

Humoral and cellular components in inflammation: hyper-reactivity and decreased feed-back inhibition of asthmatic neutrophils

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INTRODUCTION

Regardless of their cause, most forms of inflammation are amplified and propagated as a result of the recruitment of humoral and cellular components of the immune system. The binding of an antigen to a recognition unit (immunoglobulin, T cell receptor) of the immune system leads to activation of an amplification system, initiating the production of proinflammatory mediators. The latter alter blood flow, increase vascular permeability, augment adherence of circulating leukocytes to vascular endothelium, promote migration of leukocytes into tissues, and enhance their functional capabilities so that the inciting agent is destroyed. Most immune responses are ongoing so that elimination of foreign antigen occurs without producing clinical disease. However, excessive inflammation or a failure to terminate the inflammatory process may lead to significant tissue damage and chronicity. Such may be the case in patients with asthma and/or atopic diseases.

The distinctions in physiologic functioning between atopic and non-atopic individuals are often subtle and have not been well defined. Although the neutrophil plays a supporting role in atopic disease, several differences in neutrophil function have been noted when atopic and non-atopic subjects were compared. For example, decreased enzyme release (16) and a decreased cAMP response to adrenergic agents (2) have been reported in leukocytes of patients with atopic dermatitis, but the relevance of this to atopic conditions such as rhinitis and asthma

is not clear. Impairment of neutrophil chemotaxis (14), phagocytic activity (11), and Fc and complement receptor activity (8) has been noted in asthma, with decreased chemotaxis also reported in neutrophils from patients with other atopic disorders (15). My colleagues Drs. B. Styrt and M. Klempner and I have examined the granulocyte respiratory burst in patients with respiratory allergy (18). These studies measured both resting and stimulated generation of toxic oxygen metabolites, using three soluble stimuli which act by different pathways. The feed-back inhibition by histamine on this response was also determined.

RESULTS

Superoxide production by neutrophils from patients with allergic rhinitis and/or asthma

Measurement of superoxide by neutrophils from patients with respiratory allergy and controls in response to different stimuli revealed that unstimulated atopic cells reduced 5.0 ± 0.7 nmol cytochrome C as compared with 3.0 ± 0.4 nmol for same-day controls ($P < 0.05$). When stimulated with 0.5 micromolar calcium ionophore A23187, superoxide production by atopics was $43.8 \pm 13.7\%$ higher than same-day controls ($P < 0.01$ by single-sample t-test). In response to 0.1 micromolar f-met-leu-phe, atopic cells generated $44.9 \pm 18.3\%$ more superoxide than controls ($P < 0.05$ by single-sample t-test). The response to 0.5 micromolar

f-met-leu-phe or to 5 micrograms/ml PMA was nearly identical in granulocytes from atopic and nonatopic subjects.

To determine whether the differences in total stimulated superoxide production were due simply to increased spontaneous production, spontaneous release was also subtracted out from stimulated production for those experiments in which both were measured simultaneously. When this was done, atopic cells were still "normal" in PMA-stimulated production ($7.1 \pm 2.8\%$ less than control) but had increased production in response to A23187 ($27.2 \pm 16.2\%$ greater than control). Furthermore, after subtraction of baseline production, an enhanced response to 0.5 micromolar f-met-leu-phe was noted ($23.9 \pm 10.2\%$ greater than control, $P < 0.05$).

To study further stimulus specificity, the response to a lower concentration of PMA (5 ng/ml) was measured in a small number of subjects (4 atopic, 3 nonatopic). Superoxide production in atopics was $9.5 \pm 6.8\%$ greater than control in this group, a nonsignificant difference.

Inhibition of neutrophil superoxide production by histamine

In accordance with the findings of others (17), histamine did not inhibit superoxide production in response to PMA even when histamine was used at millimolar concentrations and submaximal stimulation was used. Cytochrome C reduction by granulocytes treated with histamine (1 mM) and stimulated with PMA (5 ng/ml) was 98.1%, 96.4%, and 100% of that in cells not treated with histamine, in one nonatopic and two atopic subjects, respectively. Histamine likewise did not interfere with measurement of superoxide as assessed by the xanthine-xanthine oxidase assay (data not shown).

In contrast, histamine significantly reduced superoxide production in response to f-met-leu-phe at concentrations as low as 10 micromolar. A comparison of atopics with normals showed that with 0.5 micromolar f-met-leu-phe as the stimulus, slightly less inhibition in atopics by 1 mM histamine ($44.9 \pm 4.8\%$ inhibition in

atopics, $52.8 \pm 2.9\%$ inhibition in nonatopics) and 0.1 mM histamine ($35.2 \pm 3.7\%$ inhibition in atopics, $42.7 \pm 3.4\%$ in nonatopics) occurred. However, the total amounts of superoxide produced did not differ significantly when this dose of stimulus was used.

In neutrophils stimulated with 0.1 micromolar f-met-leu-phe the differences between atopics and nonatopics were more pronounced. While there was substantial overlap between the two groups, 11 of 16 atopics showed less inhibition to 1 mM histamine than the least-inhibited control, and mean inhibition differed significantly ($39.8 \pm 4.6\%$ in atopics, $60.1 \pm 3.0\%$ in controls, $P < 0.02$).

DISCUSSION

The potential contribution of neutrophils to allergic respiratory disease is suggested by the release of neutrophil chemotactic factor in association with asthma - which has been demonstrated in both atopic and nonatopic subjects after appropriate stimuli (10, 12). It is supported by the demonstration that ovine bronchial reactivity to histamine aerosol is decreased by neutrophil depletion (7) and that the late asthmatic response in an animal model is dependent on neutrophil availability (13).

We examined the relative susceptibility of atopic and nonatopic neutrophils to both stimulation and inhibition, assessing differences in the release of toxic oxygen metabolites. Our findings demonstrate several differences which could contribute to tissue damage in allergic respiratory disease.

Atopic neutrophil produced more superoxide in the absence of stimuli than nonatopic neutrophils. Because an excess of eosinophils contaminating the neutrophil preparation might have contributed to this difference, we examined the relationship between eosinophil percentage and spontaneous superoxide production. These two quantities were not significantly correlated and therefore could not adequately explain the differences observed.

Eosinophilia similarly could not explain the increased superoxide production by atopics in response to f-met-leu-phe since eosinophils

reportedly do not produce superoxide when treated with this stimulus (20). However, this difference was pronounced only when a concentration of f-met-leu-phe giving submaximal stimulation was employed, and little difference was seen between atopics and controls at higher concentrations of f-met-leu-phe or after PMA stimulation. This suggests that the total capacity of atopic cells for oxidative metabolic activity is not abnormal but there may be a greater responsiveness to low levels of stimulation. Alternatively, the difference between atopic and nonatopic cells may involve an early step in the stimulation pathway which can be bypassed by direct PMA activation of protein kinase C (19). The mechanism is not clear but is probably not a matter of altered f-met-leu-phe receptor number or affinity since similar results were obtained after nonreceptor-mediated stimulation with the calcium ionophore A23187.

Part of the heightened total superoxide production in stimulated atopic cells could be due to their increased activity in the basal state. However, when resting superoxide production was subtracted out, the net response to some stimuli was still significantly greater in atopics than in controls. This suggests a genuine increase in stimulus responsiveness in addition to hyperactive baseline oxidative metabolism.

When we examined the response of atopic and nonatopic neutrophils to histamine, there was less inhibition in atopic cells stimulated with 0.1 micromolar f-met-leu-phe than in nonatopic cells which received the same stimulus. Although only a small number of controls were assessed in this part of the study, the differences reached statistical significance. This suggests that atopic cells may be both easier to stimulate and more difficult to suppress than normals, a suggestion which finds some support in previously published studies.

The decreased inhibition of oxidative metabolism by histamine which we observed in f-met-leu-phe-stimulated neutrophils from atopics is compatible with the decreased inhibition of zymosan-stimulated degranulation reported by Busse and Sosman (3) and Katagiri et al. (9) in asthmatics. Several possible mechanisms could be invoked to explain these findings. Firstly,

increases in circulating histamine could lead to down-regulation of receptors in a manner analogous to that reported for beta-adrenergic receptors (5, 6). However, down-regulation of histamine receptors was shown not to be a likely reason to explain decreased atopic histamine-induced suppressor cell activity (1). Secondly, this could be a medication effect, again as noted in asthmatics treated with beta-agonists, in whom decreased histamine inhibition of lysosomal enzyme secretion has been reported (4). This seems unlikely as the full explanation since the atopics showing decreased histamine inhibition were studied out of season and were not on medication. Thirdly, if atopic neutrophils are genuinely hyperactive in the "resting" state, this may represent a baseline level of NADPH oxidase activation which is not susceptible to inhibitors.

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