ANTI-INFLAMMATORY AND IMMUNOSUPPRESSIVE EFFECTS OF RECOMBINANT SOLUBLE COMPLEMENT RECEPTORS

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BACKGROUND

Despite the devastation wrought by the human immunodeficiency virus (HIV) which serves as a reminder of our dependency on the immune sytem for our viability, there is still an imperative need for developing anti-inflammatory and immunosuppresive therapies for the purpose of preventing tissue damge associated with autoimmune diseases. In fact, the infectious complications of HIV emphasize that an important goal for investigators is to target such therapy in a way that does not cause generalized suppression of host defense capabilities. The complement system is especially relevant in this regard, because we know that individuals having inherited deficiencies of complement proteins are not usually susceptible to viral infections, and may be adequately protected from bacterial infections by prophylactic antibiotics or specific immunizations. Nevertheless, there is substantial evidence for the activation of the complement system in many human diseases and for the dependency on the complement system of animal models of these human diseases (Table 1). Therefore, inhibitors of the complement system may be extremely useful therapeutic agents in inflammatory and autoimmune diseases.

Table I
Animal Models of Human Diseases in which Tissue Injury is Complement Dependent[a]

Autoimmune model	"Nonspecific" tissue injury
Myasthenia gravis	Burn
Experimenal allergic encephalitis	Myocardial ischemia
Heymann's nephritis	
Immune complex-induced vasculitis	
Collagen-induced arthritis	

[a]Suppressed by cobra venom factor or does not occur in C5-deficient mice

The complement system has two distinct functions: the induction of an inflammatory response, and the enhancement of the primary immune response. The former probably is the phylogenetically oldest function of the complement system and is mediated by products of C3 and C5 activation that cause changes in vascular permeability (C3a, C5a), leukocyte adhesion, migration, biosynthetic and secretory activities (C5a), and direct membrane damage (C5b-9) (reviewed in 1-4). The latter function is mediated by the interaction of C3dg that is bound to complement-activating antigen with the complement receptor, CR2 (CD21), on B lymphocytes. Our strategies for interrupting these two processes of the complement system are the use of soluble forms of complement receptors, CR1 (CD35) for inhibition of complement-dependent inflammation, and CR2 for blocking complement-dependent augmentation of B cell activation.

SOLUBLE CR1 AS AN INHIBITOR OF COMPLEMENT ACTIVATION

The first molecular identification of CR1 was as a membrane protein of erythrocytes that inhibited the alternative complement pathway in a manner similar to that of factor H: dissociating Bb from the C3b,Bb convertase, and promoting the cleavage of C3b by factor I to iC3b and C3dg (reviewed in 5). CR1

differed from H in three important respects, however, that suggested that it may be even more effective at suppressing complement activation at the C3/C5 convertase step: it was more active on a molar basis; it interacted equally well with C3b on alternative pathway activating and non-activating surfaces, whereas H bound well only to C3b on a non-activating surface; and it had the functions of C4-binding protein (C4-bp). Thus, CR1 combined all the functions of both the soluble and membrane regulatory proteins in being capable of dissociating the convertases of both pathways, and of promoting the factor I-mediated cleavage of C3b and C4b (Table 2). The biologic rationale for these inhibitory functions of CR1 may be that the receptor serves to capture complement-activating complexes when sufficient amounts of C3b and C4b have been covalently attached, after which further complement activation is not required and the C3b and C4b would be processed to non-complement-activating forms. In the case of C3b, the inactivation also results in the generation of iC3b and C3dg that are ligands for the signal transducing receptors, CR2 and CR3. This sequence of uptake, processing and transfer would require potent C3b- and C4b-inactivating functions and probably accounts for the role of CR1 in the immune complex clearance function of erythrocytes, and in the CR1-CR2 molecular complex on B lymphocytes. The full potential of CR1 for blocking complement activation could be assessed when a soluble form of the receptor was engineered by introducing a translational stop codon at the junction of the extracytoplasmic and the carboxyterminal transmembrane domains (6). This recombinant protein, termed sCR1, bound dimers of C3b or C4b bivalently with nM Kd's, consistent with the simultaneous use of both C3b-binding sites or of the single C4-binding site in combination with one of the C3b-binding sites. The sCR1 also was a co-factor for the factor I-mediated cleavage of both C3b and C4b. Most importantly, sCR1 suppressed alternative and classical pathway activation in whole serum in a nM concentration range, fulfilling the prediction that it would be more effective than the endogenous regulatory proteins of the C3/C5 convertases, factor H and C4-bp, this finding indicated that sCR1 is more than 100-fold more effective than these two proteins.

Table II
Regulatory Functions of the RCA Family of Proteins

Protein	Dissociation of C3/5 convertases		Factor I-cofactor		Restriction by alternative pathway activators
	Alternative	Classical	C3b	C4b	
Factor H	+	-	+	-	Yes
C4-bp	-	+	-	+	Not applicable
DAF	+	+	-	-	Not known
MCP	-	-	+	+	Not known
CR1	+	+	+	+	No

The first *in vivo* study of the effects of SCR1 on complement-dependent inflammation was of the myocardial reperfusion injury (6). In this model, sCR1 or buffer was administered to rats immediately before the left anterior descending coronary artery was ligated for 35 minutes. After the ligation was released, the rats were assessed two hours later for evidence of complement activation and leukocyte infiltration in the myocardium at risk, or one week later for quantitation of the size of myocardial infarction. Complement activation, as assessed by immunoperoxidase staining with monoclonal antibody to the C5b-9 complex, occurred along endothelial cells in the injured myocardium of the control rats, and this was suppressed to an undetectable level in the sCR1-treated rats. Many leukocytes appeared to be attached to endothelial cells in the control rats, and their number was decreased by two-thirds in the sCR1-treated rats. This suppression of complement activation and inflammation was associated with a 45% decrease in the size of the myocardial infarction. Thus, sCR1 was an effective complement inhibitor and anti-inflammatory agent in this model of non-immunologic tissue injury.

Several problems may limit the number of complement-dependent diseases in which sCR1 may be a practical *in vivo* agent, the principal issue being its rapid clearance from blood. However, this problem

may be solved by creating chimeric proteins with the active sites of CR1 fused to other proteins having longer half-lives, such as immunoglobulin. A chimeric construct containing short consensus repeats (SCRs) 8-11, one of the two C3b-binding sites of CR1, and an $F(ab')_2$ has been created and shown to be as effective as sCR1 for binding to dimers of C3b, promoting the cleavage of C3b by factor I, and inhibition of alternative pathway activation (7). In addition to having extended plasma half-lives, CR1-Ig chimeras with appropriate antibody specifities could be targeted to certain sites at which complement activation was occurring. A second solution might be the tissue-specific expression of wild type, membrane-associated CR1 by the use of constructs in which transcription is driven by tissue specific promotors. The expression of membrane CR1 at sites of complement activation would negate the need to repeatedly administer a soluble form of the receptor.

SOLUBLE CR2 AS AN IMMUNOSUPPRESIVE AGENT

The absence of C3 or of the classical pathway components, C4 and C2, that form the C3-activating enzyme, C4b,2a, impairs the humoral immune response to T-dependent and T-independent antigens, the impairment manifesting as a requirement for higher doses of antigen to elicit a primary antibody response and to prime for a secondary immune response (reviewed in 5,8). Inherited deficiencies of C1, C4 and C2 also are associated with low plasma concentrations of IgG3 in man, higher concentrations of rheumatoid factor in the guinea pig, and the occurrence of an autoimmune syndrome in man closely resembling systemic lupus erythematosus. The immunoregulatory functions of complement are almost certainly mediated by the interaction of C3 fragements covalently bound to antigen with cellular C3 receptors, and CR2 is the best candidate for this receptor: it binds the terminal cleavage fragment, C3dg, and it is expressed on cell types involved in immune responses: all mature B cells, follicular dendritic cells, and some T cells and thymocytes. Three recent studies support this conjecture.

First, Heyman et al. suppressed the primary response to T-dependent antigen in mice by the administration of rat monoclonal antibodies to murine CR1 that crossreact with CR2 (9). The suppression was complete at lower doses of antigen, was observed with both particulate antigen, erythrocytes, and soluble antigen, KLH, and was most effective with an antibody that blocked C3d binding to CR2 *in vitro*. However, this study could not exclude some contribution of CR1; indeed, as CR2 may be a truncated form of CR1 in the mouse; it would not be possible to develop monoconal antibodies reactive only with CR2. Therefore, this study indicated that complement receptors were important to the enhancing function of complement in the immune response, and suggested that CR2 was the critical receptor, but could not exclude the participation of CR1.

The second study supporting a role for CR2 rather than CR1 is biochemical, involving an analysis of the membrane proteins on B cells that associate with these two receptors (10,11). Immunoprecipitation of CR1 and CR2 from digitonin lysates of surface labelled B cells revealed that CR1 was associated with CR2, and that CR2 was associated with CR1 and with a complex of membrane proteins containing CD19, a B cell-specific member of the immunoglobulin superfamily, TAPA-1 and Leu-13 (12), proteins which are not specific for B cells. However, anti-CR1 did not co-precipitate the CD19 complex, nor did anti-CD19 co-precipitate CR1 although CR2 was present in this immunoprecipitate. These findings indicated that CR2 is present in two distinct complexes on the B cell: a CR1-CR2 complex which is presumed to have a role in capturing C3b-containing immume complexes for proteolytic processing and transfer to CR2, and a CD19-CR2 complex which has signal transducing functions. CR1 does not associate with the CD19 complex directly or through its interaction with CR2, accounting for its inability to enhance synergistically activation of phospholipase C (PLC) by membrane Ig, a function of CR2. A central role for the CD19 complex in the biology of the B cell is likely: CD19 is a member of the Ig superfamily (13,14); it is expressed throughout the ontogeny of the B cell, except at the plasma cell stage; and ligation of CD19 with antibody induces the activation of an unkown protein tyrosine kinase and of PLC. Thus, the CD19-CR2 complex represents a molecular link between the complement and immune systems, with CR2 serving as a complement ligand-binding subunit and CD19 as an endogenous component of the immune system with both signal transducing function and binding activity for an as yet unknown ligand of the immune system.

The third study identifying CR2 as the relevant receptor mediating the immune enhancing function of complement used a soluble form of CR2 for suppression of *in vivo* immune responses in the mouse (15). In this recombinant protein, the amino terminal two SCRs of CR2, which carry the C3dg binding site of the receptor, were fused to the amino termini of the two heavy chains of a non-complement activating, IgG1 anti-nitrophenacetyl antibody. The CR2-IgG1 bound polymers of C3dg bivalently with a Kd in the nM

range, and competed with cellular CR2 for C3dg polymers in this concentration range. As would be anticipated based on the prior mapping of the binding site in CR2 for the Epstein-Barr virus (EBV) to these same repeats, this chimeric protein also blocked the infection of B cells by EBV. Administration of CR2-IgG1 to mice at the time of immunization with sheep erythrocytes (E) suppressed the generation of specific IgM and IgG anti-sheep E to the same extent as did depletion of C3 by treatement of other mice with cobra venom factor; in control mice receiving IgG1 lacking the CR2 domains, the immune response occurred. Inhibition by CR"-IgG1 of antibody responses to KLH, a soluble T-dependent antigen, and fluorescein-Ficoll, a T-independent antigen, was also observed. As IgG antibody to soluble antigens enhance the immune response, the immunosuppressive effects of CR2-IgG1 are not based on interaction with Fc receptors on B cells. Therefore, the specific inhibition of the interaction of ligand with CR2, which was achieved without perturbing the B cell as occured with anti-receptor antibodies, blocked complement-dependent immune responses, indicating that the CD19-CR2 complex promotes the B cell response to antigen. Determination of the molecular biology of signal transduction of the CD19 complex, and of the immune system ligand for CD19 will define the contribution of this complex to the immune response. It is likely that this contribution will be critical as this complex apparently was selected during evolution by the complement system.

SUMMARY

CR1 and CR2 have served as unusual probes for the analysis of the two major functions of the immune system involving inflammation and the immune response, respectively. CR1, or some construct containing its active site SCRs, may find a role in the therapy of complement-dependent tissue injury, and may be used to define which diseases are caused by the inappropriate or excessive activation of this system. Although soluble forms of CR2 may be shown to have potential clinical utility when foreign antigen is given prospectively, as in monoclonal antibody therapy, perhaps the most important finding emanating from the analysis of this receptor is the recognition of a previously unrecognized membrane protein complex whose role in B cell development is yet to be determined. It is reasonable to predict that the function of the CD19 comlex will be significant as it serves as the link between two evolutionarily distinct systems that share a common purpose of anti-microbial host defense.

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