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Research Article

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Mechanisms of Activation of the Classical Pathway of Complement by Hageman Factor Fragment

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ABSTRACT The mechanism by which a fragment of activated Hageman factor (HFf) activates the classical pathway of complement in serum or platelet-poor plasma has been further delineated. When serum or platelet-poor plasma was incubated with various concentrations of HFf, the total complement hemolytic activity was reduced in a dose-dependent manner. This activation appears to be due to the direct interaction of HFf with macromolecular C1, since incubation of purified C1 with HFf resulted in dissociation of the subunits with concomitant reduction of C1r antigenicity that is indicative of C1 activation. HFf-dependent activation was prevented by prior treatment of HFf with the active site-directed inhibitor, H-D-proline-phenylalanine-arginine chloromethyl ketone or with a specific inhibitor of activated HF derived from corn. Incubation of HFf with highly purified C1r also resulted in activation of C1r as assessed directly using a synthetic substrate or indirectly by activation of C1s and consumption of C2. However, incubation of HFf with highly purified C1s resulted in formation of activated C1s ($C1\bar{s}$) but this was less efficient than HFf activation of C1r. We therefore conclude that activation of C1 in macromolecular C1 is the result of HFf conversion of C1r to $C\bar{1}r$; activation of C1s then occurs primarily by $C\bar{1}r$ and to a lesser degree by the direct action of HFf.

INTRODUCTION

The biological significance of the interaction between the proteins of the complement system and the various Hageman factor-dependent pathways leading to blood coagulation, fibrinolysis, and kinin formation has been the subject of considerable interest. Both systems are capable of generating mediators of vascular permeability that play a role in certain inflammatory processes. Hageman factor (HF, coagulation Factor XII)[1] activates plasma prekallikrein to kallikrein, which in turn cleaves higher molecular weight kininogen to produce bradykinin, a potent mediator of vascular permeability (1). Bradykinin may play a major role in the pathogenesis of hereditary angioedema (2, 3). Activation of the complement system on the other hand, leads to the production of a number of biologically active peptides, such as the anaphylatoxins, C3a (4, 5), C4a (6), and C5a (7). The interaction of both systems may thus produce mediators of inflammation and tissue damage. In previous reports (8, 9), we have shown that a fragment of activated Hageman factor, HFf, can activate the classical pathway of complement by interacting with macromolecular C1 and that this activation was both dose and time dependent. In this report we demonstrate that activation by HFf is enzymatic and does not require the participation of either prekallikrein or plasminogen. Further, we present evidence that HFf activates macromolecular C1 by cleavage and activation of C1r and that it is capable of activating C1r, and to a lesser degree, C1s, in purified systems.

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[1] *Abbreviations used in this paper:* CBZ-, benzyloxycarbonyl-; CHFI, corn Hageman factor inhibitor; CMK, H-D-Pro-Phe-Arg chloromethyl ketone; DFP, disopropyl fluorophosphate; DTNB, 5,5'-dithiobis (2-nitrobenzoic acid); GPS-E, guinea pig serum diluted 1/50 in 0.04 M EDTA-GVB; GVB, veronal-buffered saline containing 0.1% gelatin; HF, Hageman factor (coagulation Factor XII); NHS, normal human serum; NHS-E, NHS containing 0.01 M EDTA; PNA, paranitroanilide.

METHODS

Buffers. The following buffers were used: VB, isotonic veronal-buffered saline, pH 7.4; VB^{++}, VB containing 0.15 mM $CaCl_2$ and 0.5 mM $MgCl_2$; GVB^{++} containing 0.1% gelatin; GVB-E, VB containing 0.1% gelatin and 0.01 M EDTA; $SGVB^{++}$, GVB^{++} containing 2.5% (wt/vol) sucrose; Mg-EGTA, 0.1 M $MgCl_2$, 0.1 M EGTA adjusted to pH 7.0; and Mg-GVB, VB containing 0.5 mM $MgCl_2$ and 0.1% gelatin. Each buffer was prepared according to published procedures (10).

Purified proteins. Macromolecular C1 was purified according to a procedure described by Gigli et al. (11) except that the serum, containing 1 ml of 2.5 M-DFP was first made free of plasminogen by passage over a lysine-Sepharose 6B immunoadsorbent column (12) equilibrated with VB pH 7.0, containing 0.15 M NaCl, 0.02 M sodium barbital, 0.01 M $MgCl_2$, 5 mM $CaCl_2$ and 1 ml of 2.5 M diisopropylfluorophosphate. Highly purified Hageman factor (HF), activated Hageman factor (HFa), and Hageman factor fragment (HFf) were prepared as described (13). C1q (14), C1r (15), C1s (16), C2 (17), C3 (18), and C4 (19) were isolated according to published procedures. The functional activity of the isolated C1q, C1r, and C1s subunits was assessed by their ability to form a functionally active macromolecular C1. Briefly, 5 μg C1r, 5 μg C1s, and 12 μg C1q were incubated for 15 min at 30°C in the presence of 0.001 M calcium as described by Ziccardi and Cooper (20). The ability of such reconstituted C1 to form EAC14 was tested by incubating various dilutions to EAC4 in $SGVB^{++}$ (15 min, 30°C). The hemolytic activity of the generated EAC14 was then assayed by sequential addition of C2 (10 min, 39°C) and GPS-EDTA (60 min, 37°C) as described elsewhere (20).

Aggregated IgG. Human IgG that had been isolated from serum by a single step on a column of DEAE Affi-Gel Blue (Bio-Rad Laboratories, Richmond, CA) was aggregated by incubation at 63°C for 20 min at 10 mg/ml concentration. Particulate aggregates were removed by centrifugation at 200 *g* for 5 min. The supernate was used as soluble aggregated IgG.

Protein determination. The protein concentration was determined either by the method of Lowry et al. (21) or, for C1q, by measuring the optical density at 280 nM and using an extinction coefficient $E^{1\%}_{1cm}$ of 6.8 (14).

Inhibitors of activated HF. The active site-directed inhibitor of activated HF, H-D-proline-phenylalanine-arginine chloromethyl ketone (CMK), was a gift from Dr. Elliot Shaw and Dr. Charles Kettner of Brookhaven National Laboratory (Long Island, NY) and Corn Hageman factor inhibitor (CHFI) was a gift from Dr. Yoshio Hojima and Dr. John Pisano of the National Institutes of Health (Bethesda, MD).

Antisera. Monospecific antisera to C1q, C1r, and C1s, were raised in rabbits and characterized previously (9).

Immunochemical analyses. Radial double immunodiffusion (Ouchterlony) analyses were carried out in 1% agarose in barbital-buffered saline pH 8.6, containing 0.05 M NaCl and 0.01 M EDTA. Immunoelectrophoresis was carried out for 2 h at 4.7/cm at 4°C. Electrophoresis in the presence of sodium dodecyl sulfate (SDS PAGE) was performed according to the method of Weber and Osborn (22) using the modifications of Harpel and Mosesson (23).

Preparation of EA, EAC4, and EAC14. The preparation of sheep erythrocytes sensitized with rabbit antibody (EA), and complement-carrying cellular intermediates, EAC1, EAC4, and EAC14 were prepared according to established methods (24). Briefly, 5 ml of EA (1×10^9 ml) was incubated with 0.5 ml of C1 for 15 min at 37°C. The cells were washed once with warm GVB^{++} (kept at 30°C) and resuspended to original volume as EAC1. Then 1 ml of EAC1 (1×10^9/ml) was further incubated with 100 μg of isolated and active C4 for 30 min at 37°C, washed once with 0.01 M EDTA-GVB, followed by two washes with GVB^{++} and resuspended to original volume in GVB^{++}. EAC14 cells were prepared by further incubation with C1 as described above (24).

Hemolytic assays. Various concentrations of HFf were tested for their effect on serum complement by incubation with 10 μl of normal human serum (NHS) for 60 min at 37°C. Then 0.2 ml of sensitized sheep erythrocytes (EA 5 $\times 10^8$/ml) were added, the volume was adjusted to 0.4 ml with GVB^{++}, and further incubated for 60 min at 37°C. The reaction was stopped by addition of 1 ml of cold GVB^{++}, the mixture was centrifuged, and free hemoglobin was measured in the supernate at 412 nM.

Determination of CH_{50} (25) and individual complement titrations of C1 (10) and C4 (24) were performed according to previously published methods.

Assay for the activity of HFf. Assays for HFf activity were conducted with a Gilford recording spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, OH) using an automated cell changer thermostated to 37°C as described earlier (13). Semicro plastic cuvettes (Precision Cells, Inc., Hicksville, NY) were used for routine assays of HFf. The reaction mixture consisted of 0.4 ml of 50 mM Tris/HCl, pH 7.8 containing 0.117 M sodium chloride (to give I = 0.15) and 500 μM H-D-Pro-Phe-Arg-PNA (S2302, paranitroanilide; Kabi Group, Inc., Greenwich, CT).

Treatment of HFf with inhibitors. To determine whether the ability of HFf to activate the classical pathway was dependent upon the availability of its enzymatic site, HFf was first treated (60 min 37°C) with either 10 μM of the active site-directed CMK (26) or 100 μg/ml of a specific inhibitor obtained from corn (CHFI) (27) as described elsewhere. Such treated or untreated HFf was then incubated with NHS followed by incubation with 0.2 ml EA (5×10^8/ml) for 60 min at 37°C. After centrifugation, the percent hemolysis was determined spectrophotometrically.

Determination of C1r and C1s activation by HFf. Highly purified C1r (100 μg/ml) was incubated with either 10 μg/ml HFf for 60 min at 37°C in the presence of 0.001 M calcium or with $GVB^{=}$ alone in the presence of 0.01 M EDTA for 15 min at 37°C. The ability of such treated C1r to generate $C1\bar{s}$ from C1s was examined by incubating the mixture with proenzyme C1s (100 μg/ml) at 37°C for 30 min. The conversion of C1s to $C1\bar{s}$ was in turn tested either by the fluid phase destruction of C2 or C4 by activated C1s; or the conversion of C1s to the anodally faster migrating $C1\bar{s}$ on immunoelectrophoresis. In all experiments, untreated proenzyme C1r (in the presence of 0.001 M calcium) and C1s were included as controls. Alternatively, spectrophotometric assays were conducted at room temperature using a Beckman DU8 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA). The enzyme solution (5 μl) was added to 0.4 ml of assay mixture comprising 0.05 M Tris HCl, pH 7.8 containing 0.112 M NaCl, 2.5 mM $CaCl_2$, 0.2 mM CBZ-L-lysine thiobenzyl ester and 1 mM DTNB (Vega Biochemical, Tucson, AZ). The rate of change of absorbance at 412 nm was determined and converted to the value of nanomoles substrate cleaved per minute using $E_{412} = 13{,}600\ M^{-1}cm^{-1}$ as described (28). The assay was linear in response to the amount of C1s offered.

Immunochemical determination of C1r activation in serum. The ability of HFf to activate C1r in serum was tested by incubating various concentrations of HFf with NHS-E (normal human serum containing 0.01 M EDTA) at

37°C for 60 min. The amount of C1r activated was than analyzed by the single radial immunodiffusion technique (29), as modified by Ziccardi and Cooper (30). The immunodiffusion slides contained appropriate dilutions of monospecific antiserum to C1r in 1% agarose in 0.03 M potassium phosphate pH 8.0, containing 0.1 M NaCl and 0.01 M EDTA. Serum C1-INA readily binds to activated C1r ($C1\bar{r}$) but not to native C1r, and masks its antigenicity (31). The disappearance of C1r antigenicity in this assay is therefore a measure of C1r activity and the degree of activation could be quantitated as described (30).

RESULTS

Effect of HFf on total complement hemolytic activity in normal and deficient plasma. As described in our earlier work (8, 9) and confirmed in these studies, incubation of HFf with normal serum results in the sequential depletion of serum C1, C4, C2, and C3 indicative of activation of the classical pathway of complement. This depletion is reproducible, dose dependent, and is maximal after an incubation period of 60 min at 37°C (9). Fig. 1 is a representative of five such experiments in which NHS was first incubated with various concentrations of purified HFf for 60 min at 37°C, and the residual complement hemolytic activity determined as described in Methods. A concentration of 12 μg/ml HFf was sufficient to cause the reduction of complement hemolytic activity by 100%, while 2.5 μg/ml of HFf caused a 36% reduction. Table I demonstrates the results of comparative studies of the effects of HFf on NHS; citrated, (0.38%) platelet-poor, plasminogen-free plasma; prekallikrein-deficient plasma, or Factor XI-deficient plasma and is a mean of three replicate experiments. When the sample sera or plasma were incubated with a high concentration of HFf (10 μg/ml), which produces maximal reduction in complement hemolytic activity (Fig. 1), a reduction of 92–100% of their total hemolytic activity was observed and was consistent throughout these studies.

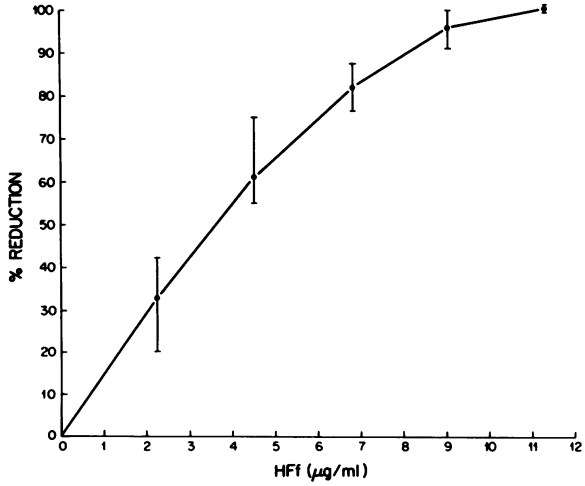

FIGURE 1 Dose-dependent consumption of complement in serum by HFf. NHS was incubated with various concentrations of HFf. The residual total complement hemolytic activity was determined as described in Methods.

TABLE I
*Comparison of Effect of HFf on Serum and Plasma**

Reaction mixture	Reduction
	%
NHS + GVB^{++}	0–
NHS + HFf	93±1.1
NHP + GVB^{++}	0–
NHP + HFf	78±1.5
Prekall. DP + GVB^{++}	0–
Prekall. DP + HFf	72±4.3
Factor XI-DP + GVB^{++}	0–
Factor XI-DP + HFf	68±1.9

* NHS, citrated, (0.38%) platelet-poor, plasminogen-free plasma (NHP) or prekallikrein-deficient platelet-poor plasma (Prekall. DP) or Factor XI-deficient plasma (Factor XI-DP) was incubated with HFf (10 μg/ml) in the presence of calcium or GVB^{++} for 60 min at 37°C. 10 μl of such treated serum or plasma was further incubated with EA. The released hemoglobin was measured in the supernate at 412 nm as described in Methods. Data represent a mean of three replicate experiments±SEM.

Abrogation of the effect of HFf on serum complement by active site-directed inhibitors. To determine whether activation of complement by HFf was an enzymatic event requiring the availability of an active site, 6 μg HFf was first incubated with either 10 μM of the active site-directed CMK or 100 μg/ml of CHFI. Then a fivefold excess (0.5 μg) of such inhibitor-treated or untreated HFf was incubated with 10 μl NHS for 60 min at 37°C. After further incubation (60 min at 37°C) with EAs the amount of hemolysis was determined and as shown in Table II, the ability of HFf to activate complement in serum was totally abrogated by both CMK and CHFI, indicating that the active site of HFf is required for such activation. Incubation of NHS with either CMK or CHFI alone on the other hand, did not have any effect on its hemolytic activity.

Quantitative analysis of C1r activation. It is known that when C1 is activated in serum, C1-inactivator (C1-INA) readily binds to active C1r ($C1\bar{r}$) thereby masking its antigenicity (30). A single radial immunodiffusion method has therefore been developed by Ziccardi and Cooper (30) exploiting this interaction. The ability of HFf to activate C1r in serum was analyzed by incubating various concentrations of HFf with serum containing 0.01 M EDTA and sub-

TABLE II

*Abrogation of Effect of HFf on Serum Complement by Active Site-directed Inhibitors**

Reaction mixture	Reduction
	%
NHS + GVB	0
NHS + HFf	96
NHS + (HFf + CMK)	0.9
NHS + (HFf + CHFI)	1.0
NHS + CMK	2.0
NHS + CHFI	0

* HFf was first inactivated by incubation with either the active site-directed (10 μM) CMK or 100 μg/ml of CHFI. Treated HFf was then incubated with NHS and its activity compared with that of untreated HFf after further incubation with 0.2 ml EA (5×10^8/ml). Percent hemolysis was determined spectrophotometrically. Data represent a mean of duplicate and reproducible results.

TABLE III

*Quantitation of Clr Activation by HFf in EDTA Serum**

Reaction mixture	Residual Clr	Activation
	μg/ml	%
NHS-E + $GVB^{=}$	50	—
Clr	134	—
NHS-E + 5 μg/ml HFf	36	28
NHS-E + 10 μg/ml HFf	21	58
NHS-E + 20 μg/ml HFf	16	68

* NHS-containing 0.01 M EDTA was incubated with $GVB^{=}$ with various concentrations of HFf at 37°C for 60 min and applied to Mancini plates containing monospecific anti-Clr as described in the legend to Fig. 2. Untreated, isolated Clr (134 μg/ml) was used as control for the specificity of the antibody. Assay was run in duplicate. Each datum is a mean.

jected to immunodiffusion analysis as described. Fig. 2 demonstrates the dose-dependent reduction in Clr precipitin ring that is indicative of Clr activation by HFf. Clr in untreated serum however remained without a change. Table III is a calculated representation of Fig. 2 and shows the residual Clr antigen (micrograms per milliliter) after incubation with HFf. Untreated Clr was used as control.

Activation of Clr and Cls by HFf. The homogeneity of representative preparations of Clq, Clr, and Cls used in these studies are shown in Fig. 3. In order to elucidate the mechanism by which HFf enzymatically initiates the activation of macromolecular Cl, highly purified Clr (100 μg/ml) was incubated with either 5 μg/ml HFf for 60 min or alone in the presence of 0.001 M EDTA for 15 min at 37°C. Then the ability of such treated Clr to generate activated Cls ($Cl\bar{s}$) from Cls was examined by incubating with 100 μg/ml proenzyme Cls at 37°C for 30 min. The conversion of Cls to $Cl\bar{s}$ was in turn tested by one of several methods as described in Methods. In all the experiments conducted, it was found that Clr was converted into enzymatically active Clr ($Cl\bar{r}$), since such treated Clr was capable of activating highly purified Cls. However, untreated Clr was not capable of converting Cls into $Cl\bar{s}$. Table IV is the result of triplicate experiments in which the fluid phase destruction of C2 by $Cl\bar{s}$ was used to detect conversion of Cls by activated Clr. In addition, a shift in electrophoretic mobility to the region of activated Clr and Cls was also observed when HFf activated-Clr or Cls, activated by

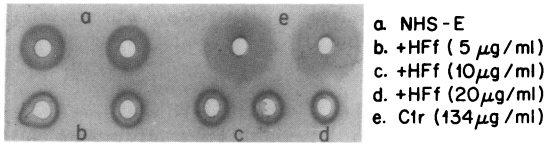

FIGURE 2 Quantitative single radial immunodiffusion analysis. NHS containing 0.01 M EDTA was incubated with or without HFf. Plates contained monospecific antiserum to Clr in 1% agarose. After staining with Amido Schwartz the sizes of precipitation rings were measured and converted to micrograms per milliliter Clr remaining. The photo depicts duplicate radial diffusions except for condition "d". The results are tabulated in Table III, and are the mean of duplicate experiments.

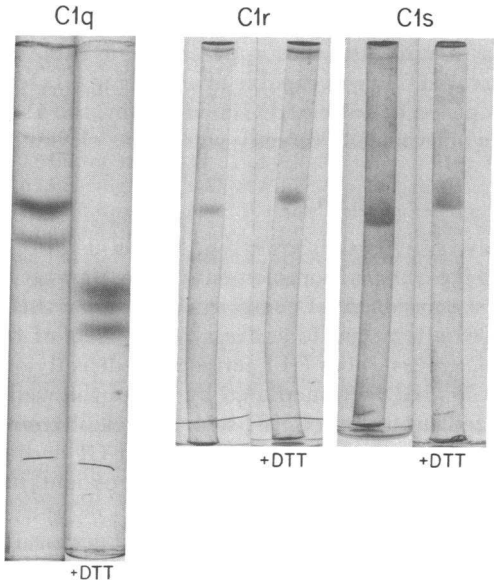

FIGURE 3 SDS-polyacrylamide gel electrophoresis of Clq, Clr, Cls (20 μg) in the absence and in the presence of 0.014 M DTT. Gels were stained with Coomassie Blue.

TABLE IV

*Demonstration of Activation of Cls by HFf and HFf-treated Clr (Clr̄)**

Reactants		Hemolysis‡
		%
C2 + buffer	+EAC14	75±1.4
C2 + Clr	+EAC14	73±2.1
C2 + Cls	+EAC14	70±1.8
C2 + [Cls + Clr]§	+EAC14	69±2.1
C2 + [Cls + Clr̄]‖	+EAC14	0–
C2 + [Cls + Clr + HFf]¶	+EAC14	5–
C2 + [Cls + HFf]	+EAC14	30±1.5

* The amount of C2 which was sufficient to produce 75% lysis in the absence of Cl̄s̄, was incubated with buffer or with Cls that had been pretreated in various ways as indicated.

‡ The values represent percent C2 hemolytic activity remaining as result of C2 consumption by Cl̄s̄.

§ Cls (100 μg/ml) incubated at 37°C for 30 min with Clr (100 μg/ml), in the presence of 0.001 M calcium, prior to addition to EAC14.

‖ Clr̄ was obtained by incubation of proenzyme Clr at 37°C for 15 min in the presence of 0.001 M EDTA.

¶ Clr, incubated first with HFf for 60 min at 37°C in the presence of Ca^{++} then with Cls for 30 min at 37°C prior to addition to C2. Each datum is a mean of duplicate experiments±SEM.

TABLE V

*Demonstration of Activation of Cls by HFf-treated-Clr Using CBZ-L-Lysine Thiobenzyl Ester as Substrate**

Reactants	Cls
	nmoles/min/μg
Cls + buffer	1.35
Cls + Clr‡	1.47
Cls + [Clr + HFf]§	6.47
Cls + trypsin‖	6.02
Cls + HFf	2.64

* Cls (100 μg/ml) was incubated with or without pretreated Clr for 30 min at 37°C.

‡ Cls (100 μg/ml) was incubated with Clr (100 μg/ml) in the presence of 0.001 M Ca^{++}.

§ Clr (100 μg/ml) preincubated with 5 μg/ml HFf 30 min at 37°C in the presence of calcium.

‖ Cls (100 μg/ml) preincubated with 1% (wt/wt) trypsin 10 min at 37°C. The reaction was stopped by addition of 2% (wt/wt) soybean trypsin inhibitor.

such a mixture (HFf + Clr), was subjected to immunoelectrophoretic analysis (not shown). These results were also confirmed by spectrophotometric means in which the ability of Clr̄ to convert proenzyme Cls into Cl̄s̄ was measured by using CBZ-L-lysine thiobenzyl ester as a substrate. It is apparent from Table V that HFf-treated Clr was capable of activating Cls fourfold as compared to untreated Clr. However, the ability of HFf to activate Cls directly was not as elevated.

DISCUSSION

We have previously shown that HFf is capable of initiating the classical complement pathway by activating the first component of complement (8, 9). In this paper we have attempted to address two aspects of this interaction. First, does HFf activate C1 directly or is C1 activation indirect, mediated by one of the well-characterized substrates of HFf such as prekallikrein (32)? Second, if the interaction of HFf and C1 is direct, is it enzymatic, and which of the subcomponents of complement are involved?

HFf is a weak coagulant enzyme because it lacks the binding site for surfaces that initiate the intrinsic coagulation pathway (33). Thus Hageman factor-dependent activation of Factor XI (34) was not considered to play a role in the activation of C1. This notion is further supported by the fact that HFa, the major Factor XI activator (35), does not cause complement activation (9). HFf, however, is a potent prekallikrein activator (36), thus it is possible that kallikrein mediates HFf-dependent C1 activation. Further, kallikrein has been shown to activate the C1r and C1s subcomponents of complement (37) so there is precedent to consider such an interaction. However, our studies with prekallikrein-deficient plasma (Table I) demonstrate that prekallikrein is not a requirement of complement activation of HFf and, in fact, C1 activation in this plasma is indistinguishable from that seen in normal plasma. Plasmin has also been shown to activate C1s and we considered the possibility that it is involved in HFf-dependent C1 activation (38). The major activators of plasminogen in Hageman factor-dependent fibrinolysis are kallikrein (32, 39) and Factor XIa (40, 41). Yet prekallikrein as well as Factor XI-deficient plasma behave normally (Table I). Furthermore, HFf is a poor Factor XI activator. Thus it is not surprising that HFf activation of C1 is also normal in plasminogen-free plasma. These data all suggest that HFf might activate C1 directly and our data support this conclusion. When HFf and purified C1 are incubated together C1 becomes activated and can digest C4 and C2. That the process is enzymatic and dependent upon the active site of HFf is indicated by the abrogation of C1 activation when Hff is incubated with either CMK or CHFI (27).

Since C4 and C2 digestion is a consequence of C1 activation by HFf, we can conclude that the C1s subcomponent has become activated (17). This could occur either by HFf activation of C1s directly or by

conversion of C1r to C1r̄ and activation of C1s by C1r̄. By single radial immunodiffusion analysis of C1r in serum C1, we demonstrated loss of C1r antigenicity after incubation with HFf indicating conversion to C1r̄ and complex formation with C1̄-INA (27, 28). We also demonstrate C1r activation upon incubation of purified C1r with HFf (Table V). However, incubation of HFf with C1s, in the absence of C1r also lead to C1s activation (Tables IV and V). It appears that HFf converts C1r to C1r̄ in macromolecular C1 and that both HFf and C1r̄ may then activate C1s. However, the interaction of HFf with C1r appears to be the predominant reaction. It is difficult to quantitate how much C1r or C1s is activated from these studies. That such treatment of C1r or C1s with HFf triggers complement activation however, is quite clear.

The activation of the classical pathway is usually triggered by the ability of C1q to recognize and bind to activating substances such as immune complexes. C1q is a collagen-like glycoprotein (42), which, together with C1r and C1s forms the C1 macromolecule (43). Since C1q is known to bind to negatively charged molecules (reviewed in 44, 45), it is possible that an interaction between HFf and C1q might facilitate activation of C1r and/or C1s. Nonetheless, our data indicate that C1q is not requisite for the reaction to proceed.

Our data may be important in disease states in which simultaneous activation of contact activation and the classical complement pathway is observed such as endotoxic shock (46) or gout (47). It may also be of particular importance in hereditary angioedema. In this disorder, there is evidence of activation of both cascades (48, 49) and the absence of C1̄-INA, the major control protein of activated Hageman factor (50, 51) might facilitate conversion of HFa to HFf and thereby perpetuate kinin formation as well as complement activation.

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