Brief communications

Effects of fractions of skin chamber fluids from human allergic reactions on neutrophil activation

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Using a skin chamber approach, we have found an accumulation of activated polymorphonuclear neutrophils (PMNs) and their released components in the sites of developing human late-phase IgE-mediated skin reactions.[1] We recently found that the overlying chamber fluid obtained during the first hour of pollen antigen challenge could directly stimulate resting blood PMNs in vitro.[2]

The nature of the stimulatory component or components in such fluids is not known. Attempts at characterization are limited by the amount of fluid that can be obtained from an individual subject. A reasonable initial characterization would be the determination of in vitro effects of ultrafiltration fractions of such fluids on blood neutrophils.

Abbreviations used
PMNs: Polymorphonuclear leukocytes
CF: Chamber fluid
HBSS: Hanks' balanced salt solution
UF: Ultrafiltration from fraction

METHODS

We obtained skin chamber fluid (CF) overlying the blister sites of pollen-reactive subjects during the first hour of antigen challenge as described previously.[2] Ultrafiltration was performed with a Centricon 10 apparatus (Amicon Inc., Beverly, Mass.) with a 10 kd separation cutoff. The ultrafiltration technique used was first validated with N-formyl-methionyl-leucyl-phenylalanine (f-MLP), a low molecular weight (324 d) PMN stimulant. In initial studies we found that the ultrafiltrate of an f-MLP solution centrifuged for 90 minutes contained more than 95% of the stimulatory activity when the fractions were subsequently incubated with blood neutrophils in the presence of cytochalasin B and when superoxide generation was assessed by the reduction of cytochrome C, as previously described.[2] To be sure that all the material that weighed less than 10 kd was removed from the retentate after ultrafiltration, 1.0 ml of Hanks' balanced salt solution (HBSS) was added on two subsequent occasions to the retentate, each followed by centrifugation at 5000 *g* for 30 minutes. The retentate at this time was then diluted with HBSS to the volume originally placed in the Centricon apparatus.

Duplicate aliquots of 2^6 resting blood PMNs were incubated in 1.0 ml total reaction volumes that contained 100 μl of one of the following: (1) 1:2 diluted unfractionated CF, (2) ultrafiltrate fraction (UF) of 1:2 diluted CF, (3) retentate of 1:2 diluted CF, (4) additional HBSS. The solution also contained ferritocytochrome/C^{++}/Mg^{++}/gelatin with superoxide dismutase added to one of each pair, as previously described.[2] After incubation for 1 hour at 37° C (to stimulate in vivo events in the skin chamber), the tubes were centrifuged (1000 rpm for 10 minutes). The supernatant was removed and analyzed immediately for superoxide levels, as reported previously.[2] An aliquot of the supernatant was also stored at −20° C for lactoferrin analysis.[2]

RESULTS

Because there was a varying degree of spontaneous superoxide secretion by PMNs from different subjects, the stimulatory effects of CF and their UF and retentate fractions were expressed as net effects by subtracting the spontaneous superoxide secretion from other PMN aliquots incubated at the same time. With this assessment, unfractionated CF induced a net superoxide secretion of 2.6 ± 0.6 nmol (Fig. 1, *A*). In compar-

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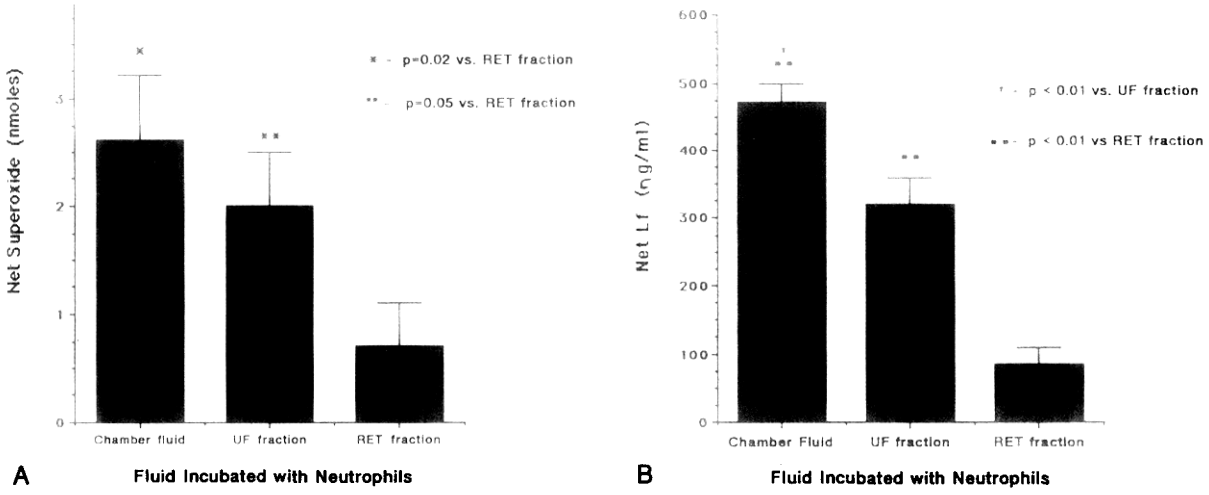


FIG. 1. A, Superoxide secretion stimulated by unfractionated CF, and the ultrafiltrate (*UF,* < 10 kd) and retentate (*RET,* > 10 kd) of such fluids. Net superoxide = stimulated superoxide minus spontaneous secretion by aliquots of the same neutrophils in individual experiments. **B,** Net lactoferrin *(Lf)* secretion stimulated by unfractionated CF, ultrafiltrate (*UF,* < 10 kd) and retentate (*RET,* > 10 kd). Net lactoferrin = stimulated lactoferrin minus spontaneous secretion by aliquots of the same neutrophils in individual experiments.

ison, the UF stimulated secretion of 2.0 ± 0.5 nmol, which is slightly (but not significantly) less. However, the net superoxide secretion stimulated by the retentate fractions (0.7 ± 0.4) was significantly lower than that stimulated by unfractionated CF ($p = 0.02$). The difference between the stimulatory effects of the UF and retentate fractions was of borderline significance ($p = 0.054$).

Net lactoferrin secretion stimulated by unfractionated CF was 472 ± 26 ng/ml, significantly more than that stimulated by the UF fractions (320 ± 39; $p < 0.01$) (Fig. 1, *B*). Lactoferrin release induced by the retentate fractions (85 ± 24) was significantly less than lactoferrin secretion stimulation by unfractionated CF or the UF fractions ($p < 0.01$). This stimulation of lactoferrin secretion was not accompanied by any increased release of lactic dehydrogenase, suggesting that there was no toxic effect of CF or its fractions on PMNs.

DISCUSSION

The findings reported here extend our earlier observations in that most of the PMN stimulatory activity we have found in the CF in chambers overlying the challenge sites during the first hour of antigen skin challenge of reactive subjects appears to be in an ultrafiltration fraction with a molecular weight of less than 10 kd. We have not yet obtained sufficient amounts of this CF to further characterize this stimulatory activity. However, we do know from our previous study that the activity was stable in fluids heated at 56° C for 1 hour. Also several of the components present in such fluids (histamine, heparin, tryptase, the challenge antigen, autologous serum) were not stimulatory, either alone or in combination.[2] Other PMN agonists can also be considered. The level of leukotriene B_4 (molecular weight = 337 d) released in the first hour of antigen challenge would generally not stimulate superoxide or lactoferrin secretion in the absence of enhancing materials such as cytochalasin B.[3] We have also found that platelet activating factor (molecular weight = 560 d) can stimulate PMNs by itself.[3] However, in our previous studies increased platelet activating factor levels were not detected in CF until well after the first hour of antigen challenge.[4] Other known PMN activators such as cytokines are generally greater than 10 kd in size and would be unlikely to be in the ultrafiltrate of the CF evaluated here. Further attempts at characterization may require use of pooled CF to afford sufficient material for study.

The in vitro stimulatory activity reported is probably much less than that present at the site of release in the underlying dermis. There is inevitable dilution of such components as they diffuse into the overlying CF. There was a further 1:20 dilution of the materials in the in vitro incubation with PMNs. Therefore we can readily postulate a

strong stimulation of PMNs entering the microenvironment of the allergic reaction, of possible great pathogenic importance in IgE-mediated reactions.[5]

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Factors determining bradykinin bronchial responsiveness and refractoriness in asthma

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The mechanism by which bradykinin narrows the airways in human beings is believed to be an indirect one.[1, 2] In contrast, histamine and methacholine both cause bronchoconstriction directly by stimulating the corresponding receptors on airway smooth muscle. Therefore it is perhaps surprising that three studies[2-4] have shown a correlation in the responsiveness between the two stimuli.

The aim of the present study was to reexamine the relationship between bronchial hyperresponiveness induced by bradykinin and histamine. We also assessed the incidence and factors that influence the development of refractoriness to bradykinin.

Abbreviations used

FEV_1	Forced expiratory volume in 1 second
PC_{20}	Provocative concentration causing a 20% fall in FEV_1

METHODS

Subjects

Twenty-one nonsmoking, atopic, subjects with mild asthma (13 men) with a mean age of 28.1 years participated in the study. The subjects were chosen without any prior knowledge about their bronchial responsiveness to histamine or bradykinin. The criteria used for selection has been described previously.[2, 5]

Procedure

The protocols for bronchial challenge with mediators and measurement of forced expiratory volume in 1 second (FEV_1) have been described previously.[2, 5]

The study consisted of two visits separated by at least 7 days, during which two consecutive concentration-response studies with either inhaled bradykinin or histamine were undertaken in a double-blind, randomized manner. An initial bronchial provocation test with either bradykinin or histamine was performed until FEV_1 fell

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