

Preface

Therapeutic Interventions in the Complement System focuses on the manipulation of complement-system activity in a therapeutic setting. Many basic texts discuss complement and a number of reviews have been published that address specific disease areas; however, no other source offers the comprehensive reviews of therapeutic applications presented here.

We envision that someone who has little if any current knowledge of complement will be able to read our text and come away with substantial insights derived from some of the leading authorities in the area today. This is particularly relevant and timely because there are many investigators with a rudimentary knowledge of complement who are considering entering this area. Through our interactions with scientists from outside the complement field, it is clear that there is a great deal of misinformation regarding this system. Because there are relatively few active experts in the area of complement biology, the ability of an investigator to obtain accurate and timely information from a wide array of research and clinical settings is very limited.

Each chapter in Therapeutic Interventions in the Complement System focuses on a specific area of basic and/or applied complement biology. One focus is on critical mediators, such as C5a and C3a, that are generated in the complement pathway. New information on receptors for these important inflammatory mediators is also presented. Other exciting new studies in Therapeutic Interventions in the Complement System demonstrate that the membrane attack complex, itself, can activate cells and act as a proinflammatory mediator. Informative animal models are discussed in detail, including the relative values of each and the large potential for important interspecies differences that have obvious relevance to the interpretation of preclinical studies.

In addition to a comprehensive discussion of the activation pathway and complement receptors, Therapeutic Interventions in the Complement System presents an up-to-date discussion of the natural complement inhibitors as well as small-molecular-weight complement inhibitors such as compstatin that regulate the activation of this pathway. Recombinant forms of many natural inhibitors are being developed as therapeutics, and pros and cons for the use of each in specific disease settings is discussed.

We believe one particular strength of *Therapeutic Interventions in the Complement System* is its disease-oriented focus. The clinical evidence for the role of complement in ischemia-reperfusion injury is addressed in addition to systemic and organ-specific inflammatory and autoimmune diseases. Here, emerging knowledge about specific complement inhibitors and discussions of the use of such inhibitors in each disease are integrated. Important caveats about infection risk, immune complex alterations, and untoward effects on autoimmunity are also explored.

We have also included a comprehensive section on the major current techniques used in measuring complement. Many methods have previously been utilized, most commonly hemolytic assays, but with the development of new ELISA methods, the potential for high-throughput assay has greatly increased. However, there are many issues regarding the sensitivity and specificity of assays that must be considered, and we review these issues in some detail.

We envision that *Therapeutic Interventions in the Complement System* will be of interest to biotechnologists and scientists—at both large pharmaceutical firms and smaller research laboratories—who have specific interests in pursuing a complement-related project. In addition, clinical investigators will be able to find information pertinent to the use of inhibitors in disease processes and gain insight into measuring complement in various types of clinical trials.

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Chapter 2

The Regulation of C1 Activation and Its Role in Disease

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Introduction

Inflammatory reactions contribute to the pathogenesis of numerous diseases. These reactions result from the release and activation of endogenous inflammatory mediators, such as cytokines and the major plasma cascade systems. The latter include the coagulation, fibrinolytic, contact, and complement systems. Proinflammatory activity of the complement system is mainly attributable to the release of peptide fragments, such as the anaphylatoxin, and the generation of macromolecular protein complexes, such as the C5b-9 complexes, during activation. Modulation of the biological effects of these complement activation products constitutes a therapeutic option in human disease.

Pathological activation of the complement system often occurs via the classical pathway. Activation of the first complement component, C1, is the earliest step in classical pathway activation. Hence, inhibition of this activation is an attractive approach to reduce the inflammatory activities of complement *in vivo*, the especially because this inhibition does not affect the alternative pathway, and hence does not completely abrogate the antibacterial activities of the system. In this chapter we will discuss the activation of C1, the nature

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of the activators *in vivo*, the biochemistry and biology of the main inhibitor of activated C1, C1 esterase inhibitor (C1-Inh), and clinical and experimental studies evaluating C1-Inh therapy.

Activation of C1

Binding of the first component of complement, C1, is the first event during activation of the classical complement. C1 is a macromolecular protein complex consisting of one C1q molecule and a Ca^{2+} -dependent tetramer of two C1r and two C1s molecules (1,2). C1r and C1s are homologous serine proteases, which are inactive zymogens in the tetramer. Activation of C1 starts with the binding of C1q to an activator. Subsequently autoactivation of C1r is triggered. Activated C1r, in turn, activates C1s (3). C1q has an unusual structure in that it consists of three polypeptide chains—A, B, and C—of which six copies are present in each C1q molecule. The C-termini of A, B, and C chains together form a globular structure (the “head”), whereas the N-termini are arranged as a collagenous strand (the “stalk”). Overall the structure of C1q resembles a bouquet of six flowers, with the heads constituting the flowers. The globules and part of the collagenous stalks of C1q are arranged as a cone. The C1r-C1s tetramer is intertwined between the stalks. In unbound C1, the tetramer is arranged in such a way that the two C1s molecules prevent close contact between the catalytic sites of the C1r molecules, and hence prevent autoactivation of pro-C1r (2). On binding to an activator the conformation of the C1q cone changes, resulting in a spatial rearrangement of the C1r₂C1s₂ tetramer in such a way that the catalytic sites of pro-C1r come in close contact. This changed conformation of C1q facilitates autoactivation of C1r and subsequent activation of C1s (2). The inhibitory action of C1s on C1r autoactivation in unbound C1 is not complete, and in time C1 will become autoactivated, even in the absence of an activator (“spontaneous autoactivation”). At physiological concentrations, C1-Inh (which is present at about sevenfold molar excess) also can interact with the catalytic sites of nonactivated C1r, and prevent autoactivation of C1 (4). There is no evidence that C1-Inh can inhibit autoactivation of C1 induced by an activator. During activation both C1r and C1s are cleaved into two-chain proteins. Under physiological conditions the active molecules are inactivated within seconds by C1-Inh, resulting in the release of stable complexes consisting of C1rC1s(C1-Inh)₂

from the activator (5). Plasma levels of these complexes reflect activation of C1 *in vivo* (6). C1q remaining bound to the activator may perform other functions, for example, interaction with C1q receptors.

Activators of C1

C1q binds to immunoglobulins in immune complexes via specific binding sites in the Fc portion of immunoglobulin G (IgG) or IgM. Activation of C1 requires the binding of at least two globular heads of the C1q molecule to the immune complex. The affinity of C1q for the IgG subclasses varies, that for IgG3 being the highest, that for IgG4 the lowest, whereas that for IgG1 or IgG2 is in between. C1q does not interact with IgA, IgE, or IgD. Owing to this low affinity IgG4 is not able to induce activation of C1. The potential of IgG or IgM-containing immune complexes to activate C1 *in vivo* is supported by numerous studies showing codepositions of immunoglobulins and C1q in inflamed tissues.

In some diseases activation of the classical pathway occurs without apparent involvement of immune complexes, for example, during therapy with high doses of interleukin-2 (IL-2) (7–9). At a tissue level, classical pathway activation has been observed in acute myocardial infarction and in Alzheimer's disease, without apparent involvement of immunoglobulins (10,11). Thus, classical pathway activation may be induced *in vivo* by activators other than immune complexes. These activators may include viral proteins, A β -protein in Alzheimer's disease (12), mitochondrial constituents released by damaged cells (13,14), serum amyloid P (15), and probably others. Several *in vitro* studies have demonstrated that the acute phase C-reactive protein (CRP), bound to a ligand, can activate the classical complement pathway (16–18). We have recently shown that CRP-induced activation of complement also occurs *in vivo*, for example, following surgery (19,20), during sepsis (21) and during immunotherapy with IL-2 (G. J. Wolbink, C. E. Hack, et al., unpublished observations). We have also found codepositions of CRP and activated complement fragments in infarcted human myocardium (22). The ligand for CRP in these conditions is not known. We have speculated (23) that phospholipid microparticles or cells with flip-flopped membranes (in these cells or microparticles phospholipids of the inner and outer leaflets of the membrane have exchanged [24,25]) may be involved. Notably, the sites on C1q mediating binding to

CRP, serum amyloid P, or the β A-protein may be different than those involved in binding to immune complexes (15,26–28).

C1 Inhibitor

C1-Inh belongs to the superfamily of serine proteinase inhibitors (serpins) (29–32). It is a major inhibitor of factor XIIa, kallikrein, and factor XIa of the contact system, and the only known inhibitor of activated C1s and C1r from the classical pathway of complement (1,32–38). C1-Inh is therefore an important regulator of inflammatory reactions, and to a lesser extent of (factor XI-dependent) clotting activation.

The molecular mass of C1-Inh is approx 105 kDa, its plasma concentration is approx 270 mg/L, or 1 unit (U)/mL (32,39). C1-Inh inhibits proteinases by binding to the active site of the proteinases via its reactive center (33,36,37), yielding stable complexes. These complexes are removed from the circulation with an apparent half-life of clearance ranging from 20 to 47 min (40–43), via receptors specific for complexed serpins on hepatocytes (44,45). C1-Inh is an acute phase protein, plasma levels of which may increase up to twofold during uncomplicated infections (46). Woo et al. (47) reported that the synthetic rate of C1-Inh increases up to 2.5 times the normal rate in patients with rheumatoid arthritis. This increased synthesis, presumably by the liver, is induced by various cytokines, including interferon- γ (48).

C1-Inh, like most other serpins, can be inactivated by elastase released from activated neutrophils to yield so-called modified C1-Inh (49–51). This cleavage likely occurs between the amino acids at the P4 and P5 positions and between those at the P2 and P3 positions (52). Proteolytic inactivation of C1-Inh may occur in inflamed tissues and contribute to local complement activation. Plasma levels of modified C1-Inh are increased in sepsis (39). C1-Inh mutants with a decreased susceptibility for inactivation by elastase have been developed (52), but their therapeutic efficacy remains to be established.

In normal volunteers the fractional catabolic rate (FCR) of C1-Inh is 2.5% of the plasma pool per hour, yielding an apparent plasma half-life of clearance of about 28 h (47,53). The half-life of clearance of human C1-Inh in rabbits is comparable, that is, 26 h (54), whereas in rats it is considerably shorter, that is, about 4.5 h (40). The apparent half-life of clearance in patients with hereditary angioedema (HAE)

has been reported to be considerably longer, that is, more than 48 h (55,56). However, clearance of C1-Inh in these patients is often determined by assessing the course of plasma levels following the intravenous administration of exogenous C1-Inh. This may not be correct, as at lower plasma levels of C1-Inh (as occurs in untreated HAE patients) autoactivation of C1 is poorly controlled (see earlier) (4), which causes consumption of functional C1-Inh. At higher concentrations of C1-Inh (as occur after administration of C1-Inh) this autoactivation is inhibited, leading to a decreased consumption of C1-Inh. Hence, following a therapeutic dose of C1-Inh, plasma concentrations of C1-Inh increase as a result of the administration of exogenous C1-Inh as well as because of a reduced consumption of endogenous C1-Inh.

C1-Inh is heavily glycosylated, the protein portion of the molecule constituting only 51% of its molecular mass (57). It probably contains 20 carbohydrate groups, that is, six glucosamine-based, five galactosamine-based, and the others linked to threonine residues. Most carbohydrate groups are located at the N-terminal region (57). Their function is unknown. Removal of sialic acids greatly enhances the clearance of C1-Inh from the circulation, yielding an apparent half-life of 3–5 min (54), presumably via binding to asialoglycoprotein receptors in the liver. The enhanced clearance of asialo-C1-Inh is due to exposure of penultimate galactosyl residues, as the subsequent removal of the latter prolongs the clearance rate up to a value similar to that of normal C1-Inh (54). Removal of sialic acid or galactose groups does not impair the functional activity of C1-Inh *in vitro* (54).

We have administered high doses of C1-Inh (up to 12,000 U for 2–5 d; 1 U is the amount present in pooled normal plasma) to 12 patients with severe septic shock (58,59), and measured plasma C1-Inh concentration at various time points during the study period. The recovery of C1-Inh in these patients was calculated according to a pharmacokinetic model. In this model it was assumed that (a) the FCR of C1-Inh is 2.5% that of the plasma pool per hour; (b) the endogenous production of C1-Inh is constant; (c) each C1-Inh dose is distributed immediately in one central plasma compartment and is constantly eliminated therefrom following a first-order process; and (d) the plasma volume in patients with sepsis is approx 45 mL/kg of body weight. The overall correlation between the course of C1-Inh

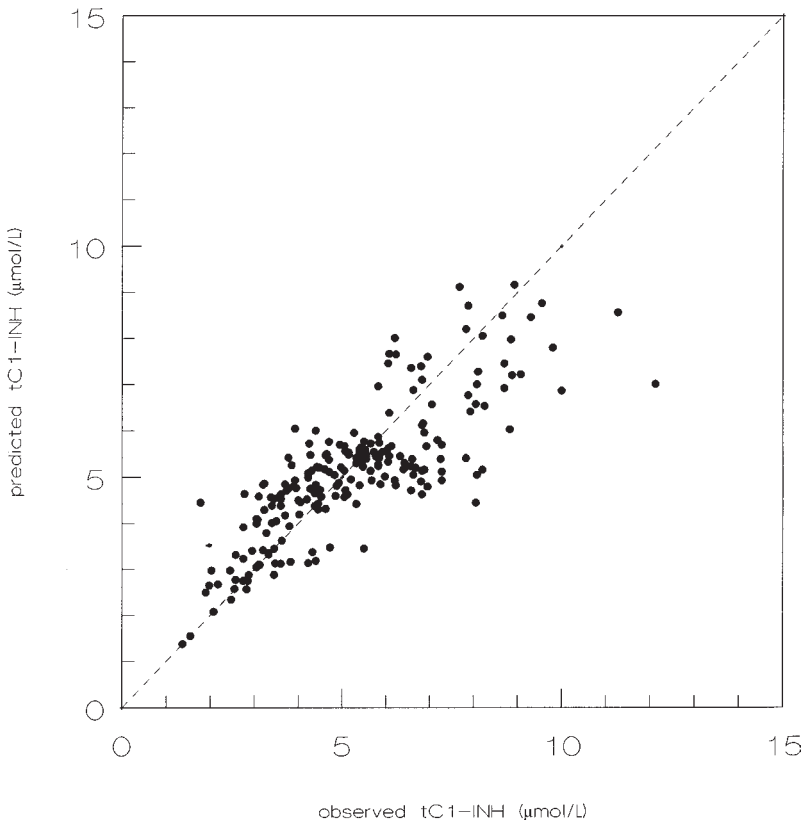


Fig. 1. Relation between observed (x-axis) and predicted C1-Inh concentration (y-axis) in septic shock patients receiving C1-Inh therapy.

levels after the various doses of C1-Inh calculated according to this model with those actually measured in the patients was very good ($R = 0.7807$, $p < 0.001$; see Fig. 1), although occasionally recovery was less than expected, possibly owing to a higher FCR (58). These results indicate that the clearance data observed with radiolabeled C1-Inh in human volunteers (47,53), may be used to estimate the dose of C1-Inh to be administered to patients. Also the course of human C1-Inh following intravenous administration in septic baboons was as predicted by the pharmacokinetic model described earlier (60), again demonstrating the validity of this model.

The function of C1-Inh is potentiated by glycosaminoglycans such as heparin (61,62), which enhance the inactivation of factor XIa and C1s by C1-Inh up to more than 100-fold, but have no effect on

the inactivation of factor XIIa or kallikrein (63,64). In particular, dextran sulfate species are promising as drugs to enhance C1-Inh function (63). Recent studies (I. Bos et al., manuscript submitted) indicate that this enhancing effect of dextran sulfate also can be achieved in vivo, although noncovalently linked complexes of dextran sulphate and C1-Inh slowly dissociate in vivo. These findings not only demonstrate the feasibility to develop a C1-Inh preparation with strongly enhanced inhibitory activity, but also show that the inhibitory spectrum of C1-Inh can be shifted, that is, toward a strong inhibitor of complement and of clotting (via inhibition of factor XIa), and a poor inhibitor of contact system proteinases and possibly also of fibrinolytic proteinases.

C1-Inhibitor Therapy in Animal Models and in Clinical Disease

Hereditary Angioedema

HAE may be caused by a heterozygous deficiency of C1-Inh, and is characterized by occurrence of painless local swelling of soft tissues resulting from a local increase of vasopermeability. An excessive release of vasoactive peptides owing to unbalanced activation of the contact and complement systems likely is the pathogenic explanation for these phenomena. Attacks of HAE may be precipitated by minor trauma, and can be treated by intravenous administration of C1-Inh purified from pooled plasma (55,56,65,66). Usually a dose of 1000 U of C1-Inh will be sufficient. This therapy in general is well tolerated. Alternatively, patients may be treated with antifibrinolytic agents such as ϵ -aminocaproic acid and related drugs. The efficacy of antifibrinolytics as a treatment for HAE shows that the generation of plasmin, which occurs during attacks (67), contributes to the pathogenesis of the angioedema, presumably by generating the C2-derived vasoactive peptide (68). Attacks of HAE can be prevented by attenuated androgenic steroids, which increase the synthesis of C1-Inh by the normal gene. However, long-term use of these drugs may have several disadvantages such as mild virilization and production of liver tumors.

Sepsis

Sepsis is often induced by bacterial infections and is a major cause of mortality in intensive care units. Sepsis results from the

excessive release and activation of endogenous inflammatory mediators, which include the complement and contact systems. Activation of the contact system during sepsis was convincingly demonstrated by studies in primates showing levels of various contact system proteins decrease during sepsis, whereas levels of activation products of the contact system increase (69). Treatment (before the bacterial challenge) with a monoclonal antibody that blocks activation of factor XII had no effect on clotting activation but largely prevented the irreversible hypotension and slightly improved survival of baboons challenged intravenously with a lethal dose of *E. coli* (70). Moreover, this treatment also reduced classical pathway activation, suggesting involvement of factor XII in this activation (71).

The role of complement activation during sepsis seems to be dual. Some activation is necessary for an efficient clearance of bacteria or their products: Dogs with a genetic C3 deficiency are more susceptible to endotoxin than healthy littermates owing to an impaired clearance of endotoxin (72). Similarly, mice totally deficient in C3 or C4 also have an increased susceptibility to endotoxin (73). On the other hand, inhibition of the biological effects of C5a in baboons suffering from sepsis attenuates lethal complications (74), illustrating that the proinflammatory effects of complement activation, in particular those of C5a, may contribute to the complications of sepsis. This proinflammatory effect of complement during sepsis is supported by observations that C5-deficient mice tolerate endotoxin better than their C5-sufficient littermates (75). The proinflammatory effects of C5a may include enhancement of cytokine-release: C5-deficient mice exhibited a twofold lower tumor necrosis factor (TNF) response and a slower increase of pulmonary vasopermeability than C5-sufficient animals (76). Administration of human soluble complement receptor-1 did not affect circulating TNF levels, although this treatment improved pulmonary responses during endotoxemia (77). Thus, although complement is required for a rapid clearance of bacteria or their products during sepsis, it also may enhance inflammatory reactions via the release of C5a and possibly other phlogistic fragments.

C1-Inh does not interfere with alternative pathway activation and does not completely block activation of the classical pathway. Hence, C1-Inh administration may allow opsonization of the infect-

ing microorganisms or their products. In baboons with lethal *E. coli* sepsis, the administration of C1-Inh at a dose that increased plasma levels 5- to 10-fold reduced activation of C4, and to a lesser extent that of C3 (60). This treatment had some beneficial effect: three of seven animals challenged with a lethal dose survived 64 hours, two of them were long-term survivors. Though studies in C4-deficient mice have indicated that an intact classical pathway is required for efficient clearance of endotoxin (73), C1-Inh treatment did not interfere with the clearance of the bacteria in the baboon model (60). Similarly, a favorable, though mild, effect of C1-Inh has been found in several endotoxin models in rats, dogs, rabbits, and in mice deficient in C4 and C3 (73,78–80). Whether these beneficial effects of C1-Inh in sepsis are due to its effect on complement or on the contact system, or on both, is not known.

Limited experience exists with C1-Inh therapy in septic shock patients (58,81). Initially, five patients treated with mechanical ventilatory support, volume substitution, vasopressor medication, and positive inotropic drugs received C1-Inh for 5 d, starting with a dose of 2000 U, subsequently followed by 1000 U every 12 h. No patient died during the study period of 5 d. Four of the patients needed less and one patient needed more vasopressor therapy during this period. No side effects of C1-Inh treatment were observed. Both complement and contact activation decreased in four of these five patients. Later, six other patients with severe septic shock were treated with C1-Inh: Three received a starting dose of 4000 U followed by two doses of 2000 U and four doses of 1000 U, each given at 12-h intervals. Three other patients received 6000 U of C1-Inh followed by 3000, 2000, and 1000 U (all doses given at 12-h intervals). Effects comparable to those obtained with the other dose regimen were seen, that is, no toxic side effects and a slight reduction of complement and contact activation. These results (no toxic side effects; no sepsis-related mortality during the study period; attenuation of complement and contact activation; beneficial effect on hypotension) were confirmed by several open uncontrolled studies, each performed in a limited number of septic shock patients. These patients received C1-Inh according to the schemes outlined earlier (second workshop on C1 esterase inhibitor, Dusseldorf, Germany, 24–26 April, 1997). Therefore, double-blind controlled studies in a larger number of patients are warranted to confirm these promising effects.

Vascular Leak Syndrome

A vascular or capillary leak syndrome (VLS) may complicate sepsis (82), or occur independently of this disease, for example, during therapy with cytokines, or following a bone marrow transplantation or open heart surgery (83–86). Occasionally it develops in the absence of any known precipitating event. The pathogenic mechanisms underlying the VLS are increased vasopermeability and vasodilation. The molecular mechanisms causing these phenomena are poorly understood, although endothelial damage resulting from interactions with activated neutrophils and/or natural killer cells is likely at their basis (9,87,88). Studies of the VLS induced by IL-2 suggest that other mediators, including cytokines (89,90), complement (7–9,91) and coagulation and fibrinolysis (92,93), are involved as well. Activation of the classical pathway of complement correlates with the development of side effects (7–9,86). As discussed earlier, complement activation during VLS likely is induced by CRP, which is confirmed by observations that this acute phase protein binds to IL-2-activated lymphocytes that subsequently activate and fix complement (91). The effects of C1-Inh administration on patients receiving high doses of IL-2 (94) have been evaluated in six patients who received 72×10^6 IU of recombinant IL-2 (Chiron Corp., Amsterdam, the Netherlands) during treatment of either metastatic melanoma or renal cell carcinoma. These patients received C1-Inh at a dose of 2000 U initially, subsequently followed by 1000 U every 12 h, for 4 d (treatment cycle). As controls, the same patients received fourfold lower doses of IL-2 given four wk after the first cycle. In addition, four other patients who received escalating doses of IL-2, starting with 18×10^6 IU and increasing by 18×10^6 IU every 2–3 d were studied (8). The clinical toxicity of IL-2 was comparable in all patients, despite the fact that the C1-Inh treatment group had received considerably more IL-2 (at least 2.7 times greater) (94). Thus C1-Inh therapy may reduce IL-2 toxicity, probably via inhibition of IL-2-induced complement activation. C1-Inh may attenuate other forms of VLS as well: A newborn baby with sepsis-associated VLS was treated with C1-Inh for 3 d (300, 100, and 50 U per kilogram on d 1, 2, and 3, respectively) (82). Although the patient died 15 d later from liver failure, the effect of C1-Inh administration was judged to be beneficial as the patient had been able to stop vasopressor medication

and his body weight had normalized. VLS following bone marrow transplantation was treated with C1-Inh therapy initially in two patients who received 60 U/kg as a loading dose, then two doses of 30 U/kg given at 12-h intervals, and finally, four doses of 15 U/kg of C1-Inh (95). Body weight normalized in each patient, as did the increased levels of C4d. In a later report, the same authors describe 15 patients treated with C1-Inh because of VLS induced by bone marrow transplantation (96). The 1-yr-survival rate was 57% in the treated patients vs 14% in a control group consisting of seven patients (the study was not randomized placebo-controlled). Treatment was accompanied by normalization of circulating C4d and C5a levels. These effects suggest a beneficial effect of C1-Inh therapy in this severe complication of bone marrow transplantation, but need to be confirmed by a double-blinded placebo controlled study.

Twenty-nine children with mild to severe VLS induced by open heart surgery were also treated with C1-Inh (starting dose of 300 U/kg, followed by two doses of 150 U/kg, three doses of 100 U/kg, and finally three of 50 U/kg, each dose given at 8- h intervals). In most children the effect of C1-Inh therapy was judged to be favorable in that hemodynamic, respiratory, and laboratory parameters improved. However, in eleven patients arterial blood pressure did not respond, in three leakage continued, and in six children diuresis did not improve (85). Notably, in two patients possible adverse side effects were recorded: superior vena cava thrombosis in one patient with a transposition of the great vessels, and extended renal vein thrombosis in a neonate.

Together these studies indicate that C1 inhibitor therapy is a promising approach for the management of patients with VLS, but double-blinded placebo-controlled studies are needed to confirm this.

Acute Myocardial Infarction

Acute myocardial infarction (AMI) is a major cause of mortality and morbidity in the Western world. Mortality is often due to arrhythmia, cardiac rupture, and acute heart failure, whereas morbidity often results from chronic heart failure. The latter is mainly determined by the amount of necrotic tissue in the jeopardized myocardium. Studies in animals indicate that irreversible myocardial cell injury starts about 30 min after occlusion of coronary vessels and proceeds for hours. The later phase of myocardial cell injury likely results from an acute

inflammatory reaction ensuing in the ischemic myocardium, as it can be effectively reduced by antiinflammatory agents. For example, corticosteroids given as late as 6 h after coronary occlusion reduce infarction size by about 35% as compared to untreated control animals (97). The local inflammatory response ensuing in the infarcted myocardium is characterized by the local production of chemotactic factors and cytokines; the infiltration and activation of neutrophils; the expression of adhesion molecules, which enhance adherence of neutrophils to cardiac myocytes; and local activation of the complement system (98).

Hill and Ward were the first to show that complement is activated by ischemic myocardium. They demonstrated that complement activation products generated in the infarcted myocardium were responsible for the infiltration of neutrophils (99). Later studies in animals as well as in patients showed that several complement components become localized in the infarcted myocardium, either reperfused or not, whereas membrane-bound complement inhibitors decrease (22,100–108). Furthermore, plasma levels of activated complement components are increased in patients with AMI and correlate with myocardial damage (109,110). Although some studies claim that the activation of complement in ischemic myocardium occurs via the alternative pathway (111), the involvement of C1q and C4, and hence the classical pathway, has been repeatedly demonstrated (22,102–104,107). The molecular mechanism of the observed activation of complement during AMI is not clear, although mitochondrial constituents in particular have been implied as activators (13,14,112,113). Our own studies in humans suggest a contribution of the acute phase protein CRP (see below).

Complement activation products such as the anaphylatoxins and the terminal complement complexes have deleterious effects on the myocardium via mechanisms dependent and independent of neutrophils, which result in vasoconstriction, impaired microcirculation, an increase in coronary perfusion pressure, ischemia, contractile failure of the myocardium, tachycardia, and impairment of atrioventricular conduction (98,114–116). These deleterious effects of complement on the myocardium presumably explain the observations that in animal models complement depletion by administration of cobra venom factor prior to or shortly after permanent occlusion of a coronary vessel significantly reduces the amount of myocardial necrosis (101,107,117).

Early reperfusion of ischemic myocardium is a main goal in treatment of AMI. However, reperfusion of ischemic myocardium itself may induce an inflammatory reaction, which among others involves activation of complement (98,103,118). This ischemia/reperfusion injury may damage the cardiac tissue and limit the beneficial effects of a restored circulation. Inhibition of complement activation induced by reperfusion of ischemic myocardium or inhibition of C5a activity reduces the extent of myocardial infarction considerably in animals (111,119). Taken together these studies provide convincing evidence that ischemic myocardium induces activation of complement and that inhibition of this activation may reduce the inflammatory damage to ischemic myocardium, either reperfused or not.

C1-Inh administration has been shown to reduce the extent of infarction in ischemia/reperfusion models of AMI in animals. In a cat model (90 min of ischemia, 270 min of reperfusion) C1-Inh administered intravenously shortly before reperfusion at a dose of 15 mg per kg of body weight (about 60–100 U/kg), reduced infarction size by 65% as compared to cats receiving vehicle (120). Part of this cardioprotective effect of C1-Inh occurred via a diminished infiltration of neutrophils. A similar dose of C1-Inh was shown to reduce the extent of infarction in a rat model (20 min of ischemia, 24 h of reperfusion) by 60–70%, and to prevent the influx of neutrophils in the ischemic myocardium (121). The effect of C1-Inh was slightly better than that of soluble complement receptor-1 (sCR-1) given at a dose of 15 mg/kg of body weight. Intracoronary application of C1-Inh at a much lower dose (20 U/kg of body weight) reduced infarction size by about 33% in a pig model (60 min of ischemia, 120 min of reperfusion) (122). Increases of circulating C3a, and to a lesser extent C5a, were attenuated by C1-Inh treatment (122). In cooperation with Dr. A. Kleine and Dr. W. Th. Hermens, Cardiovascular Research Institute Maastricht, the Netherlands) we evaluated the effect of C1-Inh in a dog model of AMI induced by permanent occlusion of a main coronary vessel. The intravenous administration of human C1-Inh at a dose of 500–1000 U per dog (weighing approx 25 kg) at 2 h and 8 h after permanent coronary artery occlusion significantly (up to 50%) reduces infarct size as assessed at 48 h after the occlusion. These studies thus show an important cardioprotective effect of C1-Inh in various experimental models of AMI. Whether a similar effect may

occur in humans with AMI is currently under study. Finally, although there is no doubt that complement is activated by ischemic myocardium, it remains to be established that inhibition of this activation is at the basis of the beneficial effects of C1-Inh.

Other Diseases

C1-Inh has been explored as a therapy in animal models of various other diseases as well. Four studies have evaluated the efficacy of C1-Inh in pancreatitis. In a short-term pig model (6-h observation period) pancreatitis was induced by retrograde injection of sodium taurocholate in the pancreatic duct. C1-Inh reduced mortality and improved hemodynamic performance in this model, presumably because of improved inhibition of kallikrein and trypsin (123). In another model using rats C1-Inh decreased mortality and reduced massive necrosis (124). A mild beneficial effect of C1-Inh (250 U/kg) was also shown in rats with hemorrhagic pancreatitis (125). Combined treatment with antithrombin III (also 250 U/kg) further improved survival in this model of severe acute experimental pancreatitis (125). Beneficial effects were, however, not found in three other models, two in mice and one in rats, although relatively high doses of C1-Inh were administered (about 400 U/kg of body weight) at various time intervals (124). Thus, the efficacy of C1-Inh in experimental pancreatitis is inconsistent, and it is therefore difficult to judge whether therapy with this inhibitor may be of clinical benefit.

C1-Inh administration to the donors (500 U/20–32-kg dog) 30 min before cardiac inflow in the lungs was arrested, as well as to the recipients (500 U per animal) 30 minutes before reperfusion, largely prevented a deterioration of respiratory and hemodynamic parameters in dogs after orthotopic allogeneic lung transplantation. In addition, C1-Inh prevented a fall in circulating contact and complement parameters (126). Hence, it was concluded that by inhibiting complement and contact system activation, C1-Inh prevented early, ischemia reperfusion-induced dysfunction of transplanted lungs. Notably, C1-Inh may also be a useful drug for the management of xenotransplantation, as in an *in vitro* model relevant to hyperacute xenograft rejection, C1-Inh is capable of protecting endothelial cells against complement-mediated destruction (127,128).

In a porcine model of thermal injury C1-Inh (given at a dose of 100 U/kg initially, followed by three lower doses given at 12-h inter-

vals) reduced organ alterations, improved microcirculation, and largely prevented bacterial translocation in the gastrointestinal tract (129,130). Favorable effects of C1-Inh administration to patients with burns have been reported in a preliminary communication (A. Jansen et al., presented at the second Workshop on C1-esterase inhibitor, 24–26 April 1997, Dusseldorf, Germany).

Finally, one study reported on the effect of C1-Inh in a murine model of traumatic shock (131). Treatment with 15 mg/kg intravenously 10 min posttrauma reduced mortality from 83% to 33%. This was accompanied by reduced activation of neutrophils.

Summary

The classical pathway of complement is activated in various (patho)physiological conditions. A main inhibitor of this pathway is the serpin C1-Inh, which regulates classical pathway activation at the level of C1. C1-Inh also inhibits the mannan binding lectin associated serine proteases 1 and 2. C1-Inh purified from plasma has been used for 25 yr as a replacement therapy for patients with hereditary angioedema caused by a heterozygous deficiency of C1-Inh. Animal studies and preliminary clinical studies suggest that C1-Inh may also be used for the treatment of various other diseases, such as sepsis, cytokine-induced vascular leak syndrome, or acute myocardial infarction, as well. In this chapter we will discuss the activation process of C1, the nature of possible activators *in vivo*, the biochemistry and biology of C1-Inh, and experimental and clinical studies evaluating the therapeutic efficacy of C1-Inh therapy in disease have been discussed.

Conclusions

C1-Inh therapy is well tolerated and may be beneficial in a number of clinical disease states. We suggest that CRP-mediated activation of complement is a basic pathogenic mechanism in these diseases, and constitutes a major target for C1-Inh therapy. Future studies should definitely show the clinical benefit of this novel anti-inflammatory therapy.

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