

# C1-Esterase Inhibitor: An Anti-Inflammatory Agent and Its Potential Use in the Treatment of Diseases Other Than Hereditary Angioedema

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**Abstract**—C1-esterase inhibitor (C1-Inh) therapy was introduced in clinical medicine about 25 years ago as a replacement therapy for patients with hereditary angioedema caused by a deficiency of C1-Inh. There is now accumulating evidence, obtained from studies in animals and observations in patients, that administration of C1-Inh may have a beneficial effect as well in other clinical conditions such as sepsis, cytokine-induced vascular leak syndrome, acute myocardial infarction, or other diseases. Activation of the complement system, the contact activation system, and the coagulation system has been observed in these diseases. A typical feature of the contact and complement system is that on activation they give rise to vasoactive peptides such as bradykinin or the anaphylatoxins, which in part explains the proinflammatory ef-

fects of either system. C1-Inh, belonging to the superfamily of serine proteinase inhibitors (serpins), is a major inhibitor of the classical complement pathway, the contact activation system, and the intrinsic pathway of coagulation, respectively. It is, therefore, endowed with anti-inflammatory properties. However, inactivation of C1-Inh occurs locally in inflamed tissues by proteolytic enzymes (e.g., elastase) released from activated neutrophils or bacteria thereby leading to increased local activation of the various host defense systems. Here we will give an overview on the biochemistry and biology of C1-Inh. We will discuss studies addressing therapeutic administration of C1-Inh in experimental and clinical conditions. Finally, we will provide an explanation for the therapeutic benefit of C1-Inh in so many different diseases.

## I. Introduction

Clinical signs and symptoms of several diseases result from the release and activation of endogenous inflammatory mediators. Among these mediators are plasma cascade systems such as the contact and complement system. Activation of these systems has indeed been demonstrated in a variety of human diseases. A typical feature of both the contact and the complement system is that on activation they give rise to vasoactive peptides such as bradykinin (contact system) or the anaphylatoxins (complement system), which in part explains the proinflammatory effects of either system (Mason and Melmon, 1965; Vogt, 1986).

C1-inhibitor (C1-Inh)<sup>2</sup> is a major inhibitor of both the complement and the contact system (Sim et al., 1979; Van den Graaf et al., 1983; Pixley et al., 1985; Wuillemin et al., 1995b), and, therefore, is endowed with anti-inflammatory properties (Table 1). The important physiological role of C1-Inh is best demonstrated by hereditary C1-Inh deficiency and its association with hereditary angioedema (HAE) (Lander mann et al., 1962; Donaldson and Evans, 1963). The first detailed description of the clinical signs and the dominant inheritance of HAE was done by Quinke and Osler (Osler, 1888; Quinke, 1882). Lepow and colleagues demonstrated that human serum contained a heat-labile protein that inhibited the esterolytic activity of the first complement component C1, which they named "C1-Inhibitor" and subsequently achieved its partial purification (Lepow et al., 1956; Levy and Lepow, 1959; Pensky et al., 1961). Finally, it was shown that serum of patients with HAE

<sup>2</sup> Abbreviations: C1-Inh, C1-inhibitor; AMI, acute myocardial infarction; CRP, C-reactive protein; F, factor; GAG, glycosaminoglycans; HAE, hereditary angioedema; HK, high-molecular weight kininogen; IFN, interferon; IL, interleukin; MAC, membrane attack complex; MBL, mannan-binding lectin; MASP, MBL-associated serine proteinase; PAGE, polyacrylamide gel electrophoresis; PAI, plasminogen activator inhibitor; serpin, serine proteinase inhibitor; TNF, tumor necrosis factor; t-PA, tissue-type plasminogen activator; u-PA, urokinase-like plasminogen activator; VLS, vascular leak syndrome.

TABLE 1  
Characteristics of C1-Inh

Synonyms	$\alpha$ 2-neuramino-glycoprotein, C1-esterase inhibitor, C1s-inhibitor, C1-inactivator
Classification	Serine proteinase inhibitor (serpin)
Abbreviation	C1-Inh
Molecular weight	Native glycosylated molecule, 104,000 to 105,000 (analytical ultracentrifugation and SDS-PAGE); protein backbone, 52,869 to 52,880 (478 amino acids according to complementary DNA sequencing)
Substrates	C1s, C1r, $\alpha$ FXIIa, $\beta$ FXIIa, kallikrein, FXIa, plasmin, MASP-1, MASP-2
Half-life of clearance <sup>a</sup>	67 to 72 h
Concentration	0.24 g/l (range 0.18–3.4) [ $\sim$ 2.3 $\mu$ m]
Enzyme activity	None
Coenzymes/cofactors	GAG <sup>b</sup>

<sup>a</sup> Determined in hereditary angioedema patients; half-life of clearance in other patients and in normal individuals is probably  $\sim$ 28 h.

<sup>b</sup> GAGs enhance inhibition of C1s, C1r, and FXIa but not that of FXIIa or kallikrein.

was deficient in kallikrein inhibitory capacity and that patients with HAE had significantly decreased serum levels of C1-Inh (Lander mann et al., 1962; Donaldson and Evans, 1963). HAE is characterized by episodes of painless local swelling of soft tissues resulting from a local increase of vasopermeability. Only heterozygous conditions are known to be associated with the disease and a homozygous deficiency is apparently lethal (Späth, 1997).

Attacks of HAE can be treated effectively by intravenous administration of C1-Inh purified from pooled human plasma. Long-term prophylactic substitution with pasteurized C1-Inh was demonstrated to be safe and of clinical benefit with few negative effects (Gadek et al., 1980; Bergamaschini et al., 1983; Bork and Witzke, 1989; Agostoni and Cicardi, 1992; Waytes et al., 1996).

Because of its anti-inflammatory (and anti-clotting) activity and the possibility of its large scale preparation

with a high degree of purity, biological activity, and viral safety, C1-Inh concentrates may be useful for the treatment of other diseases as well (Pouille et al., 1994). Recent studies support this idea. Here, we will summarize the biochemistry and biology of C1-Inh. We then will review the results of experimental and clinical studies evaluating the therapeutic efficacy of C1-Inh therapy in several diseases other than angioedema. Finally, we will discuss a hypothesis that may explain the efficacy of C1-Inh in these diseases.

## II. Biochemistry and Biology of C1-Inh

### A. Biochemistry

C1-Inh is a heavily glycosylated single-chain polypeptide of 478 amino acid residues, the protein portion of the molecule constituting only 51% of its molecular mass (Bock et al., 1986). It probably contains 13 carbohydrate groups, i.e., 6 glucosamine-based and 5 galactosamine-based, whereas the remainder is linked to threonine residues. Most carbohydrate groups are located at the N-terminal region (Perkins et al., 1990). The precise function of these carbohydrate groups is unknown. Removal of sialic acids from C1-Inh (asialo-C1-Inh) enormously enhances its clearance from the circulation yielding an apparent half-life time of 3 to 5 min in a rabbit model (Minta, 1981), presumably via binding to asialoglycoprotein receptors in the liver. The enhanced clearance of asialo-C1-Inh is due to exposure of penultimate galactosyl residues, because the subsequent removal of the latter prolongs the clearance rate up to a value similar to that of normal C1-Inh (Minta, 1981). Removal of sialic acid or galactose groups does not impair the functional activity of C1-Inh in vitro (Minta, 1981).

The molecular mass of C1-Inh is approximately 105 kDa, and plasma concentration  $\sim 240$  mg/l, corresponding to 1 U/ml (Schapira et al., 1985; Nuijens et al., 1989).

By sequence homology C1-Inh belongs to the superfamily of serine proteinase inhibitors (serpins), which also includes, e.g.,  $\alpha$ 1-antitrypsin, antithrombin, and plasminogen activator inhibitor type I (Travis and Salvesen, 1983; Carrell and Travis, 1985; Schapira et al., 1985; Carrell and Boswell, 1990). As with other serpins, the sequence homology is not distributed throughout the molecule but is restricted to the carboxyl-terminal end (serpin domain) (Carter et al., 1988; Coutinho et al., 1994). Near the carboxyl-terminal end of the serpin domain is the protease recognition region, which is termed the "reactive center loop" (Coutinho et al., 1994).

### B. Synthesis

Several cells, including hepatocytes, fibroblasts, monocytes, macrophages, endothelial cells, amnionic epithelial cells, and perhaps cells in the mikrogliia are capable of C1-Inh synthesis (Bensa et al., 1983; Yeung

Laiwah et al., 1985; Katz and Strunk, 1989; Katz et al., 1995; Walker et al., 1995; Zahedi et al., 1997a). In rats, spontaneous expression of C1-Inh gene was found in Kupffer cells, whereas peritoneal macrophages and blood monocytes expressed C1-Inh only after treatment with interferon- $\gamma$  (IFN- $\gamma$ ) (Armbrust et al., 1993). Human platelets contain C1-Inh in their  $\alpha$ -granules, and the platelet levels of C1-Inh correlate with plasma C1-Inh levels (Schmaier et al., 1993). Activated platelets can express C1-Inh on their external membrane (Schmaier et al., 1993). Because plasma C1-Inh does not bind and get absorbed onto platelets, it was suggested that the platelet C1-Inh level depends on synthesis of C1-Inh in megakaryocytes. In agreement herewith, human megakaryocytes contain C1-Inh mRNA (Schmaier et al., 1993).

Synthesis of C1-Inh is stimulated by IFN- $\gamma$  and, to a lesser extent, by several other cytokines including tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), IFN- $\alpha$ , monocyte colony-stimulating factor, and interleukin-6 (IL-6) (Lotz and Zuraw, 1987; Katz and Strunk, 1989; Heda et al., 1990, 1996; Zuraw and Lotz, 1990; Schmidt et al., 1991; Lappin et al., 1992; Schwogler et al., 1992). Studies on human erythroleukemia cells and on plasma specimens of patients receiving intravenous IFN- $\gamma$  because of metastatic colorectal carcinoma indicated that IFN- $\gamma$  can increase C1-Inh protein expression in vitro and in vivo (Heda et al., 1990). In purified blood monocytes, the release of functional C1-Inh was markedly increased in the presence of IFN- $\gamma$ , but less with IFN- $\alpha$  or IFN- $\beta$  (Lotz and Zuraw, 1987). It was shown that induction of C1-Inh mRNA in IFN- $\gamma$ -stimulated cells is primarily due to the enhanced transcription rate of its gene (Zahedi et al., 1994). The IFN- $\gamma$ -responsive element has been characterized and located in the 5'-flanking region of the C1-Inh gene (Zahedi et al., 1997b). Studies with human hepatoma cell line cultures (HepG2) indicated that phosphatase 2A is required to dephosphorylate a substrate to allow IFN- $\gamma$  to induce transcriptional up-regulation of C1-Inh mRNA (Heda et al., 1996). However, in a small study with six volunteers and two patients with type I angioedema, a 4-day course of administration of IFN- $\gamma$  failed to influence plasma levels of C1-Inh (Gluszko et al., 1994).

C1-Inh is an acute phase protein, the plasma levels of which may increase up to 2-fold during uncomplicated infections (Kalter et al., 1985). The synthetic rate of C1-Inh may increase to up to 2.5 times the normal rate in patients with rheumatoid arthritis (Woo et al., 1985). This increased synthesis during acute phase responses is probably the result of the release of IL-6, because in vitro studies with human HepG2 cells have shown IL-6, and to a lesser extent IL-1, to increase the biosynthesis of C1-Inh as well as of the complement components C3 and of factor B (Falus et al., 1990).

### C. Genetics

C1-Inh is encoded by a 17-kilobase single-copy gene on chromosome 11, which consists of eight exons separated by seven introns (Davis et al., 1986; Theriault et al., 1990; Carter et al., 1991). The first intron contains IFN- $\gamma$ -responsive elements that are functional in vitro and may play a role in IFN- $\gamma$ -mediated induction of C1-Inh synthesis (Zahedi et al., 1997a). The second exon contains the translation initiation site, whereas the DNA encoding the reactive center sequence is situated within exon 8 (Donaldson and Bissler, 1992). Approximately 20% of described mutations of the C1-Inh gene are large deletions or duplications, and a substantial proportion of mutations are localized within exon 8, the coding region for the reactive center of C1-Inh (Bissler et al., 1997).

### D. Interaction with Target Proteinases

Interaction of C1-Inh with target proteinases results in the formation of SDS-stable enzyme-inhibitor complexes and proteolytically cleaved C1-Inh (Schapira et al., 1988). Analogous to other serpins, C1-Inh inhibits a target proteinase by presenting a peptidyl bond (P1-P1') lying on an exposed loop within the reactive center that matches the substrate specificity of the proteinase. Attack on this peptidyl bond, connecting residues P1 (Arg-444) and P1' (Thr-445), results in the formation of a complex between inhibitor and proteinase (Sim et al., 1979; Van den Graaf et al., 1983; Pixley et al., 1985). The importance of the P1 residue and this peptidyl bond with regard to the binding capacity to target proteinases was demonstrated by construction of various P1 substitutions that resulted in nonfunctional molecules in the majority of cases (Eldering et al., 1992).

The complexes formed between C1-Inh and proteinase are removed from the circulation with an apparent half-life time of clearance ranging from 20 to 47 min (Nuijens et al., 1988; De Smet et al., 1993; Wuillemin et al., 1996a). The complexes were found to be removed via receptors specific for complexed serpins, such as the low-density lipoprotein receptor-related protein on hepatocytes or fibroblasts (Pizzo et al., 1988; Perlmutter et al., 1990; De Smet et al., 1993; Storm et al., 1997). Neutrophils and monocytes have been shown to express a not yet completely characterized serpin-enzyme complex receptor, which binds, internalizes, and degrades several serpin-proteinase complexes, including those with  $\alpha$ 1-antitrypsin,  $\alpha$ 1-antichymotrypsin, antithrombin, and to a lesser extent C1-Inh (Perlmutter et al., 1990).

### E. Inactivation

C1-Inh, like most other serpins, can be inactivated by elastase released from activated neutrophils by limited proteolytic cleavage resulting in the production of several different and characteristic derivatives, so called "modified C1-Inh" (Brower and Harpel, 1982; Carrell et

al., 1987; Weiss, 1989). C1-Inh mutants with a decreased susceptibility for inactivation by elastase have been developed, but their therapeutic efficacy remains to be established (Eldering et al., 1993). Human proteinase 3, isolated from human leukocytes, cleaves and inactivates human C1-Inh in a time- and dose-dependent manner (Leid et al., 1993). Likewise, bacterial elastases and proteinases were shown to proteolytically cleave and inactivate C1-Inh (Catanese and Kress, 1984). Finally, plasmin was found to play a role in the local cleavage and degradation of C1-Inh in inflammatory processes (Wallace et al., 1997). Also thrombin may inactivate C1-Inh (M. Cugno, I. Bos, and C. E. Hack, unpublished results), although the significance of this process remains to be established.

Thus, the inactivation of C1-Inh may predominantly occur locally in inflamed tissues and, therefore, contribute to increased local complement activation and consumption as well as to local potentiation of pathological proteolysis (Brower and Harpel, 1982; Leid et al., 1993). This conclusion is supported by the demonstration of increased plasma levels of modified C1-Inh in patients with sepsis (Nuijens et al., 1989).

### F. Half-Life and Clearance

In normal volunteers the fractional catabolic rate of C1-Inh is 2.5% of the plasma pool per hour, yielding an apparent plasma half-life time of clearance of  $\sim$ 28 h (Quastel et al., 1983; Woo et al., 1985). The half-life time of clearance of human C1-Inh in rabbits is comparable, i.e., 26 h (Minta, 1981), whereas in rats it is considerably shorter, i.e.,  $\sim$ 4.5 h (De Smet et al., 1993). The apparent half-life time of clearance has been reported to be considerably longer in patients with HAE, in whom it may be 48 h (Agostoni et al., 1980; Gadek et al., 1980). It is to be noted, however, that the clearance half-life times of C1-Inh in these patients are often determined by assessing the course of plasma levels following the intravenous administration of  $\sim$ 1000 U. Presumably it is not correct to determine the half-life time of clearance in these patients in this way, because it is not considered that at lower plasma levels of C1-Inh (as occurs in untreated HAE patients), the first component of complement, C1, is autoactivated, 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 because of the administration of exogenous C1-Inh as well as a reduced consumption of endogenous C1-Inh.

We have administered high doses of C1-Inh (up to 12,000 U over a period of 2 to 5 days) to 12 patients with septic shock (Hack et al., 1993; Hack, 1996). Plasma C1-Inh concentration was measured at various time-points during the study period. To calculate the recovery of C1-Inh in these patients, we used a pharmacokinetic

model assuming that, a) the fractional catabolic rate of C1-Inh is 2.5% of the plasma pool per hour; b) the C1-Inh concentration at a given time after administration of exogenous C1-Inh is described by the sum of a constant concentration due to endogenous production and a concentration increase resulting from the C1-Inh administration; c) the C1-Inh increase resulting from the administration of exogenous C1-Inh is equal to the summation of the concentration effects of each subsequent administered exogenous dose, distributed immediately in one central plasma compartment, and constantly eliminated from there following a first order process; and d) the plasma volume in patients with sepsis is approximately 45 ml/kg of body weight. The overall correlation between the course of C1-Inh levels after the various administrations of C1-Inh calculated according to this model and those actually measured in the patients was very significant ( $r = 0.7807$ ,  $P < .0001$ ; A. C. Ogilvie, C.E.H., L. G. Thijs, J. Wagsteff, unpublished observations), although on some occasions in individual patients, recovery was less than expected, possibly due to a higher fractional catabolic rate (Hack et al., 1993). These results indicate that the clearance data observed with radiolabeled C1-Inh in human volunteers (Quastel et al., 1983; Woo et al., 1985) may be used to calculate the dose of C1-Inh to be administered to patients. Recently, we have evaluated the effects of high doses of C1-Inh in septic baboons.

C1-Inh was administered to yield 5- to 10-fold increased levels over a period of 8 h. The observed course of C1-Inh exactly matched that calculated from the clearance data described above (Jansen et al., 1998), again demonstrating the validity of the pharmacokinetic model.

### III. C1-Inh As Inactivator of Plasma Cascade Systems and Leukocytes

C1-Inh is the only known inhibitor of the activated serine proteinases C1s and C1r from the classical pathway of complement, and is a major inhibitor of activated factor FXII (FXIIa) from the contact system, as well as an inhibitor of kallikrein and activated factor XI (FXIa) (Chan et al., 1977; Sim et al., 1979; Ziccardi, 1981; Schapira et al., 1982, 1985; Van den Graaf et al., 1983; Cooper, 1985; Pixley et al., 1985; Scherer et al., 1996; Wuillemin et al., 1995b) (see Fig. 1). C1-Inh is, therefore, an important regulator of inflammatory reactions and of the intrinsic pathway of coagulation.

#### A. Complement System

The complement system consists of more than 30 serum and cellular proteins linked in three biochemical cascades, the classical and the alternative pathway (Makkrides, 1998) and the mannan-binding lectin (MBL) pathway (see Fig. 2). The classical pathway is

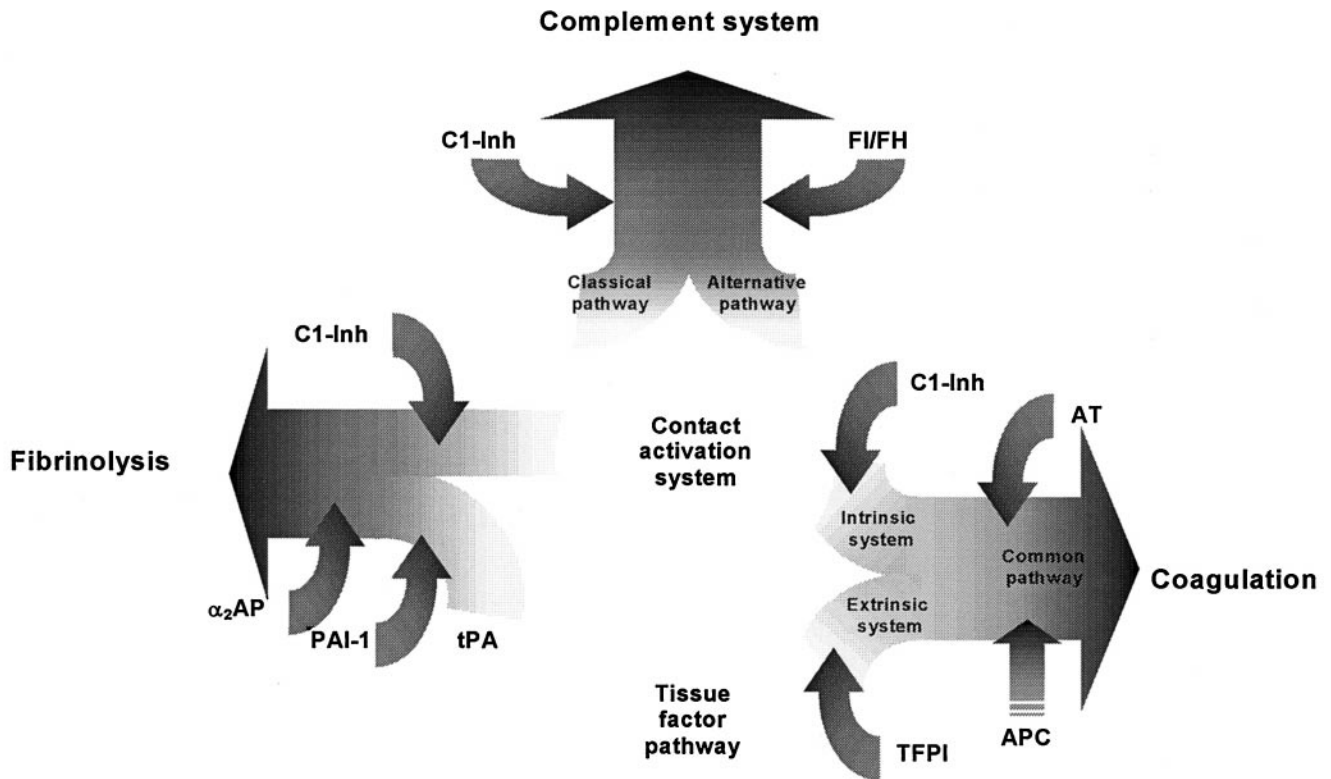


FIG. 1. Activation of plasma cascade systems. During host defense reactions, complement, coagulation, and the fibrinolytic system are activated. C1-Inh is the only known inhibitor of C1s and C1r of the classical pathway of the complement system, as well as a major inhibitor of FXIIa and kallikrein of the contact system and of FXIa, which links the contact activation system to the coagulation cascade. FI, Factor I; FH, Factor H; Ag, antigen; Ab, antibody; LPS, lipopolysaccharides; AT, antithrombin; TFPI, tissue factor pathway inhibitor; APC, activated protein C; t-PA, tissue plasminogen activator;  $\alpha_2$ -AP,  $\alpha_2$ -antiplasmin; shaded down arrow, action of C1-Inh (or other inhibitors).

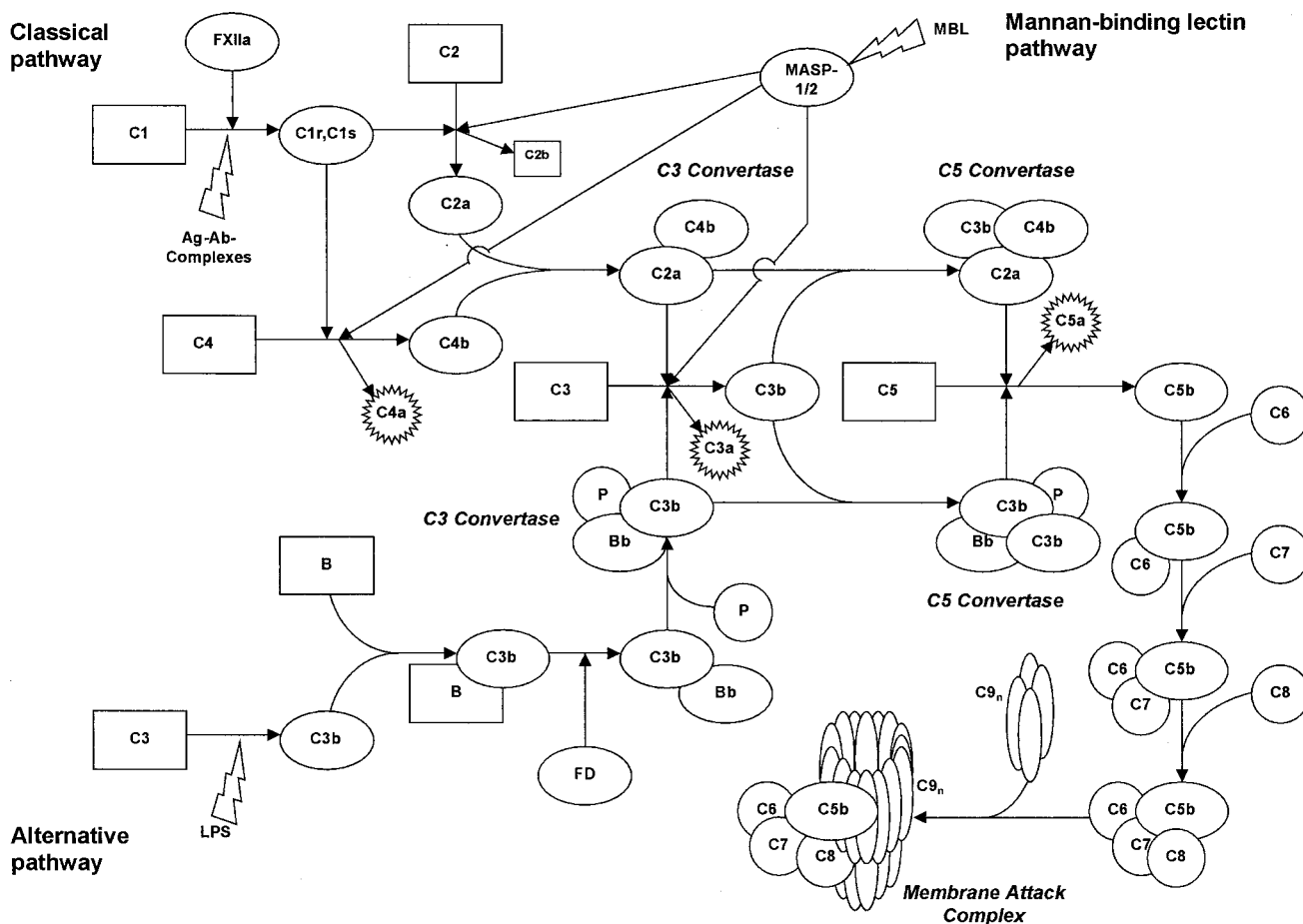


FIG. 2. The complement system. The complement system consists of three plasmatic cascades each leading to the formation of the C3 and C5 convertases. The classical pathway is initiated when antigen-antibody complexes bind to C1 or by cleavage of C1 by FXIIa. The alternative pathway is triggered by microbial surfaces and complex polysaccharides. The MBL pathway is triggered by polysaccharides in particular those occurring on bacterial surfaces. The common final step of complement activation is the activation of C5 by the C5 convertases that leads to the formation of the MAC C5b-9, which finally inserts into target cell membranes and causes cell lysis. The peptides C3a, C4a, and C5a are known as anaphylatoxins and mediate several reactions in the inflammatory response, including smooth muscle cell contractions, changes in vascular permeability, chemotaxis for human mast cells, histamine release from mast cells, neutrophil chemotaxis, and platelet activation and aggregation. Ag-Ab complexes, antigen-antibody complexes; LPS, lipopolysaccharides; C, complement.

usually initiated when a complex of antigen and immunoglobulin M (IgM) or IgG antibody binds to the first component of complement, C1. Activated C1 cleaves both C4 and C2 to generate C4a and C4b, and C2a and C2b, respectively. The C4b and C2a fragments combine to form the classical C3 convertase, which cleaves C3 to form C3a and C3b. The binding of C3b to the C3 convertase yields the C5 convertase, which cleaves C5 into C5a and C5b, the latter becoming part of the membrane attack complex (MAC; Makkrides, 1998).

The alternative pathway of the complement system is triggered by microbial surfaces and a variety of complex polysaccharides. C3b, formed by the spontaneous low-level cleavage of C3, can bind to nucleophilic targets on cell surfaces and forms a complex with factor B that is subsequently cleaved by factor D. The resulting alternative C3 convertase is stabilized by the binding of properdin. Cleavage of C3 and binding of an additional C3b to the C3 convertase give rise to the C5 convertase. The C3 and C5 convertases of the alternative pathway are con-

trolled by complement receptor type 1, decay-accelerating factor, membrane cofactor protein, and by factor H (Makkrides, 1998). The C5 convertase cleaves C5 to produce C5a and C5b. Thereafter, C5b sequentially binds to C6, C7 and C8 to form C5b-8 that catalyzes the polymerization of C9 to form the MAC, which inserts into target membranes and causes cell lysis (Hu et al., 1981; Podack et al., 1982; Tschopp et al., 1982). Vitronectin and similarly clusterin control fluid phase MAC by binding to the C5b-7 complex to prevent its insertion into membranes (Podack et al., 1977; Jenne and Tschopp, 1989). C8 binding protein (CD59) blocks MAC formation by binding to C8 and C9 (Rollins et al., 1991).

The MBL pathway is triggered by binding of MBL to polysaccharides of various microbes (Turner, 1996). Subsequently, MBL stimulates the activation of MBL-associated serine proteinase-1 (MASP-1) and MASP-2 (Matsushita and Fujita, 1992; Thiel et al., 1997). MASP-1 and MASP-2 can activate C4 (International Complement Workshop, Rhodes, Greece, October 1998), leading

to classical pathway activation. MASP-1 and MASP-2 can be inhibited by C1-Inh.

The peptides C3a, C4a, and C5a are known as anaphylatoxins (Hugli and Müller-Eberhard, 1978). They mediate several reactions in the inflammatory response, including smooth muscle contractions, changes in the vascular permeability, chemotaxis for human mast cells, histamine release from mast cells, neutrophil chemotaxis, and platelet activation and aggregation (Hugli and Müller-Eberhard, 1978; Morgan, 1986; Gerard and Gerard, 1994; Hartmann et al., 1997). The anaphylatoxins are rapidly inactivated by carboxypeptidase N (Bokisch and Müller-Eberhard, 1970).

Antibody-mediated complement activation at the cell surface has been demonstrated to result in increased tissue factor activity, indicating that complement fixation on the cell surface can have a direct stimulatory effect on the coagulation cascade (Carson and Johnson, 1990).

Activation of the classical pathway of complement is regulated by C1-Inh. It is the only known inhibitor of the activated serine proteinases C1s and C1r from the classical pathway of complement (Sim et al., 1979; Schapira et al., 1985). C1-Inh either binds reversibly to proenzymic C1r and C1s within intact C1 to prevent the autoactivation of these proteinases (Bianchino et al., 1988) or binds to activated C1r and C1s and dissociates them from C1q in the form of a C1-Inh–C1r–C1s–C1-Inh tetramer (Sim et al., 1979; Liszewski et al., 1996). The rate of inhibition of C1r by C1-Inh is significantly slower than that of C1s (Sim et al., 1980). Interaction of C1-Inh with either activated C1r or C1s results in the formation of cleaved C1-Inh and an SDS resistant enzyme-inhibitor complex (Harpel and Cooper, 1975; Reboul et al., 1977; Arlaud et al., 1979; Ziccardi and Cooper, 1979; Chesne et al., 1982; Salvesen et al., 1985).

C1-Inh–C1s complexes were shown to be finally removed by the low-density lipoprotein receptor-related protein of murine fibroblasts and probably of hepatocytes but did not bind to the serpin-enzyme complex receptor of HepG2 cells, neutrophils, or monocytes nor to the hepatic asialoglycoprotein receptor (Storm et al., 1997).

### B. Contact Activation

FXII, prekallikrein, high-molecular weight kininogen (HK), and FXI are grouped together as “contact system,” because they require contact with negatively charged surfaces for zymogen activation (Schmaier, 1997) (see Fig. 3). In vitro, FXII and prekallikrein reciprocally activate each other on contact with macromolecules such as kaolin, glass, celite, or dextran sulfate (Colman, 1984; Kaplan and Silverberg, 1987). In addition, FXII is able to autoactivate (Kaplan and Silverberg, 1987). Activation of FXII leads to the formation of the activated fragments  $\alpha$ FXIIa (or FXIIa) and  $\beta$ FXII (or FXIIf). The strictly surface-dependent  $\alpha$ FXIIa converts FXI to FXIa,

whereas  $\beta$ FXIIa is an effective prekallikrein activator (Kaplan and Silverberg, 1987). Soluble  $\beta$ FXIIa has been shown to activate the first component of complement (Ghebrehiwet et al., 1983). Activated FXII has the ability to cleave plasminogen, rendering it to a weak activator of the fibrinolytic system (Colman et al., 1975).

HK is a nonenzymatic cofactor that augments reciprocal activation of FXII and prekallikrein as well as the rate of FXI activation by FXIIa (Griffin and Cochrane, 1976; Meier et al., 1977). It is the pivotal protein for contact protein assembly on endothelium (Schmaier, 1997).

The zymogen prekallikrein becomes activated to kallikrein when it binds to HK on endothelial cells. The resulting proteinase kallikrein is the major activator of FXII (Van den Graaf et al., 1982). Kallikrein activates the fibrinolytic system either by activation of single-chain urokinase or of plasminogen (Colman, 1969; Motta et al., 1998). Kallikrein has been shown to prime neutrophils for superoxide production (Zimmerli et al., 1989). Finally, kallikrein cleaves HK at two sides to liberate the potent vasoactive nonapeptide bradykinin (Kaplan and Silverberg, 1987; Schmaier, 1997). Bradykinin is known to stimulate endothelial cell prostacyclin synthesis, leading to inhibition of platelet function (Hong, 1980; Crutchley et al., 1983), to increase superoxide formation (Holland et al., 1990), to release tissue-type plasminogen activator (t-PA) (Smith et al., 1985), as well as to induce the formation of nitric oxide (Palmer et al., 1987). HK and bradykinin are selective inhibitors of  $\alpha$ -thrombin-induced platelet activation (Meloni and Schmaier, 1991; Hasan et al., 1996).

In an intact vessel the sum of bradykinin activities is to keep blood flowing and vessels patent; in the absence of endothelium, bradykinin stimulates repair of vessels, which leads to smooth muscle proliferation and intimal hypertrophy (Schmaier, 1997). Bradykinin is thought to play a major role in the symptomatology of acute attacks in patients with HAE (Fields et al., 1983; Al-Abdullah and Grealley, 1985; Kaplan et al., 1989; Shoemaker et al., 1994; Cicardi et al., 1998; Nussberger et al., 1998). Activation of the kallikrein-kinin system during acute attacks of HAE indeed has been demonstrated by a significant increase in the cleavage of HK (Fields et al., 1983).

Degradation of bradykinin into inactive fragments depends on the activity of the angiotensin-converting enzyme, which in turn has been documented to be severely decreased in patients with septic shock and septic adult respiratory distress syndrome (Fourrier et al., 1985).

C1-Inh is a major inactivator of  $\alpha$ FXIIa and  $\beta$ FXIIa and besides  $\alpha$ 2-macroglobulin a major inactivator of kallikrein (Nilsson, 1983a; Pixley et al., 1985; Kaplan and Silverberg, 1987; Schapira et al., 1988). Incubation of radiolabeled FXIIa with various plasma proteinases in purified systems and in human plasma demonstrated FXIIa–C1-Inh complex the predominant complex to be formed confirming the major inhibitory role of C1-Inh

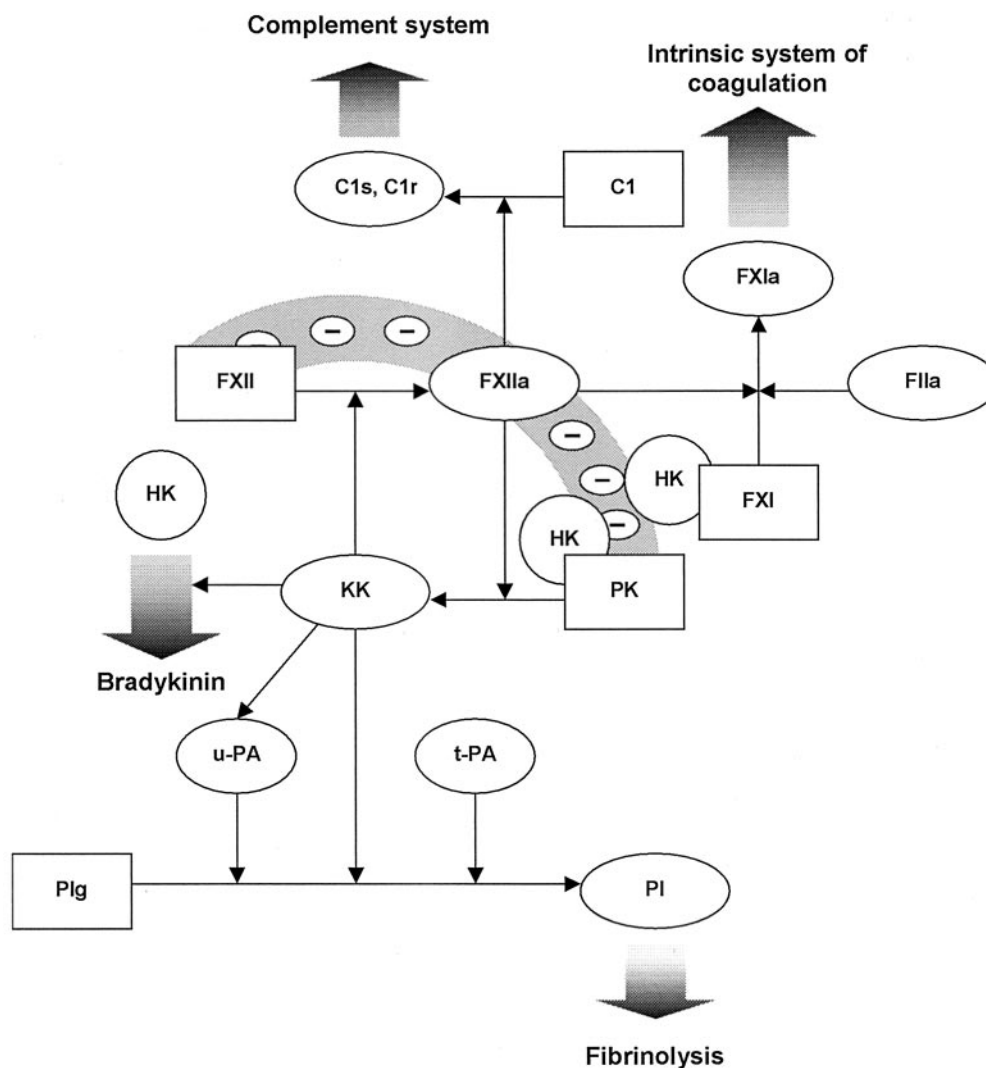


FIG. 3. Contact activation system. The contact system consists of the proteins FXII, prekallikrein, HK, and FXI. FXII and prekallikrein activate each other upon contact with negative surfaces (kaolin, glass, dextran sulfate). The activation of C1 by FXIIa activates the classical pathway of the complement system. FXIIa activates prekallikrein and FXI, the latter forming the link to the intrinsic coagulation system. Kallikrein activates the fibrinolytic system by direct conversion of plasminogen to plasmin or by activation of u-PA. HK is a nonenzymatic cofactor for PK and FXI activation. Cleavage of HK by kallikrein liberates the potent vasoactive nonapeptide bradykinin. F, coagulation factor; "a", activated coagulation factor; C, complement; PK, prekallikrein; KK, kallikrein; Plg, plasminogen; PI, plasmin.

for FXIIa (Pixley et al., 1985). The majority of the additional inhibitory capacity toward FXIIa is provided by  $\alpha$ 2-macroglobulin (Davis, 1998).

C1-Inh and  $\alpha$ 2-macroglobulin are the predominant inhibitors of kallikrein (Schapira et al., 1988). In acute attacks of HAE, despite activation of the contact system, the C1-Inh-kallikrein complexes did not increase, leading to the suggestion that  $\alpha$ 2-macroglobulin may compensate, to a certain extent, for the lack of inhibitory capacity of C1-Inh toward kallikrein (Waage Nielsen et al., 1996).

### C. Intrinsic Pathway of Coagulation

FXI is the coagulation protein that links contact activation to intrinsic blood coagulation (see Fig. 4). FXI circulates in plasma complexed to HK (Thompson et al., 1977). It is activated by FXIIa into the active form FXIa

(Bouma and Griffin, 1977; Kurachi and Davie, 1977). Because patients deficient in FXII, prekallikrein, or HK do not suffer from a bleeding tendency, contrary to the variable bleeding disorder of patients with FXI deficiency, an alternative pathway for the activation of FXI has been assumed (Gailani and Broze, 1991; Naito and Fujikawa, 1991). Recently it has been demonstrated that thrombin and FXa can activate FXI in the presence of dextran sulfate or activated platelets (Gailani and Broze, 1991; Naito and Fujikawa, 1991; Roberts et al., 1998).

A current concept of blood coagulation depicts the tissue factor-FVIIa complex as the initiator of all subsequent reactions: The cell-based tissue factor-FVII complex activates both, FIX and FX, the latter interacting subsequently with FV. The FXa-FVa complex then converts prothrombin to thrombin. The amount of thrombin



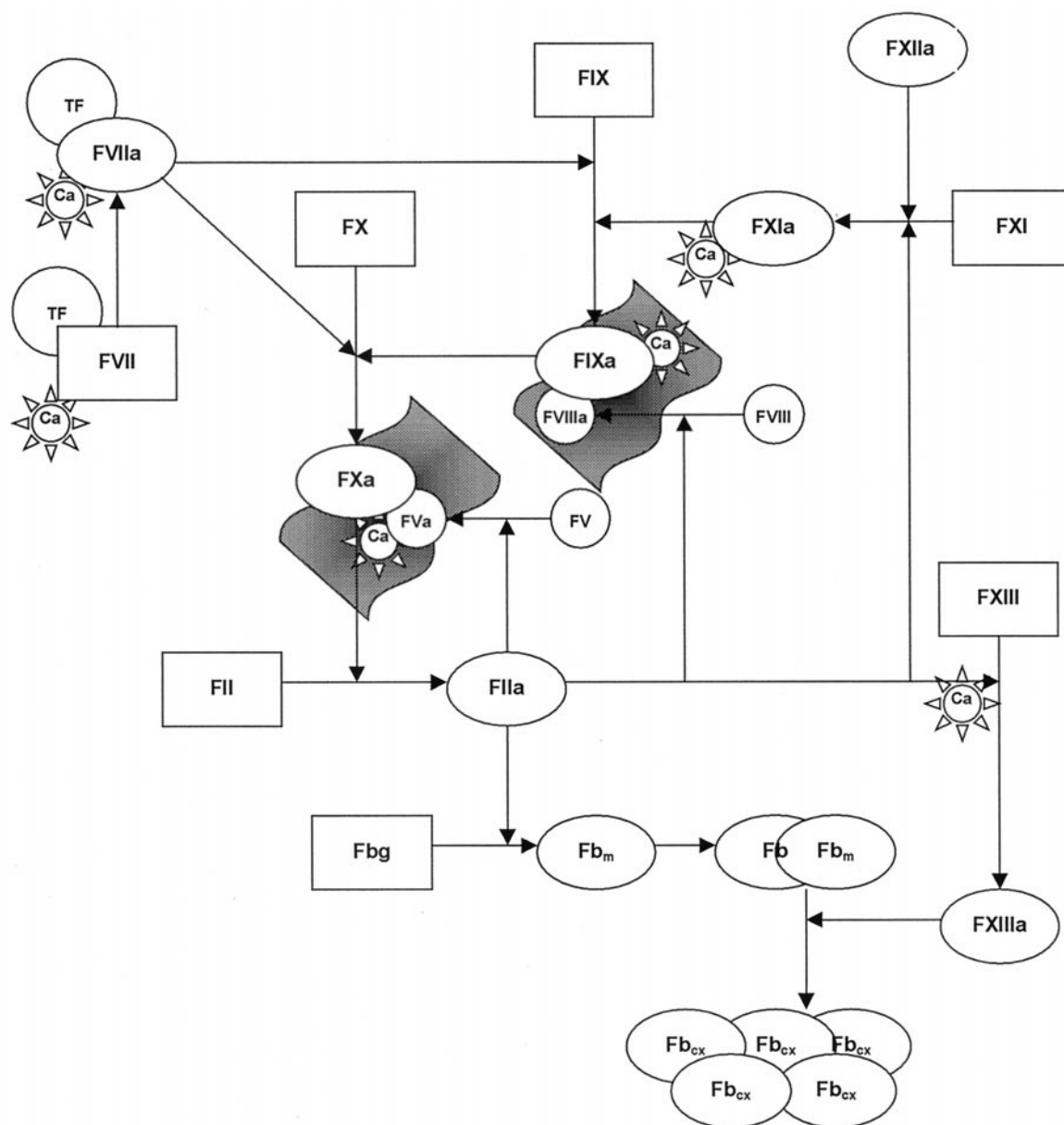


FIG. 4. Coagulation system. A current concept of blood coagulation depicts the tissue factor-FVIIa complex to be the initiator of primary thrombin generation. This initial amount of thrombin is sufficient for the activation of platelets FV, FVIII, and FXI, but it is insufficient for the formation of a stable fibrin clot. Only the subsequent burst of thrombin generation by activated FXI, FIX, and FVIII leads to quantitative thrombin generation and to the formation of a stable fibrin clot (Roberts et al., 1998) (Fig. 4). These data suggest that activation of FXI by thrombin is an alternative pathway for the activation of blood coagulation and that the role of FXI is to enhance and maintain thrombin generation.

generated in this way is sufficient to activate platelets and to activate FV, FVIII and FXI. However, it is insufficient for the formation of a stable fibrin clot (Roberts et al., 1998). Only the subsequent burst of thrombin generation by activated FXI, FIX, and FVIII leads to quantitative thrombin generation and to the formation of a stable fibrin clot (Roberts et al., 1998) (Fig. 4). These data suggest that activation of FXI by thrombin is an alternative pathway for the activation of blood coagulation and that the role of FXI is to enhance and maintain thrombin generation.

Experiments in plasma milieu demonstrated C1-Inh to be the main inhibitor of FXIa, contributing to 47% of

FXIa inactivation in EDTA plasma, followed by  $\alpha$ 2-antiplasmin (24.5%),  $\alpha$ 1-antitrypsin (23.5%), and antithrombin (5%) (Wuillemin et al., 1995b). This had been confirmed in chimpanzees given a bolus infusion of FXIa, where C1-Inh was shown to be the main inactivator of FXIa, followed by  $\alpha$ 2-antiplasmin,  $\alpha$ 1-antitrypsin, and antithrombin (Wuillemin et al., 1996c).

More evidence that C1-Inh plays a role in the regulation of blood coagulation raised from studies on patients with hereditary or acquired angioedema, where it has been shown that levels of coagulation FVIIa, prothrombin fragments F1 + 2, and thrombin-antithrombin complex significantly increased during acute attacks, al-

though remaining normal in remission (Waage Nielsen et al., 1995, 1996).

#### D. Fibrinolytic System

The physiologic activators of the fibrinolytic system are t-PA and urokinase-like plasminogen activator (u-PA). Both serine proteinases are quite capable of converting plasminogen to plasmin (Vassalli et al., 1991). Cleavage of single-chain t-PA to the two-chain form increases its binding affinity for fibrin (Husain et al., 1989). Single-chain u-PA can be rapidly converted to two-chain u-PA by plasma kallikrein and plasmin. Two-chain u-PA has greater enzymatic activity but is less fibrin-specific (Zamarron et al., 1984). More recent studies suggested that the activation of single-chain u-PA by kallikrein can best occur on a platelet or endothelial surface (Gurewich et al., 1993; Loza et al., 1994). Kallikrein-activated single-chain u-PA is considered the major physiological activator of plasminogen (Hauert and Bachmann, 1985; Motta et al., 1998).

Kallikrein, FXIIa, and FXIa have the ability to cleave plasminogen directly, albeit much less efficiently than t-PA or u-PA (Schmaier, 1997). However, activation of plasminogen by FXIIa is considerably potentiated in the presence of cofactors (Schousboe, 1997) with the result that at plasma concentrations FXIIa is as potent as u-PA in clearing plasminogen. In agreement herewith, Levi et al. (1991) demonstrated that the plasminogen-converting activity induced by DDAVP is 50% dependent on t-PA, 25% on u-PA and 25% on FXIIa.

In patients with HAE, antigenic levels of t-PA and u-PA as well as t-PA-C1-Inh complexes remained normal during both remission and acute attacks. However, during acute attacks plasmin-antiplasmin complexes were significantly increased, supporting an enhanced activation of the fibrinolytic system via direct activation of plasminogen by kallikrein and FXIIa in a C1-Inh deficiency state (Waage Nielsen et al., 1996).

The main inhibitor of single-chain and two-chain t-PA and two-chain u-PA is plasminogen activator inhibitor type-1 (PAI-1) (Kruithof et al., 1986). PAI-2, originating from human placenta and macrophages and being distinct from PAI-1, inhibits two-chain t-PA and two-chain u-PA (Lecander and Astedt, 1986). The principal physiologic inhibitor of plasmin is  $\alpha$ 2-antiplasmin, which is present in plasma and in platelets. C1-Inh as well as  $\alpha$ 2-macroglobulin, antithrombin, and  $\alpha$ 1-antitrypsin only play a limited physiologic role as plasmin inhibitors (Levi et al., 1993).

C1-Inh contributes only slowly and to a minor extent to the inactivation of t-PA when t-PA levels are normal (Huisman et al., 1995). However, when t-PA circulates at high concentrations, e.g., during thrombolytic therapy or escapes rapid liver clearance, e.g., in case of venous occlusion, an increase of circulating t-PA-C1-Inh complexes has been demonstrated (Huisman et al., 1995).

#### E. Leukocytes

C1-Inh has been shown to inhibit the activation of CD4- and CD8-positive T-lymphocytes by specific cleavage of the major histocompatibility complex class 1 molecules, whereas no effect on B-lymphocytes has been demonstrated (Eriksson and Sjögren, 1995). In a model of allogene- or mitogen-activated murine or human lymphocyte cultures the addition of C1-Inh was followed by the down-regulation of the activity and proliferation of cytotoxic T-lymphocytes (Nissen et al., 1998). The addition of C1-Inh altered the production of cytokines by T-lymphocytes, increasing the production of IFN- $\gamma$ , IL-10, and IL-12 (Nissen et al., 1998). Because, inversely, IFN- $\gamma$  is capable of inducing the production of C1-Inh, we concluded that C1-Inh and IFN- $\gamma$  up-regulate each other during the maturation of the immune response, indicating a regulatory function of C1-Inh on T-cell-mediated immune functions.

#### IV. Potentiation of C1-Inh Activity by Glycosaminoglycans

The activity of a group of serpins, such as antithrombin, heparin cofactor II, and PAI-1 is potentiated by glycosaminoglycans (GAG) (Potempa et al., 1994). Early reports suggested the inhibitory activity of C1-Inh toward C1s also to be enhanced by heparin (Rent et al., 1976; Sim et al., 1980; Caughman et al., 1982; Nilsson and Wiman, 1983b; Lennick et al., 1986), whereas GAG had no effect on the interaction of C1-Inh with FXIIa (Pixley et al., 1987). Heparin and other GAGs have multiple inhibitory effects on the complement system, such as that on the binding of C1q to an activator and that on the formation of C3-convertases of the classical or the alternative pathway.

We, therefore, studied the influence of various physiological (heparin, heparan sulfate, dermatan sulfate, chondroitin sulfate) and nonphysiological (dextran sulfate) GAGs on the kinetics of the interaction of C1-Inh with its target proteinases C1s, FXIa,  $\alpha$ FXIIa,  $\beta$ FXIIa, and kallikrein. First, we showed that the inactivation of C1s by C1-Inh is increased 6- to 130-fold in the presence of GAG, with dextran sulfate being the most effective GAG to enhance inactivation of C1s by C1-Inh (Wuillemin et al., 1997). Moreover, GAGs reduced the deposition of C3 and C4 on immobilized aggregated human IgG and also reduced the fluid phase formation of C4b/c and C3b/c in recalcified plasma upon incubation with aggregated IgG (Wuillemin et al., 1997). In similar experiments we demonstrated that in the presence of GAG the rate constant of the inactivation of FXIa by C1-Inh increased up to 117-fold compared with the rate of inactivation in the absence of any GAGs (Wuillemin et al., 1996b). Recently, low-molecular weight heparins, such as dalteparin, enoxaparin, and nadroparin and low-molecular weight dextran sulfate were found to increase the inactivation of FXIa by C1-Inh up to 39-fold. More-

over, in the presence of low-molecular weight heparin or low-molecular weight dextran sulfate, FXIa was inactivated in human plasma to more than 90% by C1-Inh (Mauron et al., 1998). In contrast, we found no significant influence of the tested GAGs on the inhibition of kallikrein by C1-Inh and about a 2-fold protection of  $\alpha$ FXIIa and  $\beta$ FXIIa from inhibition by C1-Inh in the presence of dextran sulfate (Wuillemin et al., 1996b).

These findings definitely demonstrate that C1-Inh belongs to the group of GAG-sensitive serpins. Moreover, the results suggest the possibility of developing a C1-Inh preparation with strongly enhanced inhibitory activity. Interestingly, the influence of GAGs on C1-Inh function is not only a quantitative one but also modulates the inhibitory spectrum of C1-Inh; physiological GAGs selectively enhance the inactivation of the complement system (C1s) and the intrinsic coagulation (FXIa) but do not affect the activity of FXIIa and kallikrein, suggesting that GAGs may modulate the biological effects of contact activation by inhibiting intrinsic coagulation without affecting the fibrinolytic potential of FXIIa and kallikrein (Wuillemin et al., 1996b).

## V. C1-Inh Therapy in Animal Models and Clinical Disease

The treatment of acute attacks in patients suffering from hereditary angioedema with C1-Inh purified from pooled human plasma is well established (Agostoni et al., 1980; Gadek et al., 1980; Bork and Witzke, 1989; Waytes et al., 1996) and will not be discussed further. Here we will elaborate on the therapeutic application of C1-Inh in other diseases (Tables 2 and 3).

### A. Sepsis

Sepsis is often induced by bacterial infections and is a leading cause of mortality in noncardiologic intensive care units. Sepsis results from the excessive release and activation of endogenous inflammatory mediators, which include the complement and contact systems. In a study of experimental endotoxemia in healthy volunteers, activation of the contact system was suggested to occur due to endothelial injury during the septic process (De La Cadena et al., 1993). Another mechanism leading to activation of the contact factors was proposed to be a direct activation of the contact factors such as FXII and prekallikrein by bacterial lipopolysaccharides (Morrison and Cochrane, 1974; Kalter et al., 1983; ten Cate et al., 1993). Recently, as a clue to serious complications in infectious disease, the assembly and activation of the contact phase system on bacterial surfaces such as *Escherichia coli* and *Salmonella typhimurium* has been reported (Herwald et al., 1998). Although it has long been considered that the contact system is activated during sepsis, not until recently was this concept proven to be correct. Studies in primates showed decreasing levels of various contact system proteins during sepsis, accompa-

nied by increasing levels of activation products of the contact system (Pixley et al., 1992). In patients with septic shock, significantly decreased activities of FXII have been demonstrated (Kalter et al., 1985; Nuijens et al., 1988) and in children with meningococcal septic shock the plasma levels of FXII, FXI, and prekallikrein were found to be reduced to ~50% of normal (Wuillemin et al., 1995a). Definite evidence for the activation of the contact system during the septic process was provided by a study in septic baboons treated (before the bacterial challenge) with a monoclonal antibody that blocks the activation of FXII. This treatment had no effect on clotting activation but largely prevented the irreversible hypotension and slightly improved the survival of baboons challenged intravenously with a lethal dose of *E. coli* (Pixley et al., 1993). Thus, FXII activation during sepsis does not primarily contribute to clotting derangements, but probably, via the generation of kallikrein and subsequently bradykinin, contributes to the formation of nitric oxide and to vasodilation (Vane et al., 1990).

The complement system can be activated by bacteria and their products such as endotoxin even in the absence of antibodies (Morrison and Cochrane, 1974; Morrison and Kline, 1977; Kalter et al., 1983). The role of complement activation during sepsis seems to be dual. Some activation is necessary for an efficient clearance of bacteria or their products as demonstrated in animal studies. Dogs and mice with a genetic C3 deficiency are more susceptible to endotoxin than healthy littermates due to an impaired clearance of endotoxin (Quezado et al., 1994; Fischer et al., 1997). On the other hand, inhibition of the biological effects of C5a in baboons suffering from sepsis attenuated lethal complications (Stevens et al., 1986), illustrating that the proinflammatory effects of complement activation, in particular those of C5a, may contribute to the complications of sepsis. Complement component C5a and the MAC have proinflammatory effects such as accumulation and stimulation of neutrophils and may increase the permeability of endothelial cells, mediated in part by histamine, and promote coagulation by inducing expression of tissue factor (Björk et al., 1985; Hack et al., 1993). In animal models, intravenous C5a can induce a fall in mean arterial blood pressure and leukopenia, the latter probably due to aggregation and subsequent sequestration of leukocytes (Lundberg et al., 1987). Finally, C5a is able to induce or enhance production of cytokines as IL-1, TNF, and IL-6 by monocytes (Cavaillon et al., 1990; Scholz et al., 1990). The proinflammatory effects of complement components during sepsis were supported by observations that C5-deficient mice tolerate endotoxin better than their C5-sufficient littermates (Olson et al., 1985). C5-deficient mice exhibit a 2-fold lower TNF response and a slower increase of pulmonary vasopermeability than C5-sufficient animals (Barton and Warren, 1993). Notably, these effects of complement on the release of cytokines have not been observed consistently. In an endotoxin model in

TABLE 2  
C1-Inh administration in animal models

Condition	Animal	Model	Dosage of C1-Inh	Results	Reference
Sepsis	Baboons	<i>E. coli</i> -induced sepsis	500 U/kg bolus i.v. and 200 U/kg/9 h, i.v.	Less activation of contact (FXII, prekallikrein) and complement (C4b/c) system	Jansen et al., 1998
Sepsis, hypercoagulability	Rabbits	<i>E. coli</i> endotoxin, 120 µg/kg	400 U/kg bolus i.v. and 400 U/kg/4 h, i.v.	Stabilization of mean arterial pressure, increase central venous oxygen saturation, reduced fibrin deposition in microcirculation	Scherer et al., 1996
Sepsis	Mice deficient in C3 and C4	<i>Salmonella typhimurium</i> endotoxin, 40 mg/kg	200 µg	Less sensitivity to endotoxin, enhanced clearance of endotoxin, improved survival rate	Fischer et al., 1997
Pulmonary dysfunction	Dogs	<i>E. coli</i> endotoxin, 2 mg/kg i.v.	500 U bolus i.v.	Prevention of hypoxemia, increase in intrapulmonary shunts, and decrease of factors of the contact system	Guerrero et al., 1993
Hemorrhagic pancreatitis	Rats	Retrograde injection of sodium taurocholate into the pancreatic duct	250 U/kg i.v., time points 1, 3, and 5 h combined with antithrombin, 250 U/kg/dose	Reduced mortality	Yamaguchi et al., 1997
Edematous and hemorrhagic pancreatitis	Mice/rats	Induction of pancreatitis with cerulein, ethionine-supplement, choline-deficient diet, or sodium taurocholate	100 U/kg i.v. before induction of pancreatitis	No beneficial effect with respect to survival, histology, and plasma amylase	Niederrau et al., 1995
Acute pancreatitis	Rats	Retrograde injection of sodium taurocholate in pancreatic duct	400 U/kg at various time intervals	Reduction of mortality, no beneficial effect with respect to edema and histopathological lesions	Vesentini et al., 1993
Acute pancreatitis	Pigs	Retrograde injection of sodium taurocholate in pancreatic duct	50 U/kg bolus i.v. followed by 50 U/kg after 6 h	Reduction of mortality, improvement of hemodynamic performance	Ruud et al., 1986
Acute myocardial infarction	Cats	Ischemia-reperfusion model (90 min of ischemia, 270 min of reperfusion)	15 mg/kg i.v.	Reduced necrotic infarction area, more rapid recovery of myocardial contractility	Buerke et al., 1995
Acute myocardial infarction	Pigs	Ischemia-reperfusion model (60 min ischemia, 120 min of reperfusion)	20 U/kg intracoronary before reperfusion	Reduction of myocardial injury, improvement of local myocardial contractility	Horstick et al., 1997
Acute myocardial infarction	Rats	Ischemia-reperfusion model (20 min of ischemia, 24 h of reperfusion)	100 U/kg i.v. before reperfusion	Reduction of infarction size by 60 to 70%	Murohara et al., 1995
Lung transplantation	Dogs	Allogeneic transplantation	500 U/animal i.v. to recipient and donor, 30 min before occlusion or reperfusion of lung circulation, respectively	Prevention of early pulmonary dysfunction	Salvatierra et al., 1997
Trauma	Rats	Noble-collip drum trauma	15 U/kg i.v., 10 min post-trauma	Prolonged survival and reduced mortality rate from 83 to 33%	Kochilas et al., 1997
Thermal injury	Pigs	Scald injury induced by hot water	100 U/kg i.v., bolus	Reduced organ damage, improved microcirculation, prevention of bacterial translocation in the gastrointestinal tract	Henze et al., 1997; Khorram-Sefat et al., 1998

TABLE 3  
C1-Inh administration in different clinical conditions

Clinical condition	Number of patients	Dosage of C1-Inh	Results	References
Septic shock	5	2000 U i.v. bolus followed by 1000 U daily for 4 days	No side effects, less vasopressor medication, no death	Hack et al., 1992
Septic shock	6	4000 U i.v. bolus, followed by 2000, 2000, and 1000 U or 6000 U i.v. bolus, followed by 3000, 2000, and 1000 U every 12 h	No side effects, no death	Hack et al., 1993
Open heart surgery; emergency coronary artery bypass grafting after failed percutaneous transluminal coronary angioplasty	3	2000 U i.v. bolus, followed by 1000 U after 12 and 24 h, respectively	Hemodynamic stabilization, improved myocardial contractility	Bauernschmitt et al., 1998
Vascular leak syndrome following bone marrow transplantation	15	60 U/kg i.v. bolus, followed by 2 × 30 U/kg and 4 × 15 U/kg every 12 h	1 year survival, 57% vs. 14% of controls	Nürnbergger et al., 1997
Vascular leak syndrome following high dose therapy with IL-2 in cancer patients	6 (with metastatic melanoma or renal cell carcinoma)	2000 U bolus i.v. followed by 1000 U every 12 h for 4 days	Comparable side effects of IL-2 despite 4-fold higher doses during treatment	Ogilvie et al., 1994
Vascular leak syndrome following open heart surgery	29 (children)	300 U/kg bolus i.v. followed by 2 doses of 150 U/kg, 3 doses of 100 U/kg, and 3 doses of 50 U/kg every 8 h	Improved hemodynamic, respiratory, and laboratory parameters, persistent vascular leak syndrome in 3 children, no amelioration of diuresis in 6 children	Stieh et al., 1996
Severe thermal injury with septic shock	16 (with severe burns and shock, systemic inflammatory response syndrome, or septic shock)	6000 U i.v. bolus, followed by 3000, 2000, and 1000 U every 12 h	Trend to reduced mortality (50 vs. 26% in a control group)	Jostkleigreve et al., 1997

rats inhibition of complement activation by administration of human soluble complement receptor-1 did not affect circulating TNF levels, although this treatment improved pulmonary responses during endotoxemia (Rabinovici et al., 1992). Thus, these studies suggest that during sepsis complement on the one hand is required for a rapid clearance of bacteria or their products, but on the other hand via the release of C5a and possibly other phlogistic fragments may enhance inflammatory reactions. Baboons challenged with lethal and sublethal doses of *E. coli* showed a biphasic pattern of complement activation consisting of a rapid initial activation and followed by a second more pronounced activation from about 6 h up to over 24 h (De Boer et al., 1993). Although the initial activation was probably due to a direct stimulation of the complement system, e.g., via IgG or IgM antibodies, the second phase of activation coincided with increasing levels of C-reactive protein (CRP), IL-2, and IL-6, suggesting a further complement activation via cytokines (De Boer et al., 1993).

Human studies revealed that plasma levels of native complement proteins are decreased in septic patients being the lowest in patients with fatal outcome (McCabe, 1973; Kalter et al., 1985). On the other hand, elevated plasma levels of C3a in patients with sepsis and septic shock were significantly correlated with mortality and patients with septic shock had significantly higher C3a levels than normotensive patients (Hack et al., 1989). The levels of C4a and C1-Inh complexes correlated with

C3a levels and with the clinical outcome (Hack et al., 1989). It is likely that activated C5a plays the predominant role in the pathophysiology of the septic processes, because it greatly exceeds C3a in biologic activity. However, measurement of C5a is difficult due to its rapid binding to cellular receptors (Hack et al., 1989).

The role of C1-Inh in sepsis was investigated in several clinical studies. In 48 patients with sepsis, compared to healthy volunteers, a discrepancy was demonstrated in plasma levels of functional and antigenic C1-Inh that was mainly due to an increase of inactive cleaved C1-Inh (iC1-Inh) with molecular masses of 98, 96, and 86 kDa as assessed by SDS-polyacrylamide gel electrophoresis (PAGE), respectively (Nuijens et al., 1989). The extent of plasma C1-Inh proteolysis and the level of cleaved iC1-Inh appeared to be positively correlated with the mortality of the sepsis-patients. Functional C1-Inh was significantly reduced only in patients with septic shock (Nuijens et al., 1989). The similarity of the cleavage pattern of C1-Inh in vivo with the pattern of cleavage by plasmin led to the assumption that local degradation by plasmin may play a certain role in the loss of C1-Inh activity during inflammation (Wallace et al., 1997). However, in baboons challenged with lethal and sublethal doses of *E. coli* the peak values of iC1-Inh coincided with the peak values of elastase-antitrypsin complex levels and not with those of plasmin-antiplasmin complexes, suggesting that elastase may be predom-

inantly responsible for the generation of iC1-Inh (De Boer et al., 1993).

Therapeutic C1-Inh administration incompletely blocked the activation of the classical pathway and did not interfere with the clearance of bacteria in primates suffering from lethal septic shock (Jansen et al., 1998). Hence, during C1-Inh administration some opsonization of the infecting micro-organisms or their products by the complement system will be preserved.

We have evaluated the effects of therapeutic administration of C1-Inh in a baboon model for lethal *E. coli* septic shock. Administration of C1-Inh at a dose that increased plasma levels 5- to 10-fold, reduced activation of C4 (and to a lesser extent C3) and improved mortality. Three of seven animals challenged with a lethal dose of *E. coli* survived 64 h, one of them was a permanent survivor (Jansen et al., 1998). Similarly, a favorable, although mild, effect of C1-Inh has been found in several endotoxin models in rats, dogs, rabbits, and in mice deficient for C4 and C3 (Guerrero et al., 1993; Scherer et al., 1996; Fischer et al., 1997). Notably, it has to be established whether the beneficial effects of C1-Inh in sepsis are due to its effect on the complement system, on the contact system, or on both.

Preliminary evaluation of C1-Inh therapy in patients with septic shock has been performed (Hack et al., 1992, 1993). Initially, five patients treated with mechanical ventilatory support, volume substitution, vasopressor, and positive inotropic drugs, received C1-Inh for 5 days, starting with a dose of 2000 U, subsequently followed by 1000 U every 12 h. No patient died during the study period of 5 days. 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 system parameters were measured in the five patients who received C1-Inh. C3a levels tended to decrease in these patients (Hack et al., 1993), whereas FXII levels increased (Hack et al., 1992). Thus, high doses of C1-Inh are well tolerated by patients with sepsis and may attenuate ongoing complement and contact activation. We then administered C1-Inh to seven additional patients with septic shock, one of whom (with bacterial endocarditis) did not complete the study because of transfer to another hospital (for open heart surgery). Three patients received C1-Inh for 3 days, a starting dose of 4000 U followed by 2 doses of 2000 U and 4 doses of 1000 U each 12 h, the other three patients received 6000 U of C1-Inh followed by 3000, 2000 and 1000 U (all doses given at 12 h intervals). Comparable effects as with the other dose regimen were seen, i.e., no toxic side effects and a slight reduction of complement and contact activation. The overall results (no toxic side effects, no sepsis-related mortality during the study period, a possible attenuation of complement and contact activation, a possible beneficial effect on hypotension as reflected by a decreased need for vasopressor medication) were con-

firmed in several open uncontrolled studies in a limited number of septic shock patients, who all received C1-Inh according to the schemes outlined above (2nd workshop on C1-esterase inhibitor, Duesseldorf, Germany, 24–26 April 1997). Double-blind controlled studies in a larger number of patients are warranted to confirm these promising effects. Therefore, we initiated a prospective, randomized, double blind and placebo-controlled study investigating the clinical outcome and laboratory parameters of C1-Inh administration in patients with severe sepsis or septic shock. In addition to standard routine treatment on the intensive care unit, the patients were randomized to either high dose C1-Inh (starting dose of 6000 U, followed by 3000, 2000, and 1000 U at 12 h intervals) or placebo (same amount of a solution of albumin 5%). We expect the first results of this study at the end of 1999.

### B. Vascular Leak Syndrome

A vascular or capillary leak syndrome (VLS) may complicate sepsis (Nürnbergger et al., 1992) but may also occur independently of this disease. For example, VLS is induced by therapy with cytokines, such as IL-2, or following a bone marrow transplant or open heart surgery (Gaynor et al., 1988; Ognibene et al., 1988; Nürnbergger et al., 1993; Stieh et al., 1996), or develops in the absence of any known precipitating event. The pathogenetic mechanisms underlying VLS are increased vasopermeability and vasodilation. Hence, hypotension may complicate VLS. The molecular mechanisms causing these phenomena are poorly understood, although endothelial damage resulting from interactions with activated neutrophils and/or natural killer cells are likely to be at the basis (Damle et al., 1987; Damle and Doyle, 1989; Baars et al., 1992b). Studies of the VLS induced by IL-2 have suggested that this syndrome results from the release and activation of inflammatory mediators such as cytokines (Gemlo et al., 1988; Boccoli et al., 1990), activation of neutrophils (Baars et al., 1992b), complement (Thijs et al., 1989, 1990; Vachino et al., 1991; Baars et al., 1992b) and coagulation and fibrinolysis (Baars et al., 1992a). In addition, changes in the contact system proteins resembling those seen in sepsis, occur during IL-2 therapy (Hack et al., 1991). In IL-2-induced VLS, we have shown that the activation of the classical pathway of complement correlates with the development of side effects (Thijs et al., 1989; 1990; Baars et al., 1992b). Also in other forms of VLS, such as that following bone marrow transplantation, complement is activated via the classical pathway (Nürnbergger et al., 1993). The mechanism of this activation is not known but may involve binding of CRP to IL-2 activated lymphocytes, which subsequently activate and fix complement (Vachino et al., 1991). The involvement of CRP in IL-2-induced activation of complement in vivo is discussed below. Regardless of the cause for classical pathway activation during IL-2 therapy, we decided to eval-

uate the effects of C1-Inh administration in patients receiving high doses of IL-2 (Ogilvie et al., 1994). Six patients with either metastatic melanoma or renal cell carcinoma received  $72 \times 10^6$  U of recombinant IL-2 (from the former Eurocetus, Amsterdam, The Netherlands) daily with exogenous C1-Inh given at a dose of 2000 U initially, subsequently followed by 1000 U every 12 h, for 4 days (treatment cycle). As controls, the same patients receiving a second cycle of IL-2 given at 4-fold lower doses 4 weeks after the first cycle (control cycle) were studied, as were 4 other patients who received escalating doses of IL-2, starting with  $18 \times 10^6$  U and increasing by  $18 \times 10^6$  U every 2 to 3 days (Thijs et al., 1990). Four of the six patients needed vasopressor medication during the treatment cycle (one patient for reasons not related to IL-2). The degree of hypotension was comparable to that observed during the control cycle and in the patients who received an escalating dose of IL-2. Thus, 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 (Ogilvie et al., 1994). Thus the results suggested that C1-Inh therapy is also able to reduce IL-2 toxicity, probably via inhibition of IL-2-induced complement activation. Moreover, various effects of C1-Inh substitution observed in the IL-2 treated patients (attenuated complement activation, less hypotension) were comparable to the effects seen in the patients with septic shock.

The effect of C1-Inh administration in other forms of VLS has also been evaluated in preliminary studies. A newborn baby with sepsis-associated VLS was treated with C1-Inh for 3 days (300, 100, and 50 U/kg on days 1, 2, and 3, respectively) (Nürnberg et al., 1992). Although the patient died 15 days later because of liver failure, the effect of C1-Inh administration was judged to be beneficial, because the patient no longer needed vasopressor medication, and his body weight normalized. The effect of C1-Inh has also been evaluated in VLS following bone marrow transplantation. In an initial study, two patients received 60 U/kg as a loading dose, then 2 doses of 30 U/kg given at 12-h intervals, and finally, 4 doses of C1-Inh of 15 U/kg (Nürnberg and Gobel, 1996). Body weight normalized in each patient, as did the increased levels of C4 days, which parameter reflects the extent of classical pathway activation. In a later report, the same authors describe 15 patients treated with C1-Inh because of VLS induced by bone marrow transplantation (Nürnberg et al., 1997). The one year-survival rate was 57% in the treated patients versus 14% in a control group consisting of seven patients (the study was not randomized and not placebo-controlled). Treatment was accompanied by normalization of circulating C4 days and C5a levels. These effects suggest a beneficial effect of C1-Inh therapy in this severe complication of bone marrow transplantation, but needs 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 2 doses of 150 U/kg, 3 doses of 100 U/kg and finally 3 doses of 50 U/kg, each dose given at 8 h-interval). In most children the effect of C1-Inh therapy was judged to be favorable because hemodynamic, respiratory, and laboratory parameters improved. However, in 11 patients arterial blood pressure did not respond, in 3 leakage continued, and in 6 children diuresis was not ameliorated (Stieh et al., 1996). Notably, in two patients, possible adverse side effects were registered, 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-Inh therapy is a promising approach for the management of patients with VLS; however, double-blinded placebo-controlled studies are needed to confirm this.

### C. Acute Myocardial Infarction

Acute myocardial infarction (AMI) is one of the major causes of mortality and morbidity in the western world. Mortality is due to arrhythmia, cardiac rupture, and acute heart failure, whereas morbidity often results from chronic heart failure. An important determinant in the development of heart failure is the amount of necrotic tissue in the jeopardized myocardium. In patients with unstable angina pectoris, evidence for the activation of the contact system of coagulation was provided by the stimulation of the kallikrein system and the generation of bradykinin in the acute phase (Hoffmeister et al., 1995). Studies in animals have shown 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 anti-inflammatory agents. For example, corticosteroids given as late as 6 h after coronary occlusion reduce infarction size by about 35% compared with untreated control animals (Libby et al., 1973). The local inflammatory response ensuing in the infarcted myocardium is characterized by the local production of chemotactic factors, the infiltration and activation of neutrophils, the local production of cytokines (such as TNF- $\alpha$  and IL-6), the expression of adhesion molecules, which enhance adherence of neutrophils to cardiac myocytes, and local activation of the complement system (Entman et al., 1991).

Complement activation by ischemic myocardium was first demonstrated by Hill and Ward who showed that complement activation products generated in the infarcted myocardium were responsible for the infiltration of neutrophils (Hill and Ward, 1971). Later studies in animals, as well as in patients, showed that several complement components become localized in the infarcted myocardium, independent of reperfusion, whereas membrane-bound complement inhibitors de-

crease (Pinckard et al., 1980; McManus et al., 1983; Rossen et al., 1985; Schafer et al., 1986; Crawford et al., 1988; Hugo et al., 1990; Vakeva et al., 1992, 1993, 1994; Lagrand et al., 1997). Furthermore, plasma levels of activated complement components are increased in patients with AMI and correlate with myocardial damage (Langlois and Gawryl, 1988; Yasuda et al., 1990). Although some studies claim that the activation of complement in ischemic myocardium occurs via the alternative pathway (Amsterdam et al., 1995), the involvement of C1q and C4, and hence the classical pathway, has been repeatedly demonstrated (McManus et al., 1983; Rossen et al., 1985; Crawford et al., 1988; Vakeva et al., 1994; Lagrand et al., 1997). The molecular mechanism of the observed activation of complement during AMI is not clear, although mitochondrial constituents, in particular, have been implicated as activators (Pinckard et al., 1973; Giclas et al., 1979; Rossen et al., 1988; Kagiya et al., 1989). Our own studies in humans suggest a contribution of the acute phase protein CRP (see below).

Complement activation products as the anaphylatoxins and the terminal complement complexes have deleterious effects on the myocardium by 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 (Del Balzo et al., 1985; Martin et al., 1988; Ito et al., 1990; Entman et al., 1991). The deleterious effects of complement activation products on the myocardium have been substantiated by observations that in animal models, complement depletion by administration of cobra venom factor before or shortly after permanent occlusion of a coronary vessel significantly reduces the amount of myocardial necrosis (Maroko et al., 1978; Pinckard et al., 1980; Crawford et al., 1988).

Early reperfusion of ischemic myocardium is a main goal for treatment of AMI. However, reperfusion of ischemic myocardium itself may induce an inflammatory reaction, which among others involves activation of complement (Rossen et al., 1985; Entman et al., 1991; Dreyer et al., 1992). This ischemic-reperfusion injury may damage the cardiac tissue and limit the beneficial effects of a restored circulation. Reperfusion therapy in AMI, therefore, can be regarded as a "double-edged sword." Inhibition of complement activation induced by reperfusion of ischemic myocardium or inhibition of C5a activity reduces myocardial infarction size considerably in animals (Weisman et al., 1990; Amsterdam et al., 1995). 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, independent of reperfusion.

Recent studies have shown that C1-Inh administration can reduce experimental infarction size in ischemia-

reperfusion models for 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/kg of body weight (about 60–100 U/kg), reduced infarction size by 65% compared with cats receiving vehicle (Buerke et al., 1995). Because of lower myeloperoxidase activity in the ischemic myocardium of treated animals, it was suggested that part of the cardioprotective effect of C1-Inh was related to a diminished infiltration of neutrophils. A similar dose of C1-Inh was shown to reduce infarction size in a rat model (20 min of ischemia, 24 h of reperfusion) by 60 to 70%, and also in this model, C1-Inh therapy was associated with less influx of neutrophils in the ischemic myocardium (Murohara et al., 1995). The effect of C1-Inh was slightly better than that of soluble complement receptor-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) (Horstick et al., 1997). Increases of circulating C3a, and to a lesser extent C5a, were attenuated by C1-Inh treatment (Horstick et al., 1997). In our own studies (A. H. Kleine, W. L. Mullers-Boumans, B. Kop-Klaassen, A. H. L. Koendermans, C.E.H., W. Hermens, manuscript submitted) we have observed that the intravenous administration, in a dog model of AMI, of human C1-Inh at a dose of 500 to 1000 U/dog (approximately 25 kg) at 2 and 8 h after permanent coronary artery occlusion (the left anterior descending coronary artery), significantly reduced (up to 50%) the 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 for AMI. Whether a reduction of infarct size in humans with AMI may be achieved by administration of C1-Inh is being studied currently. In a report of ~3 patients undergoing emergency coronary surgery after failed percutaneous transluminal coronary angioplasty, the administration of C1-Inh (single dose of 2000 U) rapidly improved the severely impaired postoperative contraction patterns of the left ventricle of these patients and allowed weaning from the extracorporeal circuit (Bauernschmitt et al., 1998). 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.

#### D. Other Diseases

The effects of C1-Inh have been investigated in animal models for various other diseases. Four studies have evaluated the efficacy of C1-Inh in pancreatitis. In a short-term pig model (6-h observation period) where pancreatitis was induced by retrograde injection of sodium taurocholate in the pancreatic duct, C1-Inh reduced mortality and improved hemodynamic performance. This effect was suggested to be due to a better



inhibition of kallikrein and trypsin (Ruud et al., 1986). A mild beneficial effect of a combined therapy with C1-Inh (250 U/kg) and antithrombin III (250 U/kg) was demonstrated in rats suffering from experimental hemorrhagic pancreatitis (Yamaguchi et al., 1997). A reduction of mortality but no beneficial effects to edema and histopathological lesions were found in another model of acute pancreatitis in rats, although relatively high doses of C1-Inh were administered (about 400 U/kg of body weight) at various time intervals (Vesentini et al., 1993). Similarly, high doses of C1-Inh (100 U/kg of body weight) failed to cause major beneficial effects in three models of experimental edematous or hemorrhagic pancreatitis (two in mice and one in rats) in terms of histological alterations, plasma amylase levels, and survival, when compared with animals only receiving albumin (Niederau et al., 1995). Thus, the efficacy of C1-Inh in experimental pancreatitis is inconsistent, and therefore, it is difficult to judge whether therapy with this inhibitor may be of clinical benefit.

In a dog model for orthotopic allogeneic lung transplantation, C1-Inh administration to the donors (500 U/dog weighing 20–32 kg) 30 min before cardiac inflow in the lungs was arrested, and 30 min before reperfusion (500 U/animal), largely prevented a deterioration of respiratory and hemodynamic parameters. In addition, C1-Inh prevented a fall in circulating contact and complement parameters (Salvatierra et al., 1997). 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, because in an *in vitro* model relevant to hyperacute xenograft rejection C1-Inh was capable of protecting against complement-mediated destruction of endothelial cells (Dalmasso and Platt, 1993; Heckl-Ostreicher et al., 1996).

In a porcine model for thermal injury, C1-Inh (given at a dose of 100 U/kg initially, followed by 3 lower doses given at 12-h intervals) was shown to have beneficial effects in the acute stage of thermal injury, because it reduced organ alterations, improved microcirculation, and largely prevented bacterial translocation in the gastrointestinal tract (Henze et al., 1997; Khorram-Sefat et al., 1998). Favorable effects of C1-Inh administration to patients with burns have been reported in a preliminary communication (A. C. F. G. M. Janssen et al., presented at the 2nd Workshop on C1-esterase inhibitor, 24–26 April 1997, Duesseldorf, Germany). In a nonrandomized study, 16 patients with severe burn injuries and either shock, systemic inflammatory response syndrome, or septic shock received C1-Inh at an initial dose of 6000 U, followed by 3000, 2000, and 1000 U every 12 h. Eight patients (53%) survived, comparing favorably with a historical control group where only 4 of 15 patients (26%) survived (Jostkleigreve et al., 1997).

Finally, in a murine model of traumatic shock treatment with C1-Inh at a single dose of 15 mg/kg given intravenously 10 min post-trauma reduced mortality from 83 to 33% and was accompanied by a reduced activation of neutrophils (Kochilas et al., 1997).

## VI. CRP-Mediated Complement Activation: A Common Target for C1-Inh Therapy?

As discussed above, C1-Inh therapy may be beneficial in rather different diseases. So, apparently, complement and/or contact system activation occurs in these conditions. This raises the question of how these systems become activated under these conditions. Although it cannot be excluded that part of the beneficial effects in the diseases discussed above are due to inhibition of the contact system, we will limit the discussion here to complement, because virtually nothing is known about the nature of activators of the contact system *in vivo*.

According to textbooks on immunology, immune complexes are the main activators of the classical pathway of complement, and bacteria are those of the alternative and the MBL pathways; but, these activators are not likely to be involved in the diseases where C1-Inh may have beneficial effects, except for sepsis. Also in the latter disease there is evidence that part of the complement activation is independent of the infecting bacteria (De Boer et al., 1993). Hence, other molecular mechanisms should explain the activation of complement in most of the diseases discussed above. Most of the studies regarding these mechanisms have focused on ischemia-reperfusion-induced activation. Older studies have identified constituents of mitochondria, consisting of proteins with a molecular mass ranging from 23 to 53 kDa (Rossen et al., 1988; Kagiya et al., 1989), as well as cardiolipin (Rossen et al., 1994), released by ischemic cells as the main activators of the classical complement pathway during reperfusion of ischemic myocardium. More recent studies in mice deficient for immunoglobulins or C3 or C4, have demonstrated a role for natural IgM antibodies in reperfusion injury in the intestine or skeletal muscle (Weiser et al., 1996; Williams et al., 1999). In these studies, natural IgM was found to bind to ischemic cells during reperfusion and was found to be necessary for reperfusion-induced complement activation and injury. Hence, classical pathway activation induced by mitochondrial constituents released by damaged cells, or by natural IgM antibodies binding to these cells, might be a target for C1-Inh in the diseases discussed in the previous sections. Another trigger for complement activation in disease may be binding of MBL (see section III, A) to damaged cells. Although it is generally accepted that MBL can bind to bacteria and trigger the complement cascade, there is evidence that this C1q-like protein can contribute to tissue damage. For example, MBL and its associated protease MASP-1 has been found to be deposited in the glomeruli of patients

with IgA nephropathy (Endo et al., 1998; Matsuda et al., 1998).

In vitro studies show also that the acute phase protein CRP can activate the classical pathway of complement (Volanakis, 1982; Kilpatrick and Volanakis, 1991; Steel and Whitehead, 1994). Recently, we have obtained evidence that CRP-dependent activation of the classical pathway also occurs in vivo, particularly in the diseases where C1-Inh may be beneficial.

To demonstrate complement activation by CRP in vivo in humans, we took two different approaches: we studied colocalization of activated complement and CRP in acute myocardial infarction where at a tissue level complement is activated via the classical pathway; and, we investigated the feasibility of developing assays for complement activation fragments specifically generated during CRP-mediated activation.

Ischemic myocardial tissue induces activation of complement as has been shown in various animal models and in humans (see previous section). In animal models, this activation contributes significantly to infarction size. Although old immunohistochemical studies in animals suggest that CRP may localize in inflamed tissues (Kushner and Kaplan, 1961; Kushner et al., 1963), metabolic studies with labeled CRP found no evidence for this in humans (Vigushin et al., 1993). However, in an immunohistochemical study we observed colocalization of CRP and C3- and C4-activation fragments in infarcted but not in healthy myocardium of 16 of 17 patients that had died in the acute phase of myocardial infarction (Lagrand et al., 1997). The patient that had no deposits of CRP or complement had an infarction of less than 12 h duration. Thus, these data strongly support that complement can be activated by CRP in vivo, at least at a tissue level.

We also have developed assays for circulating activation products, CRP-complement complexes, that are specifically generated during CRP-mediated complement activation (Wolbink et al., 1996). Circulating levels of CRP-complement complexes increase in patients receiving IL-2; this increase coincides with the increase of complement activation products such as C3a (G.J. Wolbink, J. Wagstaff, J. W. Baars, L. G. Thijs, C.E.H., manuscript in preparation). Increased levels of CRP complexes also occur in patients with sepsis (Wolbink et al., 1998) or AMI (W. K. Lagrand, H. W. M. Niessen, C. A. Visser, C.E.H., manuscript in preparation). Together these results indicate that CRP can activate complement in vivo, at least in some clinical conditions.

To activate complement, CRP has to be bound to a ligand. Recently, we have discussed the possibility that CRP may bind to flip-flopped cells (phospholipids of the inner and outer leaflet of the membrane have exchanged in these cells (Higgins, 1994; Zachowski, 1993), probably with the help of phospholipase A2 (Hack et al., 1997), although other ligands for CRP may exist as well.

Considering the role of CRP in activating complement via the classical pathway, it can be hypothesized that the beneficial effect of C1-Inh is related to its potency in reducing CRP-dependent activation and hence to limit tissue damage. The frequent occurrence of acute phase responses and flip-flopped cells in clinical situations may explain the efficacy of C1-Inh therapy in various diseases.

## VII. Summary

C1-Inh therapy was introduced in clinical medicine about 25 years ago as a replacement therapy for patients with hereditary angioedema caused by a genetic deficiency of C1-Inh. There is, however, accumulating evidence obtained from studies in animals and patients suggesting that the therapeutic administration of C1-Inh may have a beneficial effect as well in several other conditions such as sepsis, cytokine-induced vascular leak syndrome, or acute myocardial infarction. The studies discussed above indicate that C1-Inh therapy is well tolerated and may be beneficial in several 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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