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Research Article

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Inhibition of the Activation of Hageman Factor (Factor XII) by Complement Subcomponent C1q

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Abstract

Hageman factor (HF, Factor XII) is activated by glass, collagen, and ellagic acid, and initiates blood coagulation via the intrinsic pathway. C1q inhibits collagen-induced platelet aggregation and adherence of platelets to glass, effects attributable to the collagen-like region of C1q. We examined the actions of C1q on HF activation. Incubation of C1q with HF before addition of HF-deficient plasma extended the activated partial thromboplastin time. Similarly, when glass tubes were coated with C1q before testing, the partial thromboplastin time of normal plasma was increased. C1q reduced the activation of HF by ellagic acid, as measured by the release of *p*-nitroaniline from the synthetic substrate H-D-prolyl-L-phenylalanyl-L-arginine-*p*-nitroanilide dihydrochloride, an effect inhibited by monoclonal anti-human C1q murine IgG and by digestion of C1q by collagenase. Thus, C1q inhibits activation of HF in vitro in clot-promoting and amidolytic assays and suggests a regulatory mechanism for the inhibition of coagulation.

Introduction

The initial step of the intrinsic pathway of thrombin formation is the activation of Hageman factor (HF,[1] Factor XII) by negatively charged agents, a process that can be inhibited by positively charged substances (1). Some fractions of normal plasma are known to block the activation of HF, but neither the identity nor mode of action of the inhibitory agents in these fractions has been clarified (2).

C1q, a subcomponent of the first component of complement (C1), has amino acid sequences resembling those of collagen and can inhibit collagen-induced platelet aggregation and the adherence of platelets to glass (3, 4). Both glass and some forms of collagen activate HF (1, 5, 6), although the effect of collagen on HF is controversial (6–13). The possibility was examined that, similar to its effect on platelets, C1q might block the activation of HF. The experiments to be described demonstrate that C1q can inhibit the activation of HF by glass or ellagic acid. The biological significance of this observation is yet to be determined.

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1. *Abbreviations used in this paper:* BS, barbital-saline buffer; HF, Hageman factor; HPPAN, H-D-prolyl-L-phenylalanyl-L-arginine-*p*-nitroanilide dihydrochloride; *p*-NA, *p*-nitroaniline; PTT, partial thromboplastin time.

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Methods

Plasmas. Pooled human serum and frozen citrated (acid citrate dextrose) plasma were obtained from normal human volunteers. A pool of 24 normal human plasmas was prepared from venous blood to which one-fiftieth volume of 0.5 M sodium citrate buffer (pH 5.0) had been added, and stored at −70°C as previously described (14). Human HF-deficient plasma was similarly prepared and stored. Venepuncture was performed after informed consent with the approval of the Institutional Review Board for Human Investigation, University Hospitals of Cleveland. The pooled normal plasma was arbitrarily said to contain 1 U of HF/ml, as measured in a coagulant assay.

C1q preparation. C1q was isolated from pooled human serum or frozen citrated plasma by successive chromatography upon columns of Bio-Rex 70 and Bio-Gel A5M (Bio-Rad Laboratories, Richmond, CA), using a minor modification of the method of Tenner et al. (15) in which 102 mM sodium chloride was substituted for 82 mM sodium chloride in the initial buffer used for the equilibration of the Bio-Rex 70 column (Bio-Rad Laboratories). The presence of C1q in the C1q preparation was established by Ouchterlony gel diffusion against polyclonal anti-human C1q goat antibody (Atlantic Antibodies, Scarborough, ME or Boehringer-Mannheim Biochemicals, Indianapolis, IN). The purity of C1q was assessed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), which demonstrated homogeneous subunits of C1q. The apparent molecular weights of the reduced samples were 35,000, 32,000, and 28,000, as previously described (16). Digestion of the collagenous portion of C1q was performed by a modification of the method of Pacques (17) by heating the C1q solution at 54°C in a water bath for 10 min and then adding 0.037 ml collagenase (*Clostridium histolyticum*, 10 mg/ml, 689 U/mg; Cooper Biomedical, Malvern, PA). The sample was immediately removed from the water bath and freed of traces of precipitate by centrifugation at 900 *g* for 10 min at 2°C. The sample was then precipitated at 4°C overnight by addition of $(NH_4)_2SO_4$ to one-third saturation. The precipitate was collected by centrifugation, dissolved in barbital-saline buffer (BS), and dialyzed against the buffer for 1 h. A control sample was treated in a similar manner, omitting the addition of collagenase. SDS-PAGE of reduced and unreduced samples of the collagenase-treated C1q preparation exhibited homogeneous subunits; the apparent molecular weights of the subunits in the reduced sample were 25,000, 19,000, and 18,000, similar to those previously described for this digestion (17).

HF preparation. Purified human HF, depleted of other detectable clotting factors (coagulant titer 2.56 U/ml; specific activity 58 U/mg protein) was prepared by a method described elsewhere (18) and stored at −70°C.

Antibody preparation. Murine monoclonal anti-human C1q antibody (Genzyme, Boston, MA) was filtered through Affi-gel Blue IgG purification gel (Bio-Rad Laboratories) that had been equilibrated with 70 ml 0.02 M KH_2PO_4, pH 8.0; similarly prepared monoclonal antibody against von Willebrand factor (Factor VIII:vWF) was used as a control (19). For use in these experiments the protein contents of the filtered samples were adjusted to 20 μg/ml with BS.

Reagents and buffers. Ellagic acid, synthesized by Dr. Miklos Bodanzky, Case Western Reserve University, was dissolved at a concentration of 10^{-4} M in BS using a motorized Teflon pestle fitted to a glass mortar. The solution was filtered through No. 1 paper (Whatman, Inc., Clifton, NJ) and diluted in the same buffer to 2×10^{-5} M. Ellagic acid solutions were used freshly prepared.

H-D-prolyl-L-phenylalanyl-L-arginine-*p*-nitroanilide dihydrochloride (HPPAN, S2302; Helena Laboratories, Beaumont, TX) was dissolved at a concentration of 10^{-3} M in water and diluted to 5×10^{-4} M with an equal part of Tris-imidazole–saline buffer. Crystallized bovine serum albumin (BSA; Pentex Div., Miles Laboratories, Elkhart, IN) was dissolved in BS at a concentration of 0.25 g/100 ml. Crude soybean phosphatides (Centrolex-P; a gift from Central Soya Co., Chicago, IL) were suspended at a concentration of 0.1% in 0.15 M sodium chloride.

Tris–imidazole buffer (pH 8.2) was 0.025 M *tris* (hydroxymethyl) aminomethane (Sigma Chemical Co., St. Louis, MO), 0.25 M imidazole (Matheson, Coleman and Bell, Norwood, OH), and sufficient sodium chloride to provide an ionic strength of 0.15 (20). BS, pH 7.5, was 0.025 M sodium barbital in 0.125 M sodium chloride.

Coagulation studies. The coagulant activity of glass-activated HF was tested by a modified partial thromboplastin time (PTT) technique, incubating 0.1 ml of HF (0.05 U/ml in 0.25% BSA in BS) with or without C1q with 0.1 ml Centrolex-P in 10 × 75-mm glass tubes at 37°C for 2 min. Thereafter, 0.1 ml of HF-deficient plasma was added, incubation was continued for 8 min, 0.1 ml of 0.025 M $CaCl_2$, prewarmed to 37°C, was then added, and the PTT was measured. Variations of this technique are noted in the footnotes to Tables I and III. All PTTs reported are the averages of duplicate tests with a maximum of 2 s between results, and the data illustrated are typical of replicate experiments.

Blockage of the activation of HF by glass was tested by the use of various materials to coat the clotting tubes before measuring the PTT. New 10 × 75-mm glass tubes were coated with 0.1 ml of the test materials, vortexed for 30 s, then rinsed three times with 10 ml BS and three times with 10 ml distilled water, and finally dried using an air jet. Then, 0.1 ml of normal pooled plasma and 0.1 ml of Centrolex-P were added to each tube and the mixture was incubated at 37°C. After 1 min, 0.1 ml of prewarmed 0.025 M $CaCl_2$ was added, the tubes again vortexed, and the clotting time was measured.

Amidolytic assays. The amidolytic properties of ellagic acid–activated HF were tested by incubating a "generation mixture" of 0.1 ml of HF (0.05 U/ml in 0.25% BSA in BS), 0.1 ml of 2×10^{-5} M ellagic acid in 37°C in 12 × 75-mm polystyrene tubes (Falcon Labware, Div. of Becton-Dickinson & Co., Cockeysville, MD). After 1 h, 1.0 ml of 5×10^{-4} HPPAN was added and incubation was continued for an additional hour. The reaction was stopped by addition of 0.3 ml of glacial acetic acid. The *p*-nitroaniline (*p*-NA) released from HPPAN by the generation mixture was measured by absorption at 405 nm against appropriate blanks. This assay method was used to test inhibition of the activation of HF by prior treatment of C1q versus various control substances.

Assay for protein concentrations. Protein concentrations were estimated by Lowry's method (21).

Results

Inhibitory effect of C1q on the clot-promoting activity of HF. Addition of C1q to purified HF had a slight inhibitory effect upon the clot-promoting properties of HF, as measured by the PTT. Thus, the PTT of HF-deficient plasma, after the addition of purified HF, was 118.9 s (Table I). Incubation of HF with 1×10^{-6} M C1q before the addition of HF-deficient plasma extended the PTT to 135.4 s. Incubation of HF with a lesser concentration of C1q (1×10^{-7} M) gave an intermediate value of 126.9 s. The prolongation of the PTT was not due to simple dilution of the HF, as the control time was measured with a volume of BS equal to that of the C1q used in these studies.

A similar result was noted when C1q was used to coat glass tubes before the addition of the normal plasma (Table II). The PTT of normal plasma was 75.3 s when BS was used as the coating substance, but extended to 86.4 s when 1×10^{-6} M C1q was the coating substance. This PTT approached the PTT obtained when HF-deficient plasma was the coating substance, a procedure known to inhibit the activation of HF (22). When pooled normal plasma was used as the coating substance, the PTT was 68.3 s, presumably reflecting the activation by glass of the HF present in normal plasma. A dose-response relationship was present, as decreasing the C1q concentration from 2×10^{-6} to 2×10^{-10} M resulted in a decreasing prolongation of the PTT, approaching the control value obtained with BS. The effect of C1q is in contrast to that of certain other proteins, as glass tubes coated with albumin or transferrin retain their clot-promoting properties (2).

When C1q was added to the generation mixture after HF had been incubated with Centrolex-P in glass tubes for 2 min, and thus presumably activated, the PTT was 120.7 s, not significantly different from the 118.9 s PTT obtained when BS was substituted for C1q (Table III). These findings suggest that C1q acts to block the activation of HF, but does not block activated HF.

Inhibition of the amidolytic property of HF by incubation with C1q. Under the conditions used, HF, after activation by ellagic acid, released 50.5 nmol *p*-NA/ml per h from HPPAN (Table IV). Incubation of HF with an equal volume of 1×10^{-6} M C1q before the addition of ellagic acid reduced amidolysis to 23.5 nmol *p*-NA/ml per h. When C1q was added after the activation of HF by ellagic acid, 46.3 nmol *p*-NA/ml per h were released, a value approaching the control measurement with BS.

C1q dose-related inhibition of HF activation. Incubation of

Table I. Inhibitory Effect of C1q on the Clot-promoting Property of Glass-activated HF

Incubation mixture*	PTT
	s
HF and BS	118.9
HF and 1×10^{-7} M C1q	126.9
HF and 1×10^{-6} M C1q	135.4
BS	>400

* 0.11 ml each of HF (0.05 U/ml in 0.25% BSA in BS) and test material (C1q or BS) were incubated for 10 min at 37°C in a polystyrene tube. A volume of 0.2 ml of this mixture was transferred to a glass tube and the PTT was then measured (see Methods).

Table II. Inhibition of the Clot-promoting Property of HF by C1q Coating of Glass Tubes

Coating material*	PTT
	s
Pooled normal plasma	68.3
Hageman-deficient plasma	91.1
2×10^{-6} M C1q	86.4
2×10^{-7} M C1q	86.0
2×10^{-8} M C1q	80.3
2×10^{-9} M C1q	79.2
2×10^{-10} M C1q	77.5
BS	75.3

* Glass tubes (10 × 75 mm) were coated with plasma, BS or C1q, in BS. Thereafter, 0.1 ml of normal plasma was added to each tube and the PTT was measured (see Methods).

Table III. Noninhibition of the Clot-promoting Property of Activated HF by C1q

Incubation mixture*	PTT
	s
HF and 2×10^{-6} M Clq	120.7
HF and BS	118.9
BS	>400

* 0.1 ml of HF (0.05 U/ml in 0.25% BSA in BS) was incubated with 0.1 ml of crude soybean phosphatides (Centrolex-P) in 10 × 75-mm glass tubes at 37°C. After 2 min, 0.05 ml C1q or BS was added and the activated PTT was measured (see Methods).

HF with increasing concentrations of C1q, from 2×10^{-9} to 1.8×10^{-5} M, resulted in decreasing amounts of *p*-NA released from the synthetic substrate, from 40.2 nmol/ml per h to 14.8 nmol/ml per h (Table V). These results suggest that C1q-induced inhibition of HF activation has a dose-response relationship. Furthermore, as 0.05 U/ml HF is an $\sim 2 \times 10^{-8}$-M concentration, some inhibition of HF activation occurs at equivalent concentrations of C1q and HF.

Nonactivation of HF by C1q in an amidolytic assay. Incubation of HF with varying doses of C1q in the absence of ellagic acid resulted in essentially no amidolysis (Table VI), suggesting that C1q neither activated HF, nor induced amidolysis itself.

Reversion of C1q-induced inhibition of the amidolytic property of HF by murine anti-human C1q antibody. Incubation of 2×10^{-6} M C1q with monoclonal murine anti-human C1q antibody before the addition of HF resulted in the release of 42.7 nmol *p*-NA/ml per h (Table VII), a value that approximated the *p*-NA released by activated HF in the absence of C1q (46.9 nmol *p*-NA/ml per h). This observation suggests that anti-C1q murine antibody had almost completely blocked the inhibitory effect of C1q on HF activation. A control monoclonal antibody had no effect on the capacity of C1q to inhibit the activation of HF, as the amount of *p*-NA released was similar to the values obtained when BS was used (22.4 nmol *p*-NA/ml per h vs. 22.2 nmol *p*-NA/ml per h). Similar results were seen when only 2×10^{-7} M C1q was used. The presence of either anti-C1q IgG or control IgG in the absence of C1q did not significantly affect the amidolytic property of HF.

Table IV. Inhibition of the Amidolytic Property of HF by Incubation with C1q Added Before, but Not After, Activation of HF with Ellagic Acid

Initial incubation mixture*	Addition	*p*-NA released
		nmol/ml per h
HF and C1q	Buffer	23.5
HF and buffer	C1q	46.3
HF and buffer	Buffer	50.5
Buffer	Buffer	0.2

* 0.1 ml of HF (0.05 U/ml in 0.25% BSA in BS) was incubated with 0.1 ml of 1×10^{-6} M C1q or BS at 37°C; after 10 min 0.1 ml ellagic acid was added to each tube (to activate the HF) and incubation was continued. After 1 h, 0.05 ml 2×10^{-6} M C1q or BS was added and the assay completed (see Methods).

Table V. C1q Dose-related Inhibition of HF Activation

Incubation mixture*	*p*-NA released
	nmol/ml per h
HF and BS	44.3
HF and 2×10^{-9} M C1q	40.2
HF and 2×10^{-8} M C1q	38.6
HF and 2×10^{-7} M C1q	31.6
HF and 2×10^{-6} M C1q	23.8
HF and 1.8×10^{-5} M C1q	14.8
BSA and BS	0.2
BS	0.2

* 0.1 ml of HF (0.05 U/ml in 0.25% BSA in BS) or BS was incubated with 0.1 ml BS or BSA or C1q at 37°C. After 10 min 0.1 ml ellagic acid was added to each tube (to activate any HF present) and the assay completed (see Methods).

Reversion of C1q-induced inhibition of HF by partial digestion of C1q with collagenase. 5×10^{-6} M C1q that had been heated and partially digested by collagenase before the addition of HF and ellagic acid was not inhibitory, and led to the release of 45.6 nmol *p*-NA/ml per h, approximating the baseline value of 42.4 nmol/ml per h obtained with the BS control (Table VIII). Untreated C1q, or C1q that had been heated but not treated with collagenase, inhibited activation of HF as demonstrated by the release of less *p*-NA (17.3 nmol/ml per h and 13.8 nmol/ml per h, respectively). Some amidolysis was induced by the collagenase-digested preparation of C1q itself (8.1 nmol/ml per h *p*-NA). This background amidolysis presumably resulted from the presence of amidolytic activity in the preparation of collagenase, as amidolysis was not observed with untreated C1q or C1q that had only been heated. This background amidolysis resolved upon 10-fold dilution of the C1q preparations. This dilution reduced the C1q-induced inhibition of HF activation, as reflected by the higher amount of *p*-NA released (30.5 nmol/ml per h), but collagenase digestion of C1q again abolished its inhibition of HF activation, as 42.3 nmol/ml per h *p*-NA were released.

Discussion

The activation of HF, Factor XII, is a pivotal point in the initiation of clotting via the intrinsic pathway. Moreover, this event has relevance not only to hemostasis, but also to a legion of

Table VI. Nonactivation of HF by C1q in an Amidolytic Assay

Incubation mixture*	*p*-NA released
	nmol/ml per h
HF and BS	0.0
HF and 2×10^{-8} M C1q	0.8
HF and 2×10^{-7} M C1q	0.1
HF and 2×10^{-6} M C1q	0.6

* 0.1 ml of HF (0.05 U/ml in 0.25% BSA in BS) was incubated with 0.1 ml BS or C1q at 37°C. The assay proceeded as usual except that no ellagic acid was added to the generation mixture (see Methods).

Table VII. Reversion of C1q-induced Inhibition of HF Activation by a Monoclonal Anti-Human C1q Murine IgG

Initial incubation mixture*	Addition	p-NA released
		nmol/ml per h
BS	HF	46.9
2×10^{-6} M C1q	HF	22.2
2×10^{-6} M C1q and anti-C1q IgG	HF	42.7
2×10^{-6} M C1q and control IgG	HF	22.4
2×10^{-7} M C1q	HF	37.9
2×10^{-7} M C1q and anti-C1q IgG	HF	42.5
2×10^{-7} M C1q and control IgG	HF	37.3
BS and anti-C1q IgG	HF	43.2
BS and control IgG	HF	46.7
BS	Buffer	0.2

* 0.05 ml of C1q or buffer (BS) was incubated in polystyrene tubes with 0.1 ml of monoclonal anti-human C1q murine IgG (20 U/ml in BS), control IgG, or BS at 37°C. After 30 min 0.1 ml HF (0.05 U/ml in 0.25% BSA in BS) was added to each tube and the incubation continued. After 10 min, 0.1 ml of 2×10^{-5} M ellagic acid was added to each tube and the assay procedure continued (see Methods).

other biologic events that may result from activation of HF. In this report, we present evidence that C1q, the protein responsible for initiation of activation of the classical pathway of complement, may modulate the activation of the intrinsic clotting mechanism.

Table VIII. Reversion of the C1q-induced Inhibition of HF Activation by Partial Digestion of C1q with Collagenase

Initial incubation mixture*	Addition	p-NA released
		nmol/ml per h
BS	HF	42.4
5×10^{-6} M C1q	HF	17.3
5×10^{-6} M C1q, heated only	HF	13.8
5×10^{-6} M C1q, heated and digested	HF	45.6
5×10^{-6} M C1q	Buffer	0.1
5×10^{-6} M C1q, heated only	Buffer	0.7
5×10^{-6} M C1q, heated and digested	Buffer	8.1
5×10^{-7} M C1q	HF	30.5
5×10^{-7} M C1q, heated only	HF	23.0
5×10^{-7} M C1q, heated and digested	HF	42.3
5×10^{-7} M C1q	Buffer	0.0
5×10^{-7} M C1q, heated only	Buffer	0.3
5×10^{-7} M C1q, heated and digested	Buffer	0.0
BS	Buffer	0.0
BS	BSA	0.1

* 0.1 ml of C1q (untreated, heated only, or heated and partially digested with collagenase) or BS was incubated with 0.1 ml of HF (0.05 U/ml in 0.25% BSA in BS) or BS or BSA at 37°C. After 10 min, 0.1 ml of 2×10^{-5} M ellagic acid was added to each tube and the assay procedure continued (see Methods). Due to the high background amidolysis in the digested 5×10^{-6} M C1q preparation (probably attributable to the presence of collagenase), the experiment was repeated using 10-fold dilutions of the C1q preparations; this maneuver abolished the background amidolysis.

C1q initiates activation of the classical complement pathway by attaching to IgG, IgM, or other activating substances. Under appropriate conditions, this reaction leads to stabilization of $C\bar{1}r_2C\bar{1}s_2$ complex and triggering of $C\bar{1}r$ and $C\bar{1}s$ esterase activity and classical pathway activation. C1q may play a role in other biologic processes, including stimulation of fibroblasts in areas of inflammation (23), as an Fc receptor on macrophages (24), and in enhancing killing of non-phagocytosable targets (25). Other major biologic functions have not yet been identified.

C1q is a complex macromolecule with an approximate molecular weight of 410,000 (26). It is a very basic protein, composed of 18 polypeptide chains of three types, designated A, B, and C, each containing ~ 200 amino acid residues. Amino acid analysis has demonstrated that these three types of chains are similar in that each has ~ 80 residues of typical collagen-like sequences starting close to the NH_2-terminal end. The similarity to collagen is increased by the presence of glucosylgalactosyl disaccharide substitutions on the hydroxylysine residues (26). There is evidence that a triple helix is formed between the collagen-like region of one of a pair of C chains and the corresponding regions of an AB dimer; in this manner, it is believed, three pairs of triple helices are formed (26). Then, the association of these three pairs could give the complete hexameric structure, with six globular heads formed from the COOH-terminal halves of each chain. C1q has also been shown to be susceptible to collagenase digestion (17). With regard to function, the globular head region mediates binding to the Fc region of immunoglobulin molecules, and the collagenous tail binds to most cells that have been studied (26).

HF is a single chain 80,000-mol-wt plasma protein with an internal disulfide loop. Studies by Cochrane and Revak et al. (27) demonstrated that the adsorption of HF from diluted normal human plasma to negatively charged glass surfaces brought about scission of the molecule. HF was first cleaved at the internal disulfide loop into a two-chain species and later into several fragments, notably a 52,000-mol-wt amino-terminal fragment and a 28,000-mol-wt carboxy-terminal fragment. The enzymatic properties of HF have been localized to the carboxy-terminal fragment (28).

HF is a plasma proenzyme that, when activated, can initiate clotting via both the intrinsic and extrinsic pathways of thrombin generation, and additionally brings about reactions that lead to the generation of kinins, plasmin, and angiotensin, and to the activation of certain components of the complement pathway (29–32). Activation of HF can be brought about by its exposure to negatively charged agents such as glass, kaolin (22), ellagic acid (33), a serine protease that is present in the microsomal fraction of endothelial cells (34), a combination of collagen and some other unidentified basement membrane components (27), and some but not all preparations of collagen (5–13). HF that has been activated during the "contact phase" of blood coagulation is inhibited by $C\bar{1}$ inactivator (35) and antithrombin III (36). Plasma contains undefined material that inhibits the adsorption of HF to glass and may also interfere with the surface activation of the proenzyme in the initiation of physiological blood coagulation (2). In this regard, β_2-glycoprotein I, which, like collagen, is rich in proline residues, has recently been shown to inhibit contact activation of the intrinsic coagulation pathway (37).

C1q inhibits collagen-induced platelet aggregation (3) and the adherence of platelets to glass surfaces (4). The structural similarity of collagen and C1q led to the suggestion that C1q

may occupy a collagen–receptor site on platelets, and thus inhibit aggregation. It has also been demonstrated that a murine monoclonal antibody raised against the Raji cell C1q receptor binds to human platelets and abolishes collagen-induced platelet aggregation (38). C1q also may form complexes with plasma fibronectin but this interaction does not alter C1q-mediated inhibition of collagen-induced platelet aggregation (39). The effects of glass and collagen on platelets, as altered by C1q, led us to consider the possibility that C1q could alter the effects of glass and ellagic acid on HF.

These studies demonstrate that C1q inhibits the clot-promoting and amidolytic properties of HF. C1q blocked the activating effect of glass and ellagic acid on HF but did not inhibit activated HF. The marked resemblance of the fibrillar portion of C1q to collagen and the reported activation of HF by some (but not all) preparations of collagen suggest that C1q may block a site on glass or ellagic acid that HF requires for activation. Alternately, C1q may block a site on HF itself that is required for activation. The precise mechanism of inhibition by C1q, however, remains to be clarified.

A dose-response relationship between the amount of C1q present and the degree of prolongation of the PTT, as well as inhibition of amidolysis, was noted in replicate experiments.

The reversion of the C1q inhibition of the activation of HF by prior incubation with murine monoclonal anti-human C1q antibody substantiates the conclusion that it is C1q itself that is responsible for the alteration of HF activation. That collagenase treatment of C1q also destroyed its ability to inhibit HF activation suggests that it is the collagenous, fibrillar portion of the C1q molecule that is responsible for the inhibitory effect.

HF has previously been demonstrated to have significant effects on the activation of certain components of the complement pathway, either directly or via formation of kallikrein or plasmin (30, 31). The inhibitory effect of C1q on the activation of HF demonstrated here suggests a method by which HF activation can be regulated by the complement pathway, and thus is a possible feedback inhibitory mechanism of the clotting cascade.

C1q has a molecular weight of 400,000 and has a serum concentration of 70 μg/ml or $\sim 2 \times 10^{-7}$ M. The plasma concentration of HF is 15–45 μg/ml or $\sim 4 \times 10^{-7}$ M. Demonstration of the inhibitory effect of C1q on activation of HF at concentrations as low as 2×10^{-10} M (Table II) suggests that this phenomenon could be relevant at physiologic concentrations.

The complement system and the intrinsic clotting cascade have remarkable similarities, including the requirement for initial binding to an activator to initiate a reaction sequence that then proceeds to completion. The reactions are carefully modulated or amplified, depending on the circumstances, by a complex network of control mechanisms. $C\bar{1}$ inactivator functions as a major regulatory molecule in both systems (30). It is thus perhaps not surprising that C1q, the initiating molecule in the cascade comprising the classical pathway of complement, also appears to have a potential regulatory role in the intrinsic clotting mechanism.

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References

1. Ratnoff, O. D., and H. Saito. 1979. Surface-mediated reactions. *In* Current Topics in Hematology. S. Piomelli and S. Yachnin, editors. Alan R. Liss, Inc., New York. 2:1–57.

2. Saito, H., O. D. Ratnoff, V. H. Donaldson, G. Haney, and J. Pensky. 1974. Inhibition of the adsorption of Hageman factor (factor XII) to glass by normal human plasma. *J. Lab. Clin. Med.* 82:62–73.

3. Suba, E. A., and G. Csako. 1976. C1q (C1) receptor on human platelets: inhibition of collagen-induced platelet aggregation by C1q (C1) molecules. *J. Immunol.* 117:304–309.

4. Cazenave, J. P., S. N. Assimeth, R. H. Painter, M. A. Packham, and J. F. Mustard. 1976. C1q inhibition of the interaction of collagen with human platelets. *J. Immunol.* 116:162–163.

5. Wilner, G. D., H. L. Nossel, and E. D. LeRoy. 1968. Activation of Hageman factor by collagen. *J. Clin. Invest.* 47:2608–2615.

6. Niewiarowski, S., E. Bankowski, and T. Fiedoruk. 1964. Adsorption of Hageman factor (XII) on collagen. *Experientia.* 20:367–370.

7. Niewiarowski, S., E. Bankowski, and I. Rogowicka. 1965. Studies on the adsorption and activation of the Hageman factor (factor XII) by collagen and elastin. *Thromb. Diath. Haemorrh.* 14:387–400.

8. Niewiarowski, S., I. Rogowicka-Gorzelak, K. Worowski, and M. Mysliwiec. 1967. The *in vivo* effect of the collagen or kaolin-activated contact factor on blood clotting and fibrinolysis in rabbit. *Bull. Acad. Pol. Sci. Ser. Sci. Terre.* 15(10):599–602.

9. Niewiarowski, S., and E. Bankowski. Interaction of the Hageman factor (factor XII) with insoluble collagen. *Bull. Acad. Pol. Sci. Ser. Sci. Terre.* 17(3):137–139.

10. Griffin, J. H., E. Harper, and C. G. Cochrane. 1975. Studies on the activation of human blood coagulation factor XII (Hageman factor) by soluble collagen. *Fed. Proc.* 34:860. (Abstr.)

11. Zacharski, L. R., and R. Rosenstein. 1975. Failure of collagen to activate factor XII. *Thromb. Res.* 7:929–933.

12. Niewiarowski, S. 1976. Comments on the activation of Hageman factor (factor XII) by collagen. *Thromb. Res.* 8:421–423.

13. Zacharski, L. R., and R. Rosenstein. 1977. Further comments on the failure of collagen to activate factor XII. *Thromb. Res.* 10:771–772.

14. Zimmerman, T. S., O. D. Ratnoff, and A. S. Littell. 1971. Detection of carriers of classic hemophilia using an immunologic assay for antihemophilic factor (Factor VIII). *J. Clin. Invest.* 50:255–258.

15. Tenner, A. J., P. H. LeSavre, and N. R. Cooper. 1981. Purification and radiolabeling of human C1q. *J. Immunol.* 127:648–653.

16. Knobel, H. R., W. Villinger, and H. Isliker. 1975. Chemical analysis and electron microscopy studies of human C1q prepared by different methods. *Eur. J. Immunol.* 5:78–82.

17. Paques, E. P., R. Huber, and H. Priess. 1979. Isolation of the globular region of the subcomponent q of the C1 complement. *Hoppe-Seyler's Z. Physiol. Chem.* 350:177–183.

18. Ratnoff, O. D., B. Everson, V. H. Donaldson, and B. H. Mitchell. 1986. Purification of Hageman factor (factor XII) on columns of popcorn-agarose. *Blood.* 67:1550–1553.

19. Ogata, K., H. Saito, and O. D. Ratnoff. 1983. The relationship of the properties of antihemophilic factor (factor VIII) that support ristocetin-induced platelet aggregation (factor VIIIR:RC) and platelet retention by glass beads as demonstrated by a monoclonal antibody. *Blood.* 61:27–35.

20. Svendson, L., B. Blombäck, M. Blombäck, and P. I. Olsson. 1971. Synthetic chromogenic substrates for determination of trypsin, thrombin, and thrombin-like enzymes. *Thromb. Res.* 1:267–278.

21. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.

1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193:265–268.

22. Ratnoff, O. D., and J. M. Rosenblum. 1958. Role of Hageman factor in the initiation of clotting by glass. *Am. J. Med.* 25:160–168.

23. Bordin, S., W. P. Kolb, and R. C. Page. 1983. C1q receptors on cultured human gingival fibroblasts: analysis of binding properties. *J. Immunol.* 130:1871–1875.

24. Loos, M. 1983. Biosynthesis of the collagen-like C1q molecules and its receptor functions for Fc and polyanionic molecules on macrophages. *Curr. Top. Microbiol. Immunol.* 102:1–56.

25. Greene, B. M., J. Young, and R. Chmielewski. 1984. Subcomponent C1q enhancement of granulocyte cytotoxicity. *Clin. Res.* 32: 369*a*. (Abstr.)

26. Cooper, N. R. 1985. The classical complement pathway: activation and regulation of the first complement component. *Adv. Immunol.* 37: 151–216.

27. Cochrane, C. G., S. D. Revak, B. S. Aiken, and K. D. Wuepper. 1972. The structural characteristics and activation of Hageman factor. *In* Inflammation, Mechanisms and Control. I. M. Lepow and P. A. Ward, editors. Academic Press, Inc., New York. 119–150.

28. Revak, S. D., and C. G. Cochrane. 1976. The relationship of structure and function in human Hageman factor. The association of enzymatic and binding activities with separate regions of the molecule. *J. Clin. Invest.* 57:852–860.

29. Tatemichi, S. R., and D. Osmond. 1978. Factor XII in endogenous activation of inactive renin (letter). *Lancet.* i(8077):1313.

30. Derkx, F. H. M., B. N. Bouma, M. P. A. Schalekamp, and M. A. D. H. Schalekamp. 1979. An intrinsic factor XII-prekallikrein-dependent pathway activates the human plasma renin-angiotensin system. *Nature (Lond.).* 280:315–316.

31. Donaldson, V. H. 1968. Mechanisms of C'1 esterase in hereditary angioneurotic edema in vitro. The role of Hageman factor, a clot-promoting agent. *J. Exp. Med.* 125:411–429.

32. Ghebrehiwet, B., M. Silverberg, and A. P. Kaplan. 1981. Activation of the classical pathway of complement by Hageman factor fragments. *J. Exp. Med.* 153:665–676.

33. Ratnoff, O. D. 1980. The relative amidolytic activity of Hageman factor (factor XII) and its fragments. The effect of high-molecular-weight kininogen and kaolin. *J. Lab. Clin. Med.* 96:267–277.

34. Wiggins, R. D., D. J. Loskutoff, G. C. Cochrane, J. H. Griffin, and T. S. Edgington. 1980. Activation of rabbit Hageman factor by homogenates of cultured rabbit endothelial cells. *J. Clin. Invest.* 65:197–206.

35. Forbes, C. D., J. Pensky, and O. D. Ratnoff. 1970. Inhibition of activated Hageman factor and activated plasma thromboplastin antecedent by purified serum $C\bar{1}$ inactivator. *J. Lab. Clin. Med.* 76: 809–815.

36. Stead, N., A. P. Kaplan, and R. D. Rosenberg. 1976. Inhibition of activated factor XII by antithrombin-heparin cofactor. *J. Biol. Chem.* 251:6481–6488.

37. Schousboe, I. 1985. β_2-Glycoprotein I: a plasma inhibitor of the contact activation of the intrinsic blood coagulation pathway. *Blood.* 66: 1086–1091.

38. Peerschke, E. I. B., and B. Ghebrehiwet. 1986. A murine monoclonal antibody competes for C1q receptors on human blood platelets and abolishes collagen-induced platelet aggregation. *Clin. Res.* 34:467a. (Abstr.)

39. Wautier, J. R., M. P. Wautier, H. Kaclera, and M. W. Mosesson. 1983. Plasma fibronectin-C1q complex formation and its effect on C1q inhibition of collagen-induced platelet aggregation. *Thromb. Res.* 32: 283–290.