Modulation of the Human Monocyte Binding Site for Monomeric Immunoglobulin G by Activated Hageman Factor

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Abstract

Macrophage Fcγ receptors play a significant role in inflammation and host defense. One monocyte/macrophage Fcγ receptor, FcγRI, the binding site for monomeric IgG, appears to be especially responsive to modulatory signals by hormones and mediators. Since Factor XIIa is generated during inflammation, we studied the effect of XIIa on FcγRI. Factor XIIa, in a concentration-dependent manner (0.01–0.19 μM), reduced the number of monocyte binding sites for monomeric IgG up to 80% without altering the affinity of binding. Its precursor, Factor XII, and the low molecular weight fragment of XIIa, lacking most of the heavy chain region, did not reduce the expression of FcγRI. Neither corn trypsin inhibitor (36 μM) nor diisopropylfluorophosphate (3.6 mM) diminished the effect of Factor XIIa on FcγRI, although each completely inhibited the coagulant and amidolytic activity contained on the light chain of Factor XIIa. Protein synthesis was not a requirement for this effect of Factor XIIa, nor was internalization of FcγRI necessary. In contrast to similar concentrations of IgG, Factor XIIa failed to displace significantly monomeric IgG from the monocyte surface, suggesting that Factor XIIa does not directly compete for FcγRI. The data suggest that the heavy chain of XIIa, which contains domains that may have cell hormone activity, also contains a domain that regulates FcγRI on monocytes. In addition to other hormones and mediators, Factor XIIa may serve a regulatory function in modulating Fcγ receptor expression during inflammation.

Introduction

Macrophages are an important component of the inflammatory response and their Fcγ receptors are significant in their function. Factor XII (Hageman factor) is an 80-kD proenzyme present in blood, that upon activation is responsible for initiating the intrinsic pathway of blood coagulation and is implicated in the pathophysiology of inflammation, kinin formation, complement activation, and fibrinolysis (1). Although Factor XII has been shown to activate granulocytes (2), an interaction between Factor XII and macrophages is unknown.

The primary amino acid and cDNA sequences of Factor XII have been reported and have revealed structural domains that imply physiologic functions (3–5). Proteolysis of precursor, unactivated Factor XII by its physiological activator, kallikrein, results in a cleaved form of the protein, Factor XIIa,[1] composed of two chains linked by a single disulfide bond. Factor XIIa exhibits both coagulant activity and amidolytic activity towards the synthetic substrate H-D-Pro-Phe-Arg-*p*-nitroanilide (NA). The light chain of Factor XIIa (28 kD) contains the "catalytic triad" of the amino acids serine, aspartic acid, and histidine, and is inactivated by diisopropylfluorophosphate (DFP). The heavy chain (50 kD) contains two domains homologous with epidermal growth factor, suggesting that Factor XII acts as a cell hormone. The heavy chain has been demonstrated to contain a domain that is homologous to the fibronectin, fibrin, and heparin binding region and which may function as the surface binding domain (6). A fragment of activated Factor XII (Factor XIIf), a further cleavage product of 30 kD, is composed of the light chain region of 28 kD containing the catalytic site, bonded through disulfide bridges to a 2-kD carboxyl portion of the heavy chain. Factor XIIf lacks the surface binding and putative hormonal regions of the heavy chain, but contains the catalytic triad. Factor XIIf has little coagulant activity, but has similar amidolytic activity. Factor XIIa, but not Factors XII or XIIf, has been shown to stimulate neutrophil aggregation and release of elastase (2). We, therefore, examined the effect of Factors XII, XIIa, and XIIf on another critical phagocytic cell function, expression of Fcγ receptors by blood monocytes.

Methods

Silicone oils were purchased from William F. Nye, Inc. (Fairhaven, MA), H-D-Pro-Phe-Arg-*p*NA (S-2302) from Helena Laboratories (Beaumont, TX), and DFP, cycloheximide, and chloroquine from Sigma Chemical Co. (St. Louis, MO). Polystyrene tissue culture flasks were obtained from Corning Glass Works (Corning, NY). RPMI 1640 medium, HBSS, and Dulbecco's PBS were obtained from Gibco Laboratories (Grand Island, NY). RPMI complete medium consisted of RPMI 1640 medium containing 5% heat inactivated FCS, penicillin (100 U/ml), streptomycin (100 μg/ml), gentamicin (20 μg/ml), and 2 mM L-glutamine. EDTA buffer consisted of Dulbecco's PBS containing 0.1% gelatin, 0.01 M EDTA, and 0.1% sodium azide.

Hageman factor (Factor XII) was purified as previously described using zinc chelate Sepharose and gel filtration chromatography (7) or monoclonal antibody immunoaffinity column chromatography (6). Factor XII was determined to be homogeneous with M_r 80 kD using SDS PAGE and had specific activities of 60–90 U/mg (6, 7), where 1 U is the amount of Factor XII present in 1 ml of a pool of plasma from normal individuals. Factor XII was converted to Factor XIIa by purified plasma kallikrein and the enzymes were separated by ion exchange

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1. *Abbreviations used in this paper:* DFP, diisopropylfluorophosphate; Factor XIIa, the cleaved, activated form of Factor XII; Factor XIIf, fragment of activated Factor XII; NA, nitroanilide.

chromatography as previously described (8). Factor XIIa concentrations of these fractions were determined from the difference in the value of coagulant activity and the amidolytic activity of the enzyme against the peptide substrate, H-D-Pro-Phe-Arg-*p*NA (S-2302), and confirmed by scanning on SDS PAGE. Coagulant activity was measured by a modification of the activated partial thromboplastin time (9), using Factor XII–deficient plasma. The amidolytic assay was performed using S-2302 (10) with modifications (8). Most Factor XIIa fractions used in these studies contained some zymogen Factor XII and Factor XIIf. However, the amount of Factor XIIa in all preparations used was at least 50% and was determined for each preparation used. Factor XIIf was purified to homogeneity (30 kD) by incubating the activation mixtures with 2 mg/ml kaolin in 0.01 M Tris, pH 8.0, and removing the kaolin by sedimentation. Kaolin under these conditions binds Factor XII and Factor XIIa, but not Factor XIIf, leaving Factor XIIf in homogeneous form in the supernatant. Factor XIIf activity was determined by S-2302 amidolytic activity, since it has virtually no coagulant activity.

Corn trypsin inhibitor, a specific reversible inhibitor of Factor XIIa and Factor XIIf, first described by Hojima et al. (11), was a generous gift from Dr. E. Kirby of Temple University (Philadelphia, PA), and was purified from corn as described (12).

The catalytic activity of Factor XIIa was inhibited by pretreating Factor XIIa just before the experiment with either corn trypsin inhibitor or DFP. DFP was added to Factor XIIa for a final DFP concentration of 3.6 mM and incubated 30 min at 23°C. Factor XIIa activity was then examined for amidolytic activity using S-2302 and no hydrolysis was detected.

The Factor XIIa effect was also examined in the presence of serum, using FCS which contains low amounts of IgG. Monocytes were preincubated with Factor XIIa in the presence or absence of either 10 or 50% FCS before the enumeration of monocyte IgG binding sites. FCS was obtained from Gibco Laboratories and heated at 56°C for 30 min before use.

Preparation of monocytes. We used a method to isolate monocytes in suspension based upon their adherence to serum-treated flasks and their ability to be dislodged in the cold by indirect mechanical means (13, 14). Whole blood anticoagulated with sodium heparin was obtained from normal donors and the mononuclear cells isolated by centrifugation on a Ficoll-Hypaque gradient (13). The mononuclear cells were washed once and suspended in RPMI complete medium. The mononuclear cell suspension of 25 ml (200×10^6 cells) was then added to 150 mm^2 polystyrene tissue culture flasks that had been pretreated with heat-inactivated FCS at 37°C with 5% CO_2 for 30 min. The flasks were gently agitated and the nonadherent cells removed. The flasks were then washed five to seven times with HBSS to remove residual nonadherent cells. RPMI complete medium (25 ml, 4°C) was pipetted into each flask and the adhering cells were harvested by indirect mechanical means by firmly rapping each of the two sides of the flasks against a cushioned surface three to six times. This procedure dislodged 90–95% of the adherent cells. The flasks were washed with 4°C RPMI complete medium and the cell suspensions were pooled and centrifuged at 250 *g* for 10 min, resuspended, and washed in EDTA buffer to remove residual serum or IgG. These cells were > 95% viable as assessed with trypan blue and were suspended in 10 ml of RPMI complete medium. The suspension of adherent cells morphologically appeared to be monocytes, were able to phagocytose latex particles (> 85%), stained positively with nonspecific esterase (> 85%), were able to bind IgG or C3 coated erythrocytes (90%), and reacted with the monoclonal antibody OKMI (> 90%) (Ortho Diagnostic Systems, Inc., Westwood, MA). Only 10–13% of blood mononuclear cells reacted with OKMI before adherence to the flasks.

^{125}I-IgG binding to monocytes. Human IgG was isolated from normal human serum or plasma by affinity chromatography, using anti-human IgG coupled to Sepharose and acid elution, as previously described (13, 14). The IgG fractions were pooled, and the pH was adjusted to 7.4 with 1 M NaOH. The preparation was dialyzed with PBS containing 0.02% sodium azide for 16 h and centrifuged at 105,000 *g* for 20 min. The supernatant containing soluble IgG was stored at 4°C and the protein concentration determined (15) with BSA as the standard. In selected experiments, human IgG was prepared by ammonium sulfate precipitation and gel filtration chromatography in order to obtain large amounts of IgG (13). IgG fractionated as IgG monomer when analyzed by Sephadex G-200 chromatography and was found to contain only IgG when assessed by double immunodiffusion, immunoelectrophoresis, and polyacylamide disk-gel electrophoresis (13). The isolated IgG was labeled with ^{125}I (New England Nuclear, Boston, MA) using chloramine-T followed by Sephadex G-50 chromatography as previously described (13, 14) and stored at 4°C until use. Specific activity of the final preparation was 0.16 μCi/μg of protein. Immediately before each assay, the IgG was centrifuged in an airfuge (Beckman Instruments, Inc., Palo Alto, CA) at 100,000 *g* for 15 min.

Monocytes were exposed to Factor XII, Factor XIIf, Factor XIIa, and inactivated forms of Factor XIIa in EDTA buffer for at least 30 min at 37°C before determination of IgG binding.

Equilibrium binding of ^{125}I-IgG monomer to monocytes was performed as previously published (13, 14). Enzyme-treated or control monocytes (1×10^6) were incubated with one concentration or increasing concentrations of ^{125}I-IgG in EDTA buffer (total volume 0.5 ml) at 37°C for 30 min to reach equilibrium conditions. ^{125}I-IgG input per experimental tube in the figures refers to ^{125}I-IgG per 0.5 ml of total volume. The amount of cell-associated and unbound ^{125}I-IgG was determined by rapidly sedimenting the cells through a layer (0.5 ml) of silicone oil mixture (25 ml methyl silicone + 80 ml biphenyl silicone), and the radioactivity in the cellular pellets was quantitated in a gamma counter. Nonspecific binding was defined as the cell-associated radioactivity in the presence of 100-fold excess of unlabeled IgG. Unlabeled IgG at this concentration displaced > 70% of the total cell-associated counts. Specific binding was analyzed by Scatchard plots as previously described (13, 14). The number of IgG binding sites and the affinity of IgG binding to monocytes were determined with the use of a linear regression program using the least squares method.

Fluorescence flow cytometry. Monocytes (1×10^6) were incubated 30 min at 4°C with saturating concentrations of monoclonal antibodies to the C3b receptors CR1 and CR3. We employed the anti-CR1 antibody CR1 (Becton Dickinson, Rutherford, NJ) which inhibits the binding of C3b, and the anti–CR3 antibody OKMI (Ortho Pharmaceutical, Raritan, NJ) directed at the receptor for iC3b. We also used monoclonal antibody 32.2 (generously supplied by Dr. Clark Anderson, Ohio State University, Columbus, OH, and Dr. Michael Fanger, Dartmouth University, Hanover, NH), which is directed at an epitope on FcγRI, but does not inhibit IgG ligand binding to FcγRI. The cells were washed, resuspended, and incubated at 4°C with FITC–conjugated goat anti–mouse IgG (Tago, Inc., Burlingame, CA). FACS analysis was performed using the FACStar (Becton Dickinson). Mean fluorescence intensity/monocyte was expressed in arbitrary units (16).

Results

Human monocytes were reported to express 26,200±5,900 (SD) binding sites for monomeric IgG at saturation with an affinity constant of $K_a = 4.4 \pm 1.7 \times 10^8\ M^{-1}$ (14). We observed that Factor XIIa influenced the binding of IgG to monocytes (Fig. 1). Varying concentrations of Factor XIIa were incubated with 1×10^6 monocytes for 30 min at 37°C in a total volume of 0.5 ml and the monocytes washed before assessing the number of binding sites for monomeric IgG. The effect of Factor XIIa was concentration dependent within a range of 0.01 to 0.19 μM. The concentration of Factor XIIa that reduced the number of monocyte binding sites for monomeric IgG by ~ 50% was 0.09 μM. In plasma this value represents a 24% conversion of Factor XII to XIIa based on a plasma concentration of Factor XII of 0.37 μM (29 μg/ml). We also examined the effect of Factor XIIf, the 30-kD fragment derived from

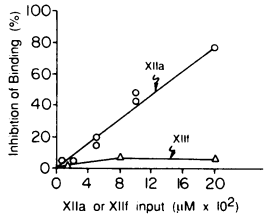

Figure 1. Concentration-dependent effect of Factor XIIa and Factor XIIf on the number of monocyte binding sites for monomeric IgG. Factor XIIa (○) or Factor XIIf (△) were added to 1 × 10^6 monocytes in a total volume of 0.5 ml to obtain the final enzyme concentrations indicated. The enzyme and cells were incubated 30 min at 37°C. The number of monocyte binding sites for monomeric IgG at each enzyme concentration was determined with a single concentration of ^{125}I-IgG (0.6 μg/ml) as described in Methods. The percent inhibition of binding was calculated in comparison with the no enzyme buffer control.

XIIa, on the Fcγ receptor–dependent binding of IgG to monocytes. As indicated in Fig. 1, Factor XIIf did not affect the binding of monomeric IgG to monocytes at concentrations between 0.01 and 0.19 μM.

When Factor XIIa (80 kD) was incubated at a final concentration of 0.09 μM with 1 × 10^6 monocytes at 37°C for 30 min, Scatchard plots of the data indicated that the number of monocyte binding sites expressed was reduced by ~ 50% (Fig. 2, *A* and *B*); however, there was no effect of Factor XIIa on the affinity of IgG binding. This effect persisted after washing the monocytes following their incubation with Factor XIIa (Fig. 2, *C* and *D*). Furthermore, Factor XIIa did not alter monocyte viability or the ability to ingest latex particles, since after incubation with XIIa > 95% of the monocytes were able to exclude trypan blue and ingest latex particles.

The presence of FCS did not alter the Factor XIIa effect. Factor XIIa (0.09 μM) diminished the number of monocyte binding sites by 62 and 50% in the presence of 10 and 50% FCS, respectively, similar to the results observed in the absence of serum.

We studied the effect of Factor XIIa on the monocyte C3 receptors CR1 and CR3, the receptors for C3b and iC3b, respectively, using two monoclonal antibodies. Factor XIIa did not diminish CR1 or CR3 expression as assessed by flow cytometry with these monoclonal antibodies. Monocytes preincubated with Factor XIIa expressed a mean fluorescence intensity of 28±5 and 32±15 (n = 5) with anti-CR1 and anti-CR3, respectively, compared with a mean fluorescence intensity of 28±6 and 31±12 in the absence of Factor XIIa.

We further examined the effect of Factors XIIf and XII on the number of IgG binding sites expressed. We incubated 0.09 μM of Factor XIIf or XII with monocytes at 37°C for 30 min and then determined the equilibrium binding of monomeric IgG over a wide concentration of IgG from 0.05 μg/ml to 2.0 μg/ml (Table I). In contrast to Factor XIIa, which inhibited the specific binding of IgG to monocytes by 46% in this experiment, XIIf did not affect the number of binding sites expressed for monomeric IgG. To determine whether activation of Factor XII is necessary to allow Factor XIIa to alter the expression of monocyte binding sites for monomeric IgG, we studied the effects of zymogen (precursor) Factor XII (Table I). We observed that Factor XII (0.09 μM) failed to alter the number and affinity of binding sites for monomeric IgG on monocytes, while in a parallel experiment, Factor XIIa inhibited the specific binding of monomeric IgG by 34% (Table I).

The proteolytic and amidolytic activity of Factor XIIa is inhibited specifically by corn trypsin inhibitor in a tight binding reversible interaction (11). To examine whether catalytically active Factor XIIa is responsible for this effect on the monocyte binding sites for IgG, Factor XIIa was preincubated with corn trypsin inhibitor. Corn trypsin inhibitor completely inhibited the coagulant activity and the amidolytic activity of Factor XIIa (data not shown). There was no change in the ability of corn trypsin inhibitor inactivated Factor XIIa to reduce the expression of monocyte IgG binding sites (Table II).

Corn trypsin inhibitor is a polypeptide of M_r = 14 kD (11) and a reversible inhibitor. Thus, its failure to alter the effect of Factor XIIa could be due to steric interference or dissociation of Factor XIIa from the enzyme-inhibitor complex. Therefore, we used as an inhibitor DFP, which is a small (184 D) irreversible alkylator of the active site serine in Factor XIIa. Similar to corn trypsin inhibitor, DFP also failed to diminish the ability of Factor XIIa to decrease the number of monocyte binding sites for monomeric IgG (Table II).

We studied whether protein synthesis is required for the

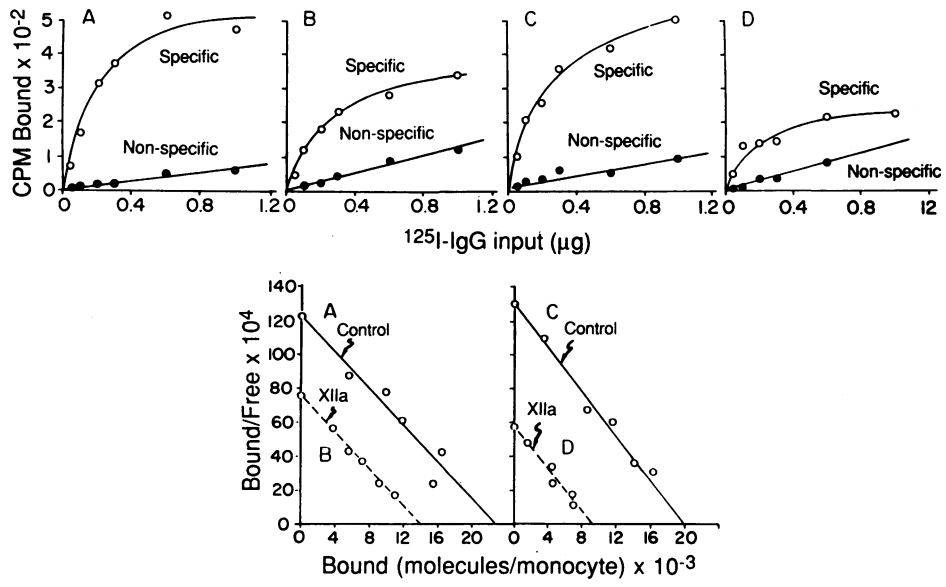

Figure 2. Effect of Factor XIIa on the number of monocyte IgG binding sites. Equilibrium binding of IgG monomer to monocytes was performed with monocytes preincubated with either 0.09 μM Factor XIIa (*B, D*) or buffer (*A, C*). In *C* and *D* the monocytes were washed after this preincubation. ^{125}I-IgG input (micrograms) refers to the amount of the IgG per 0.5 ml of total volume. Buffer control (*A*) = 22,700 IgG binding sites/cell, K_a = 2.8 × 10^8 M^{-1}; Factor XIIa (*B*) = 13,800 IgG binding sites/cell, K_a = 2.8 × 10^8 M^{-1}; buffer control (*C*) = 20,100 IgG binding sites/cell, K_a = 3.4 × 10^8 M^{-1}; Factor XIIa (*D*) = 9,200 IgG binding sites/cell, K_a = 3.2 × 10^8 M^{-1}.

Table I. Effect of Factor XII and Activated Factor XII on the Monocyte Binding Site for Monomeric IgG

Reagent	IgG binding sites/cell	K_a
		$\times 10^8\ M^{-1}$
Experiment 1		
Buffer	23,500	3.3
XII	26,600	3.1
XIIa	15,510	3.1
Experiment 2		
Buffer	34,500	3.1
XIIf	33,100	4.2
XIIa	18,630	3.6
Experiment 3		
Buffer	31,900	2.8
XIIa + chloroquine	18,000	3.1
XIIa	19,000	2.7

The number of monocyte IgG binding sites/cell and the affinity constant are shown for three experiments in which monocytes from three different donors were preincubated with either Factor XIIa or buffer and either Factor XII, Factor XIIf, or Factor XIIa + chloroquine.

effect of Factor XIIa (Fig. 3). Monocytes (1×10^6) were preincubated with cycloheximide (10 μg/ml) for 40 min at 37°C in a total volume of 0.5 ml before the addition of either 0.09 μM XIIa or buffer for an additional 30 min at 37°C. The monocytes were sedimented and resuspended in EDTA buffer (0.5 ml) containing 10 μg/ml of cycloheximide for the enumeration of Fcγ receptors. As noted in Fig. 3, Factor XIIa decreased the number of IgG binding sites on monocytes preincubated with cycloheximide or buffer equally.

Receptor-ligand interactions have been shown to possess specific mechanisms for the uptake and proteolytic degradation of the bound ligand within lysosomes (17). Since chloroquine has been shown to inhibit this process involving lysosomal proteases (17), we studied the effect of chloroquine on the action of XIIa. Monocytes were preincubated with chloroquine (10 μM) for 45 min at 37°C before assessing the effect of Factor XIIa in the presence of chloroquine. As noted in Table I, chloroquine did not impair the action of XIIa on FcγRI.

One possible explanation for the decrease in IgG binding by Factor XIIa is competition for the monocyte Fcγ binding site by Factor XIIa. We therefore compared the abilities of excess unlabeled IgG and excess Factor XIIa to displace radiolabeled IgG from the monocyte surface after equilibrium of binding was achieved. Unlabeled IgG displaced 100% of the specifically bound radiolabeled IgG within 85 min. At the same time, Factor XIIa only displaced 15% of the bound IgG, a result similar to that observed with the buffer control (Fig. 4). We also assessed whether the Fcγ receptor binding site remains decreased after removing Factor XIIa from the monocyte milieu. Monocytes were incubated with XIIa (0.09 μM) for 30 min, washed, and held at 37°C for up to 2 h in the EDTA assay buffer. During this time period there was no significant reversal of the XIIa effect (Table III).

We also examined whether Factor XIIa inhibits the reactivity with monoclonal antibody 32.2, the one available monoclonal antibody with specific reactivity for FcγRI (18). This antibody binds to an epitope of FcγRI that is distinct from the IgG ligand binding site (18). Concentrations of Factor XIIa (0.09 μM) that inhibited the number of monomeric IgG binding sites by > 50% did not alter monocyte binding of monoclonal antibody 32.2. The mean fluorescence intensity for monocytes preincubated with Factor XIIa was 25±4 (n = 4) compared with 26±2 for the buffer controls. Furthermore, > 90% of the monocytes reacted with 32.2 both before and after incubation with Factor XIIa.

Table II. Effect of DFP and Corn Trypsin Inhibitor on the Ability of Factor XIIa to Affect Monocyte Fcγ Receptor Expression

Reagent	IgG binding sites/Monocyte	K_a
		$\times 10^8\ M^{-1}$
Buffer control	14,500	3.2
Factor XIIa	4,900	1.6
Factor XIIa + DFP	6,500	2.4
Factor XIIa + corn trypsin inhibitor	6,000	3.1
Buffer + DFP	14,500	2.9

Factor XIIa (1.8 μM) was preincubated with and without DFP (3.6 mM). The equilibrium binding of IgG to blood monocytes was then determined. In a separate reaction, Factor XIIa (0.74 μM) was preincubated with corn trypsin inhibitor (43 μM) for 10 min at 37°C. Aliquots of the treated Factor XIIa were added to 1×10^6 monocytes in a total volume of 0.5 ml to obtain a final enzyme concentration of 0.18 μM.

Discussion

Macrophages are a major component of host defense systems and inflammation. One significant aspect of their function is the clearance of IgG-coated cells and IgG-containing immune complexes. The macrophage surface receptors that recognize the Fcγ fragment of IgG are an important component of this process. Present evidence indicates that fresh human monocytes have two Fcγ receptors (18). FcγRI binds monomeric IgG (13, 18) and cross-links cell bound IgG, such as IgG anti-D antibody on human erythrocytes (19). FcγRII recognizes aggregated IgG and is the only Fcγ receptor involved in the binding of aggregated IgG to human platelets (18, King, M., P. McDermott, and A. D. Schreiber, manuscript submitted for publication). Monocytes cultured in vitro for several days and tissue macrophages express FcγRI, FcγRII, and an additional Fcγ binding protein, FcγRIII (CD16), which binds aggregated, but not monomeric, IgG (20). Both FcγRI and FcγRII expression can be regulated in vitro (21). The data suggest, though, that FcγRI, the Fcγ receptor for monomeric IgG, is the Fcγ receptor on monocytes most responsive to modulating signals, including steroid hormones and γ-IFN (13, 14, 19, 22). Evidence exists that modulation of this receptor may correlate with the expression of immunologic disease manifested by the clearance of IgG-coated cells (14).

During the inflammatory response, the Hageman factor–dependent pathways are frequently activated and Factor XIIa is generated (1). We examined the effect of Factor XIIa on FcγRI. We observed that Factor XIIa reduces the number of monocyte binding sites for monomeric IgG by as much as 80%

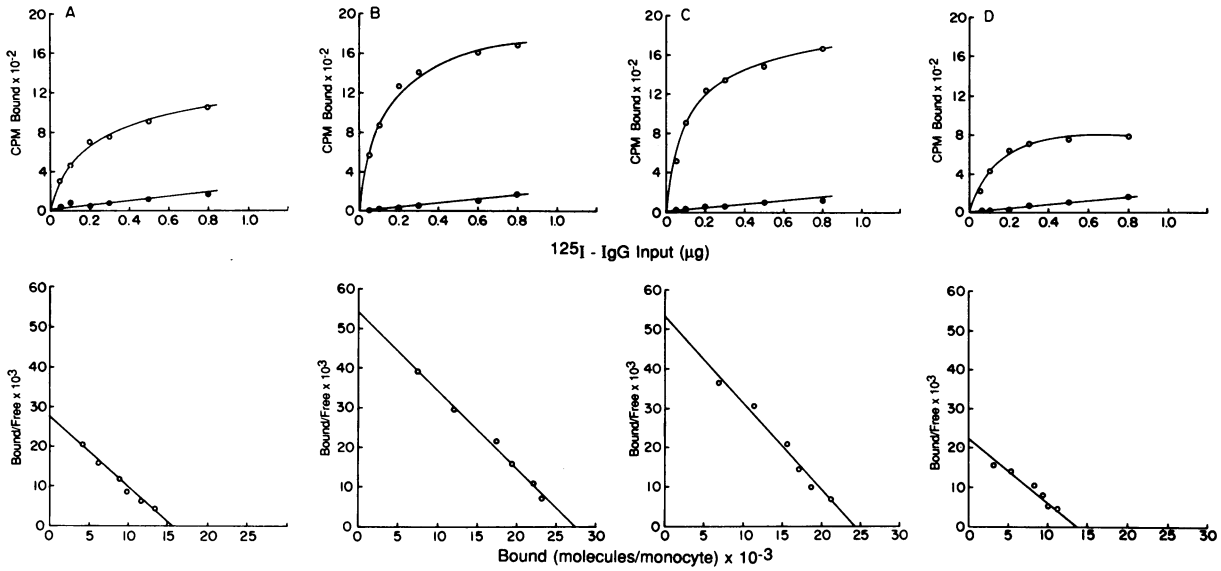

Figure 3. Equilibrium binding of IgG monomer to monocytes preincubated with cycloheximide. Monocytes were preincubated with buffer (*A, B*) or 10 μg/ml cycloheximide (*C, D*) before incubation with 0.09 μM XIIa (*A, D*) or buffer control (*B, C*). ^{125}I-IgG input (micrograms) refers to the amount of the IgG per 0.5 ml of total volume. Results with buffer + Factor XIIa = 15,500 IgG binding sites/cell, $K_a = 5.3 \times 10^8$ M^{-1}; results with buffer alone = 27,300 IgG binding sites/cell, $K_a = 6.6 \times 10^8$ M^{-1}; results with cycloheximide + buffer = 24,300 IgG binding sites/cell, $K_a = 6.6 \times 10^8$ M^{-1}; results with cycloheximide + Factor XIIa = 13,700 IgG binding sites/cell, $K_a = 5.0 \times 10^8$ M^{-1}. *Open circles,* specific binding; *closed circles,* nonspecific binding.

(Fig. 1) without altering the affinity of binding (Fig. 2). In contrast, its precursor, Factor XII, and the low molecular weight fragment of Factor XIIa lacking most of the heavy chain region, Factor XIIf, did not reduce the number of Fcγ receptors on monocytes (Fig. 1, Table I). Furthermore, this process did not require protein synthesis or intracellular processing, as evidenced by the experiments with cycloheximide (Fig. 3) and chloroquine (Table I).

Several possible mechanisms may be postulated for the reduction of FcγRI by Factor XIIa. The most direct would be competition of Factor XIIa for FcγRI on the monocyte. However, Factor XIIa, in contrast to a similar concentration of IgG, failed to significantly displace radiolabeled bound IgG from the monocyte surface (Fig. 4). Alternatively, monocytes may express a binding site for Factor XIIa that inhibits IgG binding sterically, but cannot displace IgG. An additional possibility is that Factor XIIa has a separate binding site distinct from FcγRI that causes a signal, resulting in the downregulation of the FcγRI ligand binding site, i.e., a reduction in the number of functioning receptors expressed on the cell surface. The ability of Factor XIIa to bind to the monocyte surface is further suggested by the experiments that indicate persistence of the effect after monocyte washing (Fig. 2, Table III).

Our data indicate that Factor XIIa does not alter the binding of monoclonal antibody 32.2. This monoclonal antibody recognizes an epitope on FcγRI that is not involved in the IgG ligand binding site (18). Thus, the effect of Factor XIIa occurs in the absence of an effect on this epitope. This is consistent with an effect of Factor XIIa on the FcγRI IgG ligand binding site without altering the expression of all FcγRI epitopes. There are other examples of biologic signals that alter physiologic ligand binding to a cell surface receptor without affecting monoclonal antibody binding to another epitope on the receptor. For example, the peptide RGDS can downregulate the ligand (fibrinogen) binding site on GPIIb/IIIa without altering

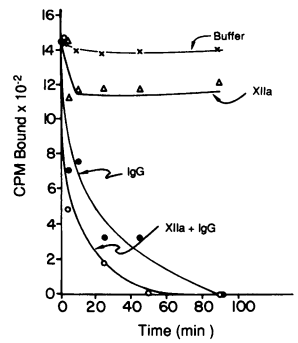

Figure 4. Kinetics of dissociation of ^{125}I-IgG from monocytes: effect of Factor XIIa. Monocytes (1 × 10^6) were incubated with 0.004 μM ^{125}I-IgG at 37°C in a total volume of 0.5 ml for 40 min at which time equilibrium of binding was attained. Either buffer, Factor XIIa (0.16 μM), unlabeled IgG (0.38 μM), or Factor XIIa (0.16 μM) plus IgG (0.38 μM) were added and the amount of ^{125}I-IgG bound to monocytes determined over the next 90 min.

Table III. Effect of XIIa on the Monocyte Binding Site for Monomeric IgG

	IgG binding sites/Monocyte	
Time	Buffer	XIIa
min		
0	20,300	10,400
30	22,100	10,600
60	23,400	12,000
120	20,600	12,100

Monocytes were incubated with buffer or Factor XIIa (0.09 μM) for 30 min at 37°C, washed, and held at 37°C. The number of IgG binding sites/monocyte was determined over the subsequent 2 h.

the binding of monoclonal antibodies reacting with other epitopes on GPIIb/IIIa (23).

Only Factor XIIa initiated the monocyte response, suggesting that the presence of the Factor XII heavy chain region and cleavage of the molecule are necessary conditions for the response. The light chain containing the serine catalytic region is not necessary for the response. This conclusion is evident from the data showing that neither Factor XII (Table I) nor Factor XIIf, which lacks most of the heavy chain (Fig. 1, Table I), affected the number of Fcγ receptor sites. Furthermore, neither corn trypsin inhibitor nor DFP blocked the effect of Factor XIIa on FcγRI (Table II). These observations raise the possibility that the heavy chain of Factor XIIa contains a domain that can interact with a distinct receptor on the monocyte, causing a downregulation or reduction in number of FcγRI ligand binding sites. The two domains homologous to epidermal growth factor present in the heavy chain region of Factor XIIa are candidates for this function (4, 5). These domains may only be available after the single proteolytic cleavage that converts Factor XII (an inactive agonist) to Factor XIIa (an active agonist).

The regulation of monocyte Fcγ receptor expression by Factor XIIa may be of pathophysiologic importance. Biologically active products of inflammation generated by plasma proteins can interact with circulating blood cells, including phagocytes. The interplay between these systems has been shown to be critical in inflammation, as evidenced by the action of C5a as a chemotaxin for phagocytic cells. During infection the weight of evidence suggests that Fcγ receptor expression on macrophages is increased as a consequence of bacterial products, and the release of lymphokines such as γ-IFN (13, 19, 22). Under this circumstance, Factor XIIa may serve a regulatory function in modulating Fcγ receptor expression.

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