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Type 1 Fimbriate *Escherichia coli* Stimulates a Unique Pattern of Degranulation by Human Polymorphonuclear Leukocytes

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Uropathogenic strains of *Escherichia coli* bearing mannose-sensitive (type 1) fimbriae promote a unique pattern of degranulation from human polymorphonuclear leukocytes (PMN). Significant quantities of the primary (1°) and tertiary (3°) granule markers, neutral protease-myeloperoxidase and *N*-acetyl-β-D-glucosaminidase, respectively, were released by PMN in a dose- and time-dependent manner when stimulated by these defined bacterial strains. Organisms bearing mannose-resistant (P) fimbriae promoted release of only the secondary (2°) granule marker, vitamin B_{12}-binding protein. When this pattern of degranulation was compared to that produced by PMN in response to a variety of soluble and particulate stimuli, only the calcium ionophore A23187 similarly triggered 1° and 3° granule marker release. All the other stimuli tested—zymosan, serum-treated and unopsonized; *n*-formylmethionyl-leucyl-phenylalanine; and phorbol myristate acetate—promoted release of only the 2° granule marker. These results demonstrate selectivity of PMN degranulation in response to a number of transmembrane signals. In addition, the capacity of *E. coli* to promote PMN degranulation is dependent on its phenotypic fimbrial expression, a surface characteristic which correlates significantly with its relative surface hydrophobicity as measured by binding to octyl Sepharose. Those bacteria demonstrating the greatest hydrophobicity were capable of triggering discharge of all three granule marker proteins. Thus, the mannose-sensitive fimbriae of uropathogenic *E. coli* may contribute significantly to their potential pathophysiologic role in renal scarring.

Chronic pyelonephritis is characterized by progressive renal scarring. Recent evidence obtained by using animal models has demonstrated that this scarring is directly proportional to the magnitude of the initial inflammatory response, which in turn is dependent on the interaction of invading bacteria with host inflammatory cells (15, 34, 36). *Escherichia coli* strains isolated from patients with urinary tract infections possess a higher frequency of defined virulence markers than do strains which compose the normal fecal flora. These urinary tract pathogens express a restricted range of O serotypes (30), resist the killing effects of serum (20), and may elaborate alpha-hemolysin (6). After isolation and subculture, these organisms usually express surface fimbriae. Type 1, mannose-sensitive (MS) fimbriae promote the mannose-dependent hemagglutination of guinea pig erythrocytes (27, 37), whereas mannose-resistant (MR) fimbriae, of which P fimbriae are a subgroup (41), promote the mannose-independent hemagglutination of human erythrocytes. Although both fimbrial types increase adherence to uroepithelial cells (7, 22), type 1 (MS) fimbriae also mediate adherence to and phagocytosis by human neutrophils (polymorphonuclear leukocytes [PMN]) (4), and there is a direct and highly significant correlation between the possession of type 1 (MS) fimbriae, relative bacterial surface hydrophobicity, and the capacity of the organism to activate the PMN respiratory burst (19).

The activation of phagocytic cells by particulate stimuli occurs as a result of either specific receptor-ligand binding (26) or a receptor-independent physicochemical interaction of a particle with the cell surface (42). This stimulus-cell interaction has been characterized in terms of respiratory burst activation (18), the release of intracellular proteins (3), and the generation of pro-inflammatory mediators (44). There is considerable evidence that the reactive oxygen metabolites and proteases released by phagocytic cells during this inflammatory response can contribute directly to tissue damage (13, 14, 23). The human PMN has a well-defined population of intracellular enzymes which are released from at least two distinct classes of cytoplasmic granule in response to particulate or soluble stimuli (3, 35). The primary (1°) (azurophil) granule contains a number of acid hydrolases and neutral proteases (such as elastase), as well as myeloperoxidase (MPO). These enzymes are discharged into the phagocytic vacuole and are believed to be responsible for microbial killing through the action of the various proteases and by the generation of hydrogen peroxide and toxic oxygen radicals (14). The secondary (2°) (specific) granule contains a number of proteins, which include lysozyme, lactoferrin, and vitamin B_{12}-binding proteins, as well as neutral proteases such as collagenase. Few of these molecules have any defined antimicrobial activity, but they may be secreted and function extracellularly to limit chemotaxis and increase PMN adhesiveness (16, 31). Isolated 2° granule protein release has been demonstrated after activation of cells by a variety of ligands (35), emphasizing the nonspecific, secretory nature of 2° granule release. A peroxidase-negative tertiary (3°) granule containing *N*-acetyl-β-D-glucosaminidase, β-galactosidase, and β-glucuronidase has also been described (35), but its function has not been defined in detail. Few studies have analyzed the degree

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of participation of each granule during degranulation in response to either phagocytic or chemical stimulation (3, 32, 46).

The present study investigates the capacity of unopsonized uropathogenic strains of *E. coli* to trigger PMN degranulation and correlates this capacity with the phenotypic expression of fimbrial type. In addition, the unique pattern of degranulation induced by certain of these strains of *E. coli* is compared with the pattern of degranulation produced by PMN stimulated with a variety of reference compounds.

MATERIALS AND METHODS

Preparation of human neutrophils. Normal human leukocytes were isolated from citrated peripheral blood by dextran sedimentation and were rendered plasma-free and platelet-poor by being washed with phosphate-buffered saline without calcium or magnesium, pH 7.3. PMN were purified by density gradient centrifugation at 400 × *g* for 35 min at 23°C on Ficoll-Paque (Pharmacia Ltd., Milton Keynes, United Kingdom). The PMN were counted in a modified Neubauer counting chamber after hypotonic lysis of the erythrocytes. The cell preparations were judged to be 98% PMN on the basis of their morphology after examination of Wright-stained centrifuged preparations (Cytospin II; Shandon Southern Products, Runcorn, Cheshire, United Kingdom).

Preparation of reference stimuli. Unless stated otherwise, all chemicals were purchased from Sigma Chemical Co. Ltd., Poole, Dorset, United Kingdom. The calcium ionophore A23187 (Cambridge Bioscience, Cambridge, United Kingdom), *n*-formylmethionyl-leucyl-phenylalanine, and phorbol myristate acetate (PMA) were stored at 10 mM concentrations in dimethyl sulfoxide at −70°C and diluted to an appropriate concentration in Krebs Ringer phosphate buffer (pH 7.4) containing 0.54 mM Ca^{2+}, 1.2 mM Mg^{2+}, and 11.0 mM D-glucose (KRPG) immediately before use. Zymosan A (500 mg) was boiled for 20 min in 50 ml of 0.9% saline, washed, and resuspended to a final concentration of 10 mg/ml (5 × 10^8 particles per ml). Serum-treated zymosan was prepared by suspending 20 mg of boiled zymosan in 1 ml of KRPG, adding 3 ml of fresh pooled human serum, and incubating this mixture with continuous mixing for 30 min at 37°C. The serum-treated zymosan was then washed three times, suspended to a volume of 4 ml in KRPG, and stored at −20°C.

Bacterial strains. Eleven uropathogenic isolates of *E. coli* were studied. Each was serially subcultured for 18 h either in nutrient broth (Oxoid No. 2) or on nutrient agar (Oxoid Ltd., Basingstoke, United Kingdom) at least three times to facilitate the maximal expression of a required fimbrial type. Bacteria were harvested by centrifugation, washed twice, and suspended to an optical density of 2.0 at 560 nm in a spectrophotometer (Unicam SP500 series 2; Pye Unicam, Cambridge, United Kingdom) phosphate-buffered saline, pH 7.3 (equivalent to 10^9 CFU/ml).

The hemagglutination of guinea pig or human erythrocytes by each bacterial strain was tested immediately prior to their use in order to assess their fimbrial expression (Table 1). Fimbriation was confirmed by transmission electron microscopy (17).

Bacterial phagocytosis. Purified human PMN were washed and resuspended in RPMI 1640 (GIBCO Ltd., Paisley, Scotland) containing 0.2% (wt/vol) bovine serum albumin at a concentration of 3 × 10^6 cells per ml. One-milliliter portions of the suspension were layered onto 35-mm tissue culture plates, incubated at 37°C in 5% CO_2 for 30 min, and then washed three times with 2 ml of RPMI 1640 (containing 5 mM $MgCl_2$). More than 97% of the adherent cells were identified as PMN on the basis of their morphology.

The PMN monolayers were then incubated for 20 min with different strains of *E. coli* suspended to a concentration of 4 × 10^8 bacteria per ml in RPMI 1640 containing $MgCl_2$. The monolayers were then washed three times with phosphate-buffered saline (pH 7.3), air dried, fixed, and stained with Wright's stain. The percentage of cells ingesting five or more bacteria was estimated by light microscopy. At least 200 PMN per plate were counted.

Measurement of bacterial hydrophobicity. Bacterial surface hydrophobicity was assessed by modified hydrophobic interaction chromatography (N. Topley, R. Steadman, R. K. Mackenzie, J. D. Williams, M. Davies, and A. W. Asscher, Rev. Infect. Dis., in press). Briefly, Octyl Sepharose CL4B columns (15 by 5 mm) (Pharmacia) were prepared, equilibrated with 15 volumes of 0.25 M ammonium sulfate in 10 mM sodium phosphate buffer, pH 6.8 (ASP buffer), and stored at 4°C until required. Samples of bacteria (optical density at 560 nm = 1.0) (100 μl) were diluted 1:10 in ASP buffer, and 500 μl of this dilution was loaded onto the column and eluted with 2 ml of ASP buffer. Bacterial ATP was extracted from eluted and from control bacterial suspensions and measured by using the firefly bioluminescence assay system (Topley et al., in press) in a Lumac M2010 biocounter (Lumac/3MbV, Schaesberg, The Netherlands). The degree of binding of bacteria to the Octyl Sepharose was then calculated and expressed as percent hydrophobicity.

Protein release experiments. One hundred-microliter volumes of KRPG containing 5 × 10^5 PMN were diluted with 300 μl of KRPG and preincubated for 5 min at 37°C before the addition of stimulus in KRPG (100 μl). Triplicate samples were incubated with 100 μl of each stimulus at the appropriate concentration for periods of up to 60 min and separated by centrifugation for 1 min at 11,000 × *g* in a Beckman Microfuge B (Beckman RIIC Ltd., High Wycombe, Buck-

TABLE 1. Surface characteristics of individual *E. coli* strains

E. coli strain	O serotype[a]	Phenotypic hemagglutination[b]		P-fimbrial expression[c]	% Binding to octyl Sepharose[d]
		MS	MR		
504	O6	+++	−	−	76.8 ± 4.5
12	O4	+++	−	−	71.1 ± 3.5
ER_2	O4	−	++	+	33.8 ± 8.6
KV	NT	++	−	−	70.5 ± 3.4
AB	NT	−	−	−	28.8 ± 6.7
49	O8	+++	−	−	84.7 ± 6.2
NK_1	O4	−	++	+	21.2 ± 8.2
SC	O1	−	++	+	13.9 ± 6.1
168	O4	+++	−	−	84.6 ± 6.4
103	AA	+++	−	−	N/A
63	NT	−	+	+	6.8 ± 2.3

[a] Abbreviations: NT, not typable (smooth but not one of the following O serotypes: 1, 2, 4, 5, 6, 7, 8, 9, 11, 17, 18, 25, or 75); AA, autoagglutinable.

[b] Hemagglutination of individual *E. coli* strains on the day of the experiment. MS represents the capacity of D-mannose (2.5%, wt/vol) to inhibit the agglutination of guinea pig erythrocytes by MS fimbriate organisms; MR represents the mannose-resistant agglutination of human erythrocytes by MR fimbriate organisms.

[c] P-fimbriation was assessed by the receptor-specific particle agglutination test for *E. coli* (BACH-test, KabiVitrum, Stockholm, Sweden) of strains grown on nutrient agar (39).

[d] Results are expressed as the means ± standard deviations for at least three separate determinations.

inghamshire, United Kingdom), and the supernatant was removed for the enzyme assays. Controls, which were performed for each experiment, consisted of supernatant from (i) unstimulated cells without incubation, (ii) cells incubated in KRPG for periods of up to 60 min without stimulation, (iii) stimuli incubated in KRPG without PMN, and (iv) a KRPG blank incubated without PMN or stimulus. Each stimulus was used in three separate experiments with cells from three different donors.

Enzyme assays. The percent release of enzymes from the PMN was calculated, after subtraction of the appropriate blank values, as a percentage of that released from cells disrupted by sonication, for two 1-min periods at an 8-μm peak-to-peak distance at 4°C in a 150-W ultrasonic disintegrator (Measuring and Scientific Equipment, Ltd., Crawley, United Kingdom). This form of disruption gave reproducible results for the maximal levels of total intracellular enzyme activities measured when compared with freeze-thawing, hypotonic lysis, and Triton X-100 extraction (data not shown).

Control incubations. Granule marker release by PMN in response to *E. coli* was compared in all cases with the appropriate control cells incubated in buffer alone. There was no detectable enzyme activity when *E. coli* strains were incubated without PMN in KRPG for 60 min at 37°C.

In all experiments, <5% of the cytoplasmic marker lactic dehydrogenase was released, indicating that most PMN remained intact throughout the 60 min of stimulation.

MPO. One hundred microliters of supernatant from each stimulation was incubated at 30°C with 1 ml of substrate consisting of 0.3 mM *o*-dianisidine, 0.03% (vol/vol) H_2O_2, and 0.05% (vol/vol) Triton X-100 (BDH Ltd., Poole, Dorset, United Kingdom) in 0.1 M citric acid (pH 5.5). The reaction was stopped after 5 min with 1 ml of 3.25 M perchloric acid. The A_{560} was measured in a spectrophotometer (Cecil CE 292; Cecil Instruments Ltd., Cambridge, United Kingdom).

Neutral protease. Fifty microliters of supernatant was added to 50 μl (0.1 mg) of [^{3}H]casein (0.41 μCi/mg) (21) in phosphate buffer (pH 7.0) containing 1 mM $CaCl_2$ and incubated for 16 h at 37°C. Ice-cold 11% (wt/vol) trichloroacetic acid (BDH Ltd.) was added. After incubation for 30 min at 4°C, the nonhydrolyzed casein was precipitated by centrifugation at 11,000 × *g* for 5 min.

One hundred and seventy-five microliters of supernatant containing the hydrolyzed product was then assayed for released radioactivity by addition to 4 ml of scintillant (Optiphase MP; LKB Instruments Ltd., Croydon, United Kingdom) and counted in an LKB Rackbeta scintillation counter for 1 min.

***N*-Acetyl β-D-glucosaminidase.** One hundred microliters of supernatant and 100 μl of substrate (0.2 mM 4-methylumbelliferyl *N*-acetyl β-D-glucosamine [Koch Light Ltd., Havehill, United Kingdom] in 0.1 M sodium citrate-phosphate buffer, pH 4.3) were incubated for 16 h at 37°C. The reaction was stopped by the addition of 2 ml of 5 mM EDTA (BDH Ltd.) in 50 mM glycine buffer, pH 10.4. Fluorescence emission at 448 nm was measured with excitation at 360 nm in an Aminco Bowman spectrophotofluorimeter (American Instrument Co. Inc., Silver Spring, Md.).

Vitamin B_{12}-binding protein. One hundred microliters of supernatant and 250 μl of [^{57}Co]cyanocobalamin (4.44 ng/ml in water, 0.067 μCi/ml) (Amersham International plc, Cardiff, United Kingdom) were incubated at room temperature for 30 min. One milliliter of activated charcoal (Norit GSX; BDH Ltd.) (5%, wt/vol) coated with 1% (wt/vol) bovine serum albumin was added and left at room temperature for 10 min before centrifugation for 10 min at 2,500 × *g* to precipitate charcoal-adsorbed, non-protein-bound vitamin B_{12}. One milliliter of supernatant was counted for 1 min in a gamma counter (Kontron Instruments, St. Albans, United Kingdom).

Lactate dehydrogenase. Three hundred microliters of supernatant was added to 200 μl of 10 mM β-NADH in 2.4 ml of 0.1 M phosphate buffer, pH 7.4. The reaction was started with 100 μl of potassium pyruvate (1 mg/ml), and the change in extinctions at 340 nm (E_{340}) was measured over 4 min in a Cecil CE292 spectrophotometer by using the reaction rate calculator.

Gelatin-degrading activity. One hundred microliters of supernatant was incubated at 37°C with 50 μl of 0.4 M Tris hydrochloride, pH 7.5 (containing 10 mM $CaCl_2$), and with 100 μl of [^{14}C]gelatin (1 mg/ml, 0.045 μCi/mg) from denatured interstitial rat skin collagen (95% type I, 5% type III) (5). Fifty microliters of ice-cold 100% (wt/vol) trichloroacetic acid was then added and incubated at 4°C for a further 30 min. The insoluble protein was pelleted in a Beckman Microfuge at 11,000 × *g* for 10 min. One hundred and fifty microliters of the supernatant was then dissolved in 4 ml of Optiphase MP (LKB Ltd.) and counted in an LKB Rackbeta scintillation counter for 1 min.

Elastase activity. One hundred microliters of supernatant was added to 100 μl of insoluble [^{3}H]elastin (25 μg/ml; 0.05 μCi/mg) (40) in 0.1 M Tris hydrochloride, pH 8.2, containing 0.1% (vol/vol) Triton X-100 and incubated at 40°C for 16 h. Nondegraded elastin was precipitated by centrifugation in a Beckman Microfuge at 11,000 × *g* for 10 min, and 100 μl of the supernatant was dissolved in 4 ml of Optiphase MP scintillant and counted in an LKB Rackbeta scintillation counter for 1 min.

Trasylol binding. Five hundred microliters of supernatant from sonicated cells or from cells stimulated by *E. coli* 504 was mixed with 0.5 ml of 0.05 M Tris hydrochloride buffer, pH 8.3, containing the serine protease inhibitor, Trasylol (Bayer Pharmaceuticals Ltd., Haywards Heath, United Kingdom), covalently bound to Sepharose beads (2). The suspension was incubated with occasional mixing for 30 min and then was centrifuged for 1 min at 11,000 × *g*. The supernatant was assayed for activity against [^{3}H]elastin. After being washed in 0.05 M Tris hydrochloride buffer, pH 8.3, the pellet was suspended in 1 ml of 0.05 M sodium acetate buffer, pH 5.0, and incubated at 4°C for 30 min. The mixture was then centrifuged for 1 min at 11,000 × *g*, and the activity against [^{3}H]elastin released into the supernatant was assayed.

Neutral protease inhibition. The serine protease inhibitor phenylmethylsulfonyl fluoride (PMSF) was stored at 4°C in propan-2-ol at a concentration of 500 mM and was used in the enzyme assays at a concentration of 5 mM. EDTA (100 mM) was used in the enzyme assays to inhibit metalloproteases at a concentration of 5 mM. *p*-Aminophenylmercuric acetate (PAMA) was prepared to a concentration of 10 mM in 0.05 M NaOH and used at 1 mM in the enzyme assays.

Statistics. All data in this paper was analyzed by using Student's *t* test with Bessell's correction unless otherwise stated in the text.

RESULTS

PMN granule marker release in response to bacterial stimulation. Stimulation of 5 × 10^5 human PMN by 10 different strains of *E. coli* of defined fimbrial type (Table 1) resulted in the release of granule marker enzymes in a dose- (Fig. 1) and

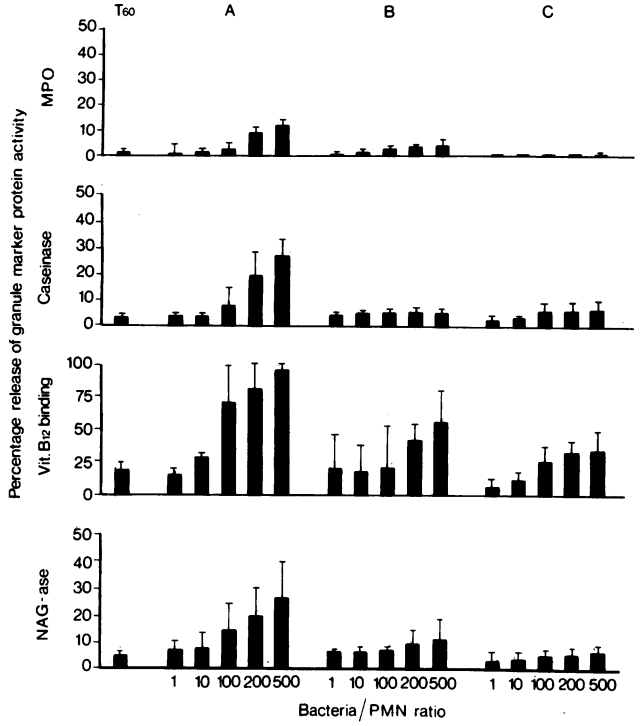

FIG. 1. Dose-dependent effect of *E. coli* on the capacity of 5×10^5 human PMN to release granule marker activity over a 60-min period. Three representative strains of *E. coli* are shown: MS fimbriate strain 504 (A), MR(P) fimbriate strain SC (B), and nonfimbriate strain AB (C). Data are expressed as the means ± standard deviations for three separate experiments with each strain using PMN from different donors. Release of granule markers from unstimulated cells was measured over a 60-min period (T_{60}).

time- (Fig. 2) dependent manner. Those strains possessing type 1 (MS) fimbriae ($n = 6$) caused significant release of marker enzymes from all three PMN granules (1° granule marker, MPO [$t = 9.4$, $P < 0.0005$] and neutral protease activity, measured as [^{3}H]casein degradation ($t = 12.0$, $P < 0.0005$); 2° granule marker, vitamin B_{12}-binding protein [$t = 7.6$, $P < 0.0005$]; 3° granule marker, *N*-acetyl β-D-glucosaminidase [$t = 4.12$, $P < 0.001$]). Those strains possessing MR(P) fimbriae alone promoted significant release of only the 2° granule marker ($t = 1.88$, $P < 0.05$, $n = 4$). MS fimbriate strains caused significantly greater release of all three granule markers compared with release by MR fimbriate strains (1°, $t = 4.11$, $P < 0.005$; 2°, $t = 2.45$, $P < 0.025$; 3°, $t = 2.48$, $P < 0.025$). Strains of *E. coli* bearing both type 1 (MS) and MR(P) fimbriae showed a pattern of release identical to that of bacteria bearing type 1 (MS) fimbriae alone (data not shown).

Bacterial phagocytosis by PMN monolayers. Five strains of *E. coli* bearing type 1 (MS) fimbriae alone were phagocytosed by between 33 and 100% in two separate experiments. In contrast, four strains expressing MR(P) fimbriae alone were phagocytosed by only 2 to 21% of PMN. One strain expressing only type 1 (MS) fimbriae was not phagocytosed, despite attachment, in either experiment.

PMN degranulation in response to stimulation with reference compounds. In a series of experiments under optimum conditions of dose and time using a variety of particulate and soluble compounds to stimulate PMN from several different donors, only the calcium ionophore A23187 at concentrations above 0.5 μM caused significant release of all granule marker proteins (1°, $t = 1.90$, $P < 0.05$; 2°, $t = 5.68$, $P < 0.005$; 3°, $t = 3.55$, $P < 0.05$). Other compounds stimulated significant release of only the 2° granule marker (Fig. 3).

Neutral protease release from human PMN. Type 1 (MS) fimbriate *E. coli* 504 and the calcium ionophore A23187 caused significant release of proteolytic activity against [^{3}H]casein ($P < 0.05$), [^{3}H]elastin ($P < 0.05$), and [^{14}C] gelatin ($P < 0.02$) (Fig. 4). All the other stimulants examined, except the MR(P) fimbriate *E. coli* SC, caused release of significant quantities of only [^{14}C]gelatin-degrading activity. This MR fimbriate organism caused release of significant quantities of vitamin B_{12}-binding protein alone (Fig. 1) in the absence of release of gelatinase activity (Fig. 4).

The activity released against [^{3}H]casein and [^{3}H]elastin from PMN stimulated with *E. coli* 504 or from sonicated PMN was totally inhibited by 5 mM PMSF, a serine protease inhibitor, but not by 5 mM EDTA, an inhibitor of metalloproteases (Fig. 5). Conversely, [^{14}C]gelatin-degrading activity released by PMN was totally inhibited by 5 mM EDTA but not by 5 mM PMSF. The inclusion of 1 mM PAMA together with 5 mM PMSF did not increase the amount of casein-, elastin-, or gelatin-degrading activity, indicating that a metalloprotease with activity against these substrates was not present in a latent form.

The release of a serine protease active against [^{3}H]elastin by PMN stimulated with *E. coli* 504 was confirmed by using Trasylol covalently linked to Sepharose beads. In three experiments, 95.5 ± 3% (mean ± standard deviation) of the activity released from PMN stimulated by *E. coli* 504 was bound to the Trasylol, of which 77 ± 12% was recovered by elution with 0.05 M sodium acetate, pH 5.0. After sonication of control cells, 93 ± 3% of the elastase activity was bound by Trasylol, of which 97 ± 4% was recovered after elution at pH 5.0.

Bacterial hydrophobicity. *E. coli* strains which demon-

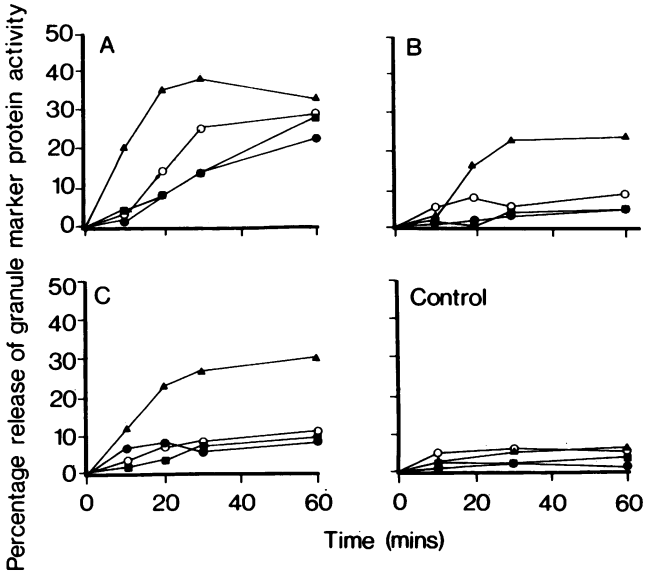

FIG. 2. Time course of release of granule marker activity (MPO [●], neutral proteinase [■], vitamin B_{12}-binding protein [▲], and *N*-acetyl β-D-glucosaminidase [○]) from 5×10^5 human PMN after incubation with: (A) MS fimbriate strain 504, (B) MR(P) fimbriate strain SC, and (C) nonfimbriate strain AB at a bacterium-to-cell ratio of 200:1. Release of granule marker protein activity from unstimulated cells was measured for times up to 60 min. Data expressed are for triplicate samples in a single representative experiment.

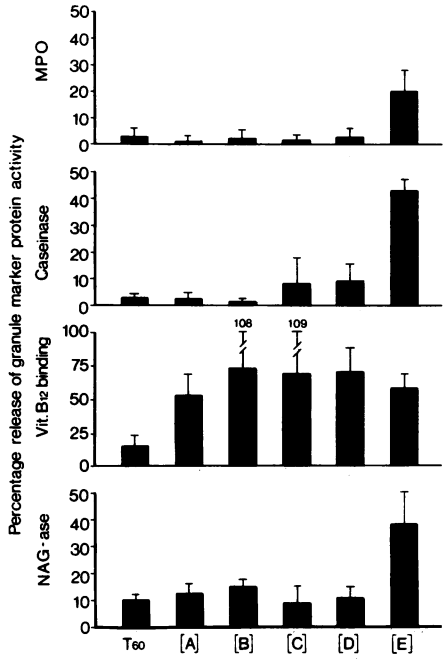

FIG. 3. Granule marker activity released from 5×10^5 human PMN after incubation for 60 min with: (A) zymosan (5 mg/ml), (B) serum-treated zymosan (5 mg/ml), (C) PMA (1 μM), (D) *n*-formyl-methionyl-leucyl-phenylalanine (10 μM), or (E) calcium ionophore A23187 (5 μM). Release of marker enzymes from unstimulated cells was measured over a 60-min period (T_{60}). Data expressed are the means ± standard deviations for three separate experiments for each ligand using PMN from different donors. NAG-ase, *N*-Acetyl β-D-glucosaminidase.

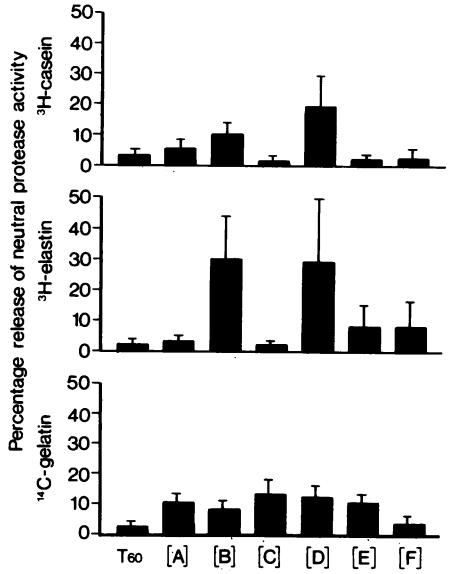

FIG. 4. Neutral protease activity (expressed as a percentage of the total activity released by cell sonication against [^{3}H]casein, [^{3}H]elastin, and [^{14}C]gelatin) released from 5×10^5 human PMN after incubation for 60 min with: (A) PMA (1 μM), (B) A23187 (5 μM), (C) zymosan (5 mg/ml), (D) MS fimbriate *E. coli* 504, (E) nonfimbriate *E. coli* AB, and (F) MR(P) fimbriate *E. coli* SC. Each organism was incubated at a bacterium-to-cell ratio of 200:1. Release of neutral protease activity from unstimulated cells was measured over a 60-min period (T_{60}). Data expressed are the means ± standard deviations for three separate experiments with each ligand using PMN from different donors.

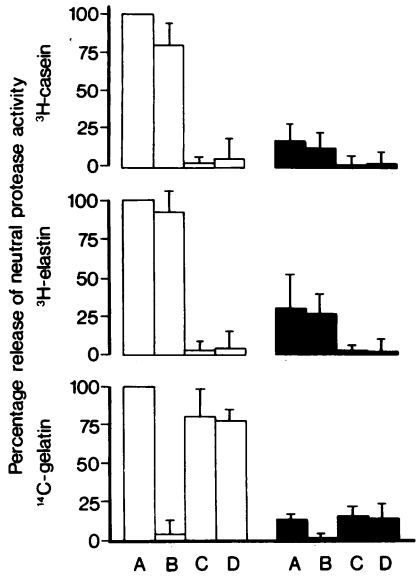

FIG. 5. Release of neutral protease activity (expressed as a percentage of total activity released by cell sonication against [^{3}H]casein, [^{3}H]elastin, and [^{14}C]gelatin) from 5×10^5 human PMN after sonication (□) and after 60 min of incubation with MS fimbriate *E. coli* 504 (■). (A) Total enzymatic activity; (B) activity in the presence of 5 mM EDTA; (C) enzymatic activity in the presence of 5 mM PMSF; (D) enzymatic activity in the presence of 5 mM PMSF and 1 mM PAMA. Results expressed are the means ± standard deviations for three experiments using PMN from different donors.

strated >50% binding to Octyl Sepharose columns (the most hydrophobic strains) stimulated significantly greater release of all the granule proteins than did those strains which bound less avidly to the column (<50%) (MPO and caseinase, $P < 0.005$; vitamin B_{12}-binding protein, $P < 0.05$; *N*-acetyl β-D-glucosaminidase, $P < 0.005$) (Fig. 6).

DISCUSSION

Human PMN challenged with defined strains of *E. coli* released significant amounts of 1° (elastase and MPO) and 3° (*N*-acetyl β-D-glucosaminidase) granule marker proteins in a time- and dose-dependent manner only when the strains possessed surface MS (type 1) fimbriae. This pattern of degranulation occurred whether these surface structures were expressed alone or in conjunction with MR(P) fimbriae. In contrast, those strains bearing MR(P) fimbriae alone did not trigger significant release of 1° and 3° granule markers. All strains examined, including nonfimbriate *E. coli*, stimulated release of the 2° granule marker, vitamin B_{12}-binding protein.

When the pattern of degranulation initiated by type 1 (MS) fimbriate strains was compared to that produced by PMN in response to a variety of soluble and particulate stimuli, only concentrations of the calcium ionophore A23187 above 0.5 μM similarly triggered 1° and 3° granule marker release. All the other stimuli tested triggered release of the 2° granule marker, vitamin B_{12}-binding protein, as well as gelatinase activity. The variety of stimuli examined were selected for their capacity to activate cells via a number of different transmembrane signals. *n*-Formylmethionyl-leucyl-phenylalanine and zymosan (either opsonized or unopsonized) are known to interact with different receptors or recognition mechanisms on the cell surface (28, 33, 45), and the chemotactic tripeptide is a potent stimulus of phospholipase C (28).

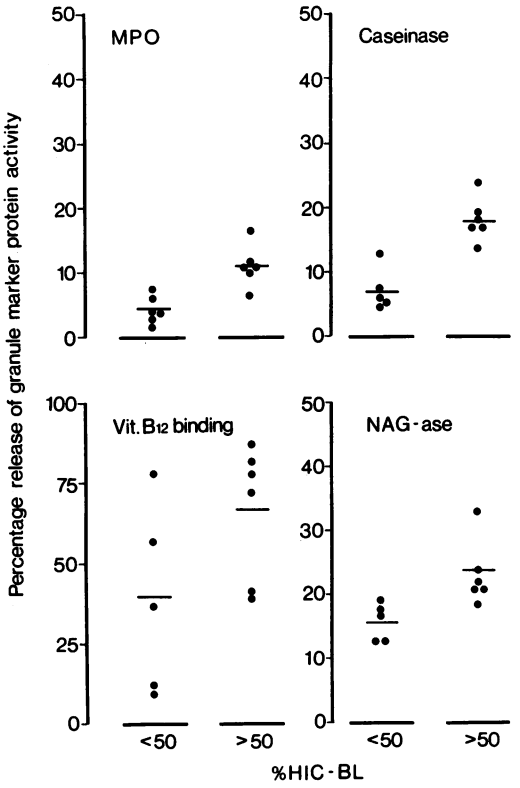

FIG. 6. Correlation of the release of granule marker activity from 5×10^5 human PMN with bacterial surface hydrophobicity expressed as percent binding to octyl Sepharose (HIC-BL). Each datum point represents the mean of triplicate samples from three separate experiments using PMN from different donors.

PMA directly activates membrane-bound protein kinase C (8), and the calcium ionophore A23187 acts nonspecifically by promoting the influx of calcium into the cell (12). These soluble stimuli directly or indirectly affect the movement of calcium into or within the cell, whereas zymosan has been shown to induce a calcium-dependent response only when opsonized with immunoglobulin G (25). All these stimuli activate the PMN respiratory burst to various extents (18). In terms of degranulation, however, only high concentrations of A23187 were capable of mobilizing all the granule markers measured, whereas at concentrations below 0.5 μM only release of the 2° granule marker was detected. Thus, even in the presence of receptor-ligand interaction or with the activation of phospholipase C or of protein kinase C, comprehensive degranulation does not necessarily occur, emphasizing the unique nature of the activation of human PMN by type 1 (MS) fimbriate *E. coli* strains.

In addition, the phagocytosis of *E. coli* strains expressing type 1 (MS) fimbriae by PMN monolayers was significantly greater than that of MR(P) fimbriate strains. However, a single type 1 (MS) fimbriate organism consistently resisted phagocytosis despite attachment to the PMN and caused a degree of degranulation similar to that of the other type 1 (MS) fimbriate organisms studied. This finding, coupled with the observation that phagocytosis of zymosan (whether serum treated or unopsonized) results in the release of only the 2° granule marker, indicates that comprehensive PMN degranulation is not simply a response to phagocytosis per se but occurs as the result of a specific surface interaction with the MS fimbriate organism.

Latent gelatinase activation could not be induced by treatment with 1 mM PAMA, an organic mercurial compound generally used to activate latent metalloproteases. Although neutral proteases are distributed among several intracellular compartments, activity against [^{3}H]elastin is confined to the specific serine protease (elastase) residing in the 1° granule (11), whereas gelatin-degrading activity may be located in a separate secretory compartment (10). By selective inhibition of released proteases with PMSF and EDTA and by their degree of binding to Trasylol (covalently bound to Sepharose beads), the contribution of either serine or metalloproteases to the total neutral protease activity (measured as activity against [^{3}H]casein) was estimated. Caseinase activity was totally inhibited by PMSF and could not be increased by treatment with PAMA, confirming that most of the neutral protease activity released by the PMN was due to serine proteases. Concomitant inhibition of specific activity against [^{3}H]elastin was also demonstrated and confirmed the release of elastase, which could be bound by Sepharose-bound Trasylol. The percentage of elastase released correlated more closely with MPO release than did total [^{3}H]casein activity, confirming that activity against [^{3}H]elastin was a true marker of 1° granule release. All stimuli tested, except the MR fimbriate *E. coli* SC, caused release of gelatinase activity, which was inhibitable by EDTA but not by PMSF, suggesting that a metalloprotease was released independently of the 1° and 3° granules. This protease was not released after stimulation by two other MR fimbriate *E. coli* strains despite a significant release of vitamin B_{12}-binding protein. This fact supports the hypothesis that this enzyme may reside in a separate, as yet undefined, secretory compartment (10).

The degranulation response of the PMN has been shown in vitro to be capable of degrading a variety of biological substrates (9, 24, 29) through the activity of the released proteases. Of the enzymes released, the neutral serine protease, elastase, is considered to be potentially the most likely protease to cause in vivo tissue damage (8, 21, 29). In the present study, we have demonstrated that PMN degranulation stimulated by defined *E. coli* strains appears to be dependent on the phenotypic fimbrial expression of the stimulating strain, that phagocytosis is not essential for extracellular marker enzyme release, and that there is a significant correlation between relative bacterial surface hydrophobicity and the pattern of PMN degranulation. We have previously discussed the importance of possession of MS fimbriae and increased relative surface hydrophobicity as virulence factors in the initiation of renal scarring by *E. coli* (Topley et al., in press). That possession of such bacterial properties also causes a unique pattern of human PMN degranulation, particularly of the potentially harmful 1° granule products, provides further evidence that MS fimbriate *E. coli* may have a causative role in the initiation of the tissue damage which precedes renal scarring.

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