The Inhibitory Effect of Factor J on the Alternative Complement Pathway*

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Factor J (FJ) is a cationic glycoprotein with inhibitory activity in C1, the first component of the classical complement pathway. This study demonstrates that FJ is able to regulate the activity of the alternative complement pathway. FJ inhibits the generation of fluid-phase and cell-bound alternative pathway C3 convertase, C3b,Bb (C3-cleaving enzyme). Thus, FJ interferes with the generation of alternative pathway C3 convertase when sheep erythrocytes bearing antibody and activated C3 and C4 (EAC,4b,3b) are incubated with the individual complement components, factors B, D, and P. FJ accelerates the decay of C3 convertase with a time course similar to that of factor H, and when both regulators are present together, the decay of enzyme activity is faster than when they are added separately. Furthermore, FJ is able to inhibit the cleavage of C3 by factor B in a fluid-phase assay. FJ prevents the initiation of alternative pathway activation in “more stabilized systems” with well known activators of alternative pathway C3 convertase such as C3 nephritic factor (an autoantibody against alternative pathway C3 convertase), cobra venom factor, and rabbit erythrocytes. In these systems, FJ has no effect on C3 convertase stabilized by rabbit erythrocytes or cobra venom factor. In contrast, FJ promotes the dissociation of C3 convertase stabilized by C3 nephritic factor, but with much lower efficiency than in preventing initiation. Direct interaction of FJ with individual components of C3 convertase was shown by a solid-phase binding assay using plates coated with C3, C3b, B, Bb, or FJ. FJ inhibitory activity in the alternative pathway can be modulated by polymeric ions such as heparin. FJ-mediated inhibition in the alternative complement pathway can be modified by surface interactions, as occurs during alternative pathway C3 convertase activation. Thus, when FJ is adsorbed by and eluted from hydroxylapatite and reverse-phase columns, its inhibitory effect on more stabilized systems is lost. This loss of inhibitory activity is fully reversed when FJ is rechromatographed on heparin-Sepharose or Sepharose columns. Taking into account these data, FJ may be included in the group of highly charged molecules that inhibit the activation of classical and alternative complement pathways (i.e., eosinophil major basic protein, proteamine, and heparin).

The complement system regulates the clearance or lysis of foreign cells, particles, macromolecules, and tissue debris. It is composed of a series of proteins, both membrane-bound and soluble, that interact with each other when the system is activated. Activation of the alternative pathway (AP) can be both antibody-dependent and antibody-independent. Antibody-independent activation can be triggered by a whole spectrum of substances located on the surfaces of bacteria, fungi, viruses, and tumor cells (1). AP is consistently activated at a low level in the blood and makes a small amount of metastable C3b (2). Metastable C3b has a reactive intramolecular thioester and can form a covalent linkage with an acceptor group on a target. C3b bound to the target surface reacts with factors B and D and becomes C3 convertase (C3b,Bb). The C3 convertase thus formed generates multiple metastable C3b molecules. Whether this C3b is inactivated or initiates amplification of C3 convertase usually depends on the affinity of bound C3b for the regulator factor H (3, 4), although other mechanisms have been described (5). The fast spontaneous decay of C3b,Bb is accelerated by H, which displaces the Bb subunit, whereas activators like properdin (P) stabilize the enzyme by binding to it. In turn, C3b-H affinity depends on the interaction of these two proteins with neutral and anionic polysaccharides on the surface (3, 4, 6–9). Heparin interacts with factor H, and this modifies its activity (10). Many diverse effects of heparin on the classical and alternative pathways of complement activation have been reported (reviewed in Ref. 11). Recently, the interaction of heparin with various complement components has been analyzed. Of the 22 proteins examined, 13 bound heparin and 9 did not (12). Other polyionic substances have the ability to regulate complement activation. Protamine, a polycation, regulates fluid-phase and cell-bound C3 convertases as well as the activity of preformed C3 convertase (13). Eosinophil major basic protein is a highly charged polycation released by mast cell degranulation and has the ability to regulate the classical and alternative pathways of complement activation (14).

Factor J (FJ) is a novel complement regulator with inhibitory activity in the classical pathway (CP) that specifically inhibits C1 activity. Functional and/or antigenic analysis indicates that FJ is distinct from other known inhibitors of C1, namely the C1 and C1q inhibitors (15). FJ was discovered in urine and is also present in serum (16, 17). Structural studies showed that FJ is a cationic glycoprotein with a content of sugar of >28% and a pI of >9.6 (16, 18). Our main purpose was to examine the ability of FJ, like other polycations, to regulate the alternative amplification pathway of C3 convertase in cellular intermediates in the presence of physiological activators of AP such as P or with potent known activators such as C3 nephritic factor (NEF), cobra venom factor (CVF), or rabbit erythrocytes (1, 2).

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Inhibitory Effect of FJ on Alternative Complement Pathway

MATERIALS AND METHODS

Buffers

VBS is Veronal-buffered saline, pH 7.4. GVB is VBS (ionic strength of 0.147) containing 0.1% gelatin, 0.5 mM MgCl₂, and 0.15 mM CaCl₂. DGVB consists of 1 part GVB and 1 part DSW, which is 5% (w/v) aqeous dextrose containing 0.5 mM MgCl₂ and 0.15 mM CaCl₂. DGVB/Mg is DGVB containing 10 mM MgCl₂. GVB/EGTA is VBS containing 0.1% gelatin, 10 mM EGTA, and 2.4 mM MgCl₂. GVB/EDTA is GVB without added Ca²⁺ and Mg²⁺ and with the addition of a stock-buffered 0.086 mM EDTA solution to give a final EDTA concentration of 40 mM.

Complement Reagents

Factor J was purified following the original scheme (15) with slight modifications (17). Rabbit polyclonal anti-factor J antisera was obtained as described previously (17). Human C1 was isolated by euglobulin precipitation (19); guinea pig C1 (20), human C2, C3 (21), B, and D (22) were purified as described. Sheep erythrocytes, sensitized with rabbit hemolysin and bearing human C4b (EAC4) (Diamedix Corp., Miami, FL), were converted to EAC1,4b by the addition of human C1 to give a final EDTA concentration of 0.86 mM. Rabbit erythrocytes (23) were purified as described. Sheep erythrocytes, sensitized with human serum in GVB/EGTA in a total volume of 300 μl. After that, the reaction was continued for 30 min at 37 °C, the reaction was stopped by the addition of GVB/EDTA (2 ml). Tubes were centrifuged, and the absorbance at 414 nm was read. The percentage of inhibition was calculated for each concentration of FJ.

Hemolytic Assays with "More Stabilized Systems"

Inhibition of the Alternative Pathway in Rabbit Cells—One-hundred-thousand cells of rabbit erythrocytes adjusted to 5 x 10⁷/ml were incubated with several concentrations of FJ (0.05-3 μg/ml) and with 20 μl of normal human serum in GVB/EDTA for a total volume of 300 μl. After that, 300 μl of guinea pig serum (C7DS), serum from a patient without C7 (25) was incubated without P at 37 °C for 30 min, after which 300 μl of GPSi/EDTA was added, and the reaction was continued as described above. The percentage of inhibition was compared in the presence and absence of FJ.

Inhibition of C3 Convertase Stabilized by C3 Nephritic Factor—C3 convertase was measured in the presence of serum from a patient with NEF antibody as described (27). Briefly, 40 μl of normal human serum, 40 μl of NEF serum, and 10 μl of GVB/EGTA containing several final concentrations of FJ (0.037-4.8 μg/ml) were incubated with 120 μl of sheep erythrocytes at 5 x 10⁶ cells/ml for 10 min at 30 °C in the presence of GVB/EDTA. The following controls were included: (a) blank tubes containing all the reagents except NEF-positive serum, and (b) positive control tubes containing all the reagents, but not FJ. After washing the sheep erythrocytes three times with GVB/EDTA, 200 μl of 1:10 diluted rat serum in GVB/EDTA was added and incubated for 1 h at 37 °C. Next, 2 ml of GVB/EDTA was added to each tube except the 100% lysis tube, which received 2 ml of water. The tubes were shaken and centrifuged, and the absorbance was determined by measuring the absorbance of the supernatant at 414 nm. After the first incubation and washing the cells, the effect of FJ on C3 convertase stabilized by NEF was measured by adding FJ for 10 min at 30 °C, after which the cells were washed, and the reaction was completed with 200 μl of diluted rat serum.

Inhibition of C3 Convertase Stabilized by Cobra Venom Factor—C3 convertases stabilized by CVF were measured in various red cells (28, 29). Rabbit, sheep, or guinea pig erythrocytes (10⁶ cells/tube) were incubated for 20 min at 37 °C with guinea pig serum (20 μl) and CVF (900 ng for rabbit or guinea pig erythrocytes and 400 ng for sheep erythrocytes) in GVB/EGTA, and different concentrations of FJ (0.09-3 μg/ml) were added to a final volume of 215 μl. After that, 1 ml of cold VBS was added, the tubes were centrifuged, and the absorbance of the supernatant was measured.

To examine the FJ effect on preformed C3 convertase, guinea pig erythrocytes (10⁶ cells/tube) were incubated for 30 min at 37 °C with CVF and 15 μl of CDC5. After washing, cells were incubated with two concentrations of FJ that induced effective inhibitions when added simultaneously with CVF; a second incubation for 10 min in 200 μl of GVB/EGTA was performed. After that, 300 μl of 1:15 diluted GPS in GVB/EDTA was added, and the incubation was continued for 30 min at 37 °C. Two ml of cold VBS was added to stop the reaction.

Effect of FJ on the Initiation of the Alternative C3 Convertase in Rabbit Erythrocytes—C3 intermediates were labeled with fluorescein isothiocyanate (FITC) (30), and a fluorescein/protein ratio of >2 was obtained. With the aim of analyzing the effect of FJ on C3 and B deposition, the experiments were carried out as described (2). Rabbit erythrocytes (100 μl, 5 x 10⁶/ml) were incubated in GVB/EGTA for 15 min at 37 °C with 40 μl of CDC5. After 15 min, 5 μl of FITC-labeled C3 or 5.5 μl of FITC-labeled C5b-9 was added. In both cases, FJ was added simultaneously to the mixture at several concentrations (0.064-3.12 μg/ml) in a total volume of 160 μl for tubes containing FITC-labeled C3 or 175 μl for tubes containing FITC-labeled C5b-9.
FITC-labeled B. After the incubation, the cells were washed with GVB/EDTA, and the pellet was resuspended in 400 μl of buffer. These experiments were also carried out by introducing FJ 10 min later than the generation of C3 convertase in the presence of the same amounts of C1DS and FITC-labeled C3 or B. Negative fluorescence controls were included in both assays: rabbit erythrocytes were incubated with FITC-labeled C3 or B in the absence of C1DS. Fluorescence was analyzed with a FACScan instrument (Becton Dickinson, Mountain View, CA) with the acquisition of 3000 individual cells.

**Fluid-phase and Solid-phase Assays**

**Fluid-phase Proteolytic Assays of C3 in the Presence of FJ**—The time- and dose-dependent appearance of the α’ chain was followed after conversion of C3a to C3b in the presence of B (31). C3 (substrate), purified from C3 (H2O) (21), was incubated at 37 °C with B (enzyme) in VBS and in the presence of 5 mM MgCl₂. In each experiment, the enzyme/substrate molar ratio was 1:1 (5 μg of C3 and 2.5 μg of B were used). The reaction was carried out in two different ways: (a) at different times (0, 1, 2, and 5 h) and in the presence or absence of a fixed concentration of FJ (33.3 pg/ml) or (b) at the presence of different concentrations of FJ (0.11–33.3 pg/ml) for 2 h. FJ was added before B was included in the reaction mixture. To stop the reaction, 15 μl of each tube was combined with 15 μl of a 2-fold concentrated sample buffer for SDS-PAGE under reducing conditions and loaded on 9% acrylamide gels with a low percentage of cross-linker (acrylamide/bisacrylamide ratio of 1:0.006) (32). Dry gels were subjected to densitometry (Scan v1.20, Molecular Dynamics, Inc.).

**Solid-phase Ligand Binding Assays**—Wells of microtiter plates (Costar Corp.) were coated overnight with C3 (0.5 pg/ml (0.26 pmol/well), 100 pm/well), B (0.24 μg/ml (0.26 pmol/well), 100 μl/well), or purified FJ (0.15 μg/ml, 100 μl/well) at 4 °C. Back-coated with skimmed milk in phosphate-buffered saline (200 μl of a 1% solution) and incubated for 60 min at 37 °C. The plates were washed with phosphate-buffered saline/Tween 20, and FJ at several concentrations ranging from 0.001 to 5 μg/ml and either C3 or B at doses ranging from 0.04 to 28.8 pmol/well (plates coated with FJ) were added and incubated for 30 min at 37 °C. After washing, to develop the added component, primary polyclonal antibodies were added: anti-FJ, anti-C3, or anti-B. The plates were incubated for another 30 min at 37 °C, followed by washing and the addition of a specific secondary peroxidase-conjugated antibody. The plates were incubated as described above and washed, and the substrate 1,2-phenylenediamine (Merck) was used for enzymatic reaction.

**Evaluation of the Possible Inhibitory Effect of FJ on D**—Cleavage of B in the presence of C3b and D was analyzed by incubation of C3b/D in VBS with 4 mM MgCl₂ at a weight ratio of 1.05:0.0025. After incubation for 3 h at 37 °C, the reaction was stopped with EDTA at a final concentration of 10 mM. The eventual inhibition induced by FJ was analyzed by incubating the same mixture in the presence of FJ at 0.5–50 μg/ml in a total volume of 40 μl. The Bb and Bn fragments were analyzed by 10% SDS-PAGE under reducing conditions (34).

**RESULTS**

**Effect of FJ on the Generation and Decay of C3 Convertases of the Alternative Complement Pathway on EAC4b,3b Cells**—Fig. 1 shows that FJ inhibited the generation of EAC4b,3b convertase in a dose-dependent manner. As shown in Fig. 2, this effect was also observed in C3 convertase stabilized by P in the same FJ concentration range. FJ inhibition in the alternative pathway was compared with that observed in the classical pathway. Inhibitory activity in CP was tested as described (15). Both inhibitions were in the same range if FJ was previously incubated with cellular intermediates: 50% inhibition was observed at -0.1 μg/ml FJ. In contrast, the amount of FJ necessary for inducing 50% lysis was ~10 times higher in AP than in CP when FJ was included during the generation of C3 convertase (Fig. 2). Furthermore, the extent of inhibition was nearly the same whether FJ was added during or after the formation of EAC3b,Bb,P (data not shown), and FJ did not act on the terminal components when EAC4b,3b cells were used as described with EAC1,4b cells (15).

FJ also accelerates the decay of C3 convertase. When three concentrations (0.44, 1.1, and 2.2 μg/ml) of FJ were added, the

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**Fig. 1. Effect of factor J on alternative C3 convertase generation**—EAC4b,3b cellular intermediates (10⁶) were incubated with factor B (1.5 μg) and factor D (15 ng) and several concentrations of FJ in DGVB/Mg for 5 min at 30 °C and then washed immediately with cold GVB/EDTA, and the incubation was continued for 60 min at 37 °C with diluted GPS/EDTA. The incubation was stopped by the addition of 2 ml saline, mixing, and centrifugation. The released hemoglobin was determined spectrophotometrically in the supernatant at 414 nm. The percentage of inhibition was calculated as follows: 100 - (Δ% of lysis in presence of FJ/Δ% of lysis in absence of FJ) x 100. Data are expressed as the mean ± S.E. of five determinations.

**Fig. 2. Comparison of the inhibition induced by factor J in EAC1,4b and EAC4b,3b intermediates before and during convertase generation and the effect of FJ on the terminal complex.** Factor J was preincubated for 10 min at 30 °C (before) with EAC1,4b or EAC4b,3b (α) and then washed and either incubated simultaneously with the same intermediates and C2 for 10 min (during) (CP (D)) or with factors B, D, and P for 30 min (AP (C)). Finally, after washing with GVB/EDTA, cells were resuspended in 200 pl of DGVB/Mg, and 500 pl of GPS/EDTA was added, followed by incubation for 60 min at 37 °C. The effect on terminal components (in the AP assay) was analyzed by adding FJ at the same time as GPS/EDTA; as shown (■), FJ did not inhibit at any dose, and thus, the closed circles are on the horizontal axis. The percentage of inhibition was determined as described in the legend of Fig. 1.
Inhibitory Effect of FJ on Alternative Complement Pathway

resulting curves demonstrated that the C3 convertase without P was dose-dependently susceptible to decay dissociation (Fig. 3A). In addition, FJ accelerated the decay of C3 convertase stabilized by P, H and FJ similarly accelerated the decay of both enzymes, and they did not interfere with each other when added simultaneously (Fig. 3B).

Inhibition of More Stabilized Systems—When rabbit erythrocytes were used as an activator, FJ inhibited the generation of stabilized C3 convertase in a dose-dependent manner as shown in Fig. 5A. When FJ was added to preformed C3 convertase with rabbit erythrocytes, we could not observe any inhibitory effect (data not shown). Similarly, inhibition of lysis was observed when C3 convertase was stabilized by NEF serum. A dose-dependent inhibition was observed when FJ was present during or after C3 convertase formation (Fig. 5B). This effect was much less evident when FJ was added after the formation

(FJ, 2.2 μg/ml; and H, 0.022 μg/ml) was incubated with EAC3bBbP cells (10⁷/tube) in 200 μl of DGVB/Mg. The rest of the experimental procedure was similar to that described for A.
Inhibitory Effect of FJ on Alternative Complement Pathway

ERYTHROCYTES was incubated with vertase-forming reagents (sheep erythrocytes, NEF-positive serum, and was tested by simultaneously including the reaction was stopped by the addition of 2 ml of GVBEGTA. Furthermore, Fig. 6 shows a dose-dependent inhibition induced by FJ of the deposition of FITC-labeled C3 and B. The results were obtained after analysis by flow cytometry of C3b and Bb deposition in rabbit erythrocytes in the presence or absence of FJ.

Effect of FJ on the Generation of Convertase in Fluid-phase and Solid-phase Assays—Cleavage was specifically induced by B, as observed by the appearance of the α' chain from C3b; the α' chain increased progressively with time (Fig. 7, lanes 3–5). In the presence of FJ, an important reduction in the appearance of α' was observed (lanes 7–9). Bands with higher molecular mass than the α chain could correspond to degraded or aggregated C3 forms, as have been previously described in C3 preparations (21). The band with a molecular mass lower than the β chain corresponds to hemopexin, a protein that copurifies with B. In another experiment, we analyzed the inhibition of C3 cleavage induced by B in the presence of different concentrations of FJ after 2 h of incubation. The percentage of C3 cleavage in each lane was defined as (α'/(α + α')) × 100. FJ concentrations up to 33.3 pg/ml produced a maximal inhibition of 60% in a dose-dependent manner. Moreover, FJ reduced the cleavage in fluid phase of B by D in the presence of C3b (data not shown).

In the solid-phase experiments, C3, C3b, and B interacted with coated FJ on plates as shown by enzyme-linked immunosorbent assay. Dose-response curves were obtained and are plotted in Fig. 8. This same interaction was evidenced when C3, C3b, B, and Bb were immobilized on the plates and incubated with different amounts of FJ (data not shown). Modulation of the Inhibitory Activity by Surfaces Such as Hydroxylapatite and Silica Matrices—During the procedure of FJ purification, FJ lost inhibitory activity in more stabilized systems (rabbit erythrocytes, CVF, or NEF) after passage through an HPHT hydroxylapatite column (7.8 × 100 mm; Bio-Rad). The same phenomenon was observed after loading onto and elution from a µBondapak™ C18 column (5.9 × 300 mm; Millipore Corp., Waters Chromatography, Milford, MA). In contrast, FJ inhibition in CP and in the generation of EAC3b,Bb,NEF convertases was invariable after these interactions. In addition, FJ-mediated inhibition in more stabilized systems was recovered when loaded onto and eluted from heparin-Sepharose or Sepharose columns.

DISCUSSION

This study suggests that factor J may play a role in vivo in regulating complement activation by either the classical and/or the alternative amplification pathway. Experimental data support the view that FJ acts with the same efficiency in C3 convertases of the classical and alternative complement pathways if FJ is preincubated with cellular intermediates. This fact may indicate an effect on the complement components bound to cells. However, if FJ is added simultaneously with C3 convertase-forming reagents (sheep erythrocytes, NEF-positive serum, and normal human serum) and incubating the mixture for 10 min at 30 °C. The effect of FJ was also tested on preformed sheep erythrocytes/C3b,Bb,NEF (AFTER). Sheep erythrocytes/C3b,Bb,NEF cells were washed with GVB/EDTA, followed by the addition of FJ and incubation for 10 min more at 30 °C. Then, rat serum diluted in EDTA was added to all the tubes, and the incubation was continued for an additional 60 min at 37 °C. FJ effect on C3 convertase stabilized by cobra venom factor. Guinea pig erythrocytes (107/tube) were incubated for 20 min at 37 °C with guinea pig serum (20 μl), 900 ng of CVF, and different concentrations of FJ in GVB/EDTA. The reaction was stopped by the addition of 1 ml of cold VBS. In all cases, the percentage of inhibition was calculated as described in the legend of Fig. 1.
Inhibitory Effect of FJ on Alternative Complement Pathway

**FITC-C3 DEPOSITION**

| NEGATIVE CONTROL | POSITIVE CONTROL |
|------------------|------------------|
| 3.12 μg/ml       | 1.04 μg/ml       |
| 0.35 μg/ml       | 0.11 μg/ml       |
| 0.04 μg/ml       | 0.015 μg/ml      |

**FITC-B DEPOSITION**

| NEGATIVE CONTROL | POSITIVE CONTROL |
|------------------|------------------|
| 2.9 μg/ml        | 0.6 μg/ml        |
| 0.11 μg/ml       | 0.02 μg/ml       |
| 0.004 μg/ml      |                  |

**Fig. 6.** Deposition of FITC-labeled C3 and B in rabbit erythrocytes in the presence of FJ. FITC-labeled C3 was incubated with adsorbed C7-deficient serum and rabbit erythrocytes in GVB/EGTA for 15 min at 37 °C. Different concentrations of FJ were simultaneously introduced with the reagents. After the incubation, cold GVB/EDTA was added to each tube and washed. Each pellet was resuspended, and the tubes were analyzed by flow cytometry. FITC-labeled B deposition was analyzed in the same way. Negative fluorescence controls were included by incubation of FITC-labeled C3 or B in the absence of C7-deficient serum. Positive fluorescence controls correspond to tubes without FJ. The figure represents the obtained histograms with the acquisition of 3000 individual cells.

**Fig. 7.** C3 cleavage in fluid phase in the presence of FJ and time course of C3 cleavage in the presence of FJ after SDS-PAGE on 8% slab gel. C3b was generated by incubation of C3 (5 μg) with B (2.5 μg) in VBS containing 5 mM MgCl₂ at 37 °C and subjected to SDS-PAGE under reducing conditions. The gel was finally stained with Coomassie Brilliant Blue R-250. Lane 1 contained molecular mass markers (myosin, 200 kDa; and phosphorylase b, 92.5 kDa). Lanes 2-9 contained a C3 and B mixture with different incubation times: lanes 2 and 6, 0 h; lanes 3 and 7, 1 h; lane 4 and 8, 2 h; lane 5 and 9, 5 h. Lanes 6-9 contained 33.3 μg/ml FJ. FJ appeared as precipitated material in lanes 6-9.

The ability of FJ to inhibit CP and AP could be related to the high PI of this protein. Other cationic proteins with regulatory effects in CP and AP have been described: eosinophil granule major basic protein (14) and myelin basic protein (35, 36). C1q (PI 9.3) has been related to the regulation of AP by direct interaction with C3b (37). Nevertheless, this property is not associated with all the cationic molecules, e.g. cytochrome c (PI 9.6; 5 μg) does not inhibit complement activation (data not shown).

Some similarities between H and FJ actions can be established. For instance, FJ accelerates the decay of C3 convertase in a similar time course as H, and the effects of both inhibitors do not interfere with each other. Furthermore, FJ acts in more...
stabilized C3 convertases" stabilized by NEF, CVF, or rabbit erythrocytes with effects analogous to those of H. In these systems, we have found some differences if FJ is added during or after the formation of C3 convertase. Thus, FJ prevents the generation, but is not able to dissociate the C3 convertases stabilized by CVF or rabbit erythrocytes. Moreover, FJ hinders C3 deposition in rabbit erythrocytes. B deposition is also affected due to the absence of C3 deposition (flow cytometry experiments). In addition, C3 convertase stabilized by CVF is completely resistant to H and I inactivation (38, 39). Rabbit erythrocytes have previously been shown to interfere with the effectiveness of the control by I and H, allowing unrestricted formation of C3 convertase (40). H is less efficient in NEF-stabilized convertase (41, 42), as is also true for FJ.

Enzyme-linked immunosorbent assay and fluid-phase experiments suggest that the observed FJ inhibition in hemolytic assays is not due to a nonspecific effect on the cellular intermediates, but rather to an effect on the specifically bound complement components. The possibility that FJ acts as an inhibitor of D could not be ruled out with our assay because D cleaves B only when B is in a Mg2+-dependent association with C3b (43). According to our data, FJ interacts with the components of C3 convertase and prevents the generation of the C3b,Bb complex.

FJ is able to neutralize the inhibitory activity of heparin in AP. This effect was observed with other regulators such as eosinophil major basic protein, and charge neutralization was suggested to explain this effect (14). Many charged molecules are present in tissues, including heparin, protamine sulfate, major basic protein, and the chondroitin sulfates. These highly charged molecules are found in areas where complement activation occurs. Therefore, complement activation may play an important role in vivo in the immune and inflammatory response. The FJ concentrations used in most of these experiments are similar to those estimated in normal human serum (5.8 ± 2.8 pg/ml) (17), suggesting that FJ may play a role in the regulation of AP in vivo. We have observed that FJ is present in many cells (data not shown). FJ, heparin, and other regulators probably act in conjunction. Furthermore, the interactions between these charged molecules may modulate their mutual functional activities. The balance between activation and inhibition of complement could be finely controlled by the relative concentrations of the polycations and polyanions.

FJ loses its inhibitory activity in more stabilized systems after chromatography on hydroxylapatite and C18 matrices. This fact could be connected to the interaction with certain surfaces. Since the inhibitory activity reappears after interaction with heparin or agarose, the effect is reversible. In contrast, we did not observe any changes in FJ inhibition in CP. The mechanisms for modulation of FJ inhibitory activity by surface interactions are not clear. In this context, the activation of the alternative pathway by an ordered surface configuration of repeating polysaccharides, e.g. dextran or other polymeric units, appears to be important. There is evidence to suggest that hydroxylapatite crystals in mineralized bone will bind the third component of the complement system (C3) on exposure to plasma (44). Recently, it has been suggested that C3 deposition in mineralized bone surfaces mediates the recruitment of mononuclear osteoclasts to the site of deposition (45).

The consequences of the modulation of FJ inhibitory activity by surface interactions could indicate that FJ might regulate C3 deposition under certain circumstances, e.g. the nature of the surface binding of C3b is crucial as to whether or not the particle will be an AP activator. In the presence of B and H (e.g. in plasma), both factors compete for bound C3b (46). The outcome of the competition is determined by the surface (47). If the C3b is protected from H, then preference is given to B, so that the convertase complex C3b,Bb is formed, and the particle becomes an AP activator capable of producing more active C3b cleavage products. If C3b is accessible to H, then the complex C3b,H allows cleavage of C3b to iC3b by factor I, and the particle is inactivated (48). H binds less strongly to C3b immobilized on particles that are AP activators (49). These observations together indicate that a comparable mechanism for FJ inhibition in AP takes place.

In conclusion, FJ is able to inhibit CP and AP. Regarding AP, FJ affects C3 convertase by interacting with C3 and B while it interferes with the generation and dissociation of this enzyme. This inhibition is evident in the presence of known activators of AP C3 convertase and can be modulated by some polyanions, such as heparin, and surfaces, such as hydroxylapatite and hydrophobic matrices.

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Inhibitory Effect of FJ on Alternative Complement Pathway

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