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PHARMACOLOGICAL MANIPULATION OF THE COMPLEMENT SYSTEM IN HUMAN DISEASES.

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1. INTRODUCTION

Complement is one of the powerful effector systems involved in the body's defense. When present in a dormant state it can, in concert with other components of immune system, protect the individual from foreign pathogens. However, inappropriately activated complement can cause disease. Several disease states such as immune complex and autoimmune diseases and deficiencies of some complement regulators are associated with inappropriate activation of complement. In some diseases complement is activated for a long or indefinite period while in others for a comparatively short time; in some it is activated systemically, in others locally; in some whole cascade is activated, in others only a few components are activated; in some classical pathway is activated, in others alternative pathway. In some diseases activation of complement takes place on cell and tissue surfaces. In many complement activating diseases biological activities of complement fragments become detrimental resulting in tissue injury and disease. Inhibition of complement by specific inhibitors is likely to arrest complement mediated disease processes.

From this point of view, some laboratories are developing low molecular weight synthetic

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inhibitors whereas others are focusing on the development of high molecular weight plasma or cell surface complement inhibitors in their natural or recombinant forms for therapeutic purposes. A review concerning development of low molecular weight inhibitors with the eventual aim of manipulating complement system in human diseases was recently published (1,2). This review is concerned with high molecular weight natural or recombinant complement inhibitory molecules in human plasma or cell membranes, some of which are already in clinical use.

2. THE COMPLEMENT SYSTEM

Complement system has recently been reviewed in detail (3-5); only a passing reference of this system will be made here. Activation of complement occurs via classical and alternative pathways. Activation of both pathways serve to covalently opsonize surfaces of foreign invading microorganisms with C3b and/or C4b. C3b and C4b on foreign cell surfaces serve as the building blocks for the formation of C3/C5 convertases of classical and alternative pathways. These convertases amplify the initial deposition of C3b and catalyze the formation of C5b fragment which causes self assembly of C5b-C9 complex, known as membrane attack complex (MAC). This complex causes osmotic lysis of the invading microorganisms. During the activation of both pathways, C3 and C5 breakdown products , C3a and C5a, are formed. These anaphylatoxins play a role in inflammation; they release histamine from mast cells. These products lead to vasodilation and increased vascular permeability. C5a chemotactically attracts neutrophils. C3b and C4b on the surface of the opsonized foreign pathogens act as ligands for complement receptors present on phagocytic cells eventually leading to phagocytosis of the pathogens.

3. REGULATION OF COMPLEMENT ACTIVATION

The complement system has powerful cytolytic activity against which individual's own cells (self cells) should be protected. Several proteins have evolved to control the extent of complement activation in fluid phase and surfaces of self cells (for recent reviews see 6-9). The proteins which inhibit complement activation in fluid phase serve to limit the generation of complement fragments such as C4b and C3b. They also render the generated fragments inactive thereby reducing the extent of cellular damage. These proteins include C1-inhibitor (C1-INH) (10,11), C4-binding protein (C4BP) (12-14), factor H (13-15) and factor I (13,14). Some of the fluid phase proteins such as clusterin (16) and vitronectin (17) inhibit the formation of cytolytic MAC. C4b and C3b which have escaped inactivation by fluid phase inhibitors of complement activation and are already fixed to self cells, are inactivated by cell membrane regulators of complement such as decay accelerating factor (DAF) (7,9,18,19), membrane cofactor protein (MCP) (7,9,20) and complement receptor 1 (CR1) (7,9,21,22). Some of the cell membrane proteins, CD59 (7,23-25) and homologous restriction factor (HRF) (7,26), render MAC non-cytolytic while it is being formed on the self cell. Thus, fluid phase inhibitors in conjunction with membrane embedded inhibitors protect cells from autologous complement. The individual functions of these fluid phase and cell surface complement regulatory proteins are summarized in Table 1.

4. THERAPEUTIC USES OF HIGH MOLECULAR WEIGHT INHIBITORS OF COMPLEMENT

4.1. C1-Inhibitor (C1-INH)

C1-inhibitor has recently been reviewed (10,11). It interacts with the activated C1. Interaction of C1-INH with activated C1 complex leads to the dissociation of C1q subunit and formation of C1r-C1s- $(C1\text{-}INH)_2$ complex. Inhibition of C1r and C1s results in the inhibition of cleavage of C4 and C2 by the activated C1s. The inhibition of C1 by C1-INH is about 100 times more effective in fluid phase than on the cell surface (27). Thus C1 activated in a fluid phase can be inhibited much more easily than the C1 activated on the target cell surface.

The regulation of complement at C1 stage is abrogated in patients with genetic deficiency of C1-INH (hereditary angioneurotic edema; HANE), patients with cancer undergoing treatment with IL-2 and septic patients. C1-INH is beneficial in these situations.

4.1.1. Treatment of hereditary angioneurotic edema (HANE)

HANE has recently been reviewed (10,11). In this disease, occasional bouts of acute edema occur in extremities, gastrointestinal tract or orificial areas. Approximately 85% of the patients have type I HANE in which C1-INH structure and function is normal but its plasma levels are low (5-30% of normal) and about 15% have type II HANE in which C1-INH protein is structurally and functionally abnormal but its plasma levels remain normal or even elevated. Anxiety or trauma can precipitate attacks of edema in these patients. The mechanism of attack of edema has not yet been firmly established. It is believed that any event that can cause local depletion of C1-INH in HANE can cause local activation of C1 in fluid phase. Local depletion of C1-INH in HANE can easily occur due to low levels of this inhibitor. Activated C1 can then cleave C4 and C2. C2 fragment is further cleaved by plasmin to a small vasoactive peptide, C2-

Clusterin	Prevents the assembly of cytolytic MAC	16
Vitronectin	Prevents the assembly of cytolytic MAC	17
Cell membrane regulators		
1. C3/C5-convertase formation stage		
DAF	Dissociates C2a and Bb from C4b and C3b	7,9,18,19
MCP	Cofactor for factor I in cleaving C4b/C3b	7,9,20
CR1	Cofactor for factor I in cleaving C4b/C3b and decay acceleration of C3/C5-convertases	7,9,21,22
2. MAC formation stage		
CD59	Inhibits formation of cytolytic MAC on self cell	7,23-25
HRF	Inhibits formation of cytolytic MAC on self cell	7,26

* for unabbreviated names see the text.

kinin. This kinin is thought to be responsible for the attack of edema in HANE.

C1-INH prepared from normal plasma in concentrated form is used as short term replacement therapy for the treatment of attacks of HANE (10,11). This therapy is life saving in laryngeal edema. Surgery can precipitate attack of edema which under certain circumstances can be life threatening. Surgery can, however, be safely performed on these patients if they are pre-treated with high doses of C1-INH and thus have acquired normal functional levels of C1-INH (28). Since the discovery that in HANE patients attenuated androgens can increase plasma C1-INH levels to normal in few days, attenuated androgens such as danazol are used on long term basis to prevent attack of edema (1,29).

4.1.2. Control of toxicity caused by Interleukin-2 (IL-2) Immunotherapy

C1-INH treatment is beneficial in patients undergoing IL-2 therapy. IL-2 treatment can induce partial or complete remission in advanced melanoma and renal carcinoma (30) but its use has been limited due to its toxicity. IL-2 treatment causes a life threatening vascular leakage syndrome characterized by hypotension and other changes similar to those seen in septic shock. During IL-2 therapy, complement (via classical pathway) as well as contact system are activated. Both these systems are regulated by C1-INH. Vascular leakage syndrome is associated with the activation of complement. In a pilot study on six patients with metastatic melanoma and renal cell carcinoma receiving high doses of IL-2, C1-INH treatment resulted in inhibition of complement activation. The authors concluded that C1-INH therapy leads to reduced IL-2 toxicity. This pilot study warrants further investigations on the ability of C1-INH alone or in combination with any of the inhibitors of complement described below to reduce IL-2 induced toxicity.

4.1.3. Treatment of sepsis

Complement and contact systems are activated by microorganisms and inhibited by high doses of C1-inhibitor. In an experimental model of endotoxic shock in dogs one group received *Escherichia coli* (*E coli*) endotoxin and the other C1-INH before *E coli* endotoxin was administered. In the latter group of animals, C1-INH prevented endotoxin-induced pulmonary dysfunction namely hypoxemia (30). High doses of C1-INH have been safely administered to patients with septic shock in whom C1-INH turnover is increased. C1-INH reduced such complications as activation of complement and contact system and hypotension. It reduced mortality in pilot studies (31). Further studies are needed to establish whether C1-INH can reduce mortality and morbidity in septic patients.

4.2. Intravenous Immunoglobulin

Intravenous immunoglobulin is prepared from plasma of a large number of healthy donors (for recent reviews see 32-34). This product consists of 95% IgG in same proportion of its subclasses as in plasma. Depending on the number of donors from whom plasma pool has been prepared, intravenous immunoglobulin contains variable amounts of IgG dimers. The higher the number of donors, the higher the content of such dimers. Intravenous immunoglobulin prepared from a large plasma pool (100,000 donations) may contain up to 30-40% dimers. These dimers are made up of idiotype (Id) IgG molecules and a complementary anti-Id antibody (35). Commercially available intravenous immunoglobulins are prepared by different methods, contain different additives, vary in pH (4-6), and may contain IgA.

Intravenous immunoglobulin exhibits a number of immunomodulating properties (32-34). Since it is prepared from the plasma of a large number of donors, it contains antibodies directed against a variety of antigens, self antigens (natural autoantibodies) and self antibodies (anti-idiotypic antibodies). *In vivo*, it inhibits B-cell activation and autoantibody production, neutralizes circulating pathogenic antibodies and suppresses antibody-dependent cellular cytotoxicity (ADCC) by FcgR blockade. *In vitro*, it inhibits pokeweed mitogen-stimulated antibody production, neutralizes multiple autoantibodies through its antiidiotype reservoir and exhibits pronounced effects on T-cell mediated immunity (36,37). It enhances CD8 positive suppressor T-cell function *in vivo* and *in vitro* (36), and reversibly inhibits proliferation of antigen specific as well as antigen independent stimulation of lymphocytes (37). Intravenous immunoglobulin also inhibits the proliferation of a wide variety of hybridomas and cell lines. Further experiments showed that it arrested the cell cycle in G0-/G1-phase; inhibiting progression into S-phase (36). Intravenous immunoglobulin also inhibits the activation and function of NK cells (38) and interferes with the cytokine network by inhibiting the release of cytokines (39). It contains autoantibodies that react with some cytokines such as IL-1 leading to the neutralization of their activities (40). In addition, intravenous immunoglobulin can upregulate IL-1 receptor antagonist (41). It can also inhibit the complement system; it inhibits complement activation at C1 stage (42) and at the stage of binding of complement fragments (C3b and C4b) to target cells (43). It is not currently known whether besides these inhibitory activities, intravenous immunoglobulin exerts similar activities at other sites of complement cascade. Besides its use in replacement therapy in primary immunodeficiencies, it has been found to be beneficial in more than 30 immunological diseases (32-34). The mechanisms for the beneficial effects of intravenous immunoglobulin in these diseases are currently unknown. Although the role of inhibition of complement as the basis for the beneficial effects of intravenous immunoglobulin has not been established as yet, it is interesting to note that most diseases in which it exerts beneficial effects are complement-mediated.

Some of the diseases in which efficacy of intravenous immunoglobulin has been tested in a relatively large number of patients and has been firmly established are described below.

4.2.1. Autoimmune thrombocytopenic purpura (ITP)

Administration of intravenous immuno-globulin is efficacious in acute ITP in children and is considered treatment of choice (for recent review see 33,44). In adults with acute ITP, treatment with intravenous immunoglobulin is likely to produce long term remission during the first six months rather than latter. In children and adults with ITP, autoantibodies to platelet glycoproteins IIb/IIa and Ib/IX are present in plasma and on platelets. Platelet counts in these patients is very low but following intravenous immunoglobulin treatment (400mg/kg/day) it rises to approximately 30 x 10^9 per liter within 48 hours of the therapy (33,45,46). If platelet count is not significantly increased during this time, additional dose is given daily for five days.

Following treatment with intravenous immunoglobulin, about 62% of children with chronic ITP undergo long term remissions. In adults with chronic ITP the response rate is lower. However, in the majority of patients a substantial but short term increase in platelet count is observed (33,45,46).

An idiotypic mechanism and blockade of FcR on reticuloendothelial cells may be the main mechanism of action of intravenous immunoglobulin in ITP (33,44). In short term, inhibition of complement and in long term, decrease in anti-platelet antibody production are likely to be the mechanisms by which intravenous immunoglobulin protects platelets from destruction.

4.2.2. Kawasaki disease

Kawasaki disease is a leading cause of acquired heart disease in children. This disease is characterized by rash, edema and erythema of hands and feet and aneurysms of coronary arteries. Intravenous immunoglobulin therapy reduces early inflammatory symptoms and decreases the incidence of coronary abnormalities in this disease (for recent reviews see 33,47-49). It has been suggested that activation of complement in this disease is mediated by bacterial toxins which results in the release of cytokines in a manner reminiscent to that seen in toxic shock syndrome (33,49,50). It has been shown that intravenous immunoglobulin neutralizes these toxins and their effects (33,49,50). Intravenous

immunoglobulin suppresses T- and B- cell activation and excessive production of cytokines such as IL-1, IL-6, TNF and T-cell cytokines (probably caused by C activation), that appear during acute phase of vasculitis in Kawasaki disease. Immediate resolution of fever by intravenous immunoglobulin therapy could be due to its ability to neutralize IL-1 and IL-6. Coronary artery damage in Kawasaki disease appears to be mediated by autoantibodies to endothelial cells. These antibodies induce complement mediated injury to cytokine-stimulated endothelial cells. Intravenous immunoglobulin can perhaps inhibit the production and reactivities of these antibodies by an idiotypic mechanism. During the first ten days of the disease, intravenous immunoglobulin (400mg/kg/day for five days) in conjunction with aspirin is the standard method of treatment for the prevention of the development of aneurysms of coronary arteries.

4.2.3. Myasthenia gravis

In myasthenia gravis, autoantibodies to acetylcholine receptor (AChR) are present in circulation and at the neuromuscular junction. Intravenous immunoglobulin (400 mg/kg/day for 5 days) results in clinical improvement in a majority of the patients within 24 hours to three weeks (for recent reviews see 33,51,52). The improvement is associated with a decrease in the levels of anti-AChR antibodies. These effects last for 40-49 days. It is currently not known as to how much of the clinical improvement is due to inhibition of complement. Treatment with intravenous immunoglobulin allows a reduction in the dose of corticosteroids and immunosuppressive drugs and reduces symptoms during the time required for these therapies to be effective.

4.2.4. Chronic inflammatory demyelinating polyneuropathy (CIDP)

CIPD is a chronic form of Guillian Barré Syndrome. Patients with CIPD have antibodies reactive with myelin, Schwann cells and other nerve structures. These antibodies can activate complement. Intravenous immunoglobulin therapy (400 mg/kg/day for five days; treatment is repeated if necessary) has been shown to be beneficial in this disease (33,53). A vast majority of these patients make a satisfactory recovery within six months of treatment.

4.2.5. Other diseases

In small groups of patients the role of intravenous immunoglobulin in the treatment of many diseases has been tested and found to have beneficial effects. For example, the results of different pilot studies have shown that intravenous immunoglobulin prevents complement-mediated destruction of fetal allografts in recurrent spontaneous abortions (for a recent review see 54). In a study, five women who among them had twenty three abortions were given 500 mg intravenous immunoglobulin per month starting before conception. Four gave birth to healthy infants and fifth was at twelve weeks of gestation (55).

Some of the diseases in which effects of intravenous immunoglobulin therapy have been tested in small groups of patients and found to have beneficial effects are listed in Table II. Since different workers have used different regimens of treatment in these diseases, in some cases in combination with other treatments, readers may refer to the original articles for methods of treatment.

4.3. Factor I, Factor H And C4-Binding Protein (C4BP)

A complement inhibitor is likely to be effective in complement-mediated diseases if it inhibits activation of complement before or at the stage of formation of C3/C5 convertase. This inhibition suppresses damage of self cell and C5a-induced recruitment of neutrophils and inflammation. Factor H and C4BP are far much less potent than the recombinant soluble CR1 (see below) in inhibiting classical and alternative pathways, thus, they have not been used in the treatment of complement-mediated diseases. Nevertheless, Factor I, Factor H and C4BP are likely to be effective in substitution therapy in patients with their deficiencies. Factor I has been used in the treatment of Factor I deficiency as described below. Although recombinant factor H has been produced (58), neither natural nor recombinant factor H has been tried in factor H deficiency (14). C4BP has also not yet been tried in the replacement therapy in C4BP deficiency (59).

4.3.1. Factor I deficiency

Several patients who suffer from factor I deficiency have been diagnosed (14,60). In this deficiency regulation of C3/C5-convertase of alternative pathway is impaired. These patients have very low plasma levels of native C3 and factor B. The underlying basis for this low plasma level of C3 and factor B seems to be increased generation of C3bBb complex whose decay is reduced due to factor I deficiency. Circulating C3bBb generates high levels of C3b which in turn leads to the generation of more C3bBb complex. Free C3b or C3b in this complex is not converted to C3bi or C3d. Administration of factor I to one of the patients with factor I deficiency normalized C3b levels within few hours (61). Normalization of the native C3, C5 and classical pathway activities, however, took about four days and these activities remained normal for an additional one week (61). Factor B and properdin levels and opsonic and bactericidal activities normalized within 24 hours and stayed normal for the subsequent five days in this patient (61).

4.4. Decay Accelerating Factor (Daf), Membrane Cofactor Protein (Mcp) And Complement Receptor 1 (CR1)

Soluble forms of DAF (62), MCP (63) and

Table II. Examples of diseases in which beneficial effects of intravenous immunoglobulin has been demonstrated in a small group of patients.

Disease	References
1. Anemias of different types	32-34
2. Neutropeneas of different types	32-34
3. Multiple sclerosis	32-34
4. Systemic lupus erythematosus	32-34
5. Polymyositis and Sjogrens syndrome	32-34
6. Rheumatoid arthritis	32-34
7. Insulin dependent diabetes	32-34
8. Pyoderma gangrenosum	32-34, 56
9. Bullous pemphigoid	32-34
10. Thyroid related eye disease	32-34
11. Uveitis	32-34
12. Asthma in children	32-34
13. Crohn's disease/ ulcerative colitis	32-34
14. Recurrent abortions	32-34
15. Epidermolysis bullosa acquisita	32-34, 57

CR1 (64) in body fluids regulate activation of complement in the fluid phase. Recombinant soluble forms of human DAF (65), MCP (66) and CR1 (67) inhibit activation of complement *in vitro*. Soluble forms of recombinant DAF and CR1 were tested and found to suppress complement-mediated disease processes in experimental animals (65,67). Therapeutic uses of recombinant forms of these inhibitors of complement have recently been reviewed (68).

4.4.1. Decay Accelerating Factor (DAF)

Moran *et al*. engineered and extracted three types of recombinant human DAF (65). These include glycosyl phosphatidyl inositol (GPI) moiety-bearing membrane DAF (mDAF), soluble DAF (sDAF) analogous to soluble DAF found in urine and a secretary form of DAF (seDAF) which lacked GPI-anchor for attachment to cell membrane. mDAF by virtue of having GPI-anchor could be incorporated into cell membranes and as compared with sDAF or seDAF was found to be a more potent inhibitor of complement on cell surfaces. In contrast, activation of complement in the fluid phase was inhibited by sDAF and seDAF but not by mDAF. seDAF inhibited CP and AP mediated generation of C5a (65).

4.4.1.1. Immune complex-induced inflammation

seDAF inhibited reverse passive Arthus reaction in guinea pigs suggesting that it inhibits immune complex-mediated inflammation (65). In spite of the fact that human DAF inhibits human complement much more effectively than guinea pig complement, human seDAF significantly slowed the development of reverse passive Arthus reaction vasculitis in guinea pigs. For 60-80% suppression of vasculitis, the effective dose of seDAF was in the range of 75-150 mg/site which was more than 200 times the effective dose of sCR1 (see below) probably because of species specificity of DAF (65). However, seDAF is likely to be much more effective in complement-mediated human diseases on a dose basis since it inhibits human complement more strongly.

4.4.1.2. Xenotransplantation

Because of the shortage of human organs available for transplantation, relentless efforts are invested to transplant animal organs (xenografts) to humans (for recent review see 69). Complement plays a major role in hyperacute rejection of xenograft (for recent reviews see 70,71). In most animal species combinations classical pathway but in some alternative pathway mediates hyperacute rejection (71). Resistance endowed upon animal cells by incorporation or transfection of these cells with human complement regulatory molecules provide hope that organs of transgenic animals expressing human complement regulatory proteins in the endothelial and other cells of various organs may allow successful transplantation of animal organs to humans. Since it is believed that pig organs are most suitable for human transplantation, pigs transgenic for human complement regulatory proteins are being produced (72). Discussion on other animals transgenic for these molecules will be avoided here.

Transplantation of vascularized organs on discordant animals results in hyperacute rejection of the organ, in part, due to presence of naturally occurring antibodies. In pig-to-human transplantation, the majority of human antibodies react with gal-(alpha 1-3)-gal epitopes of integrins on the endothelial cells of the donor pig organ. Antibodies and complement mediate a hyperacute rejection

within minutes to hours (69,71). This results in severe endothelial cell injury and the loss of natural anticoagulant surface which in turn leads to local and generalized intravascular coagulation. Since membrane regulators of complement are homologously restricted, pig MCP, DAF, CD59 and HRF present on the endothelium of pig organs can not efficiently prevent activation of human complement or effectively influence hyperacute rejection of transplanted pig organs in humans. It has therefore been suggested that the organs of pigs transgenically altered to express human membrane regulatory molecules in endothelial and other cells may be useful for transplantation to humans. Transgenic pigs have been produced that express amounts of human DAF in their organs (heart, liver, pancreas and muscle) that are comparable to that found in human tissues (73,74). These amounts were several fold more in homozygous than heterozygous transgenic pigs. Only expression in the lung was less than that seen in human lung. Expression of the protein in endothelial cells was greater than that seen in human umbilical cord vascular endothelial cells (74). The fact that the endothelial cells from such transgenic pigs were not susceptible to lysis by human complement provides hope for use of organs from these animals in xenotransplantation.

4.4.2. Complement Receptor-1 (CR1)

Soluble form of recombinant CR1 (sCR1) was produced by introducing a stop codon in CR1 cDNA before the transmembrane domain (67). sCR1 bound C3b and C4b in fluid phase and acted as a cofactor for the enzyme factor I in the cleavage of C3b and C4b (67,68). Hemolytic activities of classical and alternative pathways and generation of C3a and C5a were both inhibited by sCR1 at a very low concentration.

Since CR1 is not species specific, sCR1 could be tested in several animal models of C-mediated human diseases.

4.4.2.1. Reperfusion injury

Reperfusion of ischemic tissue causes tissue injury more than that caused by ischemia alone. Ischemia/reperfusion injury is mediated by complement (for recent review see 75). The major mechanisms responsible for the injury include C5a-dependent recruitment of neutrophils; their adherence to the site of C3b deposition on the endothelium via C3-receptors and formation of MAC. In myocardial ischemia/reperfusion injury in rats (76), sCR1 reduced the size of the infarct by approximately half. Protective effect of sCR1 was due to inhibition of complement deposition including formation of MAC, accumulation of neutrophils on endothelial cells in the ischemic zone, complement-mediated neutrophil activation, generation of free radicals and contractility failure in post-ischemic heart. Reperfusion injury to ischemic smooth muscle and mucosa of the guts in rats was also inhibited by sCR1 due to inhibition of activation of complement and neutrophil accumulation (77). Skeletal muscle ischemia and reperfusion injury, as might occur in trauma and vascular occlusion was also inhibited by sCR1 (78). In cremaster muscle ischemia and reperfusion model in the mouse, leukocyte recruitment and their vascular adherence was reduced and muscle viability was improved by treatment with sCR1 (79).

4.4.2.2. Thermal trauma

Burn injury is known to cause activation of complement (80) which presumably leads to further injury in the host. Consistent with this hypothesis, in an experimental model of thermal injury, sCR1 not only reduced dermal but also pulmonary vascular permeability and hemorrhage (81).

4.4.2.3. Hyperacute rejection

sCR1 prolonged the survival of porcine cardiac xenografts perfused with human blood (82). Beside this model, dose dependent prolongation of xenograft survival has been seen in several other animal models (83,84).

4.4.2.4. Immune complex-mediated inflammation

sCR1 inhibited the immune complex-mediated inflammation in reverse passive Arthus reaction in rats (67). Dermal administration of sCR1 in a dose dependent manner reduced deposition of C3 and C5-C9 (C9 neoantigen) and the extent of reverse passive Arthus reaction vasculitis (67). The concentration of human sCR1 required to suppress deposition of complement and vasculitis in rats (0.3 mg/site) was far lower than the effective concentration of human seDAF needed to inhibit reverse passive Arthus reaction in guinea pigs probably because sCR1 is not species-specific.

4.4.2.5. Other experimental models

Treatment with sCR1 has produced promising results in experimental models of many C-mediated diseases including experimental alveolitis, allergic encephalomyelitis and glomerulonephritis (for recent reviews see 68,85,86). According to the studies carried out so far it appears that human sCR1 is not antigenic in humans and thus is safe for therapeutic purposes. sCR1 is being tested in clinical trials in ARDS and in myocardial infarction (85,86). These and other studies may establish the role of sCR1 in the treatment of many complement-mediated human diseases.

4.4.3. CD59

4.4.3.1. Inhibition of hyperacute rejection phase of Xenotransplantation

Fodor *et al.* (87) have produced transgenic pigs which express in their organs high levels of human CD59 on a variety of cells including endothelium of large vessels and capillaries. These

cells were significantly resistant to a high titer of anti-porcine antibodies and human complement. Since organs of pigs transgenic for human DAF (see above) or CD59 exhibit a higher resistance to human complement than those of non-transgenic pigs, it is highly desirable to produce pigs transgenic for multiple human complementary regulatory molecules, including DAF, MCP, CD59, HRF and CR1. Efforts are being made to identify promotors that provide higher level of expression of these molecules in endothelial cells. The organs of pigs transgenic for multiple regulators of complement with a high degree of expression of all the regulators may perhaps find their way in organ transplantation.

5. CONCLUDING REMARKS

High molecular weight natural inhibitors of complement have found clinical use in many diseases but may also prove useful in other complement-mediated diseases. The most promising of these are C1-INH, intravenous immunoglobulin and sCR1. These appear to be non-toxic and non-immunogenic. Complete inhibition of complement by these molecules appears to have little effect, if any, on susceptibility to infection.

In addition to these molecules, membrane inhibitors of complement may also find their way into the clinic. It is hoped that in future, organs of transgenic pigs expressing high levels of multiple human membrane regulators of complement may prove to be useful for transplantation into humans. It is apparent from this review that it is possible to control complement mediated human diseases by use of high molecular weight complement inhibitors of human origin.

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