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C1 inhibitor: Biologic activities that are independent of protease inhibition

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Abstract

C1 inhibitor therapy improves outcome in several animal models of inflammatory disease. These include sepsis and gram negative endotoxin shock, vascular leak syndromes, hyperacute transplant rejection, and ischemia-reperfusion injury. Furthermore, some data suggest a beneficial effect in human inflammatory disease. In many inflammatory conditions, complement system activation plays a role in pathogenesis. The contact system also very likely is involved in mediation of damage in inflammatory disease. Therefore, the beneficial effect of C1 inhibitor has been assumed to result from inhibition of one or both of these systems. Over the past several years, several other potential anti-inflammatory effects of C1 inhibitor have been described. These effects do not appear to require protease inhibition and depend on non-covalent interactions with other proteins, cell surfaces or lipids. In the first, C1 inhibitor binds to a variety of extracellular matrix components including type IV collagen, laminin, entactin and fibrinogen. The biologic role of these reactions is unclear, but they may serve to concentrate C1 inhibitor at extravascular inflammatory sites. The second is a noncovalent interaction with C3b that results in inhibition of formation of the alternative pathway C3 convertase, a function analogous to that of factor H. The third is an interaction with E and P selectins on endothelial cells that is mediated by the Lewis^x tetrasaccharides that are expressed on C1 inhibitor. These interactions result in suppression of leukocyte rolling and transmigration. The fourth interaction is the binding of C1 inhibitor to gram negative bacterial endotoxin that results in suppression of endotoxin shock by interference with the interaction of endotoxin with its receptor complex on macrophages. Lastly, C1 inhibitor binds directly to gram negative bacteria, which leads to suppression of the development of sepsis, as demonstrated in the cecal ligation and puncture model. These observations suggest that C1 inhibitor is a multi-faceted anti-inflammatory protein that exerts its effects through a variety of mechanisms including both protease inhibition and several different non-covalent interactions that are unrelated to protease inhibition.

Keywords

C1 inhibitor; cecal ligation and puncture model; endotoxin shock; gram negative bacterial sepsis; selectins; serine proteinase inhibitors

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Introduction

Treatment with C1 inhibitor improves outcome in a number of animal models of inflammatory disease including gram negative bacterial sepsis and endotoxin shock, vascular leak syndromes, hyperacute transplant rejection, and ischemia-reperfusion injury (Akita et al., 2003; Buerke et al., 1995; Buerke et al., 1998; Dalmasso and Platt, 1993; De Simoni et al., 2003; De Simoni et al., 2004a; Fiane et al., 1999; Fukuta et al., 2003; Giebler et al., 1999; Guerrero et al., 1993; Hecker et al., 2002; Henze et al., 1997; Horstick et al., 1997; Horstick et al., 2001a; Jansen et al., 1998; Khorram-Sefat et al., 1998; Matsunami et al., 2000; Nielsen et al., 2002; Przemeck et al., 2002; Radke et al., 2000; Schelzig et al., 2001; Scherer et al., 1996; Schmidt et al., 1999a; Schmidt et al., 1999b)(Table 1). For example, in myocardial ischemia-reperfusion injury, treatment with C1 inhibitor resulted in decreased infarct size, decreased myocardial neutrophil accumulation, decreased plasma levels of creatine kinase, C3a and C5a, and decreased expression of P-selectin and ICAM-1 within the cardiac endothelium (Buerke et al., 1995; Buerke et al., 1998; Horstick et al., 1997; Horstick et al., 2002). Similar effects have been observed in skeletal muscle and middle cerebral artery ischemia-reperfusion injury (Akita et al., 2003; De Simoni et al., 2003; Nielsen et al., 2002). One study suggested that the C1 inhibitor-mediated effect was independent of inhibition of classical pathway activation; a role for inhibition of lectin pathway activation was not examined (De Simoni et al., 2004b). In addition, limited experience in human disease suggests that C1 inhibitor may prove to be a useful addition to therapy of diseases including vascular leak syndromes, sepsis, and shock syndromes (Bauernschmitt et al., 1998; Bauernschmitt et al., 2000; de Zwaan et al., 2002; Hack et al., 1993; Hack et al., 1994; Niederau et al., 1995; Ogilvie et al., 1994; Tassani et al., 2001) (Table 1). Two studies have suggested a beneficial effect in humans with ischemia-reperfusion injury (Bauernschmitt et al., 1998; de Zwaan et al., 2002). Because complement system activation plays a role in the mediation of many of these conditions, these beneficial effects have been thought to be a product of inhibition of activation of the complement system. Protease inhibition clearly is the major biologic role of C1 inhibitor. It is the only protease inhibitor that inactivates C1r and C1s (Sim et al., 1979; Ziccardi, 1981), the initial proteases activated via the classical complement pathway. It also regulates lectin pathway activation via inhibition of MASP2 (Ambrus et al., 2003; Matsushita et al., 2000a; Matsushita et al., 2000b; Matsushita et al., 2001), the initial protease activated in this pathway. However, C1 inhibitor may share this latter role with α_2 macroglobulin (Ambrus et al., 2003). In the contact or kinin system, C1 inhibitor is the primary inhibitor of both plasma kallikrein and factor XIIa (de Agostini et al., 1984; Kaplan et al., 1985; Pixley et al., 1985). In this instance, α_2 macroglobulin clearly plays a lesser role than does C1 inhibitor. It is certain that activation of these pathways have important inflammatory effects, and that their inhibition, whether by C1 inhibitor or other inhibitors, is anti-inflammatory. However, over the past several years, several other interactions of C1 inhibitor with other proteins or cells have come to light. These interactions do not require protease inhibition. Initial data suggest that, in some instances, these reactions may be significant contributors to the anti-inflammatory activity of this important protease inhibitor. These include at least four different binding reactions (Table 2): (1) An interaction with a variety of extracellular matrix components (Patston and Schapira, 1997); (2) inhibition of alternative complement pathway activation via binding of C1 inhibitor to C3b which prevents its interaction with factor B, in a manner analogous to that of factor H (Jiang et al., 2001); (3) an interaction with both E and P selectins on endothelial cells that is mediated by one or more Lewis^x tetrasaccharides expressed on C1 inhibitor, and which results in suppression of leukocyte rolling and transmigration (Cai and Davis III, 2003; Cai et al., 2005); (4) binding to endotoxin from several different gram negative bacteria that results in suppression of endotoxin shock by interference with the interaction of endotoxin with its receptor complex on macrophages (Liu et al., 2003; Liu et al., 2004; Liu et al., 2005a; Liu et al., 2005b); (5) direct binding to gram negative bacteria that results in suppression of the development of sepsis (unpublished data). This review will briefly summarize the limited

information available on the first two reactions and will describe in more detail the data relating to the interactions with selectins and with endotoxin lipopolysaccharide (LPS).

The interaction of C1 inhibitor with extracellular matrix components

C1 inhibitor binds to a number of extracellular matrix components including type IV collagen, laminin, and entactin, as was illustrated by ligand blotting assays and by ELISA (Patston and Schapira, 1997). Half-maximal binding to collagen and laminin was at approximately 8 μg/ml. Digestion of C1 inhibitor with *Crotalus atrox* α-proteinase, which cleaves at Pro^{36}, had no effect on binding. This implies that this binding has quite different requirements than the binding to gram negative endotoxin (Liu et al., 2005a)(see below). Binding had no effect on the rate constant of inhibition of C1s by C1 inhibitor. The interaction with type IV collagen resulted in a slight increase in the stoichiometry of inhibition in the reaction between C1 inhibitor and C1s, but the other proteins had no effect. Neither the biochemical mechanism nor the biologic relevance of these reactions have been defined. Tissue transglutaminase, but not factor XIII, has been shown to cross-link C1 inhibitor to immobilized fibrin via Gln^{453} (Hauert et al., 2000). Such cross-linked C1 inhibitor retains the ability to inactivate target proteases. The biology of this reaction also remains to be investigated. It seems likely, however, that these reactions with extracellular matrix proteins serve at least to concentrate C1 inhibitor at extra-vascular sites of inflammation in order to maximize regulation of local complement and contact system activation.

Suppression of alternative complement pathway activation

C1 inhibitor also has been shown to inhibit alternative pathway activation via an incompletely defined mechanism which, however, does not require protease inhibition (Jiang et al., 2001). The data suggested that the findings might be of biologic relevance because removal of C1 inhibitor from plasma resulted in enhanced alternative pathway activation. The mechanism appears to result from inhibition of binding of factor B to C3b via an interaction of C1 inhibitor with C3b. Binding to C3b does not appear to require protease inhibitor activity. This mechanism, if correct, would be quite similar to the mechanism of inhibition of C3 convertase formation by factor H and CR1. The region of the molecule required for this activity remains uninvestigated and it is not known whether the binding to C3b has any effect on protease inhibition by C1 inhibitor. The potential role of this activity *in vivo* has not been determined.

The interaction of C1 inhibitor with selectins on endothelial cells

The beneficial effect of C1 inhibitor in inflammatory disease is accompanied by a decrease in leukocyte transmigration to the site of inflammation and by decreased selectin expression (Buerke et al., 1998; Horstick et al., 2001b; Lehmann et al., 2000; Lehmann et al., 2004). These effects are probably largely due to a suppression of complement activation; C3a, C5a and C5b-9 all may enhance selectin expression and leukocyte adhesion (Hattori et al., 1989; Jagels et al., 2000; Monsinjon et al., 2003). However, additional potential mechanisms have not been evaluated previously. Selectins are adhesion molecules that are responsible for leukocyte rolling on the endothelium (Butcher and Picker, 1996). E-selectin is expressed on endothelial cells; expression is induced by cytokines (TNF-α, IL-1) and gram negative lipopolysaccharide. P-selectin is expressed on platelets and endothelial cells; it is mobilized to the cell surface from intracellular granules in response to a variety of agents including histamine and thrombin. Selectins bind to fucosylated mucin-like glycoproteins. A number of selectin ligands bind via sialyl $Lewis^x$, which is a fucose-containing tetrasaccharide expressed on N-linked carbohydrate of a number of cell surface and plasma proteins. All selectins recognize the sialyl $Lewis^x$ moiety (Frenette and Wagner, 1996a; Frenette and Wagner, 1996b; Vestweber and Blanks, 1999). C1 inhibitor previously has been reported to bind to endothelial cells that have been incubated in the cold (Bergamaschini et al., 2001a; Bergamaschini et al., 2001b). Because of these

observations, and because it is heavily glycosylated and is fucosylated (Strecker et al., 1985), we tested the hypothesis that C1 inhibitor might express the sialyl Lewis^x tetrasaccharide and thereby bind to selectins. On immunoblots following separation by SDS-polyacrylamide gel electrophoresis, C1 inhibitor reacted with two different monoclonal antibodies that react with sLewis^x (Cai and Davis III, 2003). Reactivity was lost following removal of N-linked, but not O-linked, carbohydrate.

C1 inhibitor was shown to bind to both E- and P-selectins, as demonstrated by FACS analysis of CHO/K1 cells transfected with either E- or P- selectin and by co-precipitation with soluble E- or P-selectin IgG chimeras (Cai and Davis III, 2003). Furthermore, C1 inhibitor, following incubation with human umbilical vein endothelial cells, co-precipitated with both E- and P-selectin after lysis of the cells and immunoprecipitation with antiserum to C1 inhibitor (unpublished data)(Fig. 1). The interaction with selectins had no discernible effect on the ability of C1 inhibitor to form stable complexes with C1s, although reaction kinetics have not been evaluated. Using the macrophage-like cell line, U937, C1 inhibitor suppressed both adhesion to, and transmigration across, TNF-α-treated endothelial cell monolayers (Cai and Davis III, 2003). Carcinoembryonic antigen (CEA), which expresses the sialyl Lewis^x tetrasaccharide, is an E-selectin ligand. C1 inhibitor suppressed the binding of CEA to E-selectin expressed on the surface of CHO cells in a dose dependent manner (Cai et al., 2005). In addition, reactive center cleaved C1 inhibitor, which retains no protease inhibitor activity, also suppressed the interaction to as great a degree as did active native C1 inhibitor. However, C1 inhibitor with its N-linked carbohydrate removed, lost the ability to interfere with binding.

The effect of C1 inhibitor on leukocyte rolling was analyzed both *in vitro* and *in vivo*. Using a flow chamber in which purified E-selectin was immobilized, C1 inhibitor at concentrations similar to those that occur during acute inflammation (300 μg/ml) increased rolling velocity two fold (Cai et al., 2005)(Fig. 2). Using P-selectin transfected cells cultured on the chamber, rolling velocity was increase by 2.5 fold. With both E- and P-selectin, reactive center cleaved C1 inhibitor, but not N-deglycosylated C1 inhibitor, increased velocity to the same degree. As visualized by intravital microscopy, C1 inhibitor also was shown to suppress TNF-α induced leukocyte rolling *in vivo* in mice. As with the *in vitro* experiments, reactive center cleaved C1 inhibitor retained full activity. Lastly, in the thioglycollate peritonitis model, C1 inhibitor (300 μg) suppressed leukocyte infiltration to background levels (Cai et al., 2005). Previous data suggest that leukocyte influx in this model is approximately 50% dependent on the presence of C5 and 50% dependent on leukotriene B(4) (Segal et al., 2002). Therefore, complete suppression of infiltration by C1 inhibitor, together with the effectiveness of inactive C1 inhibitor, suggest that its mechanism is not solely via complement inhibition.

Several plasma proteins, in addition to C1 inhibitor, express sialyl Lewis^x. These include α_1-acid glycoprotein, α_1-antichymotrypsin and haptoglobin (Brinkman-van der Linden et al., 1998; De Graaf et al., 1993). All these proteins are acute phase reactants. The normal plasma concentration of C1 inhibitor (80–195 μg/ml), for example, may increase up to 2.5-fold during an acute inflammatory response (Kirschfink and Nurnberger, 1999; Tietz, 1995). The data discussed here suggest that, at these concentrations, C1 inhibitor, together with these other acute phase reactants, might interfere with the leukocyte-selectin interaction, resulting in the inhibition of migration of cells to inflammatory sites. This may represent a normal physiologic mechanism that participates in down-regulation of the acute inflammatory response.

The interaction of C1 inhibitor with Gram negative bacterial endotoxin

In a variety of different animal models of Gram negative endotoxin shock and sepsis, survival is significantly improved, together with several physiologic measurements, by treatment with C1 inhibitor (Fischer et al., 1997; Giebler et al., 1999; Guerrero et al., 1993; Jansen et al.,

1998; Liu et al., 2003; Scherer et al., 1996; Schmidt et al., 1999a; Schmidt et al., 1999b). C5a very likely plays a role in the pathogenesis of septic shock (Barton and Warren, 1993; Czermak et al., 1999; Strachan et al., 2000). On the other hand, C3 and C4 deficient mice are quite susceptible to endotoxin shock, which supports the hypothesis that C3 may be required for normal endotoxin clearance (Fischer et al., 1997). Interestingly, survival of C3 deficient mice is improved by treatment with C1 inhibitor, suggesting that the effect of C1 inhibitor is not mediated via inhibition of complement activation (Fischer et al., 1997). The contact system may be involved in the mediation of some aspects of endotoxin shock (Colman, 1999; Jansen et al., 1996; Pixley et al., 1993). Other data suggest that contact system activation does not play an important role (Feletou et al., 1996). Our data indicated that not only did intact, active C1 inhibitor protect from endotoxin shock, but that treatment with inactive, reactive center cleaved C1 inhibitor also was effective (Liu et al., 2003). Both active and inactive C1 inhibitor also prevented both local and systemic vascular permeability changes in response to endotoxin (Liu et al., 2005b). C1 inhibitor prevented the binding of *Salmonella typhimurium* LPS to the murine macrophage cell line, RAW 264.7, and to human blood cells. Direct binding assays showed that C1 inhibitor interacted with LPS, thereby blocking LPS-induced TNF-α expression (Liu et al., 2003). LPS, a component of the outer membrane of Gram negative bacteria, consists of an inner lipid A component, which is responsible for the pathophysiology of endotoxin shock, and an outer polysaccharide component. In blood, LPS binds to LPS-binding protein (LBP), an acute phase plasma protein, that transfers the LPS to CD14 on the surface of monocytes and macrophages (Fenton and Golenbock, 1998; Kitchens, 2000; Poltorak et al., 1998; Schumann and Latz, 2000). The LPS-CD14 complex then initiates intracellular signalling by binding to Toll-like receptor 4 (TLR4) (Aderem and Ulevitch, 2000). At least one other protein, MD-2, is associated with CD14 and TLR4 on the cell surface; together, these make up the LPS receptor (da Silva Correia and Ulevitch, 2002; Juan et al., 1995; Stelter et al., 1997; Stelter et al., 1999; Viriyakosol and Kirkland, 1995; Viriyakosol et al., 2000). The binding of C1 inhibitor to LPS (or lipid A) was inhibited by fetal bovine serum, which contains LBP, and by an LBP peptide that includes the binding site for LBP (Liu et al., 2003).

A recombinant C1 inhibitor from which the amino terminal non-serpin domain was deleted (amino acids 1–97) failed to bind to LPS (Liu et al., 2003). N-deglycosylated C1 inhibitor did not protect mice from endotoxin shock, did not bind to LPS and did not suppress the binding of LPS to RAW 264.7 or to human blood cells (Liu et al., 2004). Therefore, N-linked carbohydrate within the amino terminal domain appears to be required for the interaction with LPS. The lipid A-binding motifs of other LPS-interacting proteins contain positively charged amino acids that interact with the negatively charged phosphate groups within lipid A (Ferguson et al., 2000; Lamping et al., 1996; Mancek et al., 2002; Schumann et al., 1997; Visintin et al., 2003). We, therefore, in addition to analyzing the requirement for N-linked carbohydrate, tested whether the four basic amino acids within the amino terminal domain of C1 inhibitor (Arg^{18}, Lys^{22}, Lys^{30}, Lys^{55}) might be involved in the interaction with lipid A. Using site-directed mutagenesis, single amino acid substitutions and combinations of substitutions were performed in which Ala was introduced at each of the above sites and and the three N-linked sites within the amino terminal domain (Asn^{3}, Asn^{30} and Asn^{55}). Substitution of Asn^{3} resulted in virtually complete loss of the ability to interact with LPS, while little or no effect was observed following replacement of the other Asn residues (Liu et al., 2005a)(Fig. 3). However, the effect of the substitution of the individual positively charged residues was additive; replacement of any single residue reduced binding to 75–80% of that of the wild type control protein, replacement of 2, 3 and 4 residues reduced binding progressively to 10–12% of the control with replacement of all four (Fig. 4). These results were confirmed by the observations that the mutants that lost the ability to bind also lost the abilities to inhibit binding of LPS to RAW264.7 cells and to inhibit LPS-induced TNF-α mRNA expression. A likely explanation of these findings is that the binding site for LPS consists of

Arg^{18}, Lys^{22}, Lys^{30}, and Lys^{55}, while the carbohydrate at Asn^{3} is required to maintain a conformation of the amino terminal domain in which the binding site is exposed.

More recently, experiments have been initiated to analyze the effect of C1 inhibitor in the mouse cecal ligation and puncture (CLP) model of peritonitis and sepsis. These experiments demonstrate that C1 inhibitor also improves survival in this model. A single dose (600 μg) increased survival from approximately 15% to 45%, while three doses at 0, 12 and 24 hours increased survival to 65% (unpublished data). In agreement with the findings in LPS-induced shock, treatment of CLP mice with reactive center cleaved C1 inhibitor was at least as beneficial as was intact, active C1 inhibitor. Fewer bacteria could be recovered from the peritoneal fluid and blood of treated than from untreated mice. C1 inhibitor did not have a direct effect on bacterial growth *in vitro*, but it did enhance bacterial phagocytosis and/or killing by both neutrophils and macrophages. Furthermore, C1 inhibitor treatment enhanced H_2O_2 release by neutrophils and macrophages from CLP mice. Although C1 inhibitor does interact with the bacteria from CLP mice, it has not yet been determined whether the binding site is the LPS or if it is some other site on the bacterial surface. Furthermore, the mechanism of the enhanced phagocytosis/killing remains to be defined.

Conclusions

The data discussed here suggest that C1 inhibitor is a multi-faceted anti-inflammatory protein. Protection from inflammatory diseases, sepsis and septic shock very likely is a result of multiple mechanisms. These obviously include inhibition of both complement and contact system activation. In the complement system, the most important effect of C1 inhibitor in inflammatory disease very likely reflects decreased generation of C5a, while in the contact system, decreased generation of bradykinin is probably the most important effect. In addition to protease inhibition, direct effects of C1 inhibitor via interactions with endotoxin, with selectins and possibly with extracellular matrix components very likely also are important.

Abbreviations

CEA
: carcinoembryonic antigen

CLP
: cecal ligation and puncture

LBP
: LPS-binding protein

LPS
: lipopolysaccharide

TLR4
: Toll-like receptor 4

References

Aderem A, Ulevitch RJ. Toll-like receptors in the induction of the innate immune response. Nature 2000;406:782–787. [PubMed: 10963608]

Akita N, Nakase H, Kaido T, Kanemoto Y, Sakaki T. Protective effect of C1 esterase inhibitor on reperfusion injury in the rat middle cerebral artery occlusion model. Neurosurgery 2003;52:395–400. [PubMed: 12535370]

Ambrus G, Gal P, Kojima M, Szilagyi K, Balczer J, Antal J, Graf L, Laich A, Moffatt BE, Schwaeble W, Sim RB, Zavodszky P. Natural substrates and inhibitors of mannan-binding lectin-associated serine

protease-1 and -2: a study on recombinant catalytic fragments. J Immunol 2003;170:1374–1382. [PubMed: 12538697]

Barton PA, Warren JS. Complement component C5 modulates the systemic tumor necrosis factor response in murine endotoxic shock. Infect Immun 1993;61:1474–1481. [PubMed: 8454352]

Bauernschmitt R, Bohrer H, Hagl S. Rescue therapy with C1-esterase inhibitor concentrate after emergency coronary surgery for failed PTCA. Intensive Care Med 1998;24:635–638. [PubMed: 9681790]

Bauernschmitt R, Schreiber C, Lange R. C1 esterase inhibitor concentrate during surgery with cardiopulmonary bypass: is there an indication beyond substitution therapy in patients with hereditary angioneurotic edema? J Thorac Cardiovasc Surg 2000;120:427–428. [PubMed: 10917977]

Bergamaschini L, Gatti S, Caccamo L, Prato P, Latham L, Trezza P, Maggioni M, Gobbo G, Fassati LR. C1 inhibitor potentiates the protective effect of organ preservation solution on endothelial cells during cold storage. Transplant Proc 2001a;33:939–941. [PubMed: 11267137]

Bergamaschini L, Gobbo G, Gatti S, Caccamo L, Prato P, Maggioni M, Braidotti P, Di Stefano R, Fassati LR. Endothelial targeting with C1--inhibitor reduces complement activation in vitro and during ex vivo reperfusion of pig liver. Clin Exp Immunol 2001b;126:412–420. [PubMed: 11737055]

Brinkman-van der Linden EC, de Haan PF, Havenaar EC, van Dijk W. Inflammation-induced expression of sialyl Lewis^x is not restricted to alpha1-antichymotrypsin and haptoglobin. Glycoconj J 1998;15:177–182. [PubMed: 9557878]

Buerke M, Murohara T, Lefer AM. Cardioprotective effects of a C1 esterase inhibitor in myocardial ischemia and reperfusion. Circulation 1995;91:393–402. [PubMed: 7805243]

Buerke M, Prufer D, Dahm M, Oelert H, Meyer J, Darius H. Blocking of classical complement pathway inhibits endothelial adhesion molecule expression and preserves ischemic myocardium from reperfusion injury. J Pharmacol Exp Ther 1998;286:429–438. [PubMed: 9655888]

Butcher EC, Picker LJ. Lymphocyte homing and homeostasis. Science 1996;272:60–66. [PubMed: 8600538]

Cai S, Davis AE III. Complement regulatory protein C1 inhibitor binds to selectins and interferes with endothelial-leukocyte adhesion. J Immunol 2003;171:4786–4791. [PubMed: 14568956]

Cai S, Dole V, Bergmeier W, Scafidi J, Feng H, Wagner DD, Davis AE III. A direct role for C1 inhibitor in leukocyte adhesion. J Immunol 2005;174:6462–6466. [PubMed: 15879149]

Colman RW. Biologic activities of the contact factors in vivo. Potentiation of hypotension, inflammation, and fibrinolysis, and inhibition of cell adhesion, angiogenesis and thrombosis. Thromb Haemost 1999;82:1568–1577. [PubMed: 10613636]

Czermak BJ, Sarma V, Pierson CL, Warner RL, Huber-Lang M, Bless NM, Schmal H, Priedl HP, Ward PA. Protective effects of C5a blockage in sepsis. Nature Med 1999;5:788–792. [PubMed: 10395324]

Dalmasso AP, Platt JL. Prevention of complement-mediated activation of xenogeneic endothelial cells in an in vitro model of xenograft hyperacute rejection by C1 inhibitor. Transplantation 1993;56:1171–1176. [PubMed: 8249120]

da Silva Correia J, Ulevitch RJ. MD-2 and TLR4 N-linked glycoproteins are important for a functional lipopolysaccharide receptor. J Biol Chem 2002;277:1845–1854. [PubMed: 11706042]

de Agostini A, Lijnen HR, Pixley RA, Colman RW, Schapira M. Inactivation of factor-XII active fragment in normal plasma: predominant role of C1-inhibitor. J Clin Invest 1984;93:1542–1549. [PubMed: 6725552]

De Graaf TW, Van der Stelt ME, Anbergen MG, van Dijk W. Inflammation-induced expression of sialyl Lewis X-containing glycan structures on alpha 1-acid glycoprotein orosomucoid in human sera. J Exp Med 1993;177:657–666. [PubMed: 7679706]

De Simoni MG, Storini C, Barba M, Catapano L, Arabia AM, Rossi E, Bergamaschini L. Neuroprotection by complement (C1) inhibitor in mouse transient brain ischemia. J Cereb Blood Flow Metab 2003;23:232–239. [PubMed: 12571454]

De Simoni MG, Rossi E, Storini C, Pizzimenti S, Echart C, Bergamaschini L. The powerful neuroprotective action of C1-inhibitor on brain ischemia-reperfusion injury does not require C1q. Am J Pathol 2004a;164:1857–1863. [PubMed: 15111332]

De Simoni MG, Rossi E, Storini C, Pizzimenti S, Echart C, Bergamaschini L. The powerful neuroprotective action of C1-inhibitor on brain ischemia-reperfusion injury does not require C1q. Am J Pathol 2004b;164:1857–1863. [PubMed: 15111332]

de Zwaan C, Kleine AH, Diris JH, Glatz JF, Wellens HJ, Strengers PF, Tissings M, Hack CE, van Dieijen-Visser MP, Hermens WT. Continuous 48-h C1-inhibitor treatment, following reperfusion therapy, in patients with acute myocardial infarction. Eur Heart J 2002;23:1670–1677. [PubMed: 12398824]

Feletou M, Jamonneau I, Germain M, Thurieau C, Fauchere JL, Villa P, Ghezzi P, Canet E. Bradykinin B2 receptor involvement in rabbit and murine models of septic shock. J Cardiovasc Pharmacol 1996;27:500–507. [PubMed: 8847866]

Fenton MJ, Golenbock DT. LPS-binding proteins and receptors. J Leukocyte Biol 1998;64:25–32. [PubMed: 9665271]

Ferguson AD, Welte W, Hofmann E, Lindner B, Holst O, Coulton JW, Diederichs K. A conserved structural motif for lipopolysaccharide recognition by procaryotic and eucaryotic proteins. Structure 2000;8:585–592. [PubMed: 10873859]

Fiane AE, Videm V, Johansen HT, Mellbye OJ, Nielsen EW, Mollnes TE. C1-inhibitor attenuates hyperacute rejection and inhibits complement, leukocyte and platelet activation in an ex vivo pig-to-human perfusion model. Immunopharmacology 1999;42:231–243. [PubMed: 10408384]

Fischer MB, Prodeus AP, Nicholson-Weller A, Ma M, Murrow J, Reid RR, Warren HB, Lage AL, Moore JFD, Rosen FS, Carroll MC. Increased susceptibility to endotoxin shock in complement C3- and C4-deficient mice is corrected by C1 inhibitor replacement. J Immunol 1997;159:976–982. [PubMed: 9218619]

Frenette PS, Wagner DD. Adhesion molecules-First of two parts. N Engl J Med 1996a;334:1526–1529. [PubMed: 8618609]

Frenette PS, Wagner DD. Adhesion molecules--Part II: Blood vessels and blood cells. N Engl J Med 1996b;335:43–45. [PubMed: 8637541]

Fukuta D, Miyagawa S, Yamada M, Matsunami K, Kurihara T, Shirasu A, Hattori H, Shirakura R. Effect of various forms of the C1 esterase inhibitor (C1-INH) and DAF on complement mediated xenogeneic cell lysis. Xenotransplantation 2003;10:132–141. [PubMed: 12588646]

Giebler R, Schmidt U, Koch S, Peters J, Scherer R. Combined antithrombin III and C1-esterase inhibitor treatment decreases intravascular fibrin deposition and attenuates cardiorespiratory impairment in rabbits exposed to *Escherichia coli* endotoxin. Crit Care Med 1999;27:597–604. [PubMed: 10199542]

Guerrero R, Velasco F, Rodriguez M, Lopez A, Rojas R, Alvarez MA, Villalba R, Rubio V, Torres A, Castillo Dd. Endotoxin-induced pulmonary dysfunction is prevented by C1-esterase inhibitor. J Clin Invest 1993;91:2754–2760. [PubMed: 8514883]

Hack CE, Ogilvie AC, Eisele B, Eerenberg AJ, Wagstaff J, Thijs LG. C1-inhibitor substitution therapy in septic shock and in the vascular leak syndrome induced by high doses of interleukin-2. Intensive Care Med 1993;19(Suppl 1):S19–S28. [PubMed: 8053997]

Hack CE, Ogilvie AC, Eisele B, Jansen PM, Wagstaff J, Thijs LG. Initial studies on the administration of C1-esterase inhibitor to patients with septic shock or with a vascular leak syndrome induced by interleukin-2 therapy. Prog Clin Biol Res 1994;388:335–357. [PubMed: 7831367]

Hattori R, Hamilton KK, McEver RP, Sims PJ. Complement proteins C5b-9 induce secretion of high molecular weight multimers of endothelial von Willebrand factor and translocation of granule membrane protein GMP-140 to the cell surface. J Biol Chem 1989;264:9053–9060. [PubMed: 2470750]

Hauert J, Patston PA, Schapira M. C1 inhibitor cross-linking by tissue transglutaminase. J Biol Chem 2000;275:14558–14562. [PubMed: 10799541]

Hecker JM, Lorenz R, Appiah R, Vangerow B, Loss M, Kunz R, Schmidtko J, Mengel M, Klempnauer J, Piepenbrock S, Dickneite G, Neidhardt H, Ruckoldt H, Winkler M. C1-inhibitor for prophylaxis of xenograft rejection after pig to cynomolgus monkey kidney transplantation. Transplantation 2002;73:675–677. [PubMed: 11907409]

Henze U, Lennartz A, Hefemann B, Goldmann C, Kirkpatrick CJ, Klosterhalfen B. The influence of the C1-inhibitor BERINERT and the protein-free haemodialsate ACTIHAEMYL20% on the evolution of the depth of scald burns in a porcine model. Burns 1997;23:473–477. [PubMed: 9429024]

Horstick G, Heimann A, Gotze O, Hafner G, Berg O, Bohmer P, Becker P, Darius H, Rupprecht HJ, Loos M, Bhakdi S, Meyer J, Kempski O. Intracoronary application of C1 esterase inhibitor improves cardiac function and reduces necrosis in an experimental model of ischemia reperfusion. Circulation 1997;95:701–708. [PubMed: 9024160]

Horstick G, Berg O, Heimann A, Gotze O, Loos M, Hafner G, Bierbach B, Petersen S, Bhakdi S, Darius H, Horstick M, Meyer J, Kempski O. Application of C1-esterase inhibitor during reperfusion of ischemic myocardium: dose-related beneficial versus detrimental effects. Circulation 2001a; 104:3125–3131. [PubMed: 11748112]

Horstick G, Kempf T, Lauterbach M, Bhakdi S, Kopacz L, Heimann A, Malzahn M, Horstick M, Meyer J, Kempski O. C1-esterase-inhibitor treatment at early reperfusion of hemorrhagic shock reduces mesentery leukocyte adhesion and rolling. Microcirculation 2001b;8:427–433. [PubMed: 11781815]

Horstick B, Berg O, Heimann A, Gotze O, Loos M, Hafner G, Bierbach B, Petersen S, Bhakdi S, Darius H, Horstick M, Meyer J, Kempski O. Application of C1-esterase inhibitor during reperfusion of ischemic myocardium: dose-related beneficial versus detrimental effects. Circulation 2002;104:3125–3131. [PubMed: 11748112]

Jagels MA, Daffern PJ, Hugli TE. C3a and C5a enhance granulocyte adhesion to endothelial and epithelial cell monolayers: epithelial and endothelial priming is required for C3a-induced eosinophil adhesion. Immunopharmacology 2000;46:209–222. [PubMed: 10741901]

Jansen PM, Pixley RA, Brouwer M, de Jong IW, Chang AC, Hack CE, Taylor FB Jr, Colman RW. Inhibition of factor XII in septic baboons attenuates the activation of complement and fibrinolytic systems and reduces the release of interleukin-6 and neutrophil elastase. Blood 1996;87:2337–2344. [PubMed: 8630396]

Jansen PM, Eisele B, de Jong IW, Chang A, Delvos U, Taylor JFB, Hack CE. Effect of C1 inhibitor on inflammatory and physiologic response patterns in primates suffering from lethal septic shock. J Immunol 1998;160:475–484. [PubMed: 9552006]

Jiang H, Wagner E, Zhang H, Frank MM. Complement 1 inhibitor is a regulator of the alternative complement pathway. J Exp Med 2001;194:1609–1616. [PubMed: 11733575]

Juan TS, Hailman E, Kelley MJ, Busse LA, Davy E, Empig CJ, Narhi LO, Wright SD, Lichenstein HS. Identification of a lipopolysaccharide binding domain in CD14 between amino acids 57 and 64. J Biol Chem 1995;270:5219–5224. [PubMed: 7534291]

Kaplan AP, Gruber A, Harpel PC. Assessment of Hageman factor activation in human plasma: quantification of activated Hageman factor-C1 inactivator complexes by an enzyme-linked differential antibody immunosorbent assay. Blood 1985;66:636–641. [PubMed: 3875374]

Khorram-Sefat R, Goldmann C, Radke A, Lennartz A, Mottaghy K, Afify M, Kupper W, Klosterhalfen B. The therapeutic effect of C1-inhibitor on gut-derived bacterial translocation after thermal injury. Shock 1998;9:101–108. [PubMed: 9488254]

Kirschfink M, Nurnberger W. C1 inhibitor in anti-inflammatory therapy: from animal experiment to clinical application. Mol Immunol 1999;36:225–232. [PubMed: 10403475]

Kitchens RL. Role of CD14 in cellular recognition of bacterial lipopolysaccharides. Chem Immunol 2000;74:61–82. [PubMed: 10608082]

Lamping N, Hoess A, Yu B, Park TC, Kirschning CJ, Pfeil C, Reuter D, Wright SD, Herrman F, Schumann RR. Effects of site directed mutagenesis of basic residues (Arg 94, Lys 95, Lys 99) of LPS-binding protein on binding and transfer of LPS and subsequent immune cell activation. J Immunol 1996;157:4648–4656. [PubMed: 8906845]

Lehmann TG, Heger M, Munch S, Kirschfink M, Klar E. In vivo microscopy reveals that complement inhibition by C1-esterase inhibitor reduces ischemia/reperfusion injury in the liver. Transpl Int 2000;13(Suppl 1):S547–550. [PubMed: 11112070]

Lehmann C, Birnbaum J, Luhrs C, Ruckbeil O, Spies C, Ziemer S, Grundling M, Pavlovic D, Usichenko T, Wendt M, Kox WJ. Effects of C1 esterase inhibitor administration on intestinal functional capillary density, leukocyte adherence and mesenteric plasma extravasation during experimental endotoxemia. Intensive Care Med 2004;30:309–314. [PubMed: 14586496]

Liu D, Cai S, Gu X, Scafidi J, Wu X, Davis AE III. C1 inhibitor prevents endotoxin shock via a direct interaction with lipopolysaccharide. J Immunol 2003;171:2594–2601. [PubMed: 12928411]

Liu D, Gu X, Scafidi J, Davis AE III. N-linked glycosylation is required for C1 inhibitor-mediated protection from endotoxin shock in mice. Infect Immun 2004;72:1946–1955. [PubMed: 15039314]

Liu D, Cramer CC, Scafidi J, Davis AE III. N-linked glycosylation at Asn3 and the positively charged residues within the amino terminal domain of C1 inhibitor are required for its interaction with *Salmonella typhimurium* lipopolysaccharide and lipid A. Infect Immun 2005a;73:4478–4487. [PubMed: 16040958]

Liu D, Zhang D, Scafidi J, Wu X, Cramer CC, Davis AE III. C1 inhibitor prevents gram negative bacterial lipopolysaccharide-induced vascular permeability. Blood 2005b;72:1946–1955.

Mancek M, Pristovsek P, Jerala R. Identification of LPS-binding peptide fragment of MD-2, a Toll-receptor accessory protein. Biochem Biophys Res Commun 2002;292:880–885. [PubMed: 11944896]

Matsunami K, Miyagawa S, Yamada M, Yoshitatsu M, Shirakura R. A surface-bound form of human C1 esterase inhibitor improves xenograft rejection. Transplantation 2000;69:749–755. [PubMed: 10755521]

Matsushita M, Endo Y, Fujita T. Cutting edge: complement-activating complex of ficolin and mannose-binding lectin-associated serine protease. J Immunol 2000a;164:2281–2284. [PubMed: 10679061]

Matsushita M, Thiel S, Jensenius JC, Terai I, Fujita T. Proteolytic activities of two types of mannose-binding lectin associated serine protease. J Immunol 2000b;165:2637–2642. [PubMed: 10946292]

Matsushita M, Endo Y, Hamasaki N, Fujita T. Activation of the lectin complement pathway by ficolins. Int Immunopharmacol 2001;1:359–363. [PubMed: 11367522]

Monsinjon T, Gasque P, Chan P, Ischenko A, Brady JJ, Fontaine MC. Regulation by complement C3a and C5a anaphylatoxins of cytokine production in human umbilical vein endothelial cells. FASEB J 2003;17:1003–1014. [PubMed: 12773483]

Niederau C, Brinsa R, Niederau M, Luthen R, Strohmeyer G, Ferrell LD. Effects of C1-esterase inhibitor in three models of acute pancreatitis. Int J Pancreatol 1995;17:189–196. [PubMed: 7622941]

Nielsen EW, Mollnes TE, Harlan JM, Winn RK. C1-inhibitor reduces the ischaemia-reperfusion injury of skeletal muscles in mice after aortic cross-clamping. Scand J Immunol 2002;56:588–592. [PubMed: 12472670]

Ogilvie AC, Baars JW, Eerenberg AJM, Hack CE, Pinedo HM, Thijs LG, Wagstaff J. A pilot study to evaluate the effects of C1 esterase inhibitor on the toxicity of high-dose interleukin 2. Br J Cancer 1994;69:596–598. [PubMed: 8123494]

Patston PA, Schapira M. Regulation of C1-inhibitor function by binding to type IV collagen and heparin. Biochem Biophys Res Commun 1997;230:597–601. [PubMed: 9015369]

Pixley RA, Schapira M, Colman RW. The regulation of human factor XIIa by plasma proteinase inhibitors. J Biol Chem 1985;260:1723–1729. [PubMed: 2578463]

Pixley RA, De La Cadena R, Page JD, Kaufman N, Wyshock EG, Chang A, Taylor FB Jr, Colman RW. The contact system contributes to hypotension but not disseminated intravascular coagulation in lethal bacteremia. In vivo use of a monoclonal anti-factor XII antibody to block contact activation in baboons. J Clin Invest 1993;91:61–68. [PubMed: 7678610]

Poltorak A, He X, Smirnova I, Liu MY, van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, Beutler B. Defective LPS signaling in C3H/HeJ and C57Bl/10ScCr mice: mutations in the Tlr4 gene. Science 1998;282:2085–2088. [PubMed: 9851930]

Przemeck M, Lorenz R, Vangerow B, Klempnauer J, Winkler M, Piepenbrock S. Pretreatment with C1-esterase inhibitor improves cardiovascular stability in small primates undergoing porcine kidney xenotransplantation. Transplant Proc 2002;34:2383. [PubMed: 12270448]

Radke A, Mottaghy K, Goldmann C, Khorram-Sefat R, Kovacs B, Janssen A, Klosterhalfen B, Hafemann B, Pallua N, Kirschfink M. C1 inhibitor prevents capillary leakage after thermal trauma. Crit Care Med 2000;28:3224–3232. [PubMed: 11008986]

Schelzig H, Simon F, Krischer C, Vogel A, Abendroth D. Ex-vivo hemoperfusion (eHPS) of pig-lungs with whole human blood: effects of complement inhibition with a soluble C1-esterase-inhibitor. Ann Transplant 2001;6:34–39. [PubMed: 11899895]

Scherer RU, Giebler RM, Schmidt U, Paar D, Kox WJ. The influence of C1-esterase inhibitor substitution on coagulation and cardiorespiratory parameters in an endotoxin-induced rabbit model of hypercoagulability. Semin Thrombosis Hemostasis 1996;22:357–366.

Schmidt W, Stenzel K, Walther A, Gebhard MM, Martin E, Schmidt H. Influence of C1-esterase inhibitor on tissue oxygenation of jejunal mucosa during endotoxemia. Int J Surg Investig 1999a;1:277–283.

Schmidt W, Stenzel Z, Gebhard MM, Martin E, Schmidt H. C1-esterase inhibitor and its effects on endotoxin-induced leukocyte adherence and plasma extravasation in postcapillary venules. Surgery 1999b;125:280–287. [PubMed: 10076612]

Schumann RR, Lamping N, Hoess A. Interchangeable endotoxin-binding domains in proteins with opposite lipopolysaccharide-dependent activities. J Immunol 1997;159:5599–5605. [PubMed: 9548502]

Schumann RR, Latz E. Lipopolysaccharide-binding protein. Chem Immunol 2000;74:42–60. [PubMed: 10608081]

Segal BH, Kuhns DB, Ding L, Gallin JI, Holland SM. Thioglycollate peritonitis in mice lacking C5, 5-lipoxygenase, or p47(phox): complement, leukotrienes, and reactive oxidants in acute inflammation. J Leukoc Biol 2002;71:410–416. [PubMed: 11867678]

Sim RB, Reboul A, Arlaud GJ, Villiers CL, Colomb MG. Interaction of 125I-labelled complement components C1r and C1s with protease inhibitors in plasma. FEBS Lett 1979;97:111–115. [PubMed: 761607]

Stelter F, Bernheiden M, Menzel R, Jack RS, Witt S, Fan X, Pfister M, Schutt C. Mutation of amino acids 39–44 of human CD14 abrogates binding of lipopolysaccharide and *Escherichia coli*. Eur J Biochem 1997;243:100–109. [PubMed: 9030727]

Stelter F, Loppnow H, Menzel R, Grunwald U, Bernheiden M, Jack RS, Ulmer AJ, Schutt C. Differential impact of substitution of amino acids 9–13 and 91–101 of human CD14 on soluble CD14-dependent activation of cells by lipopolysaccharide. J Immunol 1999;163:6035–6044. [PubMed: 10570291]

Strachan AJ, Woodruff TM, Haaima G, Fairlie DP, Taylor SM. A new small molecule C5a receptor antagonist inhibits the reverse-passive Arthus reaction and endotoxic shock in rats. J Immunol 2000;164:6560–6565. [PubMed: 10843715]

Strecker G, Ollier-Hartmann MP, van Halbeek H, Vliegenthart JF, Montreuil J, Hartmann L. Primary structure of the glycan chains of normal C1 esterase inhibitor (C1-INH) after NMR analysis at 400 MHz. C R Acad Sci III 1985;301:571–576. [PubMed: 3936597]

Tassani P, Kunkel R, Richter JA, Oechsler H, Lorenz HP, Braun SL, Eising GP, Haas F, Paek SU, Bauernschmitt R, Jochum M, Lange R. Effect of C1-esterase-inhibitor on capillary leak and inflammatory response syndrome during arterial switch operations in neonates. J Cardiothorac Vasc Anesth 2001;15:469–473. [PubMed: 11505352]

Tietz, NW. Tietz Clinical Guide to Laboratory Tests. W. B. Saunders; Philadelphia: 1995.

Vestweber D, Blanks JE. Mechanisms that regulate the function of the selectins and their ligands. Physiol Rev 1999;79:181–213. [PubMed: 9922371]

Viriyakosol S, Kirkland TN. A region of human CD14 required for lipopolysaccharide binding. J Biol Chem 1995;270:361–368. [PubMed: 7529231]

Viriyakosol S, Mathison JC, Tobias PS, Kirkland TN. Structure-function analysis of CD14 as a soluble receptor for lipopolysaccharide. J Biol Chem 2000;275:3144–3149. [PubMed: 10652298]

Visintin A, Latz E, Monks BG, Espevik T, Golenbock DT. Lysines 128 and 132 enable lipopolysaccharide binding to MD-2, leading to toll-like receptor-4 aggregation and signal transduction. J Biol Chem 2003;278:48313–48320. [PubMed: 12960171]

Ziccardi RJ. Activation of the early components of the classical complement pathway under physiological conditions. J Immunol 1981;126:1768–1773.

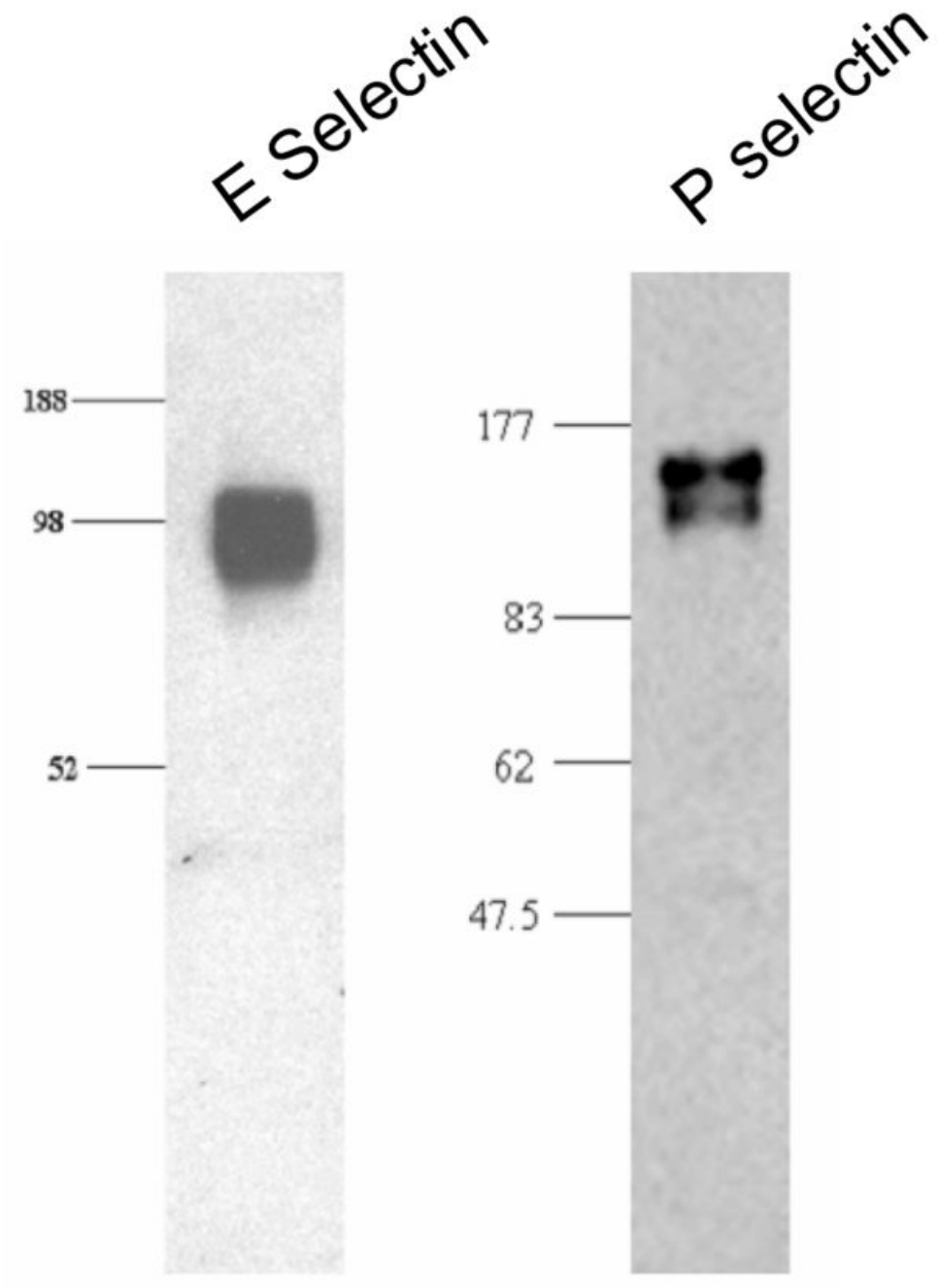

Fig. 1.

Endothelial cell E- and P-selectin co-immunoprecipitate with C1 inhibitor. Human umbilical vein endothelial cells were treated with TNF-α and H_2O_2 and were then incubated with C1 inhibitor. The cells then were lysed and immunoprecipitated with rabbit anti-C1 inhibitor antiserum, following which SDS-polyacrylamide gel electrophoresis was performed and blots were probed with antiserum to E-selectin or P-selectin.

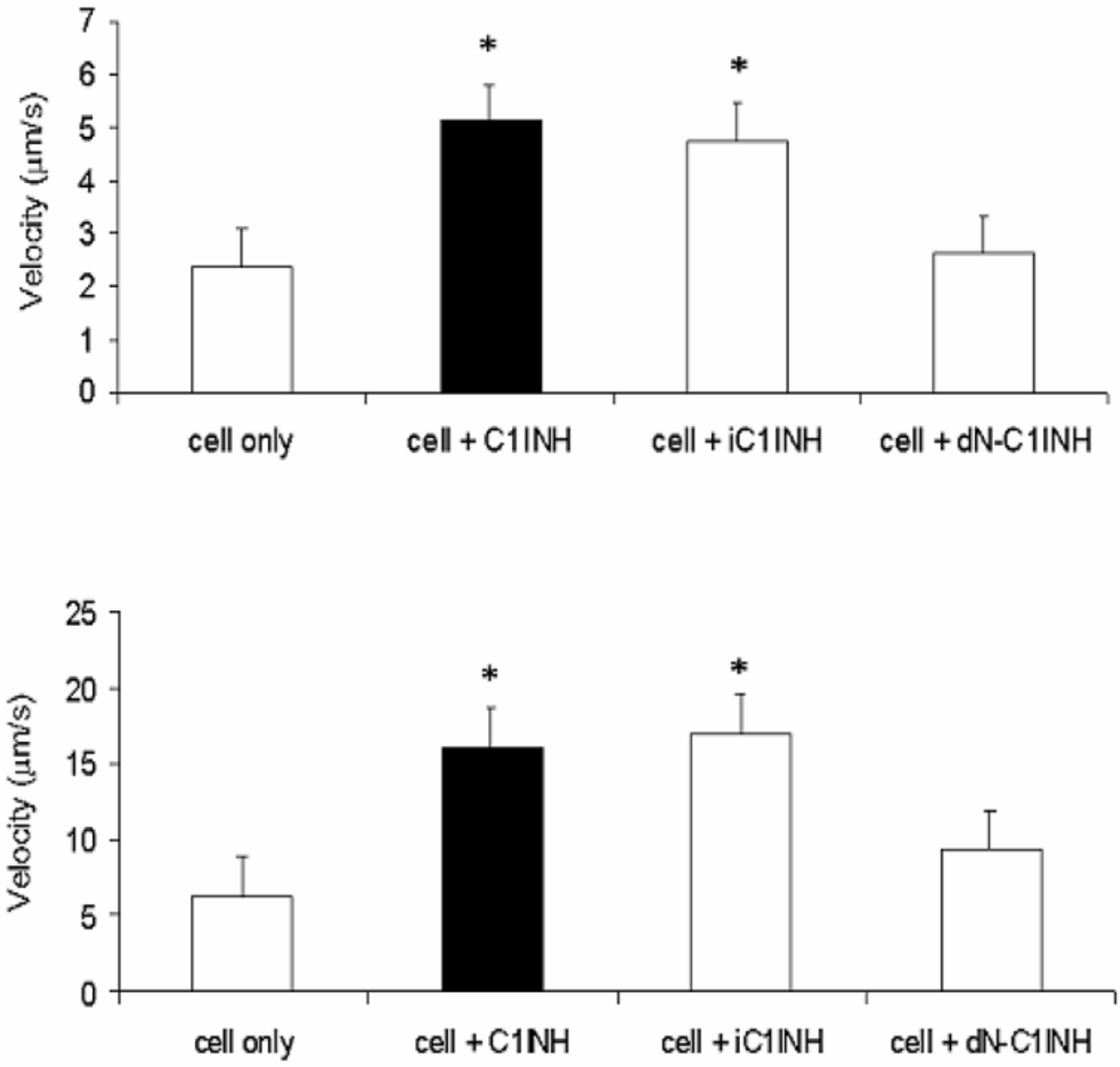

Fig. 2.

C1 inhibitor blocks the rolling of HL-60 cells on E- and P-selectin under flow conditions *in vitro*. Human E-selectin (top) was immobilized and CHO cells that express recombinant P-selectin (bottom) were grown to a monolayer on a plate. HL-60 cells (1×10^6/ml) were perfused for a 15 min period in the absence or presence of various forms of C1 inhibitor (300 μg/ml). The velocity of 30 rolling cells was determined.

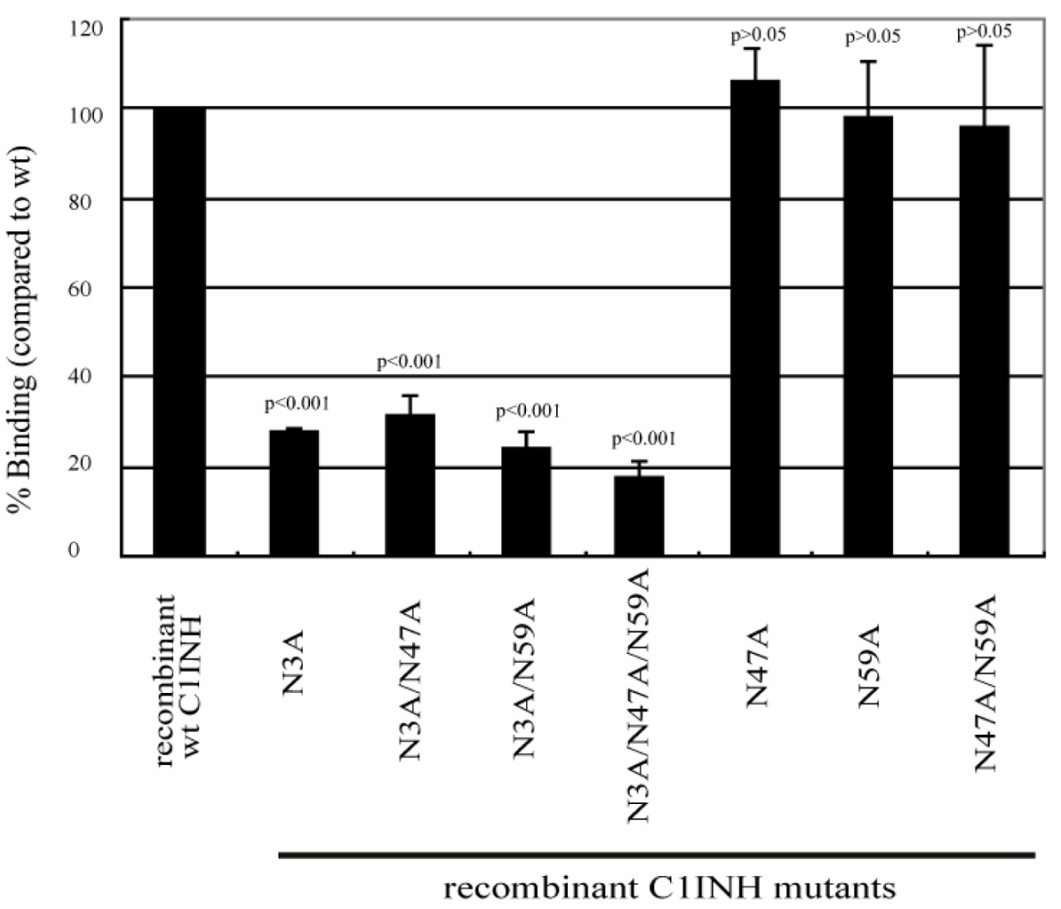

Fig. 3.

Binding of C1 inhibitor Asn substitution mutants to immobilized LPS. LPS was immobilized on the surfaces of polyvinyl chloride plates as described in Materials and Methods. Binding was detected using an anti-human C1 inhibitor antibody followed by goat anti-rabbit IgG conjugated with horseradish peroxidase, with absorbance measured at 490 nm. Results (level of binding as a percentage of the binding of recombinant wild type C1 inhibitor [wt C1INH] were expressed as the means ± SD from at least three independent experiments.

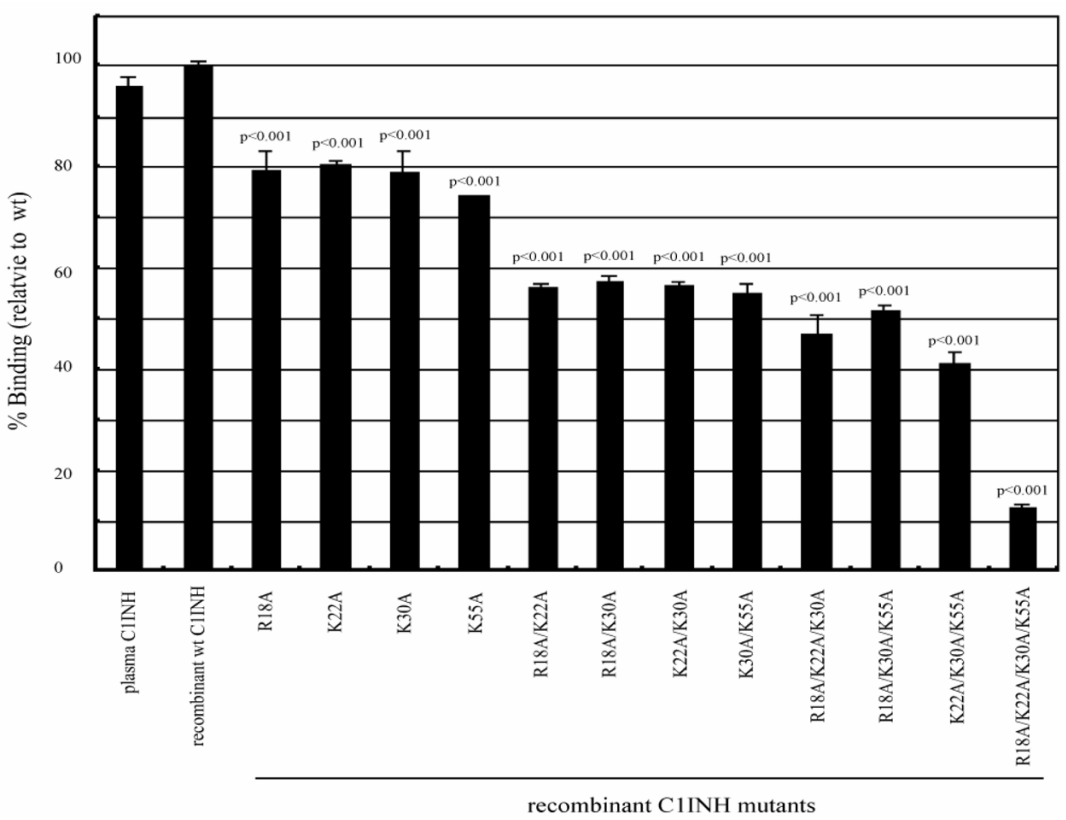

Fig. 4.

Binding of C1 inhibitor Arg and Lys substitution mutants to immobilized LPS. Assays were performed and results expressed as described in Fig. 3.

Table 1

C1 inhibitor-mediated modulation of inflammatory disease.

Animal Models	Human Disease
Ischemia-reperfusion injury (myocardial, brain, skeletal muscle)	Myocardial infarction
Vascular leak associated with thermal injury	Capillary leak associated with IL-2 therapy
Gram negative bacterial sepsis and endotoxin shock	Sepsis
Hyperacute transplant rejection	Cardiopulmonary bypass
	Traumatic and hemorrhagic shock

Table 2

C1 inhibitor: interactions unrelated to protease inhibition.

Component	Potential Biologic Function
Extracellular matrix proteins (type IV collagen, laminin, entactin, fibrin)	Retain C1 inhibitor at inflammatory sites
C3b	Alternative pathway regulation
E and P selectins	Inhibition of leukocyte rolling and transmigration
Gram negative bacteria and endotoxin	Protection from sepsis and endotoxin shock