrocytes of SLE patients is not lost (at least in vitro) due to the interaction with blood enzymes or leukocytes [39], contrary to what occurs in the shedding of the complement regulatory protein DAF (decay accelerating factor) from the surface of neutrophils [41].

As discussed above, IC bound to erythrocytes are delivered to the liver and cleared by local Kupffer cells [16]. This is the most probable site of CR1/E reduction in SLE. When large amounts of IC are bound to CR1/E, they are transferred to hepatic macrophages, resulting in a partial loss of the carrier molecule, CR1. In support of this data are recent findings utilizing an artificial model of processing of IC [42] showing that perfusion of IC bearing erythrocytes through a monocyte-macrophage column resulted in the transfer of the IC to the latter cells. During this process CR1 were significantly reduced from the erythrocyte surface.

It has recently been shown that transfusion of erythrocytes with high density of CR1 was followed by a decrease in CIC levels in patients with SLE and rheumatoid arthritis [43]. This study suggests a functional role for CR1, and may provide the rationale for this therapeutic approach in autoimmune diseases.

The previous studies define the association between low CR1 and SLE, but did not address this receptor in the chronic form of this disease, discoid lupus erythematosus (DLE). We investigated [44] whether CR1/E could be useful in differentiating DLE from SLE. Patients classified as having DLE, expressing very low CR1/E, indistinguishable from SLE, had systemic signs and symptoms, although not conforming to the criteria for SLE. We concluded that these were patients with a mild form of SLE who require closer follow-up. In contrast, those patients with DLE that were totally asymptomatic expressed CR1/E which did not differ from the normal population (Table I). The results of this study suggested that the evaluation of CR1/E in patients with DLE may be a useful parameter in detecting potential systemic involvement.

An example of how CR1 expression correlated inversely with the presence of immune complexes [45] and immunosuppression can be observed in studies of CR1/E in patients with leprosy [46]. This is a disease characterized by the spectrum that spans from the lepromatous pole, knowing to have high levels of CIC, to the tuberculoid pole, with low or absent CIC, and an intermediate form, borderline leprosy. As shown in Fig 2, the tuberculoid patients did not differ from normal controls, whereas there is a loss of CR1 from erythrocytes of lepromatous patients. A comparable spectrum of CR1 expression was observed in patients infected with HIV [47–49]. As shown in Fig 3, there was a progressive decrease in CR1 on erythrocytes, which correlated with the severity of the disease. Patients with full-blown AIDS presented with the lowest number of CR1/E (185 ± 93), compared to normal controls (509 ± 140). Concomitant with the reduction in CR1, patients with AIDS have high levels of complement activation and CIC. Pedigree studies designed to examine the nature of the reduction of CR1/E in AIDS suggested that it is acquired. Moreover, follow-up of two individuals show a progressive loss of CR1/E as they developed symptoms of AIDS.

CR1 expression on neutrophils of patients with AIDS [50] revealed that following stimulation with the chemotactic factor F-Met-Leu-Phe these cells had a marked defect in the translocation of cytoplasmic pools of receptors to the membrane, when compared to healthy controls (Table II). This defect was selective, because the study of the expression of DAF on neutrophils [50] showed that patients with AIDS actually had higher levels after activation with F-Met-Leu-Phe than normal controls.

### Table I. CR1 on Erythrocytes of Patients with SLE and DLE, and those with DLE and Mild Systemic Symptoms

<table>
<thead>
<tr>
<th>Condition</th>
<th>CR1/E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls</td>
<td>512 ± 174</td>
</tr>
<tr>
<td>DLE</td>
<td>480 ± 107</td>
</tr>
<tr>
<td>DLE</td>
<td>236 ± 113</td>
</tr>
<tr>
<td>SLE</td>
<td>210 ± 122</td>
</tr>
</tbody>
</table>

* Values represent the mean ± SD of the number of receptors per erythrocyte measured by radioimmunoassay with monoclonal 125I-Fab anti-CR1.

* Patients with DLE without systemic involvement.

* Patients classified as having DLE with mild systemic signs and symptoms, which did not conform to the criteria for SLE.

### Figure 2. CR1 on erythrocytes of patients with leprosy: 25 patients with lepromatous leprosy had a statistically significant reduction in CR1/E (p < 0.001) when compared to 77 normal controls. CR1/E in 12 tuberculoid and 18 borderline patients did not differ statistically from the controls. Bars, mean of each group ± SEM.

### Figure 3. CR1 on erythrocytes of patients with AIDS: patients with AIDS (n = 29) had a significant decrease in CR1/E when compared to 55 healthy males (p < 0.0001). The reduction of CR1/E in four patients with AIDS-related complex (ARC) and 49 with generalized lymphadenopathy (Gen. Lymph.) was also statistically significant (p < 0.002 and p < 0.0001, respectively). A moderate decrease in CR1/E was observed in 17 healthy male homosexuals (X = 434 ± 193), which did not differ significantly from the normal heterosexuals (p = 0.08). Bars, the mean ± SD of each group.
Table II. Analysis of CR1 on Neutrophils

<table>
<thead>
<tr>
<th></th>
<th>Resting (R)</th>
<th>Stimulated (S)</th>
<th>Ratio (S/R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy controls</td>
<td>1,550</td>
<td>19,400</td>
<td>12.5</td>
</tr>
<tr>
<td>AIDS</td>
<td>2,000</td>
<td>10,300</td>
<td>5.1</td>
</tr>
</tbody>
</table>

* CR1 was measured by a radioimmunoassay utilizing a monoclonal 125I-Fab anti-CR1.
* Neutrophils held at 4°C.
* Neutrophils incubated at 37°C with 10^7 M F-Met-Leu-Phe for 20 min prior to CR1 detection.

The deficient expression of CR1 by cells of patients with diseases such as SLE, leprosy, and AIDS may result in an impairment of the function of this receptor, such as clearance of C1c and phagocytosis, contributing to the immunosuppression which characterizes these diseases.

REFERENCES

25. Jack RM, Fearon DT: Altered surface distribution of both C3b receptors and Fc receptors on neutrophils induced by anti-C3b receptor or aggregated IgG. J Immunol 132:3028–3033, 1984
type 1 levels: Reduced levels in patients with SLE are acquired, not inherited. Clin Exp Immunol 59:547–554, 1985


