

Role of neutrophils in mediating human epithelial cell detachment from native basement membrane

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Epithelial cell detachment from underlying basement membrane is a feature of diseases of many organs. In the lungs it is seen in disorders as diverse as bronchiectasis, allograft rejection, and asthma. The potential for different leukocytes to induce this change is not clear. In asthma both eosinophils and neutrophils are found in affected tissues, but the capacity of each of these types of cells to induce detachment of native epithelial cells from basement membrane requires clarification. Although eosinophils damage rather than detach human epithelial cells, the effects of neutrophils on epithelial cells naturally attached to basement membrane have not previously been described. Using the human amnion in vitro model, we tested the hypothesis that neutrophils have the capacity to detach intact human epithelial cells from basement membrane. The data indicate that increasing concentrations of neutrophils are able to detach epithelial cells from their underlying basement membrane. Detachment was increased when the neutrophils were activated in situ with tetradecanoyl phorbol acetate and after longer incubation periods. Platelet activating factor and opsonized zymosan showed similar boosting effects, whereas activated complement and formyl-methyl-leucyl-phenylalanine did not. Physical contact of the neutrophils with the epithelial cells was required to induce detachment. Detachment could be inhibited by glutathione and by soybean trypsin inhibitor, an inhibition pattern similar to cathepsin G and trypsin, but not collagenase, in this system. We conclude that neutrophils are capable of detaching human epithelial cells from basement membrane, which in part involves the release of chymotrypsin-like serine proteases, probably in conjunction with oxidants, and that this detachment can be inhibited. (J ALLERGY CLIN IMMUNOL 1995;95:597-606.)

Key words: Neutrophils, basement membrane, human epithelial cells, asthma

Detachment of epithelial cells (ECs) from underlying basement membrane (BM) is seen in a number of pulmonary diseases, including bronchiectasis and lung allograft rejection.^{1,2} It is particularly prominent in asthma in which EC detachment from underlying BM is seen in animal models³ even in the mildest form of the disease⁴

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Abbreviations used

α1-AT:	α1-Antiprotease
BAEE:	Benzoyl-L-arginine ethyl ester
BM:	Basement membrane
DMSO:	Dimethyl sulfoxide
EC:	Epithelial cell
EDTA:	Ethylenediaminetetraacetic acid
FMLP:	Formyl-methyl-leucyl-phenylalanine
MEC:	Minimum effective concentration
NAC:	N-acetyl cysteine
OZ:	Opsonized zymosan
PAF:	Platelet activating factor
PMN:	Polymorphonuclear leukocyte
PMSF:	Phenyl-methyl-sulfonyl-fluoride
SBTI:	Soybean trypsin inhibitor
SOD:	Superoxide dismutase
TPA:	Tetradecanoyl phorbol acetate

and can be extensive in the bronchi of persons who die of asthma.⁵

In diseases such as asthma, both eosinophils and neutrophils accumulate,⁶ and eosinophils usually predominate.⁷ Recent studies have demonstrated that the major effect of eosinophils is to damage epithelial cells rather than to detach them.⁸ Neutrophils, however, also clearly have the capacity to damage ECs,^{9,10} but the capacity of neutrophils to detach human ECs from underlying BM has never been studied. Because neutrophils contain a range of products potentially capable of producing detachment,¹¹ we conducted this study to determine, by using native human ECs and BM, whether polymorphonuclear leukocytes (PMNs) have the capacity to mediate EC detachment from underlying BM.

Previous studies evaluating potential effects of granulocytes on human ECs have been limited, largely because of the unavailability of a suitable model. Systems studied have included human tissue samples, which have almost always come from patients with disease and have been difficult to accurately quantify, and *in vitro* culture systems with human airway cells or other ECs attached to nonnative structures (e.g., tissue culture plastic or collagen mixtures).¹²⁻¹⁴ Cultured cells have some advantages in terms of homogeneity and simplicity, but tissue culture clearly modifies bronchial EC structure and responsiveness to a variety of external signals.¹² Because the attachment of ECs to BM is a sophisticated arrangement involving specific proteins and receptors,^{15,16} we have used a model of intact human amniotic ECs, which are structurally very similar to airway ECs and BM and have major advantages in terms of the nature of the tissue and the accuracy of quantification.¹⁷

METHODS

Preparation of amniotic membrane

All studies were performed on amniotic membranes obtained at normal delivery and isolated from term placentas, as previously described.¹⁷ Briefly, term placentas were obtained from the King Edward Memorial Hospital for Women (Perth, Western Australia) within 4 hours of delivery, and each amnion was separated from the chorion. Mucous and blood clots were removed from the stromal side of the amnion, and the membrane was washed extensively in phosphate-buffered saline. The washed membrane was then stretched on a glass microscope slide with the ECs facing upward. Viability at this stage was routinely greater than 90% (as determined by trypan blue exclusion). The slide was then installed in a microchemotaxis chamber (Neuroprobe; Elmed, Inc., Addison, Ill.), which provided 48 wells for experimental

tion. All assays were started within 1 hour of obtaining the placentas.

Preparation of neutrophils

Peripheral blood neutrophils were isolated from normal, healthy volunteers as follows. Whole blood was layered in 3 ml aliquots on two-step Percoll gradients (Pharmacia, Uppsala, Sweden), with densities of 1.096 and 1.084. Cellular bands containing more than 98% PMNs were obtained at the 1.084/1.096 interface after centrifugation at 350 *g* for 30 minutes (room temperature). The bands were pooled, and the cells were washed once in CA++- and Mg++-free NaCl and resuspended in Gey's medium (osmolality = 285 mOsm) to required concentrations.

Neutrophil-induced detachment

Neutrophils obtained as described above were resuspended to final concentrations of 1×10^6 , 2.5×10^6 , 5×10^6 , and 10×10^6 per milliliter of Gey's medium, respectively, for kinetic studies. A 50 μ l suspension of cells was added to the wells with and without tetradecanoyl phorbol acetate (TPA) at a final concentration of 1 μ g/ml. The chamber was incubated for periods of up to 18 hours at 37°C in a humidified incubator in an atmosphere of 5% CO₂/95% air. All tests were performed in duplicate, and each assay included a negative control (Gey's medium alone) and a positive control (trypsin at 100 to 2000 benzoyl-L-arginine ethyl ester [BAEE] units/ml; Sigma Chemical Company, St. Louis, Mo.).

PMN activating agents studied

To study the effects of activating agents on neutrophil-mediated detachment, 5×10^6 cells/ml were incubated at 37°C in a 5% CO₂/95% air-humidified incubator for 4 hours in the 48-well chamber with various activating agents added to the wells at different doses. TPA and formyl-methyl-leucyl-phenylalanine (FMLP) (both from Sigma Chemical Company) were dissolved in 1% dimethyl sulfoxide (DMSO) and stored in aliquots at -85°C or -20°C until required for use. Zymosan-activated serum and opsonized zymosan (OZ) were prepared by incubating 10 mg of zymosan (Sigma Chemical Company) per milliliter of fresh human serum for 1 hour at 37°C then 30 minutes at 56°C. Normal human serum was obtained from laboratory volunteers. The solution was then centrifuged; the pellet was stored at 10 mg/ml in Gey's medium (OZ), and the supernatant (zymosan-activated serum) was stored in 0.1 ml aliquots. Platelet activating factor (PAF) was purchased from Sigma Chemical Company. The range of the final concentration in the test well for each agent was as follows: TPA (0.01 to 10 μ g/ml), FMLP (10^{-5} to 10^{-11} mol/L), zymosan-activated serum (0.01% to 10%), OZ (0.01 to 10 mg/ml), and PAF (0.1 to 10,000 ng/ml). The concentration selected for study was the lowest one to produce maximal detachment.

Resting or activated neutrophils were incubated at 37° C in a 5% CO₂/95% air-humidified incubator for various periods in the 48-well chamber. Desquamation of the epithelium was assessed as described below.

Mechanisms of action of PMNs

A series of experiments was conducted to determine whether PMNs required physical contact with the epithelium to induce detachment of ECs. First, to prevent physical contact between the PMNs and the EC membrane, a 0.45 µm pore size filter was interposed between the amniotic membrane and the PMNs. Second, sonicates obtained from 5×10^6 PMNs/ml with or without TPA were applied to the amniotic membrane and incubated for 4 hours at 37° C with or without TPA. In all experiments trypsin (2000 BAEE units/ml) was used as a positive control.

Assessment and quantitation of EC detachment

Cell detachment from BM was quantified as described previously.¹⁷ Briefly, the slides bearing the amniotic membranes were removed from the chamber, washed extensively with phosphate-buffered saline, dried in air, then fixed and stained with a modified Wright-Giemsa stain (Diff-Quick; Skylab, Perth, Western Australia). Detachment was evaluated at low power ($\times 60$) with use of an eyepiece graticule. The percentage of any remaining attached cells was scored on a scale of 0% to 100% detachment in 10% increments. All slides were counted in blinded fashion by the same observer. Repeat counts of randomly selected slides produced identical results ($<10\%$ difference). As stated previously, this method is as accurate as the direct counting of individual cells in adjacent high-power fields and has excellent correlates with spectrophotometric quantification methods previously reported.¹⁷

Evaluation of inhibitors

The following protease inhibitors and antioxidants were studied. Phenyl-methyl-sulfonyl-fluoride (PMSF) (an active site-directed, irreversible inhibitor of serine proteases) and 1,10-phenanthroline (an inhibitor of metalloproteinases) were dissolved in 1% DMSO. The following inhibitors were dissolved in Gey's medium: soybean trypsin inhibitor (SBTI) (a specific inhibitor of trypsin), $\alpha 1$ -antiprotease ($\alpha 1$ -AT) (broad-spectrum antiprotease), aprotinin (broad-spectrum antiprotease), N-acetyl cysteine (NAC), and glutathione (inhibitors of bacterial collagenase and cathepsin G with antioxidant properties); ethylenediaminetetraacetic acid (EDTA) (inhibitor of metalloproteinases). Catalase and superoxide dismutase (SOD) (antioxidants), SBTI type II-S, aprotinin (A.4529), PMSF, 1,10 phenanthroline, EDTA, glutathione, NAC, catalase, and SOD were obtained from Sigma, and $\alpha 1$ -AT was from Boehringer Mannheim Corporation (Indianapolis, Ind.). Different concentrations of the inhibitor were premixed with the specific

enzyme in a 96-well, conical bottomed tissue culture plate and preincubated for 15 minutes on ice before application to the amniotic membrane installed in the microchemotaxis assembly (Neuroprobe). In this way four purified enzymes were tested: human sputum cathepsin G at 2.15×10^3 SAAPPpNA U/ml (Elastin Products Company, Pacific, Mo.), porcine pancreatic elastase at 92 elastin U/ml (Sigma, type IV), *Clostridium histolyticum* collagenase at 2×10^2 Ninhydrin U/ml (Sigma, type II) porcine pancreatic trypsin at 2×10^3 BAEE U/ml (Sigma, type IX). All inhibitors were characterized by evaluating their inhibition of enzymes at the minimum effective concentration (MEC) of enzyme producing 100% EC detachment (MEC₁₀₀), and that concentration was added to wells containing TPA-activated PMNs as described above.

Statistical analyses

When enzymes were used, the MEC required to induce 50% detachment of ECs (MEC₅₀) was calculated from each dose-response curve, and the mean MEC₅₀ value for each enzyme was expressed as numbers of units of enzyme activity $\pm$ SEM. Between-treatment data were compared with Students *t* test. Significance was defined as $p < 0.05$. PMN-induced detachment is expressed as percent of cells detaching for a particular PMN cell number, and data are compared as described above.

RESULTS

Determination of optimal assay conditions

Fig. 1 shows that 1, 2.5, 5, and 10×10^6 unactivated PMNs per milliliter induced 30% to 50% detachment of amniotic ECs after 4 hours of incubation. There was no significant difference in the detachment induced by various PMN concentrations used, except that detachment induced by 10^6 PMNs/ml remained significantly low even after 18 hours of incubation (data not shown). The addition of TPA to these cells significantly boosted the desquamative effects of both 5×10^6 cells/ml and 10×10^6 cells/ml observed at 4 hours ($p < 0.05$). Fig. 2 shows that desquamation induced by 5×10^6 TPA-activated PMNs/ml is further boosted after 20 hours incubation. After a 20-hour incubation with or without TPA, 10×10^6 PMNs/ml caused 100% desquamation (not shown).

Effects of different neutrophil-activating agents

TPA, PAF, and OZ all caused a marked increase in EC desquamation induced by PMNs at 5×10^6 cells/ml, whereas C5a and FMLP had no significant effect (Fig. 3).

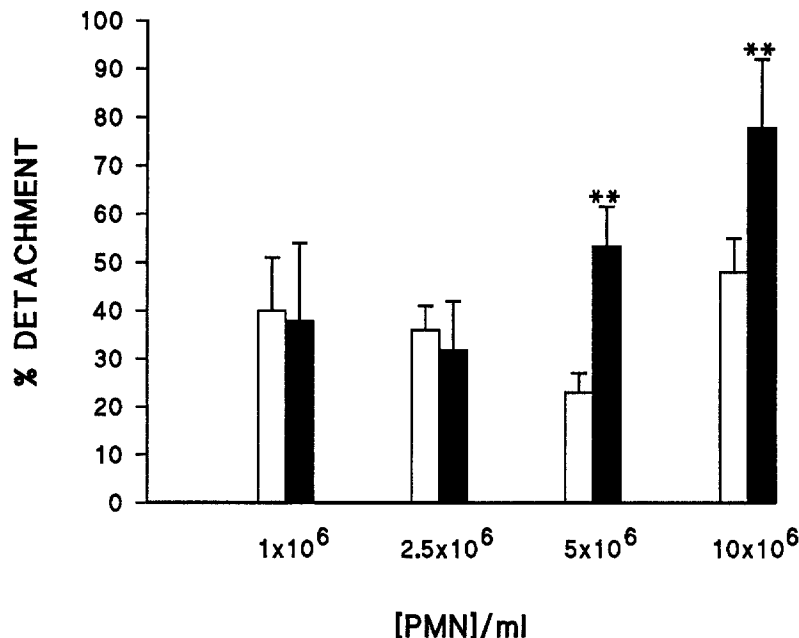


FIG. 1. Detachment of ECs with increasing numbers of PMNs in the resting state (□) or after being activated with TPA ($1 \mu\text{g/ml}$) (■) over 4 hours at 37°C , (mean $\pm$ SEM for $n = 6$; ** $p < 0.005$). Medium alone plus TPA produced no detachment.

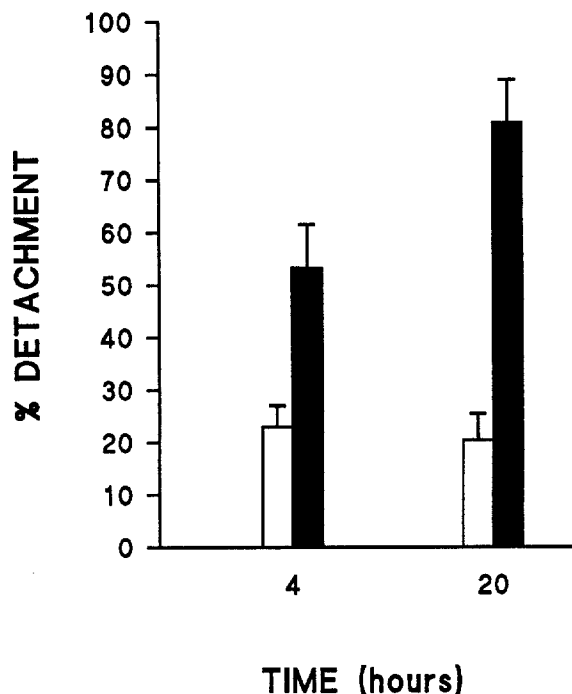


FIG. 2. Detachment of ECs by 5×10^6 PMNs/ml after 4 hours or 20 hours at 37°C in a resting state (□) or activated with TPA (■) (mean $\pm$ SEM for $n = 9$).

Mechanisms of action of PMNs

When cells were separated from the EC membrane by a $0.45 \mu\text{m}$ filter, which permitted the

passage of dissolved molecules but not cells, no desquamation was observed (Fig. 4, A). Trypsin controls did, however, permeate the filter to induce similar desquamation to trypsin, which had direct contact with the ECs, indicating that the membrane did not block the passage of soluble factors. Sonicates of resting or TPA-activated PMNs in free suspension (which included any stable effectors released by PMNs during the 4-hour incubation) also did not induce detachment (Fig. 4, B). In both cases control PMNs, which were allowed to have direct contact with the epithelium, consistently induced significant EC detachment in these experiments.

Detaching PMN proteases

PMN-induced cell detachment was not significantly reduced by the catalase and SOD mixture alone (Fig. 5). We then tested the inhibition profiles of elastase, collagenase, trypsin, and cathepsin G with a panel of protease inhibitors and antioxidants (Fig. 6). PMSF and $\alpha 1\text{-AT}$ both inhibited only elastase- and trypsin-induced detachment (by $>80\%$), phenanthroline and EDTA both inhibited only collagenase (by $>80\%$), aprotinin inhibited only trypsin-induced detachment (by $>80\%$), SBTI inhibited only trypsin- and cathepsin G-induced detachment (by $>90\%$), and glutathione and NAC inhibited only collagenase- and cathep-

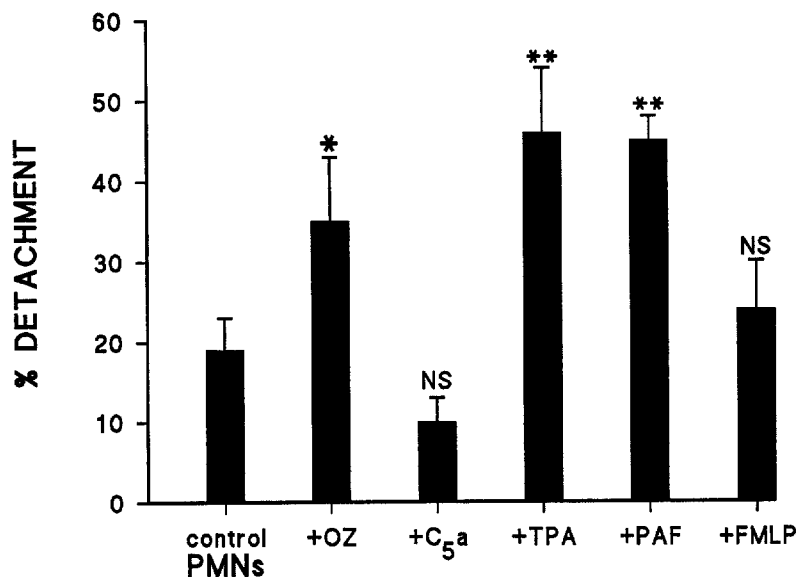


FIG. 3. Effects of various PMN-activating agents: 5×10^6 PMNs/ml alone or with OZ at 1 mg/ml, C5a at 10%, TPA at 1 μ g/ml, PAF at 100 ng/ml, and FMLP at 10^{-8} mol/L. Detachment is expressed as percent (mean $\pm$ SEM for $n = 6$). ** $p < 0.005$, * $p < 0.05$. NS, Not significant compared with controls. In the absence of PMNs, none of the activating agents produced detachment.

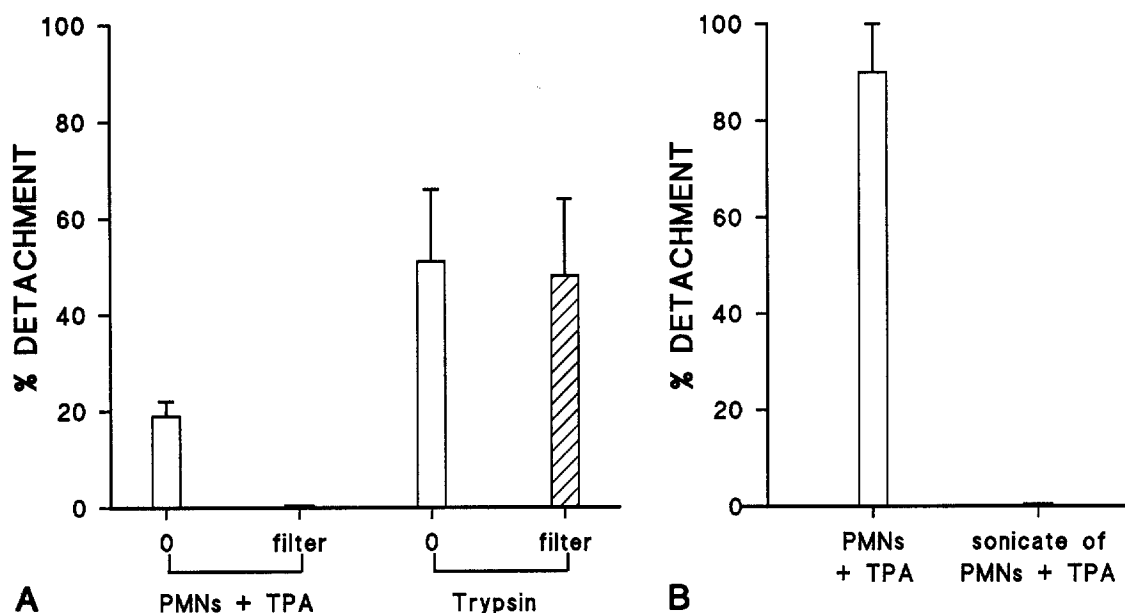


FIG. 4. **A**, Contact dependence of PMN action. EC detachment with 5×10^6 PMNs/ml (activated with 1 μ g/ml TPA) or trypsin (2000 BAEE units/ml) was evaluated with or without separation from the epithelium with a 0.45 μ m pore size filter. Results are expressed as means $\pm$ SEM for $n = 4$. **B**, Requirement for intact PMNs. Detachment with 5×10^6 PMNs/ml (activated with 1 μ g/ml TPA), used intact or sonicated. Results are expressed as means $\pm$ SEM for $n = 4$.

sin G-induced detachment (by >90%). With the panel of inhibitors, PMN-induced cell detachment was inhibited by SBTI (>75%) and glutathione (>56%) (Fig. 7). The addition of catalase and SOD did potentiate the effects of α 1-AT, aproti-

nin, NAC, and PMSF, though the latter was not above the effects of the DMSO diluent itself. Glutathione and NAC have dual properties as antiproteases, as well as antioxidants. The results of adding catalase and SOD to these inhibitors

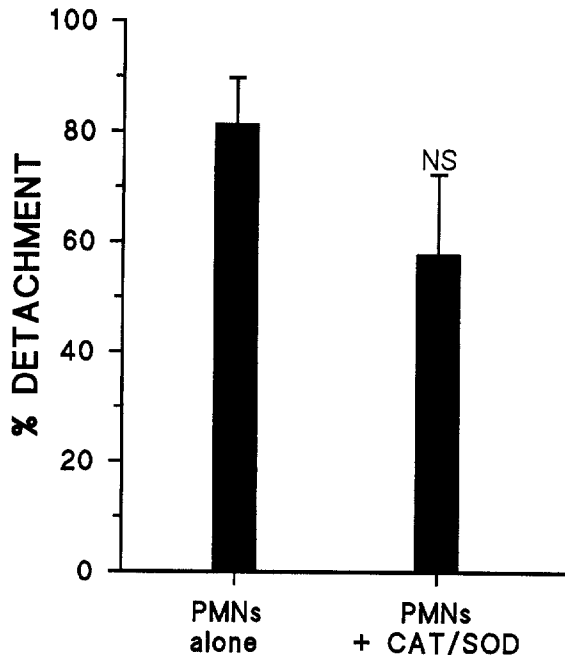


FIG. 5. Effect of catalase (2000 U/ml) and SOD (200 U/ml) when added to TPA-activated PMNs (5×10^6 PMNs/ml + 1 μ g/ml TPA). NS, Not significant. Results are expressed as means $\pm$ SEM for $n = 9$. CAT, Catalase.

indicate that their antiprotease activities are probably more important.

DISCUSSION

EC detachment from BM occurs in a variety of tissue diseases. In the lungs it is featured in diseases such as bronchiectasis¹ and lung allograft rejection² and has been recognized for many years as a prominent feature in asthma.³ In asthma, desquamated ECs are shed in the sputum, and individuals dying of asthma also demonstrate significant EC desquamation¹ in association with eosinophil and neutrophil accumulation,⁴ but a role for either eosinophils or neutrophils in causing this type of detachment has not been established. We have demonstrated that eosinophils damage rather than detach human ECs,⁸ but the effect of PMNs on human detachment has not previously been studied. Because ECs attach to BM via specific attachment proteins such as laminin, fibronectin, and type IV collagen¹⁶ and because neutrophils contain effector molecules capable of denaturing or hydrolyzing these proteins,⁹ we reasoned that intact PMNs may be able to induce EC desquamation particularly in the presence of PMN-activating agents.

We have previously described an *in vitro* model of native human ECs and BM¹⁷ and demonstrated

that EC detachment could be accurately quantified. Importantly, this model had advantages over previous models, which used precultured ECs attached to tissue culture plastic or dissected strips of human airway mucosa. In this model the ECs were human, were not modified by tissue culture, had been constituted *in vivo*, were attached to native BM rather than tissue culture plastic or artificially reconstituted BM, and exhibited tight junction formation and BM attachment factor expression similar to that in human normal and asthmatic airway tissue.¹⁵ We have also previously demonstrated detachment by cathepsin G and collagenase and synergy between oxidants and proteases in producing EC detachment when a low dose of protease did not by itself cause detachment but a high percentage of detachment was obtained at the same protease dose but with prior application of H_2O_2 to the ECs.¹⁸

Kinetics

Unactivated neutrophils at all concentrations used caused a detachment within the first 4 hours of exposure to the ECs, and although there was a progressive increase in EC detachment with time, it is possible that PMN breakdown with release of intracellular constituents contributed to the detachment seen at 18 hours, especially at the highest PMN concentration. Nevertheless, it is clear that intact neutrophils have a potent capacity to detach native ECs from BM over several hours. In view of the knowledge that *in vivo* exposure of ECs to neutrophils probably occurs over more prolonged periods, the effects seen over the short periods in these experiments are quite striking.

Effects of neutrophil activation

It was a little surprising that not all neutrophil-activating agents augmented EC detachment. The extracellular release of PMN granule contents has been the subject of much investigation. It is known that contents of the so-called specific granules (containing effectors such as collagenase and lactoferrin) and those of the azurophilic granules (containing effectors such as lysozyme, elastase, and cathepsins) are under separate control.¹¹ Various chemical stimuli, including chemoattractants, have been shown to cause the release of enzymes from granules via different pathways at the membrane level.¹⁹⁻²⁴ In this study PMNs treated with TPA showed an increased capacity to induce detachment of ECs, whereas the chemotactic stimuli C5a and FMLP did not. These observations are similar to those of Rinaldo and Basford²⁵, who evaluated

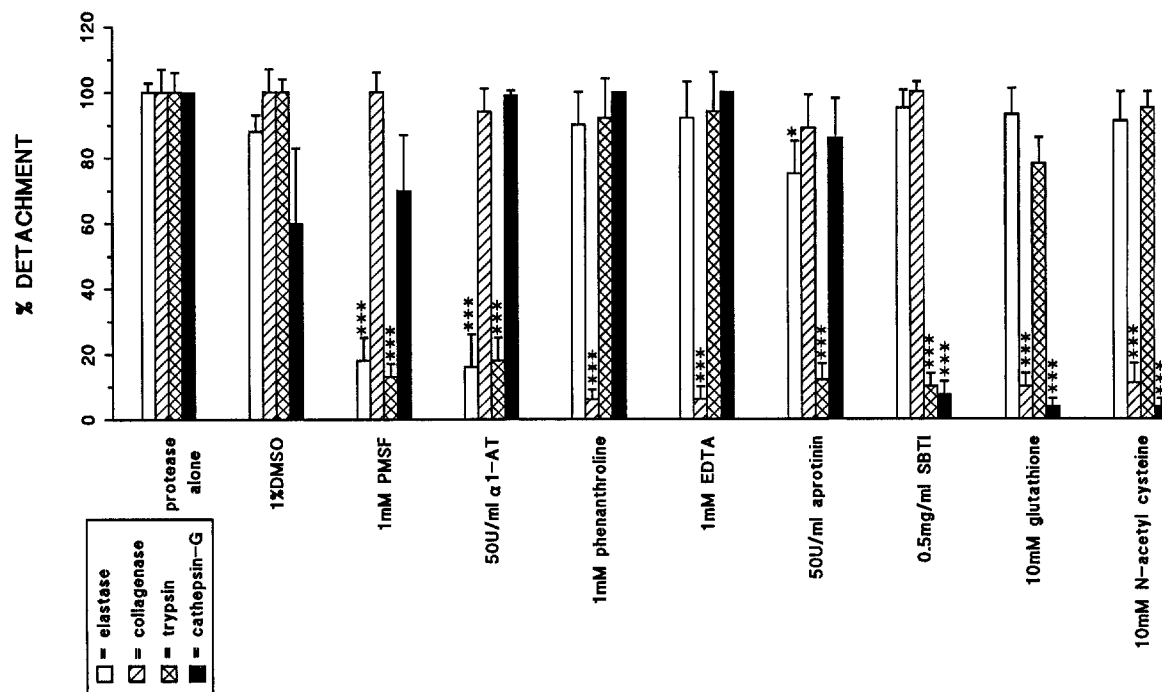


FIG. 6. Effects of inhibitors on EC desquamation induced by porcine pancreatic elastase (□), *C. histolyticum* collagenase (▨), porcine pancreatic trypsin (▩), and human sputum cathepsin G (■). (See text for details on abbreviations and concentrations used.) Results are expressed as means $\pm$ SEM for $n = 6$. *** $p < 0.0005$, ** $p < 0.005$, * $p < 0.05$.

the effect of PMNs on endothelial cell damage, reporting that although FMLP-activated PMNs did not induce damage, those activated with TPA were able to cause significant injury to endothelial cells. They suggested that this difference in response may be due to a bypass of receptor binding in the case of TPA. However, in this study we investigated the effects of two other receptor-binding molecules (PAF and OZ), both of which caused a significant increase in neutrophil-induced detachment. PAF has recently been shown to be not only a chemotactic stimulus for PMNs but also to increase protein kinase C activity in PMNs.²⁶ The other receptor-binding molecule, OZ, bears C3b fragments and is taken up by the C3b receptor, which is now well characterized.²⁷ These results suggest that the concept of receptor bypass proposed by Rinaldo and Basford²⁵ may not fully explain the different results obtained for TPA and FMLP and that the nature of the receptor and its mechanism of action may also have to be taken into consideration.

Mechanisms of action

A significant observation was the inability of PMNs to induce detachment when deprived of

physical contact with the EC membrane. Similar observations have been reported by other authors who demonstrated the requirement of the PMNs for membrane contact before degranulation.²⁸ These studies also suggest that degranulation did not occur when the PMNs were given the same stimuli in free suspension and are further supported by Vissers et al.²⁹ who demonstrated that PMNs produce oxidants only at the site of attachment. The failure of catalase and SOD to prevent detachment in this system supports the view that oxidants are not the primary agents causing detachment. Our present and previous observations in our human in vitro model suggest that stimulated PMNs adhere rapidly to the epithelium and release agents, probably oxidants, which cause structural damage to cells and the underlying BM; this may permit proteolytic enzymes released by the PMNs to gain access to the attachment proteins.

Although a number of purified proteases can detach ECs,¹⁸ it appears from our studies that serine neutral proteases (such as cathepsin G, trypsin, and chymotrypsin) are the major neutrophil-derived proteases that produce this effect when TPA-activated neutrophils are studied.

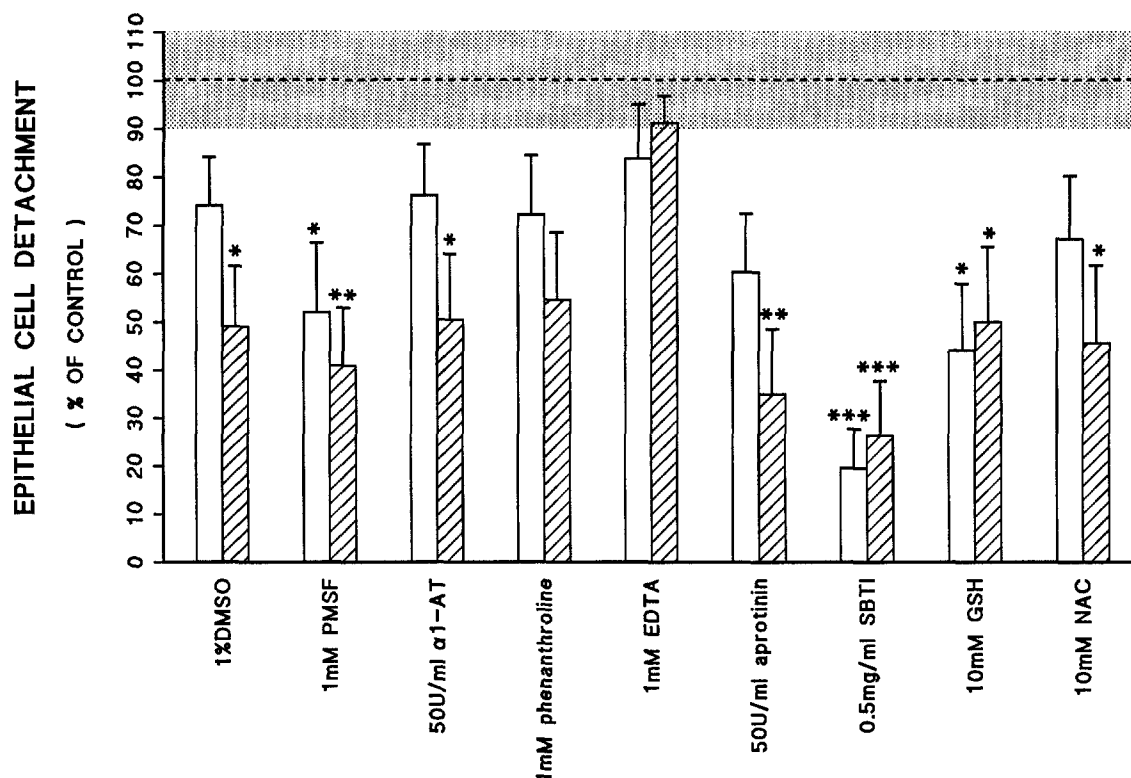


FIG. 7. Capacity of protease inhibitors to block neutrophil-mediated EC detachment (dotted line and shaded area = means $\pm$ SEM for 5×10^6 PMNs/ml activated by TPA $1 \mu\text{g/ml}$). Data are expressed as means $\pm$ SEM ($n = 9$) for PMNs plus protease inhibitors alone ($\square$) or with the addition of catalase/SOD (▨). *** $p < 0.005$, ** $p < 0.025$, * $p < 0.05$). CAT, Catalase; GSH, glutathione.

Other studies have shown that PMN-mediated cartilage matrix degradation is produced primarily by neutrophil elastase and cathepsin G.³⁰ The boosting effect of TPA in this study, which does not cause the release of cathepsin G and elastase-containing azurophilic granules, may be due to the induction of increased oxygen radical production, as well as specific granule release. The boosting effects of PMN-induced EC detachment by the other stimuli, namely OZ and PAF, were not studied further here but are likely to differ, given the different pattern of cell activation. However, it is the interaction of the cells with the epithelium that is most likely to trigger the release of the azurophilic granule contents. This release has been described in "frustrated phagocytosis"³¹ but may also be caused by fragmented peptides, as shown by Wachtfogel et al.³² who demonstrated that fibronectin degradation products are able to stimulate azurophilic granule degranulation. In our study SBTI is the major inhibitor of EC detachment, and it is known to inhibit serine neutral proteases (cathepsin G, trypsin, and chymotrypsin)

but not metalloproteases or cysteine or aspartic proteases. Importantly, it does not inhibit elastase. Glutathione has dual antiprotease and antioxidant properties, and its effects in this study may represent a combination of both activities. Aprotinin and $\alpha 1$ -AT are also serine protease inhibitors, the function of which can be affected by oxidants, and $\alpha 1$ -AT is known to inhibit elastase.

We conclude that PMN activation, boosted by TPA, results in the action of serine proteases, largely chymotrypsin-like proteases, which are important in the detachment of ECs from BM. We believe that oxidants released by PMNs may damage ECs and underlying structures in this in vitro system, given that antioxidants only seemed to limit the effect of oxygen radicals on susceptible antiproteases but by themselves were unable to prevent detachment of ECs.

Relevance of this study to tissue injury in lung disease

Neutrophils are found in the airway tissue of subjects with inflammatory diseases such as bron-

chiectasis, lung allograft rejection, cystic fibrosis, and asthma. In asthma eosinophils are usually more numerous, and in our recent studies with this model, we demonstrated that the major effect of eosinophils on ECs is to cause damage rather than detachment,⁸ a finding consistent with the known capacity of eosinophils to release proteins that are toxic to other tissues and helminths.³³ These studies confirmed that damage alone is not sufficient to induce detachment from BM but that other signals are required. The data from this study suggest that neutrophils release agents that can detach native human ECs from underlying BM and imply a role for these cells in lung inflammatory diseases, contributing to the EC detachment, which has been observed in a number of these diseases, particularly asthma and cystic fibrosis.

EC detachment in the airways has received a considerable amount of attention recently, in view of its likely contribution to the pathogenesis of inflammatory diseases. In asthma it may result in exposing irritant receptors and allow exudation of fluid into airways, thus contributing to airflow limitation. The opening up of gaps in the epithelium potentially allows increased amounts of antigen to enter the mucosal tissue, and this may trigger inflammatory and immune responses. Loss of epithelium may also remove a source of smooth muscle relaxation.³⁴ Thus the delineation of the cells and molecules responsible for EC detachment in asthma and other diseases in which a role for neutrophils in EC disruption is implied is essential for the development of new therapies aimed at effectively preventing this process.

In conclusion, we have demonstrated, by using a model of human native ECs and BM, that neutrophils exhibit a profound capacity to detach ECs from BM but that this detachment can be inhibited.

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