Therapeutic uses of recombinant complement protein inhibitors

Kimberly R. Kalli 1, Peihong Hsu 2, and Douglas T. Fearon 1,2 *

1 The Graduate Program in Immunology, and 2 Division of Molecular and Clinical Rheumatology and Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

Introduction

Complement acts in parallel and in concert with the immune system to protect the individual from microbial infection by mediating a variety of biologic reactions: increased vascular permeability, chemotaxis of leukocytes, opsonization, cytolysis of target organisms, and enhancement of the immune response. Activation of the complement system occurs via the alternative pathway in response to bacteria, yeast, virally infected cells [76, 84], or damaged tissue [10, 45], and via the classical pathway by particles or tissues recognized by classical pathway-activating antibodies [76, 84]. Both classical and alternative pathway activation serve to coat the offending surface with C3b and/or C4b by way of the metastable thiolester in these proteins. C3b and C4b are structural subunits of the C3 and C5 convertases, which proteolytically cleave C3 and C5. When unregulated, the convertases amplify the initial deposition of C3b, and allow the cascade to proceed to the assembly of fragments C5b through C9, the membrane attack complex (MAC), creating channels through the membrane which can cause cell death. Besides leading to cytolysis of activating cells, byproducts of C3 and C5 breakdown have additional inflammatory roles. The anaphylotoxins, C3a and C5a, induce histamine release by mast cells which leads to vasodilation and increased vascular permeability. C5a is a potent chemotactic factor which recruits and activates neutrophils, and both C3a and C5a induce rapid and enhanced production of leukotrienes from IL-3-primed basophils [11, 60]. Opsonization, or the deposition of C3b and C4b on an activating surface, marks the foreign particle with ligands for complement receptors involved in phagocytosis, and can lead to recognition by signaling receptors on phagocytes and lymphocytes specific for breakdown fragments of C3b.

While complement activation is a valuable first-line defense against potential pathogens, the activities of complement which promote a protective inflammato-

* Present address: Department of Medicine, University of Cambridge School of Clinical Medicine, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2SP, UK

Correspondence to: D. T. Fearon
ry reaction also carry the potential for harming the host by both indirect and direct mechanisms. For example, activated neutrophils are indiscriminate in their release of destructive enzymes, and complement components may be deposited on nearby cells as well as on targets. There is substantial evidence for the activation of the complement system in human disease, and for the dependency of tissue damage in animal models of disease on an intact complement pathway [18, 20, 23, 37, 61, 105].

Several proteins have evolved to control the extent of complement activation. The classical pathway C1 inhibitor will be discussed elsewhere in this volume, as will proteins which interfere with the insertion of the terminal components of the MAC, such as CD59 and homologous restriction factor. We will concentrate on proteins which have evolved to regulate complement activation at the level of C3 and C5 convertases, namely, members of the regulators of complement activation (RCA) gene family, and how they may be used to inhibit tissue damage due to complement in both antibody-independent and -dependent systems.

**RCA family**

The RCA gene family, summarized in Table 1 [14, 49], is a genetically linked group of proteins found in a 900-kb region on human chromosome 1, band q32 [12, 16, 66, 93, 95, 96, 106]. Two of the RCA members, factor H and C4 binding protein (C4bp) are serum proteins, while decay-acelerating factor (DAF) [65], membrane cofactor protein (MCP) [62, 65], and complement receptors type 1 and

| RCA proteins | Size (kDa) | Ligands | Binding SCRs | Decay acceleration | Cofactor activity |
|--------------|------------|---------|--------------|-------------------|-----------------|
|              |            |         |              | C3bBb            | C4b2a           | C3b | C4b |
| Factor H     | 160        | C3b     | 1–6 [4, 5]  | +                 | -               | +   | -   |
| C4bp         | 590        | C4b     | 1–3 b [80]  | -                 | +               | -   | +   |
| DAF          | 70         | C3b     | 2–4 [22]    | +                 | +               | -   | -   |
|              |            | C4b     | 2–4         |                   |                 |     |     |
| MCP          | 58–63      | C3b     | 2–4 [2]     | -                 | -               | +   | +   |
|              |            | C4b     | 1–4         |                   |                 |     |     |
| CR1          | 190 c      | C3b     | 8–11, 15–18 | +                 | +               | +   | +   |
|              | C4b        | 1–4 [55, 57–59] | + | + | + | + |
| CR2          | 140        | C3dg    | 1–2 [15, 63] | -                 | -               | d   | -   |
|              | iC3b       | 1–2 [54] |              |                   |                 |     |     |
|              | EBV        | 1–2 [15, 63] |              |                   |                 |     |     |

SCRs, Short concensus repeats; DAF, decay-acelerating factor; MCP, membrane cofactor protein; EBV, Epstein-Barr virus

a Apparent size on SDS-PAGE of nonreduced samples
b Murine C4bp
c Most common allotype
d iC3b
Therapeutic uses of recombinant complement protein inhibitors

Fig. 1. Schematic representations of members of the RCA gene family, with closed boxes representing C4b-binding regions; open boxes, C3b-binding regions; cross-hatched boxes, iC3b-/C3dg-binding regions; O, site of O-linked glycosylation; G, glycolipid anchor; U, domain with unknown function; D, disulfide bridge-containing domain; T, transmembrane domain; and C, cytoplasmic domain

2 (CR1, CR2) [3] are membrane bound. They are structurally related proteins composed of 4 to 30 of the globular domains termed short consensus repeats (SCRs) [92] (Fig. 1). Each SCR is approximately 60 amino acids long, and has several conserved residues, including 4 cysteines which are disulfide linked within each SCR in a 1 to 3, 2 to 4 manner [53, 64]. Finally, the RCA proteins are functionally similar in that they recognize activation products of C3 or C4. Factor H [109] regulates alternative pathway complement activation in the fluid phase and on nonactivating surfaces, while C4bp inactivates classical pathway-activating particles containing C4b [35, 41, 98]. CR2, which has been reported to have weak cofactor activity for the proteolytic degradation of iC3b by the serum protease factor I [74], is more interesting for its signaling role on B lymphocytes [17, 43, 44, 68, 102].

DAF, MCP, and CR1 interact with C3b and C4b and regulate their ability to form C3 and C5 convertases. DAF accelerates the dissociation of the catalytic subunits of alternative and classical pathway C3 convertases, Bb, and C2a, respectively [36], and prevents association with new catalytic subunits. MCP irre-
versibly inactivates C3 or C5 convertases by acting as a cofactor for the proteolytic digestion of C3b or C4b. CR1 has all of these functions. It has been postulated that MCP and DAF have evolved cooperatively to protect autologous cells from complement-mediated damage, and that the function of CR1 is to inactivate C3b and C4b found on complement-activating complexes [6, 99].

The structure and function of DAF, MCP (and CR1) are discussed by Morgan and Meri elsewhere in this volume and a summary of their characteristics is shown in Table 1. However, CR1 is considered in detail here because it is the molecule which has principally been used for therapy.

**CR1 (CD35).** The most common allotype of CR1 is composed of 30 SCRs, 28 of which are arranged in tandemly repeating units of 7 SCRs termed long homologous repeats, or LHRs-A through -D, followed by two additional SCRs, a hydrophobic transmembrane domain, and a 43 amino acid cytoplasmic domain [57, 58, 48]. Deletion mutant analysis showed that the first two SCRs of LHR-A were responsible for C4b binding specificity, while the first two SCRs in LHRs-B and -C specified for C3b binding [58]. Thus, the most common allotype of CR1 has a total of three active binding sites, and is bivalent for C3b. Other more rare allotypes of CR1 [26-29, 113] are generally caused by the duplication or elimination of an intact LHR with C3b-binding function, leading to allotypes with more or fewer C3b-binding sites. This alteration can affect CR1 function, as assessed by the loss of affinity in binding C3b dimers, and a reduction in the effectiveness in inhibiting the C3 and C5 convertases [112]. The genomic structure of CR1 suggests that it evolved by duplication of segments of the gene encoding LHRs, perhaps by homologous recombination with unequal crossover [50, 114]. The extensive internal homologies between LHRs would facilitate their insertion or deletion to create the different alleles encoding the polymorphic allotypes of CR1, which differ by 30–40 kDa, the predicted size of an LHR. An additional CR1-like region [48, 50, 115] is found in the RCA locus, evidence of a non-functional rearrangement of the CR1 genetic elements.

Krych and colleagues [59] have mapped the amino acids in SCRs-8 and -9 which confer C3b-binding ability when transferred to the C4b-binding site in SCRs-1 and -2. Interestingly, these changes did not affect C4b binding. The limits of the intact binding site were mapped with additional deletion mutants [55], showing that the first four SCRs of either LHR-B (SCRs-8 through -11) or -C (SCRs-15 through -18) were necessary and sufficient to reconstitute the binding affinity for polymerized C3b and the cofactor activity of full-length CR1. As the third and fourth SCRs in LHR-A are virtually identical to those in the C3b-binding LHRs, it is postulated that these SCRs play a similar role in C4b binding. When DNA encoding SCRs-8 through -11 is inserted at the amino terminus of the secreted form of an immunoglobulin heavy chain gene and expressed in a cell with an endogenous light chain, the purified chimeric protein retains all C3b-binding and cofactor functions of CR1, and has been shown in vitro to inhibit alternative pathway activation induced in human serum by zymosan [55].

CR1 is present on erythrocytes, B lymphocytes, neutrophils, monocytes, macrophages [31], and eosinophils, as well as on some T lymphocytes [111], follicular dendritic cells [94], glomerular podocytes [28, 56], Kupffer cells [46], and Schwann cells [116]. It was purified on the basis of its factor H-like ability to dissociate the alternative pathway C3 convertase and to act as a cofactor for the
factor I-mediated cleavage of C3b [30]. On a weight basis, it was eight-fold more active than factor H, and was not restricted by alternative pathway activating surfaces. Further studies showed that CR1 could act as a cofactor for C4b cleavage [51], thus inactivating the classical convertases as well as alternative pathway convertases. CR1 also accelerates the decay of each convertase [40, 51]. Unlike cells expressing MCP or DAF, CR1 expression on a cell allows it to rosette with C3b-bearing particles, or to bind soluble polymerized C3b, and to act as a cofactor for the cleavage of C3b and C4b in these particles [69, 70, 97]. Although CR1 has intrinsic activity as well as extrinsic activity [70], it is reasonable to postulate that the 90-nm extension of CR1 from the cell membrane [107] allows control of complement activation on adjacent complexes. CR1 has been implicated in controlling the solubility and clearance of immune complexes [21], participating in endocytosis of complexes and particles by neutrophils and macrophages [1], as well as having other putative immunoregulatory roles [3, 24, 108].

Potential clinical applications of RCA proteins

Complement has been shown to play a role in the pathogenesis of some types of inflammatory tissue damage initiated by both non-immunologic [39, 45] and immunologic [37, 61, 91, 105] conditions. In animal models, removal of classical pathway-activating antibodies [33, 87], depletion of complement by cobra venom factor [19, 32, 39, 61], or testing the models in animals genetically deficient in specific complement components [39, 105] abrogates or delays pathogenesis. Tissue injury can be caused by complement either directly or indirectly. The formation of the MAC on diseased or healthy cells may lead to cell lysis or even to activation, as for example occurs with endothelial cells in which MAC insertion results in calcium flux and expression of binding sites for various thrombolytic mediators [42], as well as release of heparin sulfate from endothelial surfaces to contribute to a hypercoagulable state [86]. Indirectly, the byproducts of C3 and C5 degradation recruit and activate neutrophils, and may synergize with interleukins to cause synthesis of inflammatory mediators such as leukotrienes [11, 60]. Neutrophil activation caused by the complement system has been implicated in damage to organs distinct from those in which activation has occurred, such as in adult respiratory distress syndrome (ARDS) [100].

For a complement inhibitor to be clinically useful, several requirements must be met. First, the molecule should be able to inhibit complement activation at the level of the C5 convertases of both alternative and classical pathways, not only to protect a cell from lysis, as may be achieved by CD59, but also to block the generation of C5a which would induce neutrophil influx and attendant inflammation. Secondly, an inhibitor should have a high affinity for the multivalent complexes of C3b and C4b found in the convertases. Third, it should irreversibly inactivate the convertases, and fourth, the inhibitor should be able to recycle and inhibit multiple convertases.

The members of the RCA family represent potential therapeutic inhibitors that can be evaluated according to these requirements. C4bp and factor H cause irreversible inactivation of C4b and C3b, but they are unlikely to be useful. The former acts only to prevent spontaneous, but not immune complex-induced classical pathway activation. As its major ligand is C4b, damage caused by primary
alternative pathway activation or that recruited by classical pathway activation would be unaffected. The latter serves only to prevent spontaneous but not induced activation of the alternative pathway.

Except for DAF found in the fluid phase, which functions similarly to C4bp in C4b2a decay acceleration [72], DAF and MCP are intrinsic regulators of complement, which function only when incorporated into a cell membrane. DAF does not induce irreversible inactivation of C3b and C4b, is restricted by alternative pathway activating surfaces [83], and is not associated with a clinical phenotype in its absence on Inab blood group erythrocytes [47, 73, 101], all of which suggests a modest inhibitory potential. MCP meets several requirements by acting in both pathways, by irreversibly inhibiting the function of C3b and C4b, and by being able to recycle with additional substrate. These characteristics may indicate that MCP is a suitable candidate for a cell-bound inhibitor, but its monovalency and relatively low affinity suggest that it could not serve as an effective soluble inhibitor.

CR1 meets these requirements by being capable of inhibiting both the classical and alternative pathway C3 and C5 convertases, by interacting multivalently with these complexes through its three binding sites, by irreversibly inactivating C3b and C4b through promoting their proteolysis by factor I, and by recycling for inhibition of additional C3/C5 convertases after release from degraded C3b and C4b.

Applications of soluble CR1

A soluble form of CR1 (sCR1) was made by genetically engineering a stop codon in CR1 cDNA before the transmembrane and cytoplasmic domains [107]. When purified from the media of cells transfected with the altered cDNA, this molecule was able to bind C3b and serve as a cofactor for C3b cleavage. The classical pathway-mediated lysis of sensitized sheep erythrocytes and the alternative pathway activation induced by zymosan were efficiently inhibited by sCR1 at concentrations 100-fold lower than normal serum concentrations of factor H and C4bp [107]. This molecule has been tested for its ability to inhibit damage caused by complement in several models of disease (Table 2).

Non-antibody-dependent complement activation

Reperfusion injury. The complement system was suggested to have a deleterious effect on ischemic myocardium by the finding that administration of cobra venom factor to dogs prior to coronary artery occlusion decreased the size of the myocardial infarction [67]. The maintenance of viable myocardium was correlated to reduced neutrophil infiltration. To determine whether complement activation contributed to reperfusion injury of ischemic myocardium, 1 mg sCR1 was administered as a single bolus injection to rats immediately before temporary occlusion of the left coronary artery for 35 min, after which perfusion was reestablished. Seven days later, the animals were killed and sCR1 was found to have reduced the sizes of the infarcts by 44%. In a second group of rats subjected to myocardial ischemia and reperfusion, the mechanism of the protective effect of sCR1 was shown to be inhibition of MAC deposition on endothelial cells in the
Therapeutic uses of recombinant complement protein inhibitors

Table 2. Complement-dependent disease models in which soluble CR1 reduces pathology

| Complement-dependent disease models | sCR1 effects |
|------------------------------------|--------------|
| **Antibody mediated:**             |              |
| Hyperacute rejection:              |              |
| Cardiac allografts in Lewis rats [88] | Allograft heart survival increased from 3.25 -/+/ 0.81 h to 32 -/+/ 4.47 h |
| Cardiac xenograft (guinea pigs to rats) [89, 117] | Xenograft survival increased from 17 - 23 min to 64 - 747 min in a dose-dependent manner |
| Immune complex-induced inflammation: |              |
| Reversed passive Arthus reaction [119] | Reduces vasculitis |
| Immune complex-induced alveolitis [77] | Reduces alveolitis by 68 - 72% |
| **Non-antibody mediated:**         |              |
| Reperfusion injury:                |              |
| Reperfusion injury of ischemic myocardium in rats [107] | Reduces myocardial infarction size by 44% |
| Reperfusion injury of intestine in rats [45a] | Reduces intestinal and pulmonary injury |
| Thermal trauma:                    |              |
| Acute skin and lung injury by thermal trauma [77] | Reduces damage in lung by 45 - 46% and in skin by 25% (1 hr), 44% (4 h) |

sCR1, soluble CR1

ischemic zones, and suppression of neutrophil accumulation at these sites [107]. This study demonstrated for the first time the potential utility of sCR1 as a therapeutic inhibitor of complement, and has been extended by another study showing reduction of local and remote injury after ischemia-reperfusion of the intestine [45a].

**Thermal trauma.** In a rat model of thermal injury to 25 - 30% of body surface area, sCR1 reduced dermal vascular permeability by 44%, and pulmonary vascular permeability and hemorrhage by 45% [77]. Thus, sCR1 is protective both locally at the primary site of non-immune injury, and distally in the lung in this model of ARDS.

**Antibody-dependent complement activation**

**Hyperacute rejection.** The current shortage of human organs available for transplantation is an incentive to develop systems in which xenogeneic organs could be used [7, 9]. However, the occurrence of natural antibodies directed to endothelial antigens in discordant xenografts [87], or of an alternative pathway activating capability of the xenograft [34, 75, 104] causes rapid rejection by a thrombotic process induced by MAC assembly on endothelial cells [13, 75, 85]. A similar process may account for hyperacute graft rejection that occurs in recipients having alloantibodies specific for donor antigens. The role of complement in this process was confirmed and a potential inhibitor described when human sCR1 was shown to suppress hyperacute rejection of allo- or xenografts. Hyperacute rejec-
tion of cardiac allografts in sensitized Lewis rats was delayed from 3.25 ± 0.81 h in untreated rats to 32.00 ± 4.47 h in animals that received 3 mg/kg sCR1 [88]. When administered to rats immediately prior to reperfusion of guinea pig cardiac xenografts, sCR1 extended graft survival from 17 min in untreated rats to 64 to 747 min, with the time of survival correlating with the dose of CR1 administered, ranging from 3 to 60 mg/kg in this trial [89]. Both treated and untreated animals showed significant IgM deposition in the xenografts, indicating that sCR1 inhibited damage at the level of complement activation, rather than affecting the levels of natural antibody [89, 90]. The use of recombinant RCA proteins expressed in endothelial cells of xenografts is discussed below.

**Immune complex-induced inflammation.** In a rat model of immune complex-induced alveolitis, sCR1 treatment reduced vascular permeability, hemorrhage and neutrophil accumulation by 68–72% [77]. sCR1 also inhibited the reversed passive Arthus reaction in rats, in which intravenous injection of ovalbumin (OVA) is followed by intradermal injection of anti-OVA antibodies [119].

**Other forms of complement inhibitors**

Systemic complement inhibition may be undesirable because of suppressed host defense; the targeting of an inhibitor to a particular tissue may overcome this drawback. In the mapping of the CR1-binding site [55], it was shown that the C3b-binding SCRs could be transferred to the amino termini of antibody heavy chains and retain function. This expression of SCRs near the antigen-binding site had no effect on the ability of the antibody to bind its hapten, 4-hydroxy-3-nitrophenacetyl, and allowed purification of the chimera on hapten-sepharose. Thus, if a monoclonal antibody specific for a particular tissue antigen unique to the site of complement activation were identified, a chimeric CR1/antibody inhibitor could be created that might yield effective local, rather than systemic complement inhibition.

Long-lasting suppression of complement activation by particular cells may be achieved through introduction into such cells of genes encoding complement regulatory proteins. This strategy may be applicable to the creation of endothelial cells having low complement-activating potential, the best example of potential utility being in xenografts because of the incompatibility between DAF, MCP, and CD59 expressed by xenografts and the heterologous source of complement [118].

To provide an experimental rationale for this approach, DAF was incorporated into porcine endothelial cells in a dose-dependent manner, and was shown to confer protection from lysis by 10% human serum [25], a result anticipated by earlier findings on the effects of DAF incorporated into PNH erythrocytes [71, 78], or expressed in murine/human cell hybrids [103]. A more prolonged effect of DAF was achieved in vitro by transfection of murine NIH-3T3 cells with human DAF cDNA, which suppressed their lysis by antibody and human complement [81, 110]. Similarly, high levels of expression of MCP cDNA in NIH-3T3 cells also diminished their sensitivity to classical pathway-mediated lysis [81, 110]. It is hoped that the protection in vitro of cells from lysis by heterologous complement may predict the successful creation of a transgenic donor animal for xenogeneic transplantation [8, 25, 82, 85, 103]. However, the occurrence of hyperacute allo-
Therapeutic uses of recombinant complement protein inhibitors

graft rejection, in which species-specific DAF, MCP, and CD59 [79] are incapable of suppressing complement activation induced by antibodies directed to alloantigens on graft endothelial cells suggests that the expression of these regulatory proteins in xenografts derived from transgenic animals will not avoid hyperacute rejection.

The capacity of sCR1 to suppress hyperacute rejection in a dose-dependent manner for several hours may indicate that this RCA protein should be evaluated for its potential role as a membrane inhibitor of complement activation. Accordingly, preliminary studies have demonstrated that expression of wild-type CR1 in transformed human endothelial cells confers relative resistance to lysis mediated by human complement and rabbit antibody (Braverman, Seok-Yong Kim, and Fearon, unpublished).

Conclusion

In conclusion, it is apparent that researchers are poised at the threshold of developing inhibitors of complement activation from the molecules in the RCA family. By creating soluble forms of these protective proteins for in vivo administration, or by making transgenic animals expressing these proteins or their derivatives, it may be possible to inhibit complement-mediated pathology stemming from autoimmune disease, reperfusion injuries, and physical trauma. This technology combined with current attempts to protect allografts from cellular rejection with monoclonal antibodies against members of the integrin family of adhesion molecules [52] makes it possible that the excessive mortality due to the severe shortage of human donor organs could be overcome by the use of xenografts.

Acknowledgement. We would like to thank Drs. Coyne and Lublin for sharing their data prior to publication.

References

1. Abrahamson DR, Fearon DT (1983) Endocytosis of the C3b receptor of complement within coated pits in human polymorphonuclear leukocytes and monocytes. Lab Invest 48:162
2. Adams EM, Brown MC, Nunge M, Krych M, Atkinson JP (1991) Contribution of the repeating domains of membrane cofactor protein (CD46) of the complement system to ligand binding and cofactor activity. J Immunol 147:3005
3. Ahearn JM, Fearon DT (1989) Structure and function of the complement receptors, CR1 (CD35) and CR2 (CD21). Adv Immunol 46:183
4. Alsenz J, Lambris JD, Schulz TF, Dierich MP (1984) Localization of the complement-component-C3b-binding site and the cofactor activity for factor I in the 38-kDa tryptic fragment of factor H. Biochem J 224:389
5. Alsenz J, Schulz TF, Lambris JD, Sim RB, Dierich MP (1985) Structural and functional analysis of the complement component factor H with the use of different enzymes and monoclonal antibodies to factor H. Biochem J 232:841
6. Atkinson JP, Farries T (1987) Separation of self from non-self in the complement system. Immunol Today 8:212
7. Auchincloss H Jr (1988) Xenogeneic transplantation. A review. Transplantation 46:1
8. Bach FH (1992) Discordant xenografting: a summary and look to the future. Trans Proc 24:739
9. Bach FH, Turman MA, Vercellotti GM, Platt JL, Dalmasso AP (1991) Accommodation: a working paradigm for progressing toward clinical discordant xenografting. Trans Proc 23:205
10. Baker PJ, Osofsky SG (1980) Activation of human complement by heat-killed, human kidney cells grown in cell culture. J Immunol 124:81
11. Bischoff SC, De Weck AL, Dahinden CA (1990) Interleukin 3 and granulocyte/macrophage-colony-stimulating factor render human basophils responsive to low concentrations of complement component C3a. Proc Natl Acad Sci USA 87:6813
12. Bora NS, Lublin DM, Kumar BV, Hockett RD, Holers VM, Atkinson JP (1989) Structural gene for human membrane cofactor protein (MCP) of complement maps to within 100 kb of the 3' end of the C3b/C4b receptor gene. J Exp Med 169:597
13. Calne RY (1970) Organ transplantation between widely disparate species. Trans Proc 2:550
14. Campbell RD, Law SKA, Reid KBM, Sim RB (1988) Structure, organization and regulation of the complement genes. Annu Rev Immunol 6:161
15. Carel J-C, Myones BL, Frazier B, Holers VM (1990) Structural requirements for C3d,g/Epstein-Barr virus receptor (CR2/CD21) ligand binding, internalization, and viral infection. J Biol Chem 265:12293
16. Carroll MC, Aliquot EM, Katzman PJ, Klickstein LB, Smith JA, Fearon DT (1988) Organization of the genes encoding complement receptors type 1 and 2, decay-accelerating factor, and C4-binding protein in the RCA locus on human chromosome 1. J Exp Med 167:1271
17. Carter RH, Spycher MO, Ng YC, Hoffman R, Fearon DT (1988) Synergistic interaction between complement receptor type 2 and membrane IgM on B lymphocytes. J Immunol 141:457
18. Cochrane CG (1984) The role of complement in experimental disease models. Springer Semin Immunopathol 7:263
19. Cochrane CG, Müller-Eberhard HJ, Aitkin BS (1970) Depletion of plasma complement in vivo by a protein of cobra venom: its effect on various immunologic reactions. J Immunol 105:55
20. Couser WG, Baker PJ, Adlers S (1985) Complement and the direct mediation of immune glomerular injury: a new perspective. Kidney Int 28:879
21. Cornacoff JB, Hebert LA, Smead WL, Van Aman ME, Birmingham DJ, Waxman FJ (1983) Primate-erythrocyte immune complex-clearing mechanism. J Clin Invest 71:236
22. Coyne KE, Hall SE, Thompson ES, Arce MA, Kinoshita T, Fujita T, Anstee DJ, Rosse W, Lublin DM (1993) Mapping of epitopes, glycosylation sites, and complement regulatory domains in human decay acceleration factor. J Immunol 149:2906
23. Crawford MH, Grover FL, Kolb WP, McMahon CA, O'Rourke RA, McManus LM, Pinkard RN (1988) Complement and neutrophil activation in the pathogenesis of ischemic myocardial injury. Circulation 78:1449
24. Daha MR, Bloem AC, Ballieux RE (1984) Immunoglobulin production by human peripheral lymphocytes induced by anti-C3 receptor antibodies. J Immunol 132:1197
25. Dalmasso AP, Vercellotti GM, Platt JL, Bach FH (1991) Inhibition of complement-mediated endothelial cell cytotoxicity by decay-accelerating factor. Potential for prevention of xenograft hyperacute rejection. Transplantation 52:530
26. Dykman TR, Cole JL, Iida K, Atkinson JP (1983) Polymorphism of human erythrocyte C3b/C4b receptor. Proc Natl Acad Sci USA 80:1698
27. Dykman TR, Cole JL, Iida K, Atkinson JP (1983) Structural heterogeneity of the C3b/C4b receptor (CR1) on human peripheral blood cells. J Exp Med 157:2160
28. Dykman TR, Hatch JA, Atkinson JP (1984) Polymorphism of the human C3b/C4b receptor. Identification of a third allele and analysis of receptor phenotypes in families and patients with systemic lupus erythematosus. J Exp Med 159:691
29. Dykman TR, Hatch JA, Aqua MS, Atkinson JP (1985) Polymorphism of the C3b/C4b receptor (CR1): characterization of a fourth allele. J Immunol 134:1787
30. Fearon DT (1979) Regulation of the amplification C3 convertase of human complement by an inhibitory protein isolated from human erythrocyte membrane. Proc Natl Acad Sci USA 76:5867
31. Fearon DT (1980) Identification of the membrane glycoprotein that is the C3b receptor of the human erythrocyte, polymorphonuclear leukocyte, B lymphocyte, and monocyte. J Exp Med 152:20
32. Feasby TE, Gilbert JJ, Hahn AF, Neilson M (1987) Complement depletion suppresses Lewis rat experimental allergic neuritis. Brain Res 419:97
33. Fischel RJ, Bolman RM III, Platt JL, Naharian KS, Bach FH, Matas AJ (1990) Removal of IgM anti-endothelial antibodies results in prolonged cardiac xenograft survival. Trans Proc 22:1077
34. Forty J, Hasan R, Cary N, White DJG, Wallwork J (1992) Hyperacute rejection of rabbit hearts by human blood is mediated by the alternative pathway of complement. Trans Proc 24:488
35. Fujita T, Gigli I, Nussenzweig V (1978) Human C4-binding protein. II. Role in proteolysis of C4b by C3b-inactivator. J Exp Med 148:1044
36. Fujita T, Inoue T, Ogawa K, Iida K, Tamura N (1987) The mechanism of action of decay-accelerating factor (DAF). J Exp Med 166:1221
37. Garcia-Estan J, Roman RJ, Lianos EA, Garancis J (1989) Effects of complement depletion on glomerular eicosanoid production and renal hemodynamics in rat nephrotic serum nephritis. J Lab Clin Med 114:389
38. Gelfand MC, Frank MM, Green I (1975) A receptor for the third component of complement in the human renal glomerulus. J Exp Med 142:1029
39. Gelfand JA, Donelan M, Hawiger A, Burke JF (1982) Alternative complement pathway activation increases mortality in a model of burn injury in mice. J Clin Invest 70:1170
40. Gigli I, Fearon DT (1981) Regulation of the classical pathway convertase of human complement by the C3b receptor from human erythrocytes (abstract). Fed Proc 40:1172
41. Gigli I, Fujita T, Nussenzweig V (1979) Modulation of the classical pathway C3 convertase by plasma proteins C4-binding protein and C3b inactivator. Proc Natl Acad Sci USA 76:6596
42. Hamilton KH, Hattori R, Esmon CT, Sims PJ (1990) Complement proteins C5b-9 induce vesiculation of the endothelial plasma membrane and expose catalytic surface for assembly of the prothrombinase enzyme complex. J Biol Chem 265:3809
43. Hebell T, Ahearn JM, Fearon DT (1991) Suppression of the immune response by a soluble complement receptor of B lymphocytes. Science 254:102
44. Heyman B, Wiersma EJ, Kinoshita T (1990) In vivo inhibition of the antibody response by a complement receptor-specific monoclonal antibody. J Exp Med 172:665
45. Heideman M (1979) Complement activation in vitro induced by endotoxin and injured tissue. J Surg Res 26:670
45a. Hill J, Lindsay TF, Ortiz F, Yeh CG, Hechtman HB, Moore FD Jr (1992) Soluble complement receptor type I ameliorates the local and remote organ injury after intestinal ischemia-reperfusion in the rat. J Immunol 149:1723
46. Hinglais N, Kazatchkine MD, Mandet C, Appay MD, Bariety J (1989) Human liver Kupffer cells express CR1, CR3, and CR4 complement receptor antigens. An immunohistochemical study. Lab Invest 61:509
47. Holguin MH, Martin CB, Bernshaw NJ, Parker CJ (1992) Analysis of the effects of activation of the alternative pathway of complement on erythrocytes with a isolated deficiency of decay accelerating factor. J Immunol 148:498
48. Hourcade D, Miesner DR, Atkinson JP, Holers VM (1988) Identification of an alternative polyadenylation site in the human C3b/C4b receptor (complement receptor type 1) transcriptional unit and prediction of a secreted form of complement receptor type 1. J Exp Med 168:1255
49. Hourcade D, Holers VM, Atkinson JP (1989) The regulators of complement activation (RCA) gene cluster. Adv Immunol 45:381
50. Hourcade D, Miesner DR, Bee C, Zeldes W, Atkinson JP (1990) Duplication and divergence of the amino-terminal coding region of the complement receptor 1 (CR1) gene. An example of concerted (horizontal) evolution within a gene. J Biol Chem 265:974
51. Iida K, Nussenzweig V (1981) Complement receptor is an inhibitor of the complement cascade. J Exp Med 153:1138
52. Isobe M, Yagita H, Okumura K, Ihara A (1992) Specific acceptance of cardiac allograft after treatment with antibodies to ICAM-1 and LFA-1. Science 255:1125
53. Janatova J, Reid KBM, Willis AC (1986) Involvement of disulfide bonds in the structure of complement regulatory protein C4bp. FASEB J 2: 892A
54. Kalli KR, Ahearn JM, Fearon DT (1991) Interaction of iC3b with recombinant isotypic and chimeric forms of CR2. J Immunol 147: 590
55. Kalli KR, Hsu P, Bartow TJ, Ahearn JA, Matsumoto AK, Klickstein LB, Fearon DT (1991) Mapping of the C3b-binding site in CR1 and construction of a chimeric (CR1)2-F(ab′)2 complement inhibitor. J Exp Med 174: 1451
56. Kazatchkine MD, Fearon DT, Appay MD, Mandet C, Bariety J (1982) Immunohistochemical study of the human glomerular C3b receptor in normal kidney and in seventy-five cases of renal diseases. J Clin Invest 69: 900
57. Klickstein LB, Wong WW, Smith JA, Weis JH, Wilson JG, Fearon DT (1987) Human C3b/C4b receptor (CR1). Demonstration of long homologous repeating domains that are composed of the short consensus repeats characteristic of C3/C4 binding proteins. J Exp Med 165: 1095
58. Klickstein LB, Bartow TJ, Miletic V, Rabson LD, Smith JA, Fearon DT (1988) Identification of distinct C3b and C4b recognition sites in the human C3b/C4b receptor (CR1, CD35) by deletion mutagenesis. J Exp Med 168: 1699
59. Krych M, Hourcade D, Atkinson JP (1991) Sites within the complement C3b/C4b receptor important for the specificity of ligand binding. Proc Natl Acad Sci USA 88: 4353
60. Kurimoto Y, De Weck AL, Dahinden CA (1989) Interleukin 3-dependent mediator release in basophils triggered by C5a. J Exp Med 170: 467
61. Lennon VA, Seybold ME, Lindstrom JM, Cochrane C, Ulevitch R (1978) Role of complement in the pathogenesis of experimental autoimmune myasthenia gravis. J Exp Med 147: 973
62. Liszewski MK, Post TW, Atkinson JP (1991) Membrane cofactor protein (MCP or CD46): newest member of the regulators of complement activation gene cluster. Annu Rev Immunol 9: 431
63. Lowell CA, Klickstein LB, Carter RH, Mitchell JA, Fearon DT, Ahearn JA (1989) Mapping of the Epstein-Barr virus and C3dg binding sites to a common domain on complement receptor type 2. J Exp Med 170: 1931
64. Lozier J, Takahashi N, Putnam FW (1984) Complete amino acid sequence of human plasma β2-glycoprotein I. Proc Natl Acad Sci USA 81: 3640
65. Lublin DM, Atkinson JP (1989) Decay-accelerating factor and membrane cofactor protein. Curr Top Microbiol Immunol 153: 124
66. Lublin DM, Lemons RS, LeBeau MM, Holers VM, Tykocinski ML, Medof ME, Atkinson JP (1987) The gene encoding decay-accelerating factor (DAF) is located in the complement-regulatory locus on the long arm of chromosome 1. J Exp Med 165: 1731
67. Maroko PR, Carpenter CB, Chiariello M, Fishbein MC, Radvany P, Knostman JD, Hale SL (1978) Reduction by cobra venom factor of myocardial necrosis after coronary artery occlusion. J Clin Invest 61: 661
68. Matsumoto AK, Kopicky-Burd J, Carter RH, Tuveson DA, Tedder TF, Fearon DT (1991) Intersection of the complement and immune systems: a signal transduction complex of the B lymphocyte-containing complement receptor type 2 and CD19. J Exp Med 173: 55
69. Medof ME, Nussenzweig V (1984) Control of the function of substrate-bound C4b-C3b by the complement receptor CR1. J Exp Med 159: 1669
70. Medof ME, Iida K, Mold C, Nussenzweig V (1982) Unique role of the complement receptor CR1 in the degradation of C3b associated with immune complexes. J Exp Med 156: 1739
71. Medof ME, Kinoshita T, Silber R, Nussenzweig V (1985) Amelioration of lytic abnormalities of paroxysmal nocturnal hemoglobinuria with decay-accelerating factor. Proc Natl Acad Sci USA 82: 2980
72. Medof ME, Walter EL, Rutgers JL, Knowles DM, Nussenzweig V (1987) Identification of the complement decay-accelerating factor (DAF) on epithelium and glandular cells and in body fluids. J Exp Med 165: 848
73. Merry AH, Rawlinson VI, Uchikawa M, Daha MR, Sim RB (1989) Studies on the sensitivity to complement-mediated lysis of erythrocytes (Inab phenotype) with a deficiency of DAF (deca-accelerating factor). Br J Haematol 73:248

74. Mitomo K, Fujita T, lida K (1987) Functional and antigenic properties of complement receptor type 2, CR2. J Exp Med 165:1424

75. Miyagawa S, Hirose H, Shirakura R, Naka Y, Nakata S, Kawashima Y, Seya T, Matsumoto M, Uenaka A, Kitamura H (1988) The mechanism of discordant xenograft rejection. Transplantation 46:825

76. Müller-Eberhard HJ (1988) Molecular organization and function of the complement system. Annu Rev Biochem 57:321

77. Mulligan MS, Yeh CG, Rudolph AR, Ward PA (1992) Protective effects of soluble CR1 in complement- and neutrophil-mediated tissue injury. J Immunol 148:1479

78. Ninomiya H, Abe T, Fugita T (1989) Prolongation of red-cell life-span by incorporation of decay-accelerating factor (DAF) into red cells of paroxysmal nocturnal hemoglobinuria (PNH) (letter). Br J Haematol 73:132

79. Nose M, Katoh M, Okata N, Kyogoku M, Okada H (1990) Tissue distribution of HRF20, a novel factor preventing the attack of homologous cells, and its predilection expression on endothelial cells in vivo. Immunology 70:145

80. Ogata RT, Mathias P, Bradt B, Cooper NR (1991) SCRs 1–3 of murine C4b-binding protein (C4-bp) are required for binding to C4b (abstract). Complement Inflamm 8:202

81. Oglesby TJ, White D, Tedja I, Liszewski MK, Wright L, van den Bogarde J, Atkinson J (1991) Protection of mammalian cells from complement-mediated lysis by transfection of human membrane cofactor protein and decay-accelerating factor. Trans Assoc Am Phys 104:164

82. Oglesby TJ, Allen CJ, Liszewski MK, White DJG, Atkinson JP (1992) Membrane cofactor protein (CD46) protects cells from complement-mediated attack by an intrinsic mechanism. J Exp Med 175:1547

83. Pangburn MK (1990) Reduced activity of DAF on complement enzymes bound to alternative pathway activators. Similarity with Factor H. Immunology 71:598

84. Pangburn MK, Müller-Eberhard HJ (1984) The alternative pathway of complement. Spring Semin Immunopathol 7:163

85. Platt JL, Vercellotti GM, Dalmaso AP, Matas AJ, Bolman RM, Najarian JS, Bach FH (1990) Transplantation of discordant xenografts: a review of progress. Immunol Today 11:450

86. Platt JL, Vercellotti GM, Lindman BJ, Oegema TR Jr, Bach FH, Dalmaso AP (1990) Release of heparin sulfate from endothelial cells. Implications for pathogenesis of hyperacute rejection. J Exp Med 171:1363

87. Platt JL, Fischel RJ, Matas AJ, Reif SA, Bolman RM, Bach FH (1991) Immunopathology of hyperacute xenograft rejection in a swine-to-primate model. Transplantation 52:214

88. Pruitt SK, Bollinger RR (1991) The effect of soluble complement receptor type 1 on hyperacute allograft rejection. J Surg Res 50:350

89. Pruitt SK, Baldwin WM III, Marsh HC Jr, Lin SS, Yeh CG, Bollinger RR (1991) The effect of soluble complement receptor type 1 on hyperacute xenograft rejection. Transplantation 52:868

90. Pruitt SK, Baldwin WM III, Marsh HC Jr, Lin SS, Yeh CG, Bollinger RR (1992) Effect of soluble complement receptor type I on natural antibody levels during xenograft rejection. Trans Proc 24:477

91. Quigg RJ, Cybulsky AV, Salant DJ (1991) Effect of nephritogenic antibody on complement regulation in cultured rat glomerular epithelial cells. J Immunol 147:838

92. Reid KBM, Day AJ (1989) Structure-function relationships of the complement components. Immunol Today 10:177

93. Rey-Campos J, Rubenstein P, Rodriguez de Cordoba S (1988) A physical map of the human regulator of complement activation gene cluster linking the complement genes CR1, CR2, DAF, and C4BP. J Exp Med 167:664

94. Reynes M, Aubert JP, Cohen JHM, Audouin J, Tricottet V, Diebold J, Kazatchkine MD (1985) Human follicular dendritic cells express CR1, CR2 and CR3 complement receptor antigens. J Immunol 135:2687
95. Rodríguez de Cordoba S, Dykman TR, Ginsberg-Fellner F, Ercilla G, Aqua M, Atkinson JP, Rubinstein P (1984) Evidence for linkage between the loci encoding for the binding protein for the fourth component of human complement (C4BP) and for the C3b/C4b receptor. Proc Natl Acad Sci USA 81: 789
96. Rodríguez de Cordoba S, Lublin DM, Rubenstein P, Atkinson JP (1985) Human genes for three complement components that regulate the activation of C3 are tightly linked. J Exp Med 161: 1189
97. Ross GD, Lambris JD, Cain JA, Newman SL (1982) Generation of three different fragments of bound C3 with purified factor I or serum. I. Requirements for factor H vs CR1 activity. J Immunol 129: 2051
98. Scharfstein J, Ferreira A, Gigli I, Nussenzweig V (1978) Human C4-binding protein. I. Isolation and characterization. J Exp Med 148: 207
99. Seya T, Atkinson JP (1989) Functional properties of membrane cofactor protein of complement. Biochem J 264: 581
100. Solomkin JS, Cotta LA, Satoh PS, Hurst JM, Nelson RD (1985) Complement activation and clearance in acute illness and injury: evidence for C5a as a cell-directed mediator of the adult respiratory distress syndrome in man. Surgery 97: 668
101. Tate CG, Uchikawa M, Tanner MJ, Judson PA, Parsons SF, Mallinson G, Anstee DJ (1989) Studies on the defect which causes absence of decay-accelerating factor (DAF) from the peripheral blood cells of an individual with the Inab phenotype. Biochem J 1261: 489
102. Tuveson DA, Ahearn JM, Matsumoto AK, Fearon DT (1991) Molecular interactions of complement receptors on B lymphocytes: a CR1/CR2 complex distinct from the CR2/CD19 complex. J Exp Med 173: 1083
103. Wang M-W, Wright LJ, Sims MJ, White DJG (1991) Presence of human chromosome 1 with expression of human decay-accelerating factor (DAF) prevents lysis of mouse/human hybrid cells by human complement. Scand J Immunol 34: 771
104. Wang M-W, Johnston PS, Wright LJ, Lim SML, White DJG (1992) Immunofluorescent localization of pig complement component 3, regardless of the presence or absence of detectable immunoglobulins, in hyperacutely rejected heart xenografts. Histochem J 24: 102
105. Watson WC, Townes AS (1985) Genetic susceptibility to murine collagen II autoimmune arthritis. Proposed relationship to the IgG2 autoantibody subclass response, complement C5, major histocompatibility complex (MHC) and non-HMC loci. J Exp Med 162: 1878
106. Weis JH, Morton CC, Bruns GAP, Weis JJ, Klickstein LB, Wong WW, Fearon DT (1987) A complement receptor locus: genes encoding C3b/C4b receptor and C3d/Epstein-Barr virus receptor map to 1q32. J Immunol 138: 312
107. Weisman HF, Bartow T, Leppo MK, Marsh HC Jr, Carson GR, Concino MF, Boyle MP, Roux KH, Weisfeldt ML, Fearon DT (1990) Soluble human complement receptor type 1: in vivo inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis. Scienec 249: 146
108. Weiss L, Delfraissy J-F, Vazquez A, Wallon C, Galanaud P, Kazatchkine MD (1987) Monoclonal antibodies to the human C3b/C4b receptor (CR1) enhance specific B cell differentiation. J Immunol 138: 2988
109. Whaley K, Ruddy S (1976) Modulation of the alternative complement pathway by β1H globulin. J Exp Med 144: 1147
110. White DJG, Oglesby T, Liszewski MK, Tedja I, Hourcade D, Wang M-W, Wright L, Wallwork J, Atkinson JP (1992) Expression of human decay-accelerating factor or membrane cofactor protein genes on mouse cells inhibits lysis by human complement. Trans Proc 24: 474
111. Wilson JG, Tedder TF, Fearon DT (1983) Characterization of human T lymphocytes that express the C3b receptor. J Immunol 131: 684
112. Wong WW, Farrell SA (1991) Proposed structure of the F' allotype of human CR1. Loss of a C3b binding site may be associated with altered function. J Immunol 146: 656
113. Wong WW, Wilson JG, Fearon DT (1983) Genetic regulation of a structural polymorphism of human C3b receptor. J Clin Invest 72: 685
114. Wong WW, Kennedy CA, Bonaccio ET, Wilson JG, Klickstein LB, Weis JH, Fearon DT (1986) Analysis of multiple restriction fragment length polymorphisms of the gene for the
human complement receptor type 1. Duplication of genomic sequences occurs in association with a high molecular mass receptor allotype. J Exp Med 164:1531
115. Wong WW, Cahill JM, Rosen MD, Kennedy CA, Bonaccio ET, Morris MJ, Wilson JG, Klickstein LB, Fearon DT (1989) Structure of the human CR1 gene. Molecular basis of the structural and quantitative polymorphisms and identification of a new CR1-like allele. J Exp Med 169:847
116. Vedeler CA, Nilsen R, Matre R (1989) Localization of Fe receptors and complement receptors CR1 on human peripheral nerve fibers by immunoelectron microscopy. J Neuroimmunol 23:29
117. Xia W, Fearon DT, Moore FD Jr, Schoen FJ, Ortiz F, Kirkman RL (1992) Prolongation of guinea pig cardiac xenograft survival in rats by soluble human complement receptor type 1. Trans Proc 24:479
118. Yang Q-E, Want YY, Bollinger RR, De Buyscher EV (1992) Discordant complement systems as a factor in hyperacute xenograft rejection. Trans Proc 24:481
119. Yeh GC, Marsh HC Jr, Carson GR, Berman L, Concino MF, Sciesney SM, Kuestner RE, Skibbens R, Donahue KA, Ip SH (1991) Recombinant soluble human complement receptor type 1 inhibits inflammation in the reversed passive arthus reaction in rats. J Immunol 146:250