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β_2 integrins are required for neutrophil degranulation induced by hemodialysis membranes

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β_2 integrins are required for neutrophil degranulation induced by hemodialysis membranes. An untoward consequence of hemodialysis is degranulation of peripheral blood neutrophils. The mechanisms that mediate this process, however, have not been conclusively identified. In the present study, the participation of complement activation and β_2 integrins (CD11/CD18) in hemodialysis-induced neutrophil degranulation was investigated. Incubation of neutrophils with cuprophan membrane stimulated the release of very small amounts of the cytoplasmic granular protein, elastase. This release was markedly enhanced by the addition of plasma. Inactivation of complement reduced degranulation by ~60%, but the contribution of anaphylatoxins C3a and C5a to the degranulation process was modest. Treatment of plasma with EDTA completely abolished neutrophil degranulation in the presence of cuprophan membrane. Further, when incubated with plasma and cuprophan membrane, neutrophils that are deficient in β_2 integrins released only 10% as much elastase as normal cells. Together, these observations strongly suggest that one or more members of the β_2 integrin family of receptors is essential for cuprophan membrane-induced neutrophil degranulation and that both complement-related and noncomplement-related factors serve as receptor ligands.

During hemodialysis, a variety of biologically active substances that have pathophysiological consequences are generated in the peripheral blood. These substances include proinflammatory fragments of complement proteins that are generated as a result of activation of the alternative pathway of complement (APC) [1–4]. The cellular response to acute inflammation is mediated largely by neutrophils because they have evolved a spectrum of functional characteristics that are activated by elements of the humoral immune system. As a principal mediator of humoral immunity, the complement system orchestrates many neutrophil functions. For example, C5a mediates chemotaxis [5], and binding of activation (C3b) and degradation (iC3b) products of C3 targets foreign substances for recognition by neutrophils through interactions with specific receptors (that is, complement receptor type 1, CR1; and complement receptor type 3, CR3, respectively) on the cell surface [6].

Hemodialysis has been shown to affect a variety of neutrophil functions. The neutropenia of hemodialysis has been extensively investigated, and compelling evidence indicates that the phenomenon is a consequence of C5a-induced neutrophil aggregation [7–9]. Other neutrophil dysfunctions associated with dialysis include degranulation [10–17], diminished oxidative metabolism [18], abnormal chemotaxis and aggregation [19], and impairment of phagocytic function [20]. Together, this constellation of findings suggest that neutrophils have undergone activation induced by products of immune reactions during dialysis. Indirect evidence suggests that complement related proteins participate in the induction of this dysfunctional state because the severity of the disturbance correlates with the capacity of the dialysis membrane to support complement activation [3, 20].

Stored in the primary (azurophilic) granules of neutrophils are proteins that possess proteolytic and antimicrobial properties. Release of these intracellular constituents (degranulation) in response to specific inflammatory stimuli is an essential element of host defense [21]. Neutrophil degranulation during hemodialysis has been reported [10–17], and evidence suggests that the proteolytic enzymes that are released into plasma as a result of this process contribute to the catabolic state that is observed during clinical hemodialysis [11, 12]. More recently, some investigators have postulated that neutrophil elastase release participates in the pathogenesis of carpal tunnel syndrome that is commonly seen in the dialysis patients [17]. Clinical studies show that hemodialysis using different types of membranes is associated with different degrees of degranulation [13–16]. Interestingly, cuprophan, which is associated with more elastase release than polyacrylonitrile [13], also induces more protein catabolism during sham hemodialysis in humans [22].

The mechanisms that induce dialysis-associated neutrophil degranulation have not been elucidated. Horl and colleagues found that plasma concentrations of the granular proteins did not correlate closely with the degree of complement activation as indicated by plasma $C3a_{(desArg)}$ levels [13–16], and those investigators have hypothesized that other factors contribute to the degranulation process. Shearing of the cells at the blood-dialysis membrane interphase has been proposed as one possible mechanism, but convincing data to support this hypothesis are lacking.

The studies reported herein were designed to analyze the effects of complement activation by hemodialysis membranes on neutrophil degranulation. Cuprophan membranes were used

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because their interactions with complement have been extensively characterized [2, 3, 23, 24]. As a marker of neutrophil activation, release of elastase (a constituent of primary granules) was quantitated. Our experiments have shown that degranulation is partially dependent on complement activation and partially dependent on heat-stable plasma factors that do not require complement activation but are dependent on calcium. These complement dependent and complement independent factors induce degranulation through binding to one or more members of the β_2 integrin family of receptors.

Methods

Buffers and reagents

The following buffers and reagents were used: PBS, 10 mM sodium phosphate, pH 7.4 containing 145 mM NaCl; PBS-Tween, 0.5 ml of Tween-20 (Sigma Chemical Co., St. Louis, Missouri, USA) per liter of PBS; HBSS/A, Hank's balanced saline solution containing 0.5% (wt/vol) human serum albumin (Albuminar®, Armour Pharmaceutical, Kankakee, Illinois, USA); bovine serum albumin (BSA, Sigma Chemical Co.); carbonate buffer, 0.2 M Na_2CO_3 and 0.25 M $NaHCO_3$; disodium ethylenediamine tetraacetate (EDTA, Sigma Chemical Co.); disodium ethyleneglycol tetraacetate (EGTA, Sigma Chemical Co.).

Plasma preparation and neutrophil isolation

Approximately 100 ml of blood (anticoagulated with 2 units of heparin/ml) were obtained from healthy volunteers who had not taken medication within 72 hours of the phlebotomy. Plasma was immediately separated by centrifugation. For some experiments, the separated plasma was immediately mixed with either EDTA (10 mM final concentration) or Mg-EGTA (2.5 mM $MgCl_2$ and 8 mM EGTA final concentration). After incubation at 37°C for five minutes to allow chelation of metals, the plasma was kept at 4°C prior to use in experiments. Other aliquots of plasma were heated at 56°C for 30 minutes immediately after separation from whole blood. This reagent is called heat-inactivated plasma. In some instances, heat-inactivated plasma was also treated with Mg-EGTA as described above.

Additional heparin (5.5 u/ml) was added to the portion of the blood from which the cells were isolated. Neutrophils were isolated by dextran sedimentation, hypotonic lysis of erythrocytes, and density gradient centrifugation (Ficoll-Paque®, Pharmacia LKB Biotechnology, Piscataway, New Jersey, USA) as previously described [25] and were resuspended in HBSS/A. The cell preparations contained > 95% granulocytes as determined by microscopic examination of slides stained with Wright's reagent, and the cells were 99% viable as determined by Trypan blue exclusion.

Neutrophils from a number of different donors were used in these studies. On a given day, however, the same donor's cells were used in all parallel experiments. The only exception was the experiments in which normal and CD18 deficient cells were compared (as described below).

Dialyzer membrane

Cuprophan hollow fibers were obtained from commercially available dialyzers (CF1211, Baxter-Travenol, Deerfield, Illinois, USA). They were cut into fragments of ~1 cm in length. Based on the nominal surface area of the blood compartment of a whole dialyzer (0.8 M^2), the wall thickness of the fibers (11 μM), the number of the fibers in the dialyzer (6,100), and the measured weight of the fibers, the surface area-to-weight ratio of the fibers was estimated to be 0.95 cm^2/mg. This calculation takes into account both the inner and outer surfaces of the hollow fibers. Unless specified otherwise, 14 mg of fibers (~13.3 cm^2 in total surface area) were used in each of the experimental tubes described below.

ELISA for elastase

Elastase antigen was measured using an enzyme-linked immunosorbent assay (ELISA) developed in our laboratory using standard sandwich ELISA methodology [26] and commercially available protein and antibodies. Rabbit anti-human elastase IgG (Biodesign International, Kennebunkport, Maine, USA) that had been conjugated with horseradish peroxidase (Sigma Chemical Co.) according to the method of Nakane and Kawaoi [27] was used as the detector antibody. The wells of a 96-well microtiter plate (Nunc-Immuno Plate, A/S Nunc, Roskilde, Denmark) were incubated at 37°C with 100 μl of carbonate buffer containing 20 μg/ml sheep anti-human elastase IgG (ICN ImmunoBiologicals, Lisle, Illinois, USA). After two hours, the wells were washed with PBS-Tween, and 100 μl of HBSS containing 1% BSA and either human elastase standards (Biodesign International) or a dilution of the test sample were added to the antibody-coated wells. After 90 minutes of incubation at 37°C, the wells were again washed with PBS-Tween. Next, 100 μl of peroxidase conjugated anti-elastase (diluted 1:100 with PBS containing 1% BSA) were added to the wells, and the incubation was continued at 37°C for 90 minutes. After washing with PBS-Tween, the chromogenic substrate o-phenylenediamine (Sigma Chemical Co.) was added to the wells, and the A_{492} was measured subsequently by using a Titertek Multiskan MCC spectrophotometer (Flow Laboratories, Inc., Santa Barbara, California, USA). The standard curve generated by this method was linear between 2 and 100 ng/ml of elastase suspended in either HBSS or diluted plasma that had been immunochemically depleted of elastase.

Direct effect of cuprophan membrane on neutrophil degranulation in the absence of plasma

An aliquot of 750 μl of HBSS/A containing 1×10^6 neutrophils was incubated in a 1.5 ml polypropylene tube (Life Science products, Denver, Colorado, USA) at 37°C in the presence or absence of 14 mg of cuprophan hollow fibers. After two hours, the suspension was separated from the fibers by using a Pasteur pipette. The cells in the suspension were sedimented by centrifugation, and a 500 μl aliquot of the supernate was removed for use in the elastase assay. Fluid phase elastase levels are accurate indicators of elastase release from neutrophils in these experiments since pilot experiments have demonstrated that there was no decrease in fluid phase elastase concentration when purified elastase was incubated for two hours with cuprophan fibers in a polypropylene tube in the absence of cells (that is, there was no significant adsorption of elastase to either the test tube surface or to the dialysis membrane).

Effect of normal human plasma (NHP) on neutrophil degranulation in the presence or absence of cuprophan membrane and effect of cuprophan-activated plasma

NHP (375 μl) and cuprophan fibers (14 mg) were incubated at 37°C with 375 μl of HBSS/A containing 1×10^6 neutrophils. At timed intervals between 15 minutes and four hours, the supernate was removed for elastase determination as described above. These experiments showed that elastase release increased in a linear fashion during the first hour. A plateau phase was observed after one hour and no significant increase was observed between one and four hours. Accordingly, subsequent experiments were performed using a two-hour incubation period.

In separate experiments, NHP was incubated at 37°C with cuprophan at the same ratio as described above (375 μl of NHP to 14 mg of cuprophan membrane) but cells were omitted. After two hours, the supernate was recovered and 375 μl of the cuprophan-activated plasma were incubated with 375 μl of HBSS/A containing 1×10^6 neutrophils. After two hours, the supernate was assayed for elastase. Neutrophils incubated with either NHP alone or HBSS/A alone served as controls.

Effect of heat treatment or metal chelation on plasma-induced neutrophil degranulation in the presence of cuprophan

Neutrophils (1×10^6 suspended in 375 μl of HBSS/A) were incubated in the presence of 14 mg of cuprophan fibers and 375 μl of one of the following reagents: (i) heat-inactivated plasma; (ii) EDTA-treated plasma; (iii) Mg-EGTA-treated plasma; (iv) Mg-EGTA-treated, heat-inactivated plasma. Cells incubated with NHP or buffer in the presence of cuprophan fibers served as controls. After incubation, the supernates were assayed for elastase. To determine if extracellular divalent cations are required for degranulation induced by other mechanisms, 2.5×10^6 neutrophils were stimulated with 0.5 μg/ml (final concentration) of phorbol myristate acetate (PMA, Sigma Chemical Co.) and PBS or PBS containing 10 mM EDTA for 45 minutes. The supernate was subsequently assayed for elastase.

Preparation of plasma depleted of complement C3 or C5

Plasma depleted of either C3 or C5 was prepared from NHP using immunoaffinity chromatography as previously described [28]. In brief, for C3-depleted plasma, polyclonal goat anti-human C3 IgG (Organon Teknika-Cappel, Malvern, Pennsylvania, USA) was conjugated to CNBr-activated Sepharose 4B (Pharmacia) and treated with pepsin in order to generate $F(ab')_2$ fragments according to a previously described protocol [29]. NHP was applied to the column containing this affinity adsorbent, and the A_{280} of the effluent (diluted 1:100) from the column was measured. Fractions were pooled and diluted with PBS such that the total protein concentration in the pooled plasma was equal to 75% of that of NHP.

Depletion of C3 was confirmed by a hemolytic assay as previously described [30]. In addition, the capacity of some plasma pools to generate C3a was examined by incubating 375 μl of the plasma with 375 μl of HBSS/A and 14 mg of cuprophan fibers at 37°C for two hours. The supernate was then assayed for $C3a_{(desArg)}$ using a commercial kit (Amersham Corp., Arlington Heights, Illinois, USA). The results showed that no C3a was generated during the incubation ($C3a_{(desArg)}$ levels were 145 ± 4 ng/ml and 164 ± 18 ng/ml before and after incubation, respectively; $N = 4$, $P > 0.05$). In contrast, incubation of NHP (diluted to 75% with PBS) with cuprophan fibers under the same conditions resulted in substantial generation of C3a as indicated below. The effect of C3 depletion on C5a generation was also tested. $C5a_{(desArg)}$ was measured by RIA (Amersham). In the absence of C3, C5 activation is not expected to occur. $C5a_{(desArg)}$ concentrations were unaffected by incubation of C3-depleted plasma with cuprophan membranes (35 ± 3 ng/ml and 39 ± 1 ng/ml before and after incubation, respectively; $N = 4$, $P > 0.05$). These data indicate that C3 depletion in the plasma was complete. The C3-depleted plasma was frozen at −70°C and used in experiments within seven days of preparation. An aliquot of NHP from the same donor was also frozen for comparison in the degranulation experiments described below. As a control, human serum albumin (Albuminar®, Armour Pharmaceutical) diluted to 4 g/dl with PBS was passed through the anti-C3 column using the same conditions as those used to prepare the C3-depleted plasma.

C5-depleted plasma was prepared in a manner similar to that described for C3-depletion except that polyclonal goat anti-human C5 IgG (Atlantic Antibodies, Scarborough, Maine, USA) was used to prepare the immunoadsorbent. C5 depletion was confirmed by hemolytic assay. In addition, some C5-depleted plasma pools were assayed for C5a generation following incubation with cuprophan membrane as described above for C3-depleted plasma, except that the RIA for $C5a_{(desArg)}$ was used. $C5a_{(desArg)}$ concentrations were 23 ± 2 ng/ml and 20 ± 4 ng/ml before and after incubation with cuprophan, respectively ($N = 4$, $P > 0.05$). As described below, incubation of NHP with cuprophan membrane under the same conditions resulted in substantial C5a generation.

Isolation of complement C3 and C5

C3 and C5 were isolated from normal human serum by published procedures [28]. The purity of the isolated proteins was confirmed by SDS-PAGE analyses. The functional activity of the proteins was confirmed in hemolytic assays [30].

Effect of C3 depletion on plasma-induced neutrophil degranulation

Neutrophils (1×10^6) contained in HBSS/A and 14 mg of cuprophan hollow fibers were incubated at 37°C with one of the following reagents: (i) 375 μl of NHP (diluted to 75% with PBS); (ii) 375 μl of C3-depleted plasma; (iii) 375 μl of C3-depleted plasma reconstituted with 420 μg of C3 (equivalent to plasma C3 concentration of 1.2 mg/ml); (iv) 375 μl of HBSS/A. For each experiment on a given day, NHP and C3-depleted plasma from the same blood donor were used. An equal volume of PBS was added to the tubes that did not contain purified C3. The total incubation volume remained constant at 750 μl. In separate experiments, 1×10^6 neutrophils suspended in 375 μl of HBSS/A and 14 mg of cuprophan fibers were incubated with 375 μl of either NHP or human serum albumin that had been passed through the anti-C3 column. After two hours, the supernate was removed and assayed for elastase.

Effect of C5 depletion on plasma induced neutrophil degranulation

These experiments were performed in a manner similar to that described above for C3-depleted plasma except that C5-depleted plasma and purified C5 (70 μg, equivalent to plasma C5 concentration of 200 μg/ml) were used instead of C3-depleted plasma and purified C3. For each experiment on a given day, NHP and C5-depleted plasma from the same blood donor were used.

Generation of C3a and C5a in plasma induced by contact with cuprophan

An aliquot of 375 μl of NHP (diluted to 75% with PBS) was incubated with 375 μl of HBSS/A containing either 14 mg of cuprophan fibers or 14 mg of cuprophan fibers and 3.75×10^6 neutrophils. After 120 minutes, 25 μl of 0.2 M EDTA were added to 500 μl of supernate, and the concentrations of $C3a_{(desArg)}$ and $C5a_{(desArg)}$ in the supernates were subsequently measured by RIA (Amersham).

Effect of isolated C3a and C5a on neutrophil degranulation

Isolated neutrophils (1×10^6) suspended in HBSS/A were incubated at 37°C with 15 μg/ml of purified human C3a (gift of Dr. T. Hugli, Scripps Clinic, La Jolla, California, USA) or with 500 ng/ml of recombinant human C5a (Sigma Chemical Co.) in the presence or absence of 5 μg/ml of cytochalasin B. Incubation of cells with HBSS/A or HBSS/A containing cytochalasin B served as controls. For comparison, the same number of cells were incubated with 375 μl of NHP and 14 mg of cuprophan fibers. The incubation volume was constant at 750 μl. After two hours, the supernates were assayed for elastase.

Effects of C3a and C5a in combination with heat-inactivated plasma on neutrophil degranulation

The potential synergism between anaphylatoxins and plasma noncomplement proteins in inducing neutrophil degranulation was examined as follows. Isolated neutrophils (1×10^6) suspended in HBSS/A were incubated at 37°C with a combination of 15 μg/ml of purified C3a and 500 ng/ml of recombinant C5a in the presence of 14 mg of cuprophan fibers and 375 μl of heat-inactivated plasma (the heat treatment inactivates the APC). Incubation of cells with cuprophan and heat-inactivated plasma, or with cuprophan and NHP, or with HBSS/A alone served as controls. The incubation volume was constant at 750 μl. After two hours, the supernates were assayed for elastase.

Effect of IgG on cuprophan-induced neutrophil degranulation

Fc receptor-mediated phagocytosis of IgG coated particles by neutrophils induces degranulation [21], and we have previously demonstrated that, when incubated with plasma, cuprophan membranes bind IgG [31]. The role of IgG in cuprophan-induced neutrophil degranulation was therefore examined by depletion of IgG from plasma.

Heparinized NHP from various normal donors was pooled. An aliquot of the plasma was diluted to 75% with PBS and frozen at −70°C prior to use. IgG-depleted plasma was prepared by chromatographing a 12 ml aliquot of NHP from individual donors on a 25 ml column of immobilized Protein G (Protein G Sepharose 4 Fast Flow, Pharmacia-LKB). The chromatographic fractions containing the breakthrough proteins were combined and diluted with PBS such that the total protein concentration (as determined by A_{280}) was equal to 75% of that of NHP. The residual IgG concentration in the pooled fractions was less than 0.01 g/dl (the detection limit of the assay), as determined by nephelometry using an N Immunoglobulin kit and the BN 100 Nephelometer (Behring Diagnostic Inc., Somerville, New Jersey, USA). The various IgG-depleted plasma pools were combined.

To examine the effect of IgG on neutrophil degranulation in the absence of complement activation, an aliquot of IgG-depleted plasma was incubated at 56°C for 30 minutes prior to use in the experiment described below. Neutrophils (1×10^6 cells suspended in 375 μl of HBSS/A) were incubated at 37°C with 14 mg of cuprophan fibers and 375 μl of one of the following reagents: (i) NHP; (ii) IgG-depleted plasma; (iii) IgG-depleted, heat-activated plasma; (iv) IgG-depleted plasma supplemented with 3.375 mg of IgG (Miles, Inc., West Haven, Connecticut, USA) (equivalent to plasma IgG concentration of 1.2 g/dl); or (v) HBSS/A. After two hours, the supernates were assayed for elastase.

Cytofluorometic analysis of CD18 deficient neutrophils

The neutrophils from a patient with a congenital abnormality in the expression of CD18 (the common β chain shared by the β_2 integrins, CR3, LFA-1, and p150,95) were utilized in the study of cuprophan-induced neutrophil degranulation. Expression of CD18 was analyzed by cytofluorometry. Aliquots of 50 μl of neutrophils (1×10^6 cells) from the patient or from a normal donor were incubated at 37°C with 50 μl of PBS containing 50 μg/ml of monoclonal anti-CD18 (60.3, Bristol-Myers Squibb, Seattle, Washington, USA). After 30 minutes, the cells were washed with RPMI, and incubated at 37°C with 50 μl of FITC-conjugated anti-mouse IgG (Sigma Chemical Co.) diluted 1:20 with PBS. After 30 minutes, the cells were washed and analyzed using a Cytoflurograf IIS (Ortho Diagnostics Systems Inc., Westwood, Massachusetts, USA).

Requirement of β_2 integrins for cuprophan-induced degranulation

To determine if the β_2 integrin family of receptors is required for cuprophan-induced neutrophil degranulation, 1×10^6 CD18-deficient neutrophils or normal neutrophils (suspended in 375 μl of HBSS/A) were incubated with 14 mg of cuprophan fibers and 375 μl of NHP. (To ensure that the differences in degranulation between normal and abnormal cells were due to differences in the properties of the cells and not to differences in the plasma constituents, a pool of NHP from six normal individuals was used in all these experiments). After incubation at 37°C for two hours, the supernates were assayed for elastase. As a control, neutrophils (2.5×10^6) from the patient or from volunteer donors were incubated at 37°C with 5 μg/ml of cytochalasin B. After five minutes, 250 μl of PBS containing fMLP (1 mM final concentration, Sigma Chemical Co.) was added, (total volume of the reaction mixture was 500 μl) and the incubation was continued for an additional 45 minutes. The supernate was subsequently assayed for elastase.

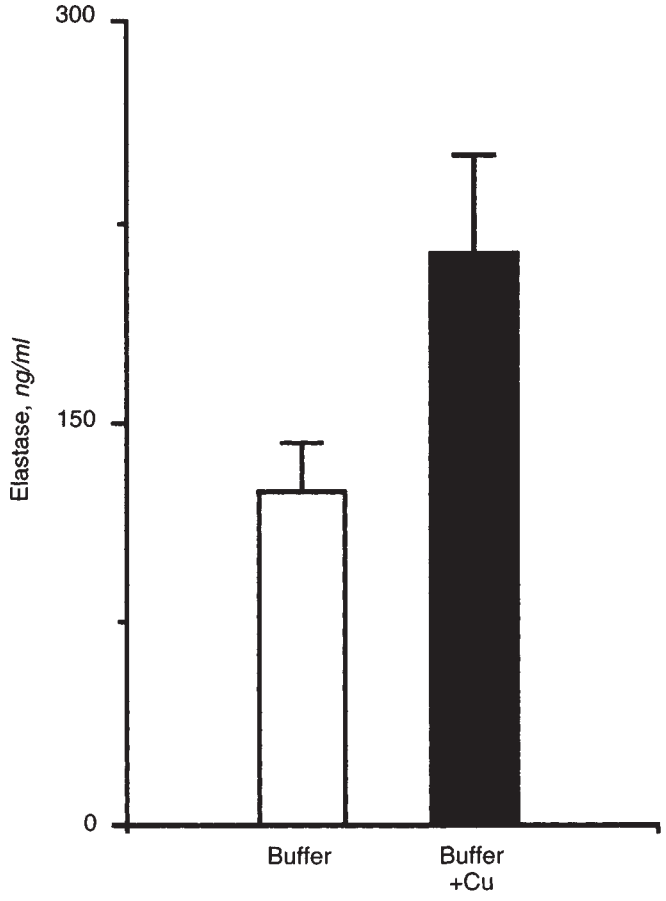

Fig. 1. *Direct effect of cuprophan membrane on neutrophil degranulation in the absence of plasma.* Incubation of neutrophils with buffer (HBSS/A) alone (□) induced a modest release of elastase from neutrophils. Addition of cuprophan fibers (Cu) to the buffer (■) increased the release ($N = 8$, $P < 0.05$) by a very modest amount.

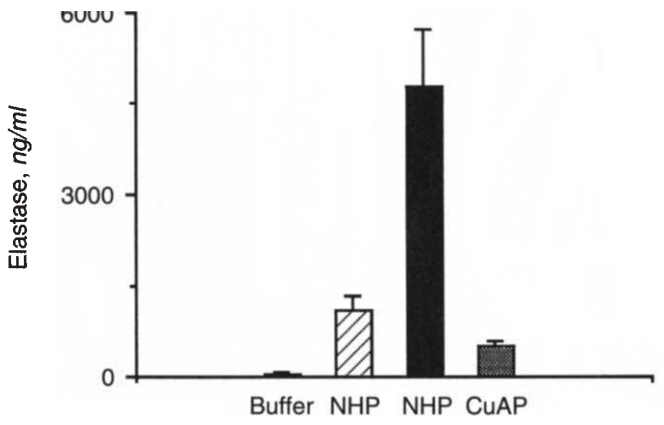

Fig. 2. *Effect of plasma on neutrophil degranulation.* Incubation of neutrophils with normal human plasma (NHP, ▨) significantly enhanced the release of elastase compared to incubation with HBSS/A (buffer) alone. Addition of cuprophan fibers (Cu) to NHP (■) in the incubation further increased the release by fivefold ($N = 12$, $P < 0.001$). Cells incubated with cuprophan activated plasma (CuAP, ▩) degranulated less than those incubated with NHP, suggesting that the degranulating activity generated during exposure of plasma to cuprophan did not reside in the fluid phase alone.

Statistical analyses

Values are reported as means ± SEM. Differences in concentrations of elastase between groups were analyzed by Student's unpaired two-tailed *t*-test. *P* values < 0.05 were considered to be statistically significant.

Results

Effect of cuprophan membrane on neutrophil degranulation in the absence of plasma

Following incubation of neutrophils in HBSS/A, a relatively modest amount of elastase was detected in the supernate (Fig. 1). When cuprophan fibers were included in the reaction mixture, a very small increase in the release of elastase was observed, although the difference was statistically significant ($N = 8$; $P < 0.05$ vs. buffer alone).

Effect of plasma on neutrophil degranulation

The effect of plasma on degranulation was studied by incubating neutrophils with plasma in the presence or absence of cuprophan membrane. The addition of NHP to cells enhanced the release of elastase significantly compared to incubation of cells with buffer alone ($N = 12$; $P < 0.001$; Fig. 2). When cuprophan membrane was added to cells and NHP, elastase release increased by approximately fivefold compared to incubation of cells with NHP without membrane ($N = 12$; $P < 0.001$). Compared to incubation of cells with cuprophan membrane alone, the addition of NHP to cuprophan and cells increased the release by ~25-fold. These observations demonstrate the importance of both plasma and cuprophan membrane in the stimulation of neutrophil degranulation. In addition, these results show that in the absence of plasma, the contribution of the membrane to neutrophil degranulation is trivial. Similar results were obtained when cuprophan flat sheets (gift of Dr. Baurmeister and Dr. Vienken, Akzo, Wuppertal, Germany) were substituted for cuprophan hollow fibers in the incubation with neutrophils and NHP (data not shown).

The importance of plasma in cuprophan-induced degranulation suggested the possibility that this process is mediated by plasma components, such as complement C5a, that are activated by the cuprophan membrane and subsequently released into the plasma. Therefore, the effect of cuprophan-activated plasma was tested. After incubation with NHP, the cuprophan fibers were discarded and neutrophils were incubated with the cuprophan-activated plasma. Compared to NHP, cuprophan-activated plasma induced less degranulation ($N = 12$; $P < 0.05$, cuprophan-activated plasma vs. NHP) (Fig. 2). These results indicate that C5a alone does not account for the degranulating activity observed when neutrophils are incubated with plasma and cuprophan. Further, these observations imply that membrane-bound proteins contribute to the degranulation process.

Effect of heat inactivation or chelation of divalent cations on neutrophil degranulation induced by plasma and cuprophan

Activation of the APC is dependent on both heat-sensitive components and Mg^{++}. In order to determine the contribution of complement activation on hemodialysis membrane-induced neutrophil degranulation, the effects of heat treatment of

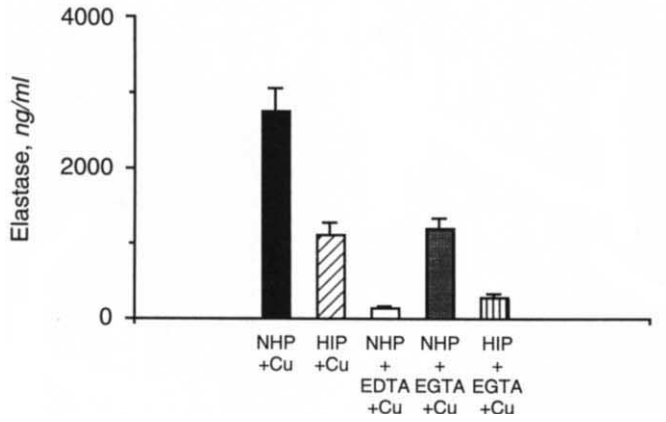

Fig. 3. *Effect of heat inactivation or chelation of divalent cations on neutrophil degranulation induced by plasma and cuprophan.* Compared to normal human plasma (NHP, ■), heat-inactivated plasma (HIP, ▨) induced 57% less elastase release from neutrophils in the presence of cuprophan (Cu) ($N = 12$; $P < 0.001$). Chelation of both Ca^{++} and Mg^{++} with EDTA (□) virtually abolished degranulation, whereas selective chelation of Ca^{++} with EGTA (▥) reduced the release by only 45% ($N = 8$, $P < 0.001$). Treatment of HIP with EGTA (▥) significantly reduced degranulation compared to either HIP without EGTA or NHP chelated with EGTA, suggesting that the heat-stable plasma degranulating activity is Ca^{++} dependent.

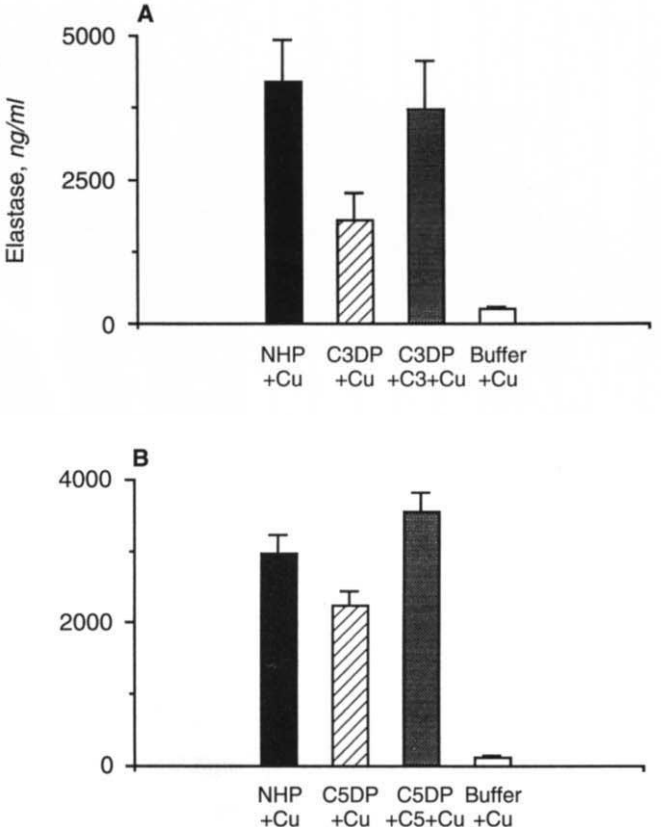

Fig. 4. *Effect of complement depletion on plasma induced neutrophil degranulation in the presence of cuprophan.* **A.** C3 depletion. Neutrophils were incubated with cuprophan fibers (Cu) in the presence of normal human plasma (NHP, ■), C3 depleted plasma (C3DP, ▨), or C3DP that had been repleted with purified C3 (▥). Cells incubated with HBSS/A (buffer) in the presence of cuprophan (□) served as control. Depletion of C3 from plasma diminished elastase release by 62% ($N = 18$, $P < 0.001$ vs. NHP). Repletion of C3DP with C3 restored the release reaction completely ($P > 0.05$ vs. NHP). **B.** C5 depletion. Neutrophils were incubated with Cu in the presence of NHP (■), C5 depleted plasma (C5DP, ▨), or C5DP that had been repleted with purified C5 (▥). Cells incubated with buffer in the presence of Cu (□) served as control. Depletion of C5 from plasma diminished elastase release by 26% ($N = 29$; $P < 0.05$ vs. NHP). Repletion of C5DP with C5 restored the release reaction completely ($P > 0.05$ vs. NHP).

plasma and chelation of divalent cations with EDTA or EGTA were analyzed. EDTA chelates both Ca^{++} and Mg^{++}, whereas EGTA chelates Ca^{++} but not Mg^{++}. Thus, plasma treated with Mg-EGTA will support activation of the APC, but heat-treated plasma or plasma treated with EDTA will not.

Compared to NHP, incubation of neutrophils with cuprophan in the presence of heat-inactivated plasma (HIP) decreased elastase release by 57% ($N = 12$; $P < 0.001$; Fig. 3). These results suggest that the majority of the degranulation process is mediated by factors that are dependent on complement activation, however, a significant portion of the process does not require complement activation. Selective chelation of Ca^{++} with EGTA reduced the release of elastase by 45% ($N = 8$, $P < 0.001$; Fig. 3). These results are consistent with the concept that over 50% of the degranulation process requires activation of the APC, however, a significant element of the degranulation process is dependent upon Ca^{++}. Chelation of both Ca^{++} and Mg^{++} with EDTA abolished degranulation (Fig. 3).

To determine whether the Ca^{++} dependent component is heat stable or heat sensitive, neutrophils were incubated with cuprophan fibers and heat-inactivated plasma that had been treated with EGTA. Elastase release under these conditions was markedly diminished compared to incubation with heat-inactivated plasma without EGTA ($N = 12$; $P < 0.001$) or incubation with NHP with EGTA ($N = 12$; $P < 0.001$). These data suggest that the heat stable (complement independent) component requires Ca^{++}.

The requirement for extracellular divalent cations for neutrophil degranulation induced by another agonist was also examined. In contrast to stimulation by NHP and cuprophan, the addition of EDTA enhanced degranulation induced by PMA (3,787 ± 877 ng/ml vs. 5,820 ± 883 ng/ml; $N = 6$; $P > 0.05$). These results demonstrate that neutrophil degranulation can occur in the absence of extracellular divalent cations, although some mechanisms have an absolute requirement for divalent cations.

Effect of complement depletion on plasma-induced neutrophil degranulation

Inasmuch as cuprophan membrane activates complement [1–4, 23, 24] and activated complement fragments are known to stimulate neutrophils [5, 6, 32, 33], the effect of complement on cuprophan-induced neutrophil degranulation was studied by using plasma depleted of C3 or C5. When C3 depleted plasma was substituted for NHP, elastase release decreased by 62% ($N = 18$; $P < 0.001$; Fig. 4A). Repleting the C3 depleted plasma with purified C3 completely restored the capacity of the plasma to induce neutrophil degranulation in the presence of cuprophan. The latter observation indicates that the affinity chromatographic procedure did not deplete substances other than

C3 that were essential in inducing neutrophil degranulation. Inasmuch as degranulation induced by repletion of C3-depleted plasma with C3 did not exceed that induced by NHP ($P > 0.05$), these data also suggest that the chromatographic procedure did not enhance the capacity of the plasma to induce degranulation.

An additional control for the effect of the chromatographic procedure was provided by subjecting human serum albumin to the same preparatory procedure as that used for C3 depletion. Incubation of neutrophils with albumin treated in this manner resulted in only very modest degranulation (189 ± 10 ng/ml; $N = 4$). These values were not significantly different compared to those obtained by incubating neutrophils with untreated serum albumin (166 ± 14 ng/ml; $N = 4$; $P > 0.05$).

Together, these data suggest that complement activation was responsible for ~60% of the degranulation when neutrophils were exposed to cuprophan membrane and plasma simultaneously.

The effect of C3 depletion on neutrophil degranulation, however, might be indirect since activation of C5 and generation of the membrane attack complex of complement (C5b-9) would not occur in the absence of C3. Therefore, the effect of C5 depletion was also examined. Substitution of NHP with C5 depleted plasma during the incubation of neutrophils with cuprophan decreased the release of elastase by 26% ($N = 29$; $P < 0.05$; Fig. 4B). Again, repletion of the C5 depleted plasma with purified C5 completely restored the capacity of the plasma to induce neutrophil degranulation. These data suggest that products of C5 activation or generation of the membrane attack complex or both contributed to the degranulation process.

Effect of C3a or C5a on neutrophil degranulation in the absence of plasma

Previous studies have shown that C3a and C5a are generated during clinical hemodialysis using cuprophan membrane [2, 3] and that, under certain circumstances, these anaphylatoxins can induce neutrophil degranulation in vitro [32, 33]. Accordingly, experiments were designed to assess the contribution of C3a and C5a to the degranulation.

First, the generation of these activation products under the present experimental conditions was quantified. Incubation of NHP in the presence of cuprophan generated 14,599 ± 1,115 ng/ml of $C3a_{(desArg)}$ and 241 ± 9 ng/ml of $C5a_{(desArg)}$ in the plasma ($N = 4$). Addition of neutrophils to the incubation did not decrease the concentration of C5a in the fluid phase significantly (217 ± 12 ng/ml, $N = 4$, $P > 0.05$), demonstrating that the amount of C5a that was bound to specific C5a receptors on neutrophil surface (~1 to 3 × 10^5 receptors/cell [34]) was so small compared to the total amount of C5a generated that it was not reflected by changes in plasma levels.

To determine directly the effects of C3a on neutrophil degranulation, cells were incubated with C3a alone, with cytochalasin B alone, or with the combination of C3a and cytochalasin B. Cytochalasin B has been used extensively in previous studies to enhance secretory events by inflammatory cells [32, 33]. In the absence of cytochalasin B, C3a (at 15 μg/ml, a concentration equivalent to that generated in plasma during incubation with cuprophan) failed to stimulate the release of elastase from neutrophils ($N = 8$; $P > 0.05$ vs. buffer; Fig. 5A). Cytochalasin B alone was also without effect ($N = 8$; $P > 0.05$ vs. buffer).

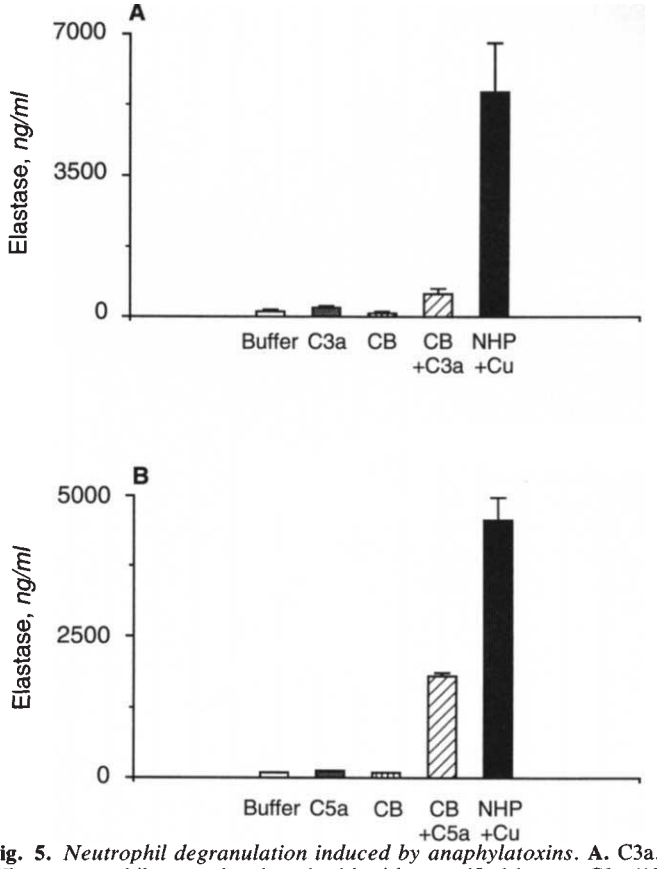

Fig. 5. *Neutrophil degranulation induced by anaphylatoxins.* **A.** C3a. When neutrophils were incubated with either purified human C3a (15 μg/ml) (▥) or cytochalasin B (CB, ▥), the amount of elastase released was not different from that released when cells were incubated with HBSS/A (buffer, □). When neutrophils were incubated with cytochalasin and C3a simultaneously (▨), elastase release increased by 4× ($N = 8$; $P < 0.01$ vs. buffer). The magnitude of release however, was significantly less than that observed when cells were incubated with NHP and cuprophan fibers (Cu, ■). **B.** C5a. Similar to C3a, recombinant human C5a (500 ng/ml, ▥) was ineffective in inducing neutrophil degranulation. When neutrophils were incubated with cytochalasin (CB) and C5a (500 ng/ml) simultaneously (▨), elastase release increased by 21× ($N = 7$; $P < 0.001$ vs. buffer).

When neutrophils were presensitized with cytochalasin B, C3a enhanced the release of elastase by fourfold ($N = 8$; $P < 0.01$ vs. buffer).

Similarly, C5a alone (at 500 ng/ml) failed to induce elastase release ($N = 7$; $P > 0.05$ compared to buffer; Fig. 5B). When cytochalasin B was added, however, C5a enhanced the release of elastase by 21-fold ($N = 7$; $P < 0.001$ vs. buffer). The magnitude of this release was, however, significantly lower than that observed during incubation of neutrophils with NHP and cuprophan simultaneously. These studies suggest that neither C3a alone or C5a alone contributes significantly to the neutrophil degranulation induced by cuprophan membrane and NHP.

Synergistic effects of C3a and C5a with other plasma proteins on neutrophil degranulation

Although neither C3a nor C5a alone induced neutrophil degranulation, the possibility that these anaphylatoxins have a synergistic effect with other plasma proteins was investigated.

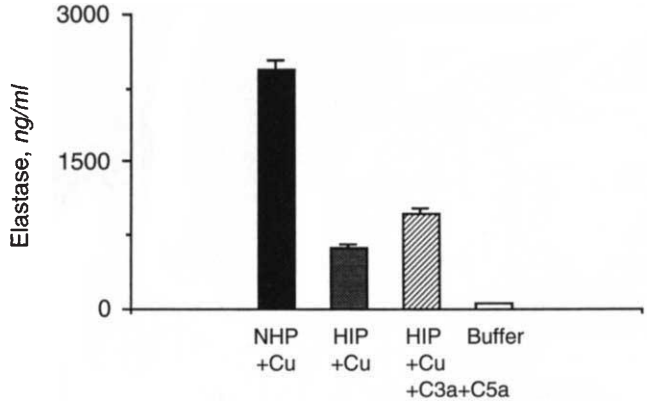

Fig. 6. *Contribution of C3a and C5a to neutrophil degranulation in the presence of heat-inactivated plasma.* When C3a (15 μg/ml) and C5a (500 ng/ml) were added simultaneously to heat inactivated plasma (HIP), cuprophan membranes (Cu), and neutrophils (▨), a statistically significant increase in elastase release was observed compared to incubation with HIP, Cu, and cells alone (▩, $N = 8$; $P < 0.001$). The magnitude of degranulation, however, was substantially lower than that observed when cells were incubated with normal plasma (NHP) and cuprophan (■). These data suggest that the anaphylatoxins may play a synergistic role with other noncomplement plasma proteins in the degranulation process, but their contribution to total degranulation induced by NHP and cuprophan is relatively small.

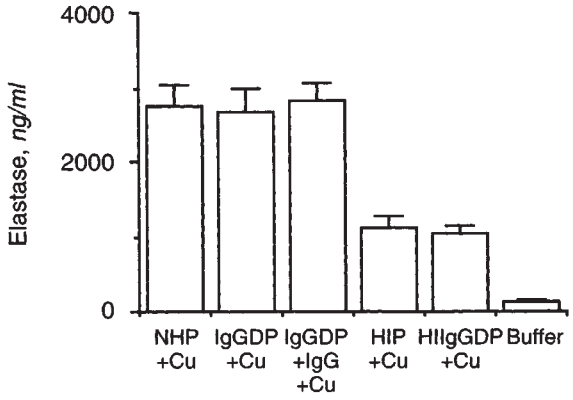

Fig. 7. *Effect of IgG depletion on plasma induced neutrophil degranulation in the presence of cuprophan.* When neutrophils were incubated with IgG depleted plasma (IgGDP) in the presence of cuprophan (Cu), the amount of elastase released was similar to that observed with normal human plasma (NHP) ($N = 12$; $P > 0.05$). Repletion of IgGDP with purified IgG did not enhance degranulation. Inactivating complement by heat treatment of plasma (heat inactivated plasma, HIP) decreased degranulation by ~60% ($N = 12$; $P < 0.001$ vs. NHP). Heat treatment of IgG-depleted plasma (HIIgGDP) produced similar results ($N = 12$; $P > 0.05$ vs. HIP). These data suggest that IgG does not participate significantly in cuprophan-induced neutrophil degranulation in the presence or absence of complement activation.

Heating plasma to 56°C × 30 minutes inactivates factor B so that the APC is not activated by cuprophan membrane. When C3a and C5a were added simultaneously to cells, heat-inactivated plasma, and cuprophan membrane, there was a statistical significant increase in elastase release compared to incubation without the addition of anaphylatoxins (966 ± 63 ng/ml vs. 617 ± 40 ng/ml, $N = 8$, $P < 0.001$). This degree of degranulation, however, was still far lower than that observed when cells were incubated with cuprophan and NHP (Fig. 6). These data suggest that anaphylatoxins may play a role in neutrophil degranulation induced by cuprophan and plasma, but the contribution is small. These results also indicate that plasma noncomplement components participate in this process.

Effects of IgG depletion on neutrophil degranulation

Fc receptor-mediated phagocytosis of IgG coated particles by neutrophils induces degranulation [21], and we have previously demonstrated that, when incubated with plasma, cuprophan membranes bind IgG [31]. To determine if interactions between membrane-bound IgG and neutrophil Fc receptors is required for cuprophan-induced neutrophil degranulation, neutrophils were incubated with cuprophan fibers in the presence of either IgG-depleted plasma or NHP. There was no difference in elastase release between these two groups ($N = 12$; $P > 0.05$; Fig. 7). Repletion of IgG-depleted plasma with purified IgG did not enhance degranulation ($N = 12$; $P > 0.05$ vs. NHP or IgG-depleted plasma). Quantitation of residual IgG in plasma following the depletion process showed that <0.01 g/dl remained. To insure that the residual IgG was not sufficient to induce degranulation, neutrophils were incubated with cuprophan membrane and either buffer or buffer containing 0.01 g/dl of purified IgG. The presence of this amount of IgG in the buffer did not stimulate degranulation (154 ± 28 ng/ml for IgG vs. 134 ± 24 ng/ml for buffer; $N = 8$; $P > 0.05$).

The effect of IgG on neutrophils could be obscured by the stimulatory effect of activated complement. To test this hypothesis, complement activity was abolished by heat treatment of plasma, and the effect of IgG depletion was examined. Heat-treated, IgG-depleted plasma induced a similar degree of degranulation in the presence of cuprophan compared to heat-treated plasma without IgG depletion ($N = 12$; $P > 0.05$; Fig. 7). These results indicate that interactions between IgG and Fc receptors are not essential for degranulation induced by cuprophan and plasma.

Effect of β_2 integrins on degranulation

Our previous studies [31] have demonstrated that neutrophil binding to cuprophan plate membranes that have been incubated with plasma is mediated through interactions between CR3 (CD11b/CD18) on the neutrophils and its counterligand, iC3b, that is present on the membranes as a result of activation of the APC. To determine if neutrophil degranulation is also dependent upon CR3, neutrophils were treated with a monoclonal antibody (60.1, a gift from Dr. P. Beatty, University of Utah) that recognizes an epitope expressed by CD11b (the α chain of CR3) [35], or with a monoclonal antibody (60.3, Bristol-Myers Squibb) that recognizes an epitope expressed by CD18 (the β chain of CR3, LFA-1, and p150,95). The antibody 60.1 has been shown to inhibit neutrophil binding to cuprophan plate membranes in the presence of plasma [31]. Neither antibody 60.1 (at concentrations up to 200 μg/ml) nor 60.3 (at concentrations up to 25 μg/ml) inhibited neutrophil degranulation (not shown). There appeared to be two plausible explanations for these observations. First, the degranulation process might not be dependent upon CR3 (or other β_2 integrins). Second, the degranulation process might be dependent upon

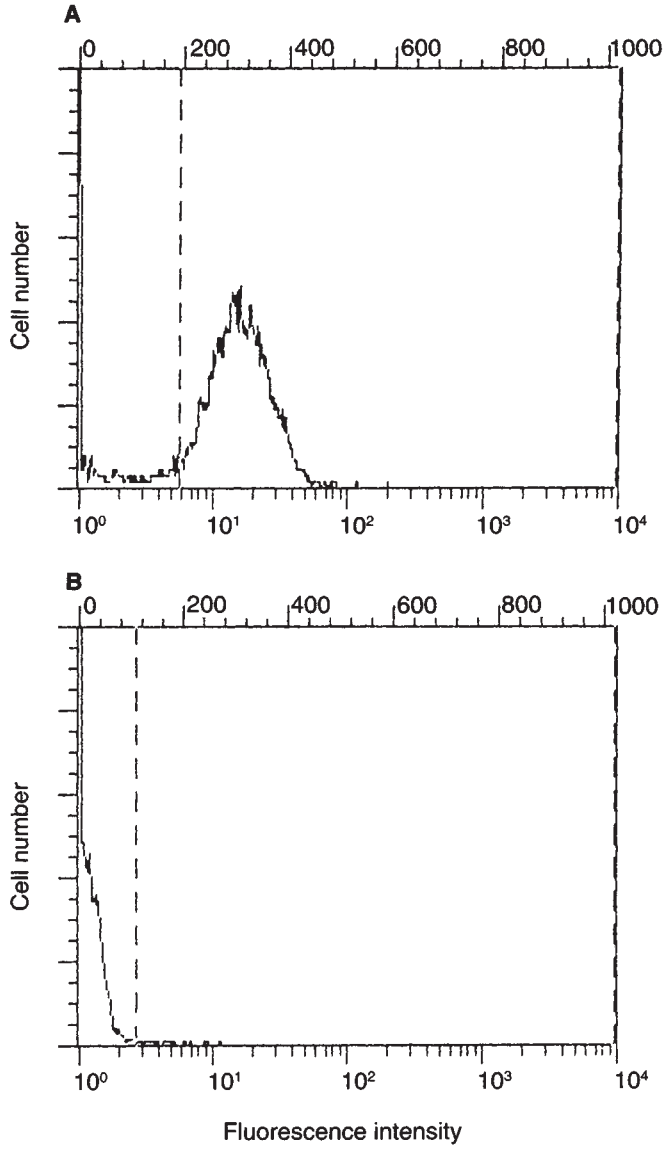

Fig. 8. *Cytofluorometric analysis of CD18 on neutrophils.* Isolated neutrophils were incubated with monoclonal anti-CD18 as primary antibody and FITC-conjugated anti-mouse IgG as secondary antibody and subjected to FACS analysis. Expression of CD18 was prominent on normal neutrophils (**A**) but was absent on neutrophils from the patient with congenital deficiency in CD18 expression (**B**).

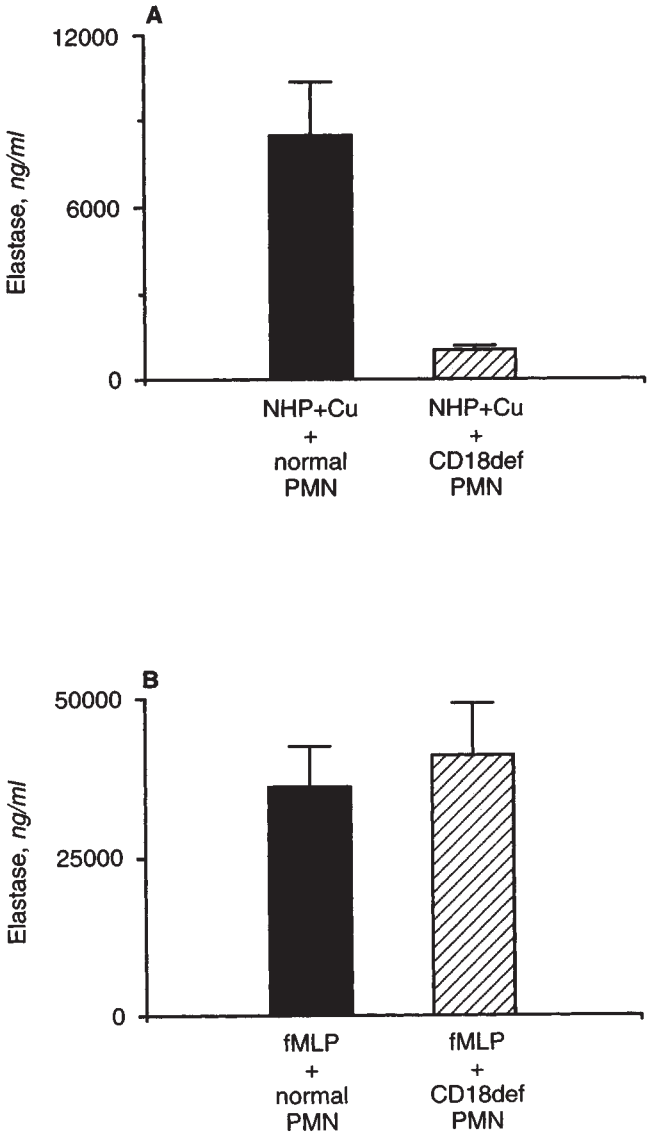

Fig. 9. *Comparison of degranulation by normal and CD18-deficient neutrophils.* **A.** Stimulation with cuprophan membranes (Cu) and NHP. Incubation of normal neutrophils (■) with NHP and Cu induced nine times more elastase release compared to incubation with CD18-deficient cells (▨) ($N = 18$ for normal cells and $N = 10$ for CD18-deficient cells; $P < 0.001$). **B.** Stimulation with fMLP. Stimulation of normal (■) and CD18-deficient cells (▨) with fMLP induced a similar degree of degranulation ($N = 18$ for normal cells and $N = 9$ for CD18-deficient cells; $P > 0.05$). Together, these results indicate that the defect in cuprophan induced degranulation is a consequence of the CD18 deficiency since the neutrophils from the patient with leukocyte adhesion deficiency degranulate normally in response to fMLP.

ligand binding to CR3 (or other β_2 integrins), but the monoclonal antibodies might not block the receptor-ligand interactions. To distinguish between these two possibilities, the degranulating properties of the neutrophils from a patient with leukocyte adhesion deficiency syndrome were studied. The neutrophils from this patient did not express CD18 (Fig. 8).

When CD18-deficient neutrophils were incubated with cuprophan and NHP, the release of elastase was only 11% of that observed when normal cells were studied under the identical conditions ($N = 10$ for CD18-deficient cells and $N = 18$ for normal cells; $P < 0.001$; Fig. 9A). In contrast, the CD18-deficient cells degranulated normally in response to stimulation with fMLP ($N = 9$ for CD18-deficient cells and $N = 18$ for normal cells; $P > 0.05$; Fig. 9B). These data show that the defect in elastase release by the abnormal cells in response to incubation with plasma and cuprophan fibers is specific for CD18-dependent mechanisms and that cuprophan-induced neutrophil degranulation requires one or more members of the β_2-integrin family of receptors.

Discussion

The studies reported here have shown that ligation of β_2 integrins is an essential component of the process by which

neutrophils are induced to degranulate when exposed to cuprophan membrane in the presence of plasma. Further, our studies suggest that both complement-dependent and complement-independent factors participate as counterligands in this process.

Exposure of neutrophils to cuprophan fibers in the absence of plasma stimulated elastase release (Fig. 1). The effect of cellulosic membranes on leukocyte activation in the absence of plasma has also been the subject of previous investigations by others. For example, cellulosic membranes have been reported to stimulate both monocytes to release interleukin-1 and PGE_2 [36, 37] and neutrophils to release oxygen radicals [37]. The effect of cuprophan membrane on neutrophil degranulation in the absence of plasma proteins, however, appears to be very modest (Fig. 1). Addition of plasma to the mixture caused a dramatic increase in degranulation (Fig. 2), demonstrating that both the dialysis membrane and plasma are essential components of the degranulation process.

Two lines of evidence indicate that complement activation is required for maximal degranulation. First, neutrophil degranulation was reduced by 57% (Fig. 3) when complement was inactivated by heating the plasma. Second, degranulation was reduced by 62% when plasma was depleted of C3 (Fig. 4A). A potential mechanism by which complement activation participates in the degranulation process is by providing a ligand through which neutrophils can adhere to the dialysis membrane. Incubation of plasma with cuprophan membrane results in activation of the APC [30, 31]. As a consequence, C3b binds to the membrane and is subsequently converted to iC3b [31] by the concert actions of factor H and factor I. iC3b is the ligand for CR3, a β_2 integrin that is expressed constitutively by neutrophils [6]. Our previous studies have shown that neutrophil adherence to cuprophan membranes is mediated through the interactions of iC3b and CR3 [31].

While both C3a and C5a are generated as consequence of cuprophan induced activation of the APC, two lines of evidence indicate that degranulation is not primarily mediated by these peptides alone. First, cuprophan activated plasma (that contained both C3a and C5a) induced less degranulation than plasma that has not been incubated with cuprophan (Fig. 2). It should be noted, however, that the C3a and C5a that had been generated during the incubation of NHP with cuprophan were likely to have undergone degradation into $C3a_{desArg}$ and $C5a_{desArg}$ by the time the neutrophils were exposed to these peptides, thus potentially diminishing their potency in stimulating the cells. Second, neither C3a nor C5a alone (when used at concentrations that were equivalent to or higher than those generated during incubation of plasma with cuprophan membrane) induced degranulation in the absence of cytochalasin B (Fig. 5). Additional studies, however, suggest that C3a and C5a may have a limited synergistic role in inducing neutrophil degranulation in the presence of heat-inactivated plasma and cuprophan membrane (Fig. 6). The mechanisms that accounts for this augmentation has not been identified, but conceivably the up-regulation of CR3 expression by the anaphylatoxins may contribute to the process.

Depletion of C5 reduces the degranulating capacity of plasma by approximately 25% (Fig. 4B). In addition to the generation of C5a, C5 may contribute to the degranulation process by participating in the formation of the membrane attack complex (C5b-9). While the primary function of the membrane attack complex is to induce cytolysis, compelling evidence indicates that sublytic amounts of C5b-9 induce biological responses in neutrophils [38]. Deppisch and colleagues have reported that during hemodialysis using cuprophan membrane, membrane attack complexes are deposited on neutrophils [4]. Whether C5b-9 complexes participate in the process of neutrophil degranulation induced by dialysis membranes, however, remains to be determined.

When plasma complement activity is eliminated by either heat inactivation or depletion of C3, approximately 40% of the degranulating activity remains. These results indicate that cuprophan membrane-associated neutrophil degranulation can be induced by heat stable constituents of plasma that are not dependent on complement activation. Our previous studies have demonstrated that during incubation with plasma, cuprophan membrane binds IgG [31], and neutrophils constitutively express receptors for the Fc fragment of IgG [6]. Furthermore, phagocytosis of particles opsonized with IgG induces neutrophil degranulation [6]. Accordingly, it seemed possible that the noncomplement, heat stable plasma component involved in the degranulation process was IgG. The results of our experiments, however, demonstrate that interactions between membrane associated IgG and neutrophil Fc receptors do not mediate cuprophan induced degranulation (Fig. 7).

Degranulation is abolished when divalent cations are chelated with EDTA (Fig. 3). Divalent cations can participate in the degranulation process in three ways. First, activation of the APC is Mg^{++} dependent [39]. Second, binding of CR3 to its counterligands is divalent cation dependent [40]. Third, extracellular cations may be involved in the signalling mechanism that induces degranulation (such as, in response to fMLP) [41]. In our present studies, selective chelation of extracellular Ca^{++} (with EGTA) reduces degranulation by 45%. Under these conditions, activation of the APC can still occur because this process requires Mg^{++} but not Ca^{++}. These results imply that the complement independent component of dialysis membrane induced degranulation is, in large part, Ca^{++} dependent. Additional support for this conclusion was provided by experiments which demonstrated that selective chelation of Ca^{++} almost completely abolished the degranulating activity of heat inactivated (that is, complement inactivated) plasma (Fig. 3).

Stimulation of neutrophils with three different endotoxins (*E. coli* 055:B5, *E. coli* 011:B4, and *S. minnesota* at 5 μg/ml) produced minimal effect. Pilot studies using flotation ultracentrifugation further indicated that virtually all of the plasma degranulating activity is associated with nonlipid bearing proteins. Together, these results indicate that neither endotoxin nor plasma lipids contribute significantly to the degranulation process.

When incubated with plasma and cuprophan membrane, neutrophils from a patient with a congenital abnormality in expression of CD18 (Fig. 8) released 90% less elastase than normal granulocytes (Fig. 9A). This defect in degranulation appears to be specific for CD18-dependent mechanisms since the abnormal cells released elastase normally in response to fMLP (Fig. 9B). CD18 is the β chain shared by the three members of the β_2 integrin family of receptors; LFA-1 (CD11a/CD18), CR3 (CD11b/CD18), and p150,95 (CD11c/CD18). CR3, however, is the predominant β_2 integrin that is present on the

neutrophil surface [6]. That heat inactivation of plasma complement or immunochemical depletion of C3 reduces elastase release substantially (approximately 60%) suggests that interactions between CR3 and iC3b are an important component of the degranulation process.

Degranulation does occur, however, in the absence of complement activation. That observation suggests that CR3 also interacts with noncomplement plasma factors. The identity of these complement independent ligands remains to be elucidated. Studies by others have shown that fibrinogen [42], fibronectin [43], serum amyloid P component [43] and coagulation factor X [44] bind to CR3, but whether these proteins contribute to hemodialysis associated degranulation remains to be determined.

The mechanism that induces the degranulation process has not been precisely identified. Conceivably, ligation of CR3 by C3-related peptides and noncomplement plasma proteins directly transduces the signal that mediates degranulation. Alternatively, CR3 mediated adhesion may be necessary but not sufficient for degranulation to occur. According to this concept, adhesion of the neutrophils to the membrane is mediated by CR3, but the signal for degranulation is induced through interactions between another neutrophil receptor and its counterligand. Further studies are required to distinguish between these possibilities.

Ward and colleagues have presented evidence implicating complement activation in the degranulation process during hemodialysis in an ex vivo model [45]. They found that single passage of whole blood through cuprophan membrane elicited activation of C3 and degranulation of neutrophils. Plasma $C3a_{(desArg)}$ level correlated with the release of neutrophil elastase, although the magnitude of response of the neutrophils varied widely among the blood donors. On the other hand, the studies of Horl, Heidland, and colleagues have suggested that the degranulation process is not necessarily complement dependent [13–16]. Our results are consistent with the hypothesis that both complement dependent and complement independent mechanisms participate in the induction of degranulation. Thus, the data reported herein appear to reconcile the apparent discrepancies between the studies of the two groups cited above. Dialysis membranes that have a high avidity for the noncomplement factors may be expected to induce significant neutrophil degranulation even if they lack the capacity to activate complement.

Acknowledgments

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Appendix. Abbreviations

APC	Alternative pathway of complement
BSA	Bovine serum albumin
CR3	Complement receptor type 3
EDTA	Ethylenediamine tetraacetate
EGTA	Ethyleneglycol tetraacetate
fMLP	Formyl-methionyl-leucyl-phenylalanine
HBSS/A	HBSS containing human serum albumin
NHP	Normal human plasma
HIP	Heat inactivated plasma
PBS	Phosphate buffered saline
PMA	Phorbol myristate acetate

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