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THERAPEUTIC INHIBITION OF THE COMPLEMENT SYSTEM. Y2K UPDATE

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1. ABSTRACT

Activation of complement is an essential part of the mechanism of pathogenesis of a large number of human diseases; its inhibition by pharmacological means is likely to suppress disease processes in complement mediated diseases. From this point of view low molecular weight synthetic inhibitors of complement are being developed and high molecular weight natural inhibitors of human origin present in plasma or embedded in cell membrane are being purified or produced in their recombinant forms. This review is concerned with high molecular weight inhibitors, some of which are already in clinical use but may be efficacious in many other diseases in which they have not yet been tried.

C1-esterase inhibitor (C1-INH) concentrate prepared from human plasma is being successfully used for the treatment of hereditary angioneurotic edema. Recently, C1-INH has been found to be consumed in severe inflammation and has been shown to exert beneficial effects in several inflammatory conditions such as human sepsis, post-operative myocardial dysfunction due to

reperfusion injury, severe capillary leakage syndrome after bone marrow transplantation, reperfusion injury after lung transplantation, burn, and cytotoxicity caused by IL-2 therapy in cancer. Factor I has been used for the treatment of factor I deficiency. Recombinant soluble forms of membrane cofactor protein (MCP), and decay accelerating factor (DAF) have not yet been tried in humans but have been shown to be effective in immune complex mediate inflammation in animals. Organs of pigs transgenic for one or more of human membrane regulators of complement namely membrane cofactor protein (MCP), decay accelerating factor (DAF) or CD59, are being produced for transplantation into humans. They have been shown to be resistant to hyperacute rejection in non-human primates; acute vascular rejection is still a problem in their clinical use. It is hoped that these observations together with future developments will make xeno-transplantation in clinical practice a reality. Several recombinant variants of complement receptor 1 (CR1) have been produced. The most effective of these appears to be sCR1-SLe x, sCR1 part of which inhibits complement and carbohydrate Sle x moiety inhibits selectin mediated interactions of neutrophils and lymphocytes with endothelium. Although clinical trials of sCR1 in humans is eagerly awaited, several of the recombinant versions of sCR1 have been shown to suppress ischemia/reperfusion injury, thermal trauma, and immune complex mediated inflammation. They have also been shown to be effective in experimental models of systemic sclerosis, arthritis, myasthenia gravis, Guillain Barré syndrome and glomerulonephritis. Intravenous immunoglobulin, three of the most prominent properties of which are neutralization of autoantibody activity, suppression of autoantibody production and inhibition of complement activity, is being used in several diseases. These include autoimmune thrombocyopenic purpura, Kawasaki disease and several neurological diseases such as myasthenia gravis and Guillain Barré syndrome. In many uncontrolled small scale studies intravenous immunoglobulin has been shown to be effective in many immunological including dermatological diseases; controlled clinical trials in a large number of patients with these diseases is needed to establish the efficacy. It is hoped that in future therapeutic inhibition of complement will be one of the major approaches to combat many human diseases.

2. INTRODUCTION

Complement is one of the powerful effector systems involved in the body's defense. When present in a dormant state, it can protect the individual from foreign pathogens. However, inappropriately activated complement can cause disease. Several disease states such as immune complex and autoimmune diseases and genetic 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 tissue or organs; in some the whole complement cascade is activated, in others only a few components are activated; in some the classical pathway is activated, in others the alternative pathway. 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 inhibitors whereas others are developing natural or recombinant forms of high molecular weight inhibitors of complement present in plasma or on cell surfaces. Development of low molecular weight inhibitors with the eventual aim of manipulating complement system in human diseases has been reviewed (1,2). This review is concerned with the current and possible future therapeutic uses of some high molecular weight natural fluid phase and cell surface human complement inhibitory molecules.

3. THE COMPLEMENT SYSTEM

Complement system has recently been reviewed in detail (3,4); only a passing reference of this system will be made here. Activation of complement occurs via classical and alternative pathways. Activation of both pathways serves 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 a number of mediators from mast cells including histamine. These mediators cause 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.

4. 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 on surfaces of self cells (4-6). The proteins which inhibit complement activation in fluid phase limit the generation of complement fragments such as C4b and C3b. They also render these fragments inactive thereby reducing the extent of cellular damage to self cells. These fluid phase proteins include C1-inhibitor (C1-INH), C4-binding protein (C4BP), factor H, and factor I. In addition, two fluid phase proteins, clusterin and vitronectin, inhibit the formation of cytolytic MAC. The genetic, structural and functional aspects of these fluid phase regulators have recently been reviewed (4-6). In case of extensive activation of complement, a proportion of

Table 1. Fluid phase and cell membrane regulators of C activation

Regulator*	Ligand specificity	Functional activity
FLUID PHASE REGULATORS		
1.C1 stage		
C1-INH	C1r/C1s	Inhibits C1r and C1s mainly in fluid phase
2. C3/C5 convertase formation stage		
C4BP	C4b	Cofactor for factor I in cleavage of C4b
Factor H	C3b	Cofactor for factor I in cleavage of C3b
Factor I	Factor H/C4BP/MCP/CR1	Cleaves C3b and C4b
3. MAC formation stage		
Clusterin	C5b-7/C8,C9	Prevents the assembly of cytolytic MAC
Vitronectin	C5b-7/C8,C9	Prevents the assembly of cytolytic MAC
CELL MEMBRANE REGULATORS		
1. C3/C5-convertase formation stage		
DAF	C3b/C4b	Dissociates C2a from C4b and Bb from C3b
MCP	C3b/C4b	Cofactor for factor I in cleavage of C4b and C3b
CR1	C3b/C4b	Cofactor for factor I in cleavage of C4b and C3b
2. MAC formation stage		
CD59	C5b-8/C9	Prevents assembly of cytolytic MAC on self cell
HRF	C5b-8/C9	Prevents assembly of cytolytic MAC on self cell

For unabbreviated names see the text. * For recent reviews see 4-6

C4b and C3b fragments may escape inactivation by fluid phase inhibitors of complement activation and can get fixed to self cells. These fragments are inactivated by cell membrane embedded regulators of complement such as decay accelerating factor (DAF), membrane cofactor protein (MCP) and complement receptor 1 (CR1). If the activation of complement is extensive, in spite of the action of DAF, MCP and CR1, a significant extent of C3/C5-convertases may be formed on self cell which can sequentially generate MAC which could cause cell lysis. Two cell membrane proteins, CD59 and homologous restriction factor (HRF), render MAC non-cytolytic while it is being formed on the self cell. Non-cytolytic MAC can activate self cell but can not lyse it. Thus, cell surface regulators of formation of C3/C5-convertases and MAC protect self cell from lysis. The genetic, structural, and functional aspects of all these membrane embedded complement regulatory proteins have been reviewed (4-6). 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.

5. THERAPEUTIC USES OF HIGH MOLECULAR WEIGHT INHIBITORS OF COMPLEMENT

5.1. C1-inhibitor (C1-INH)

C1-inhibitor (C1-INH) has recently been reviewed (4,5,7). It is a major inhibitor of two pro-inflammatory plasma cascade systems, the classical pathway of complement and the contact activation system.

During the activation of classical pathway, C1-INH interacts with the activated C1 and inhibits it Interaction of C1-INH with activated C1 complex leads to the dissociation of the C1q subunit and formation of C1r-C1s-(C1-INH)2 complex. Inhibition of activated C1r and C1s in this way 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. Thus C1 activated in a fluid phase can be inhibited by C1-INH much more easily than the C1 activated on the target cell surface.

The activation of factor XII, prekallikrein, high molecular weight kininogen (HMW kininogen), and factor XIa is known as contact activation. C1-INH is also a major inhibitor of contact activation (7). Factor XII, upon binding to certain negatively charged surfaces or molecular complexes, is activated to factor XIIa which converts pre-kallikrein to kallikrein. Kallikrein cleaves HMW kininogen to liberate bradykinin. Factor XIIa and its active fragment factor XIIf activate C1 and generate factor X1a for its participation in the intrinsic blood coagulation cascade. Factors XIIa, kallikrein, XIIf and XIa of contact system are very weak activators of plasminogen but kallikrein can convert pro-urokinase to urokinase which is a strong activator of plasminogen and converts it to plasmin to initiate the activation of the fibrinolytic system.

The regulation of activation of C1 and contact system by C1-INH is abrogated in patients with genetic deficiency of C1-INH (hereditary angioneurotic edema; HANE). Regulation of these systems is also derailed in patients with severe inflammation. C1-INH is beneficial in HANE as well as in clinical situations associated with severe inflammation some of which are described below.

5.1.1. Treatment of hereditary angioneurotic edema (HANE)

HANE has recently been reviewed (8). 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). About 15% of the patients have type II HANE in which C1-INH protein is structurally and functionally abnormal but its plasma levels remain normal or even elevated. Anxiety and trauma are two most common factors which 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 patients can cause local activation of C1 and contact system. Locally activated C1 cleaves C4 and C2. C2 fragment is further cleaved by plasmin to a small vasoactive peptide, C2-kinin. During attack, circulating C4 level reaches zero value and C2 level is decreased. Local depletion of C1-INH also causes activation of contact system leading to the generation of bradykinin whose levels are markedly increased during the episode (7,8).

Local depletion of C1-INH in HANE can easily occur due to low levels of this inhibitor. Addition of various alcohols, detergents, acetone, phenols and metal chelating agents at concentrations insufficient to activate C1s in normal plasma leads to generation of C1-esterase in HANE plasma (9). Similarly, addition of dextran sulphate at concentrations insufficient to activate normal plasma leads to cleavage of HMM kininogen with the generation of bradykinin in HANE plasma within a few minutes (10). Thus, due to deficiency of C1-INH, seemingly insufficient stimuli may be sufficient to activate C1s and factor XII and to initiate an attack in such patients (9,10). In patients this stimuli could be trauma, subclinical infection or any other factor which triggers the attack of edema.

Which of the kinins, C2 kinin or bradykinin, is responsible for the attack of edema in HANE is not well established. Attempts to produce kinin by cleavage of C2 did not succeed in our laboratory and in the laboratory of others (10). Nor has such a kinin been shown to circulate in HANE patients during attack. On the other hand, bradykinin was the major kinin formed when HANE plasma is activated and its levels are increased during attack (7,8,10). The bulk of the evidence favors a role of bradykinin in causing the symptoms of HANE. Whichever kinin is responsible for causing the attack, the basic fact remains that the deficiency of C1-INH is the cause of HANE. A C1-INH replacement therapy should therefore normalize the complement and contact activation system and abort the attack of edema. This has indeed been observed in clinical practice; no side effects have been reported.

C1-INH prepared from normal plasma in concentrated form is used as short term replacement therapy for the treatment of attacks of HANE (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 (12,13). 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 keep the complement and contact systems dormant and prevent the occurrence of attacks of edema in these patients (1).

C1-INH replacement therapy seems to be specially useful in the treatment of attacks of HANE in children. Attacks of HANE are manifested during early childhood in two-third of patients (14). Anti-fibrinolytic agents and attenuated hormones are not recommended for treatment of children because of their side effects. Recently, acute attacks of HANE in six children were treated with a dose of 500 units of C1-INH concentrate. Progression of facial and laryngeal edema was aborted in 30-60 minutes after the infusion. Edema gradually decreased and disappeared over the next 24-36 hours. Only at two separate occasions, the dose had to be repeated after 60 minutes because laryngeal edema continued to progress. Only one patient had to be given anti-fibrinolytic therapy because of increase in frequency of attacks of laryngeal edema.

5.1.2. Treatment of severe inflammation

Excessive activation of complement in severe inflammation in many clinical disorders is often closely associated with tissue destruction with life threatening consequences. Some of these disorders are described below. Activation of complement in these clinical situations causes reduction in levels of C1-INH but administration of C1-INH concentrate protects tissues from destruction. The list of clinical situations in which C1-INH is efficacious is growing.

5.1.2.1. Treatment of sepsis

Complement and contact systems are activated by microorganisms. Septic shock caused by bacterial invasion is associated with activation of both these systems (7). High doses of C1-INH suppress septic shock in a number of experimental models of sepsis. In endotoxin induced shock in dogs, C1-INH prevented pulmonary dysfunction, namely hypoxemia (15). In endotoxin-induced hyper-coagulability in rats, C1-INH inhibited early disseminated intravascular coagulation (16). A modest beneficial effect of C1-INH on clinical course and outcome of severe sepsis has been shown in non-human primates (17). In C3 and C4 deficient mice C1-INH was effective in preventing lethal shock indicating that inhibitory effects of C1-INH on contact activation is more important than its effects on complement in endotoxin shock (18). Positive results of C1-INH in animal models suggested its possible use in humans. Studies on a limited number of patients have already been carried out (19). High doses of C1-INH (a bolus dose of 2000 units followed by 100 units every 12 hours) have been safely administered to patients with septic shock in whom C1-INH is consumed. C1-INH suppressed the activation of complement and contact system and reduced complications such as hypotension. It reduced mortality. In another study on 5 cases with streptococcal shock syndrome, administration of

high doses of C1-INH (10,000 units) rapidly decreased the need for adrenogenic agents and caused a marked fluid shift from the extra-vasal to intra-vasal space (20). Large scale controlled double blind studies are needed to firmly establish whether C1-INH can reduce mortality and morbidity in septic patients.

5.1.2.2. Cardio-protective Therapy

The role of complement in reperfusion injury, which is of great relevance to the development of myocardial infarction, is now well known (21,22). C1-INH attenuates myocardial necrosis and sustains normal cardiac performance after myocardial ischemia and reperfusion in animals. A cardio-protective role of C1-INH was shown in a cat (23) and a pig (24) model of myocardial ischemia and reperfusion. In humans, cardio-protective effects of C1-INH in post-operative myocardial dysfunction due to reperfusion injury have been demonstrated. C1-INH was administered to three patients undergoing emergency surgical revascularization after failed percutaneous transluminal coronary angioplasty (25). In these patients postoperative hemodynamic stabilization was not achieved despite prolonged reperfusion periods, high dose inotropic support, inodilators and aortic counterpulsation. A bolus dose of 2000 units of C1-INH was given followed by 1000 units at 12 and 24 hours after surgery. This treatment resulted in rapid hemodynamic stabilization of all patients; weaning of aortic counterpulsation and epinephrine support was possible within 1 day. All patients survived. These results raise the prospects of future use of prophylactic C1-INH substitution therapy in patients undergoing coronary surgery at high risk conditions.

5.1.2.3. Treatment of severe capillary leakage syndrome after bone marrow transplantation

The prognosis of patients with severe capillary leakage syndrome after bone marrow transplantation is dismal despite aggressive use of intensive care therapy. Because of activated classical pathway of complement and low levels of C1-INH, efficacy of C1-INH concentrate was evaluated in these patients (26). An initial dose of 60 units/kg, followed by two doses of 30 units/kg and four doses of 15 units/kg, every 24 hours, increased survival rate of patients with severe vascular leakage syndrome from 14% to 57% at one year after bone marrow transplantation. Plasma levels of C4d and C5a of patients were increased and C1-INH activity was decreased at the time of diagnosis. However, levels were normalized after infusion of C1-INH. The fluid status normalized within 11 days in 14 of 15 patients. These results are yet to be confirmed in a randomized, controlled trial but appear to be promising.

5.1.2.4. Transplantation

Protection from reperfusion injury is required for successful transplantation. C1-INH attenuated reperfusion injury in animals. Prophylactic treatment of dogs, undergoing lung transplantation with C1-INH, prevented early pulmonary dysfunction (27). In humans, a stable C1-INH level above 110-130 % of normal was sufficient for uneventful outcome after lung transplantation. In a recent study, three patients who developed reperfusion injury after lung transplantation were given 12,000 - 15,000 units of C1-INH concentrate (28). All of them recovered completely. Hyperacute rejection of donor organ is known to be mediated by the complement system (29,30) (also see DAF, MCP and CR1 below). In an ex vivo reperfusion system, survival of porcine kidneys perfused with human serum was prolonged upon C1-INH pre-treatment, probably due to inhibition of C and contact system. In an in vitro model of hyperacute rejection, addition of C1-INH to human serum led to a dose dependent inhibition of complement-mediated destruction of aortic porcine endothelial cells (31).

5.1.2.5. Burn

Thermal injury is known to cause activation of complement (21). Complement activation appears to consume C1-INH as plasma levels of C1-INH are often reduced in burned patients (32). In a pig model of thermal injury, treatment with C1-INH caused reduction in edema formation, diminished bacterial translocation, and a reduction in inflammatory tissue damage (33,34). Only one report has appeared so far on the beneficial effects of C1-INH on thermal injury in humans (35). Treatment of 15 severely burned patients with C1-INH caused substantial improvement in long term survival rate and clinical outcome as compared to a historical control group. Treatment of burned patients with C1-INH in combination with sCR1appears to hold promise (see the section sCR1).

5.1.2.6. Control of toxicity caused by Interleukin-2 Immunotherapy

Interleukin-2 (IL-2) treatment can induce partial or complete remission in advanced melanoma and renal carcinoma. However, its use has been limited due to its toxicity (36). It may cause a life threatening vascular leakage syndrome characterized by hypotension and other changes similar to those seen in septic shock. During IL-2 therapy, classical pathway of complement as well as contact system are activated and C1-INH is consumed (37). It was postulated that administration of C1-INH will inhibit these cascades and will attenuate the toxic effects caused by their activation. 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 (38). It led to the greater tolerance of high doses of IL-2. The incidence of therapy associated vascular leakage was reduced. 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 to reduce IL-2 induced toxicity without decreasing the efficacy of IL-2.

5.2. 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

Table 2. Several versions of recombinant sCR1

Name	Characteristics	Reference
sCR1	Lacks transmembrane and cytoplasmic domains	46
sCR1	Lacks transmembrane and cytoplasmic domains; produced under modified culture conditions; higher half life	59
sCR1[SCR*8-11]-$F(ab')_2$ chimera	SCR8-11 of CR1 fused to antibody $F(ab')_2$ fragment	60
sCR1[des LHR**-A]	Lacks seven N-terminal short consensus repeats SCR1-7	61
GPI-mini-CR1	Glycosyl-phosphadyl-inositol (GPI) moiety of DAF linked to SCRs 8-11 of sCR1	62
sCR1-BA chimera	sCR1 fused to albumin binding terminus (BA) of streptococcal protein G	63
sCR1-SLe x	sCR1 decorated with SLe x***	64

*SCR: Short consensus repeat; repeating domains of approximately 60 amino acids believed to be involved in protein-protein interaction(5,6). Extracellular domain of most common allotype of CR1 (allotype A; frequency 0.82) consists of 30 SCRs. **LHR: Long homologous repeat; 28 SCRs of CR1 are organized into four LHRs (A, B, C and D) each of which is composed of seven SCRs (5,6). ***SLe x: N-linked oligosacccharide moiety (Neu 5 Ac a2-3 Gal ß1-4(Fuc a1-3) GlcNAc-) referred to as Sialyl Lewis x

self cells 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 (39), neither natural nor recombinant factor H has been tried in factor H deficiency. C4BP has also not yet been tried in the replacement therapy in C4BP deficiency.

Besides the potential uses of fluid phase regulators in substitution therapy, a novel use of C4BP came into light when it was shown that human complement on swine endothelial cells was inhibited by a surface bound form of C4BP-GPI (consisting of short consensus repeats 1-8 (SCRs1-8) of a-chain of C4BP and a glycosyl-phosphatidyl-inositol (GPI) moiety of DAF; for a short description of SCR see Table 2) expressed on these cells by transfection of cDNA (40). Although similar studies with factor H have not yet been carried out, this study suggests that organs of pigs transgenic for not only cell surface complement regulatory proteins (see the following section) but also for more than one (C4BP and factor H) engineered surface bound fluid phase complement regulatory proteins have the chances of finding their way in the clinic for xeno-transplantation.

5.2.1. Factor I deficiency

Several patients who suffer from factor I deficiency have been diagnosed (5,41). In this deficiency regulation of C3/C5-convertase of the 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 the 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 (2,42). 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. 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.

5.3. Decay accelerating factor (DAF), membrane cofactor protein (MCP) and complement receptor 1 (CR1)

Soluble forms of DAF, MCP and CR1 are present in body fluids and appear to regulate activation of complement in fluid phase (5,6,42). Recombinant soluble forms of human DAF (43,44), MCP (44,45) and CR1 (44,46), which will be referred to here as sDAF, sMCP and sCR1 respectively, inhibit activation of complement *in vitro* and suppress complement-mediated disease processes in experimental animals (44). sCR1 is under investigation also for its possible therapeutic uses in humans (47,48).

5.3.1. Decay accelerating factor (DAF)

5.3.1.1. Immune complex-induced inflammation

Moran et al engineered and extracted three types of recombinant human DAF (hDAF) (43). These include 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 the 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 classical and alternative pathway mediated generation of C5a.

In spite of the fact that hDAF inhibits human complement much more effectively than non-human complement, seDAF significantly slowed the development of reverse passive Arthus reaction vasculitis in guinea pigs (43). For 60-80% suppression of vasculitis, the effective dose of seDAF was in the range

of 75-150 µg/site which was more than 100 times (approximately) the effective dose of sCR1 in rats (46) (see below). However, seDAF is likely to be more effective in suppressing the development of complement-mediated lesion in human skin on a dose basis because of species specificity of DAF.

5.3.1.2. Xenotransplantation

Because of the shortage of human organs available for transplantation, relentless efforts are invested to transplant animal organs (xenografts) to humans (49). One of the major obstacles in xenotransplantation is hyperacute rejection. Complement plays a major role in hyperacute rejection of xenograft (29,30). In most animal species combinations, classical pathway but in some alternative pathway mediates hyperacute rejection. Animal cells incorporated or transfected with human complement regulatory molecules become resistant to human complement. This provides hope that organs of transgenic animals in which human complement regulatory proteins are expressed in the endothelial cells may eventually be used for 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. Discussion on transplantation of organs of other animals transgenic for these molecules in other animal combinations will be avoided here as much as possible.

Transplantation of vascularized organs on discordant animals results in hyperacute rejection of the organ, in part, due to presence of naturally occurring antibodies in the host. 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 (50). Antibodies and complement mediate severe endothelial cell injury and loss of natural anticoagulant surface with resultant generalized intravascular coagulation. This results in a hyperacute rejection within minutes to hours (49). Since membrane regulators of complement are homologously restricted, pig MCP, DAF, CD59 and HRF can not efficiently prevent activation of human complement on endothelial cells of transplanted pig organs and can not prevent the destruction of these organs by hyperacute rejection. However, human MCP (hMCP), hDAF, human CD59 (hCD59) and human HRF (hHRF) expressed on endothelial cells of organs of transgenic pigs can efficiently prevent activation of human complement on endothelial cells of transplanted transgenic organs and prevent endothelial cell injury. From this point of view, transgenic pigs have been produced that express high amounts of hDAF in their tissues and organs (e.g. bone marrow cells, heart, liver, pancreas, muscle) that are comparable to that found in human tissues (51,52). Expression of hDAF in endothelial cells of organs of these transgenic pigs was greater than that seen in human umbilical cord vascular endothelial cells. Endothelial cells from such transgenic pigs were not susceptible to lysis by human complement. Hearts of pigs transgenic for hDAF transplanted to non-human primates receiving immunosuppressants were not hyperacutely rejected (53). Such hearts could support primate life for prolonged periods before graft failure. Graft failure in some recipient was due to acute vascular rejection and in others due to failure to produce cardiac output and/or dysrhythmia. Pig hearts transgenic for hDAF when transplanted into primates could survive for as long as three months (54). Kidneys from hDAF transgenic pigs were not hyperacutely rejected and could support the life of primates for prolonged periods; presence of hDAF on the kidney confers some resistance even against acute vascular rejection (55). Organs of pigs transgenic for two complement regulatory proteins, hDAF and hCD59, were even more resistant to human complement (see section CD59 below). Because of these developments some workers have gone as far as to say that the initial complement mediated immunological barrier of hyperacute rejection has largely been overcome and it is now possible to study the subsequent mechanisms of xenograft rejection in the pig-to-human combination (30).

5.3.2. Membrane cofactor protein (MCP)

Limited number of studies have so far been carried out with recombinant sMCP for its ability to inhibit complement *in vitro* and *in vivo* and with organs of animals transgenic for hMCP in the field of xenotransplantation.

5.3.2.1 Immune complex induced inflammation

A human recombinant sMCP preparation inhibited lysis of Chinese hamster ovary (CHO) cells heavily attacked by rabbit anti-CHO antibody and human or rabbit complement (56) showing that sMCP is an effective fluid phase regulator of complement activation on cell surfaces. However, in this assay system, it was found to be 160 times weaker an inhibitor on dose basis in comparison to recombinant sCR1 (44). sMCP in combination with sDAF provided more protection against classical pathway than either protein alone. sMCP (and sCR1) were about 100-fold more effective in controlling the activation of the alternative pathway on CHO cells in comparison to sDAF. The possibility that these molecules might be inhibiting complement activation by attaching themselves to the cell surface was ruled out. The ability of sMCP to inhibit complement *in vivo* was tested in reverse passive Arthus reaction model in rats. It inhibited inflammation in this model (44). Administration of sMCP in a concentration range of 2.2 to 73 µg per site reduced the size of lesion by approximately 50% without showing a clear dose dependent effect. Histological examination showed that sMCP reduced intradermal and perivascular infiltrate to a minimum. There was little indication of edema. Thus sMCP, in addition to sDAF and sCR1, may eventually prove to be a useful therapeutic agent for the treatment of complement mediated human diseases.

5.3.2.2 Xenotransplantation

Limited number of studies have been carried out with organs of animals transgenic for hMCP. There is no report on transplantation of organs transgenic for

hMCP in pig-to-primate combination. So far organs of only mice transgenic for hMCP have been studied for transplantation in rats. In a study, mice transgenic for a hMCP minigene that included promotor sequence and the first two introns of genomic MCP were produced (57). When organs of these transgenic mice were transplanted to rats, they did not undergo hyperacute rejection. Mice transgenic for two complement regulatory proteins, hMCP and hDAF, were strongly protected from human complement attack (58). It is hoped that organs of pigs transgenic for hMCP or for more than one human complement regulatory molecules will also be resistant to hyperacute rejection and will eventually find their way to clinical transplantation.

5.3.3. Complement receptor-1 (CR1)

Recombinant forms of several versions of CR1 have been produced. These are listed in Table 2. All the full length sCR1, sCR1 chimera constructs, and sCR1 derivatives were effective as cofactors for the enzyme factor I. They were also effective in inhibiting classical as well as alternative pathways at very low concentrations. sCR1 constructs lacking LHR-A (which lack C4b-binding site) were only effective in inhibiting the alternative pathway *in vitro*. Of the sCR1 versions listed in Table 2, sCR1-SLe x was prepared from the point of view that sCR1 inhibits complement (44,46) and carbohydrate moiety SLe x inhibits selectin mediated interactions of neutrophils and lymphocytes with endothelium (65), therefore, sCR1-SLe x will inhibit complement as well as neutrophil and lymphocyte infiltration and will control inflammation efficiently. Indeed, sCR1-SLe x inhibited both pathways as well as interaction of lymphocytes and neutrophils with endothelium and their migration. C5a up-regulates selectin expression (66) and sCR1-SLe x blocks selectins (64).

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

5.3.3.1. Ischemia/Reperfusion injury

Reperfusion of ischemic tissue causes tissue injury more than that caused by ischemia alone. Ischemia/reperfusion injury is mediated by complement (67). The major mechanisms responsible for the injury include complement activation followed by C5a-dependent neutrophil accumulation in microvasculature. Accumulated neutrophils adhere to the site of C3b deposition on the endothelium via C3b-receptor and subsequently damage the endothelium. sCR1 has been shown to suppress complement and neutrophil mediated reperfusion injury in several animal models. The cardio-protective role of sCR1 has been shown in animal models of ischemia/reperfusion injury. Studies using C1-INH and sCR1[des-LHR-A] have shown that both classical and alternative pathways of complement contribute to reperfusion injury in myocardial ischemia but selective inhibition of the classical pathway was slightly more effective in limiting tissue injury (68). Administration of sCR1 in a rat model of myocardial ischemia/reperfusion injury inhibited the generation of C5a and deposition of C5b-9. sCR1 reduced myocardial infarct size by 44% assessed at 7 days post dosage and minimized the accumulation of neutrophils within the infarcted area (59). sCR1 prevented contractile failure in post-ischemic heart (69). In a pig model, total inhibition of complement by sCR1 optimized the recovery during the revascularization of ischemic myocardium (70). Administration of sCR1 and the use of cardiopulmonary bypass circuits coated with a complement inhibitor, heparin, also optimized the recovery. sCR1 and sCR1-SLE x have also been shown to be effective in reducing ischemia/reperfusion injury in several other tissues and cells by inhibiting complement activation and neutrophil accumulation. These include mouse skeletal muscle (71), rat intestine (72), rat liver (73), mouse neurons after stroke (74), and remote organs after lower torso ischemia in the rat (75).

5.3.3.2. Thermal trauma

Burn injury is known to cause activation of complement (21) which presumably leads to further injury in the host at distant sites. In a rat model in which thermal injury on skin was inflicted on 25-30% of total body surface, short term (4 hours) protective effects of sCR1 on acute skin and lung injury were assessed (76). sCR1 infusion reduced dermal vascular permeability by 44%. sCR1 treated animals demonstrated significant protection against lung injury; increases in pulmonary vascular permeability and hemorrhage were reduced by 45% and 46%, respectively, and myeloperoxidase content was lowered by 39%. Thus, sCR1 offers significant protection against thermal injury in rats and protective effects are related to reduced neutrophil contents. It is hoped that sCR1 alone or in combination with C1-INH will also show protective effects in thermal injury in humans.

5.3.3.3. Xenotransplantation

Complement system is known to be involved in inflammation, injury and rejection of transplanted organs (29,30). sCR1 protects allografts against cellular infiltration and vascular injury (77). Selective inhibition of the alternative pathway of complement by sCR1[des-LHR-A] has been shown to protect the isolated hearts of rabbits from human complement mediated damage (78). As regards porcine-human combination, sCR1 has been shown to attenuate hyperacute rejection and prolong the survival of porcine cardiac xenografts perfused with human blood (79). Swine endothelial cells moderately expressing GPI-mini-CR1 (see Table 2) were appreciably resistant to lysis by human complement (62); mini-CR1 was more effective than MCP and as effective as DAF in this respect. Thus, GPI-mini-CR1 may also prove to be useful in clinical transplantation.

5.3.3.4. Immune complex-mediated inflammation

A recombinant sCR1 preparation inhibited the lysis of CHO cells heavily attacked by rabbit anti-CHO antibodies and complement showing that it can act as an inhibitor of cell surface activation of complement (44).

In this assay system, it was found to be approximately 160 and 200 times stronger an inhibitor than sMCP and sDAF, respectively. It was as effective as sMCP and approximately 100-times more effective than sDAF in inhibiting alternative pathway lysis of CHO cells. The possibility that sCR1 may be inhibiting complement by attaching itself to the cell surface was ruled out. The ability of this sCR1 to inhibit complement was tested using reverse passive Arthus reaction in rats (46). sCR1 inhibited this model of complement-mediated inflammation. Dermal administration of sCR1 in a dose dependent manner reduced deposition of C3b and C5b-C9 and the extent of reverse passive Arthus reaction vasculitis. The concentration of human sCR1 required to suppress deposition of complement and vasculitis was approximately 1µg per site. This was much lower than the effective concentration of seDAF needed to inhibit reverse passive Arthus reaction in guinea pigs which was 75-150 µg per site. Strong inhibition of this reaction in rats by sCR1 in comparison to that by seDAF may probably be because CR1 is not species-specific where as DAF is.

5.3.3.5. Other experimental models

sCR1 appears to be a potential therapeutic agent in complement mediated inflammation. Treatment with sCR1 has produced promising results in experimental models of many C-mediated diseases including experimental allergic encephalomyelitis (80), dermal vascular reactions (46), collagen induced arthritis (81), traumatic brain injury (82), myasthenia gravis (83), Guillian-Barré syndrome (84), glomerulonephritis (85), allergic reactions (86) and asthma (87).

sCR1-SLE x and sCR1 (des LHR-A)-SLE x and their SLe x devoid forms were assessed in rat models of selectin dependent lung injury following 1) systemic activation of C by cobra venom factor and 2) intrapulmonary deposition of IgG immune complexes (64). In cobra venom factor model, SLe x decorated forms of sCR1 (Table 2) caused substantially greater reductions in neutrophil accumulation and in albumin extravasation in lung as compared to undecorated forms. Similar results were obtained with sCR1[des-LHR-A]-SLe x in IgG immune complex model. In both models, sCR1 versions decorated with SLe x had enhanced ability to localize to the activated vascular endothelium and had enhanced anti-inflammatory effects in comparison to undecorated forms; protective effects correlated with increase in vascular binding.

If human sCR1 is not antigenic in humans and is safe for therapeutic purposes, it may prove to be useful in the treatment of many complement-mediated human diseases. sCR1 is being tested in clinical trials in acute respiratory distress syndrome and in myocardial infarction (47,48).

5.3.4. CD59

Soluble form of CD59 (sCD59) appears to regulate activation of complement in body fluids (5,6,42). Recombinant form of human sCD59 has been shown to inhibit activation of complement on cell surface *in vitro* (88).

5.3.4.1. Xenotranplantation

Fodor et al (89) have produced transgenic pigs which expressed high levels of hCD59 in their organs on a variety of cells including endothelium of large vessels and capillaries. These cells were significantly resistant to a strong attack by high titer anti-porcine antibodies and human complement. Since organs of pigs transgenic for hDAF and hCD59 are expected to exhibit a higher resistance to human complement than organs of pigs transgenic for hCD59 alone, it is highly desirable to produce pigs transgenic for more than one human complement regulatory molecule. Efforts are being made not only to produce pigs transgenic for more than one human complement regulatory molecule but also to identify promotors that provide higher level of expression of these molecules in endothelial cells (57,90,91). Lungs from transgenic swine expressing hDAF as well as hCD59 did not undergo hyperacute rejection when perfused with human plasma and baboon blood (92) or when transplanted to baboons (93). Combined expression of even moderate levels of hDAF and hCD59 produced under the control of heterologous promotors, was sufficient to protect pig blood cells from human and baboon complement and to block complement mediated damage of pig heart transplanted in baboons (91). The above observations suggest that transgenic organs with high levels of endothelial expression of two membrane inhibitors of complement are protected from hyperacute rejection. However, elimination of xenoantigens by knocking out genes responsible for their expression with simultaneous transgenic expression of more than one complement regulatory molecules may even be a better approach to achieve clinical application of xenogeneic vascularized organ transplantation. Such studies have not yet been performed on porcine-primate combination but elimination of xeno-antigen Gal-(a 1-3)-Gal was found to prolong the function of mouse hearts expressing high levels of two regulatory molecules, hDAF and hCD59, in an *ex vivo* model of xenograft rejection (94). The organs of transgenic pigs with a high degree of expression of more than two complement regulatory molecules and with low or no expression of xeno-antigens have much less chances of undergoing hyperacute rejection. Other innovations such as expression of more than one transgenic complement regulatory molecules decorated with SLe x moieties may perhaps prevent even other events associated with xenograft rejection such as acute vascular rejection.

5.4. Intravenous immunoglobulin

Intravenous immunoglobulin is prepared from plasma of a large number of healthy donors (95,96). This product consists of 95% IgG in same proportion of its subclasses as in plasma. Depending on the number of donors from whom the 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 (97). Commercially available intravenous immunoglobulins are prepared by different methods, contain different additives, vary in pH ranging from 4 to 6, and may contain IgA. 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).

Beneficial effects of intravenous immunoglobulin in different diseases may be mediated by their different immunomodulating properties (42,95,96,98) such as their antibody activity and their ability to 1) inhibit the release and activities of cytokines, 2) neutralize multiple autoantibodies through its anti-idiotype reservoir, 3) inhibit B-cell activation and auto-antibody production, 4) inhibit antibody dependent cellular cytotoxicity by blocking Fc-receptor for IgG (Fc?R) on effector cells, 5) inhibit activation and functions of T cells and NK cells and 6) enhance CD8 positive suppressor T-cell function.

Besides the above mentioned mechanisms, intravenous immunoglobulin may exert its beneficial effects in many diseases by inhibiting complement activation at various stages. It appears to inhibit C activation at C1 stage (99) and at the stage of binding of complement fragments (C3b and C4b) to target cells (100) or immune complexes (101). Serum from a patient treated with intravenous immunoglobulin showed reduced C3b and C4b uptake (to baseline value) onto sensitized homologous erythrocyte. Intravenous immunoglobulin may also inhibit complement activation at other sites of the cascade but this has not yet been studied. Intravenous immunoglobulin inhibits complement *in vivo* in experimental models of complement mediated diseases (101). Monomeric serum IgA and IgM in intravenous immunoglobulin preparations were also effective in inhibiting C4 uptake by sensitized sheep erythrocytes and immune complexes; a mixture of immunoglobulins of different isotypes was somewhat more active than any single isotype (102). IgM enriched intravenous immunoglobulin prevented the activation of complement *in vitro* as well as *in vivo (*103*)*. *In vitro* inhibition was seen by its ability to inhibit the uptake of C1q, C4b and C3b by aggregated IgG and *in vivo* inhibition was seen by its ability to suppress the deposition of C3, C6 and C5b-9 in rat glomeruli in a rat model of nephritis. The effect of intravenous IgG was much less pronounced.

Recently, a novel mechanism for reduction of autoantibodies by Intravenous immunoglobulin has been proposed. Since plasma concentration of IgG determines its rate of catabolism, high levels of administered intravenous immunoglobulin will accelerate the rate of IgG catabolism (98). Normally plasma IgG that enters the cells through pinocytosis binds to an Fc?R called FcRn (so named because of its initial discovery in neonatal epithelium) in endocytic vesicles. IgG bound to FcRn is protected from catabolism and is subsequently returned intact to the circulation. Unbound IgG would pass to the lysosomes and be degraded. Following administration of high doses of intravenous immunoglobulin in autoimmune diseases, most of the IgG of intravenous immunoglobulin origin presumably saturates the FcRn, permitting degradation of the pre-existing IgG, including auto-antibodies in lysosomes. It is believed that this reduces the levels of pathogenic autoantibodies (98). FcRn is found in many adult tissues including skin, muscles, and intestinal epithelium; it is present in high levels in endothelial cells. Although this mechanism explains the reduction of autoantibodies by intravenous immunoglobulin, it like others, also does not answer all the questions related to the mechanism of the action of intravenous immunoglobulin, For example, it does not explain why a proportion of patients with autoimmune diseases get permanent or prolonged remission.

Besides its use in replacement therapy in primary immunodeficiencies, intravenous immunoglobulin has been found to be beneficial in more than 30 immunological diseases (42,95, 96). The mechanisms for the beneficial effects of intravenous immunoglobulin in these diseases are currently unknown. Although 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 firmly established in a relatively large number of patients are described below.

5.4.1. Autoimmune thrombocytopenic purpura (ITP)

ITP is characterized by early platelet destruction by autoantibodies directed against platelet glycoproteins IIb/IIa and Ib/IX. Patients with bleeding signs and platelet counts below 10-20 x 10^9 per liter need treatment, patients with platelet counts 20-50 x 10^9 per liter should be treated on individual levels and patients with platelet counts above 50 x 10^9 per liter without bleeding should be observed.

Administration of intravenous immunoglobulin is efficacious in acute as well as chronic ITP. In children with acute ITP, intravenous immunoglobulin therapy is considered treatment of choice (42,95, 96,104,105). In adults with acute ITP, treatment with intravenous immunoglobulin during the first six months rather than later, is likely to produce long term remission. In children and adults with acute ITP, autoantibodies to platelet glycoproteins IIb/IIa and Ib/IX are present in plasma and on platelets. This results in destruction of platelets via splenic sequestration involving macrophages. Platelet counts in these patients are very low but following intravenous immunoglobulin treatment (400 mg/kg/day) it rises to approximately 30 x 10^9 per liter within 48 hours of therapy. If platelet counts are not

significantly increased during this time, additional doses are given daily for five days. About 75% of patients with chronic ITP demonstrate platelet bound autoantibodies and 58% maintain circulating autoantibodies. 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.

Several mechanisms for the action of intravenous immunoglobulin in ITP have been proposed. A mechanism which proposes anti-idiotype activity in intravenous immunoglobulin is supported by the facts that 1) Fab fragments of intravenous immunoglobulin but not Fc fragments inhibit the binding of anti-platelet antibodies to normal platelet and 2) intravenous immunoglobulin reacted with monoclonal antibodies to glycoproteins IIb/IIa and Ib/IX (as specific idioptype source) only through its Fab and not through its Fc portions (106). Blockade of Fc?-R on reticuloendothelial cells is also postulated to be one of the main mechanisms of action of intravenous immunoglobulin in ITP (95,96). In short term, inhibition of complement and in long term, decrease in anti-platelet antibody production with simultaneous decrease in complement activation on platelets also appear to be plausible mechanisms by which intravenous immunoglobulin protects platelets from destruction.

5.4.2. Kawasaki disease

Kawasaki disease (107) is a leading cause of acquired heart disease in children. It is characterized by rash, edema and erythema of hands and feet followed by development of aneurysm of coronary arteries. Intravenous immunoglobulin therapy reduces early inflammatory symptoms and decreases the incidence of coronary abnormalities in this disease (95,96,107). Two regimens for the treatment of Kawasaki disease with intravenous immunoglobulin are relatively more common. In the first one, during the first ten days of the disease intravenous immunoglobulin (400 mg/kg/day) in conjunction with aspirin is given for the prevention of the development of aneurysms of coronary arteries. In the second regimen a single high dose of 2 gm/kg intravenous immunoglobulin is given over 10-12 hours and 80-100 mg/kg aspirin per day in four divided doses until the patient is afebrile followed by a daily dose of 3-5 mg/kg of aspirin. The improvement in coronary artery abnormalities following treatment was highly dependent on the dose of intravenous immunoglobulin rather than the dose of aspirin (108); a higher dose appears to be more efficacious than a lower dose. Whether intravenous immunoglobulin treatment needs to be combined with aspirin is under debate.

It has been suggested that bacterial toxins activate complement (109) and complement activation products may be responsible for production of cytokines in this disease (93,96,107, 110). Complement activation products are known to release cytokines from many cell types (110). Intravenous immunoglobulin neutralizes these toxins and their effects (42,95,96). Intravenous immunoglobulin suppresses excessive production of cytokines such as IL-1, IL-6, TNF and T-cell cytokines 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 suppress the production and neutralize the activities of 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.

5.4.3. Myasthenia gravis

Myasthenia gravis (111) is an autoimmune disorder characterized by fatigue and muscle weakness that improves with rest but is made worse with activity. In this disease, autoantibodies to several different epitopes of acetylcholine receptor (AchR) complex are present in the circulation. The pathology results from an autoantibody attack and subsequent activation of complement at neuromuscular junction where deposits of autoantibodies and complement can be seen.

So far, a large scale placebo controlled intravenous immunoglobulin trials have not been conducted in myasthenia gravis. However, the consensus of an expert panel is that intravenous immunoglobulin is effective in reversing myasthenic weakness. This is based on the results of a number of small uncontrolled trials on patients in different stages of the disease which show a favorable response in at least 75% of the patients (112,113). 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. The improvement is associated with a decrease in the levels of anti-AchR antibodies. These effects last for 40-49 days. In a recent randomized study (114) with 66 patients with myasthenic crisis, intravenous immunoglobulin and plasma exchange were found to be equally effective. A multi-center chart review study to compare the efficacy and tolerance of plasma exchange and intravenous immunoglobulin in the treatment of episodes of myasthenic crisis has shown that both treatments were effective but plasma exchange was associated with higher complication rate compared with intravenous immunoglobulin (115). Juvenile myasthenia gravis has been treated with intravenous immunoglobulin and thymectomy (116). High dose intravenous immunoglobulin therapy (2.0 g/kg) has been used in transient neonatal myasthenia gravis (117). 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.

It is currently not known as to how much of the clinical improvement is due to inhibition of complement and how much due to other mechanisms.

5.4.4. Guillain-Barré Syndrome

Guillain-Barré syndrome (118) is an acute polyneuropathy in which inflammation of many nerve roots and peripheral nerves occurs and which results in progressive paralysis. The pathology is believed to be caused by both cellular and humoral immune processes. The disease has a mortality rate of 4-5%. About 30% of the patients require prolonged mechanical ventilation and hospitalization before regaining the ability to walk.

In 1980's plasma exchange and in 1990's intravenous immunoglobulin were found to be effective in the treatment of Guillain-Barré syndrome. Recently, in randomized control trials comparing plasma exchange and intravenous immunoglobulin (400 mg/kg daily for 5 days), both treatments were found to be equally effective in restoring motor function (119). Early relapse after an initial response to both treatments is seen in about 10% of treated patients for whom another treatment cycle is given; some reports describe higher frequency of relapses in plasma exchange treated patients (120). Intravenous immunoglobulin offers some advantages over plasma exchange such as being better tolerated and being easily administered without special equipment. These advantages also make intravenous immunoglobulin an initial therapy for pediatric Guillain-Barré syndrome (121). In childhood Guillain-Barré syndrome, early use of a single intravenous immunoglobulin dose prevented further progression and shortened clinical course of the disease (122).

The mechanism of action of intravenous immunoglobulin in Guillain-Barré syndrome is not known. Intravenous immunoglobulin is believed to inactivate specific anti-myelin antibodies and inhibit their production. It also causes decrease in the circulating levels of pro-inflammatory cytokines, TNF-a and IL-1ß (123). Patients treated with plasma exchange do not show decrease in circulating levels of these cytokines. Clinical improvement of intravenous immunoglobulin treated patients was associated with a reduction in circulating levels of TNF-a. Levels of anti-inflammatory mediators, TNF-a-receptor and IL-10, were not affected by intravenous immunoglobulin treatment.

5.4.5. Chronic inflammatory demyelinating polyneuropathy (CIDP)

Chronic inflammatory demyelinating polyneuropathy (118) is a chronic form of Guillain Barré syndrome. Patients with this disease have antibodies reactive with Schwann cells, myelin, 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 (112,124). In most studies, a dose 400 mg/kg per day for 5 days followed by a lower maintenance dose has been used. In long term treatment, the dose is adjusted according to patient response. In several uncontrolled trials, 60% to 80% of the patients made a satisfactory recovery within six months of treatment. In a cross-over study (125) with 30 patients, 63% improved with intravenous immunoglobulin whereas 17% improved with placebo. Intravenous immunoglobulin was as effective as plasma exchange in treating chronic inflammatory demyelinating polyneuropathy but in long term is more convenient and has fewer adverse effects.

How Intravenous immunoglobulin exerts its beneficial effects in this disease is not known. It is likely that intravenous immunoglobulin may be causing the suppression of the production of the above mentioned antibodies against Schwann cells and other nerve structures and suppression of complement activation caused by these antibodies. Inhibition of autoantibody production may also result in the dormancy of the complement system.

5.4.5. Other diseases

The efficacy of intravenous immunoglobulin in many diseases has been tested in small groups of patients. These studies have shown that intravenous immunoglobulin exerts beneficial effects in many diseases. Some examples are given below. The results of different pilot studies have shown that intravenous immunoglobulin is efficacious in many complications associated with pregnancies (126). It prevents complement-mediated destruction of fetuses in recurrent spontaneous abortions (127). 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 (128). Intravenous immunoglobulin is efficacious in alleviating maternal ITP. Treated patients concomitantly delivered infants with normal platelet counts (126). Increase in fetal platelet counts was not associated with maternal response to intravenous immunoglobulin but with the levels of IgG achieved in neonates following intravenous immunoglobulin administration (129). Intravenous immunoglobulin (combined with heparin and aspirin) treatment in pregnancies complicated with anti-phospholipid syndrome has been shown to result in safe outcome of pregnancies, both for mother and neonate.

Intravenous immunoglobulin is beginning to be studied in dermatological diseases (130,131). Dermatomyositis, in which MAC attack is important in both muscle and skin inflammation, is the most thoroughly studied dermatological condition with regard to intravenous immunoglobulin therapy. A placebo controlled trial and several uncontrolled trials and case reports covering about 100 patients have so far appeared (132). From these reports it appears that the response-rate is high. The effects are long lasting only in a minority of patients; majority requires further intravenous immunoglobulin therapy within weeks to several months. This therapy allows reduction of concomitant immunosuppressive therapies. The response rates are higher in patients receiving adjunctive treatment. Intravenous immunoglobulin has been tried in a limited number of patients with systemic lupus erythematosus (SLE) (133,134). It was useful in SLE with encephalitis, leukopenia and thrombocytopenia.

Table 3. Examples of diseases in which beneficial effects of intravenous immunoglobulin have been demonstrated in small numbers (or groups) of patients

Disease	References
Anemias of different types	139,140
Neutropenias of different types	139,141
Multiple sclerosis	142
Sjogren's syndrome	143,144
Cystic fibrosis	145
Thyroid related eye disease	146
Uveitis	147
Asthma	148,149
Ulcerative and Crohn's disease	150
Pyoderma gangrenosum	151

Hematological problems were reversed in newborns whose mothers had SLE. Maternal anti-cardiolipin antibodies were blocked and better fetal survival was seen. Renal IgG deposits were solubilized. Too few cases are available for critical analysis. In last decades, several uncontrolled small scale studies have shown that intravenous immunoglobulin treatment is beneficial in several vasculitides associated with systemic and organ specific diseases. In a recent study, ten patients with vasculitis in which other therapeutic measures had failed were given one to six treatment courses of 400 mg/kg intravenous immunoglobulin for 5 days on monthly basis (135). In six of these patients, beneficial clinical responses were seen. Anti-myeloperoxidase and anti-neutrophil cytoplasmic antibody levels decreased with clinical improvement in patients with Churg-Strauss vasculitis and Wegener's granulomatosis, respectively.

This is an example of several studies which show that when other treatments such as immunosuppressive therapy fail, intravenous immunoglobulin still remains an option with high response rate. Intravenous immunoglobulin may probably be exerting its effect through different mechanisms but inhibition of complement and decrease in autoantibody levels may be two of them. Intravenous immunoglobulin has been tried in several blistering autoimmune diseases of skin. A total of 10 patients with pemphigus and 15 with pemphigoid in conjunction with steroid or immunosuppressive therapy have been treated so far in several uncontrolled studies (131,136). All patients were unresponsive to conventional therapy and mucosal involvement was seen in all. All authors reported rapid improvement in condition with a lowering of serum IgG autoantibodies. In all cases it was possible to reduce the steroid doses promptly without relapse. Only one report describes failure but in this report only one cycle of intravenous immunoglobulin was given. Intravenous immunoglobulin was tried in a patient with epidermolysis bullosa acquisita (131,137) in which circulating autoantibodies directed against type VII collagen present in dermal-epidermal junction occur. Patient was unresponsive to several immunosuppressive regimens. Intravenous immunoglobulin therapy, 400 mg/kg for 5 days, was repeated every four weeks. After three courses, there was a marked reduction in blistering. Nine therapy courses were administered which resulted in continuous inhibition of blister formation and in enhanced healing of older lesions as well as in markedly decreased fragility. The titer of antibodies was not affected raising the possibility that beneficial effects could be due to inhibition of complement activation at dermal-epidermal junction. In another patient with severe epidermolysis bullosa acquisita of seven years duration who had been treated with many immunosuppressive drugs and plasma exchanges without any lasting effect, treatment with low dose intravenous immunoglobulin, 40 mg/kg for five days, rendered patient free of disease for ten months (138). This suggested that perhaps it may be possible to treat this disease with low doses and at lower cost.

It is obvious from the foregoing that clinical trials in a large number of patients with above mentioned diseases are needed to confirm the efficacy of intravenous immunoglobulin. Many other diseases are potential candidates for intravenous immunoglobulin therapy.

Some of many other diseases in which intravenous immunoglobulin has been tested in small groups of patients and has been found to have beneficial effects are listed in Table 3. 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.

6. CONCLUDING REMARKS

Some high molecular weight human soluble inhibitors of complement in their natural or recombinant forms, have found clinical use in many human diseases. These molecules may also prove to be useful in other complement-mediated diseases in which they have not yet been tested. 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, 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 highly likely that in future it will be possible to control complement mediated human diseases by use of high molecular weight complement inhibitors of human origin.

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