

LEUKOCYTE ADHERENCE IN ATOPIC DERMATITIS: DIMINISHED RESPONSES TO HISTAMINE AND ISOPROTERENOL

Henning Thulin, Jon M. Hanifin and Richard Bryant

*Department of Dermatology, Marselisborg Hospital, University of Aarhus, Denmark
and Departments of Dermatology and Medicine, University of Oregon,
Health Sciences Center, Portland, Oregon, USA*

Abstract. Leukocyte adherence was examined in atopic and normal subjects and the influence of histamine and the beta-adrenergic agonist, isoproterenol, evaluated. Atopics included patients with mild to severe atopic dermatitis and patients with asthma and hayfever. No inter-group differences in leukocyte adherence of untreated leukocytes could be demonstrated, but there was a significantly smaller histamine and isoproterenol effect on the adherence of cells from patients with atopic dermatitis and hayfever.

The induced inhibition of leukocyte adherence by histamine could be blocked by the H-2 histamine receptor antagonist, metiamide, suggesting that this effect may be mediated via cAMP.

Our findings support the hypothesis that atopic dermatitis patients may have a defect or blockade of their cellular response to histamine.

Key words: Atopic dermatitis; Leukocyte adherence; Histamine; Isoproterenol; H-2 histamine receptor blockade

Adherence is one of the basic functional properties of phagocytes and a requisite step in the process of inflammatory diapedesis and chemotaxis. Agents which raise intracellular cyclic AMP have been shown to reduce leukocyte adherence (1) as well as chemotaxis (6).

Various studies have demonstrated that leukocytes from patients with atopic dermatitis (AD) have a diminished cyclic AMP response to beta-adrenergic agents (2, 8) and prostaglandin E₁ (8). A decreased cAMP response to histamine in granulocytes from asthmatic patients has also been reported (3). We considered that the blunted response to agents which normally increase intracellular cAMP might serve to distinguish AD from normals. This report describes differential leukocyte adherence responses to histamine and isoproterenol in atopic and normal subjects.

MATERIAL AND METHODS

11 patients with severe atopic dermatitis (AD), 5 patients with mild AD and 6 patients with hayfever were included in the study. Severity and extent of the disease were quantitated and patients with severe AD, in the present study, were those with more than 50% of body area involved and a severity of disease including pruritus and lichenification. Patients with less than 25% body area involved and mild lichenification without pruritus were considered as having mild AD.

Our assay system utilized a modification of techniques developed by MacGregor et al. (7). Scrubbed nylon fiber (3-denier, 4 cm type 200, Fenwal Laboratories, Morton Grove, Illinois) was weighed on an analytical balance and packed by means of a wooden applicator stick into 23-cm Pasteur pipettes. The length of the packed column was adjusted to exactly 15 mm, extending from the midpoint on the pipette shoulder to the top of the column. To reduce the blood flow through the column, a 20-gauge $\times 1\frac{1}{2}$ inch needle (monojet, Sherwood Medical Industries, Deland, Illinois) was placed on the tip of each pipette. The pipettes were packed with 80 mg nylon fibers to trap approximately 90% of the granulocytes and thus make the assay sensitive to a decrease in granulocyte adherence (7).

The blood to be tested was drawn into a heparinized tube (10 units preservative-free Heparin/ml) and the total number of leukocytes counted with a Coulter Counter (Coulter Electronics Model FN). One ml of blood was added to the top of three pipettes and allowed to filter through the columns. Before applying the blood to the columns it was incubated at 37°C for 30 min with histamine dihydrochloride, theophylline, or isoproterenol dissolved in 0.9% saline and added to make final concentrations ranging from 10^{-3} to 10^{-5} M. Dose-response curves were set up for histamine and isoproterenol prior to the trial. The total numbers of effluent blood cells were counted and the following calculations were carried out:

$$\text{Percentage of adherent cells} = Pa = \frac{T_a}{T} \times 100$$

$$\text{Percentage of control adherence} = Pca =$$

$$\frac{T_a (\text{active column})}{T_a (\text{control column})} \times 100$$

Percentage of Adherent Cells (Pa)

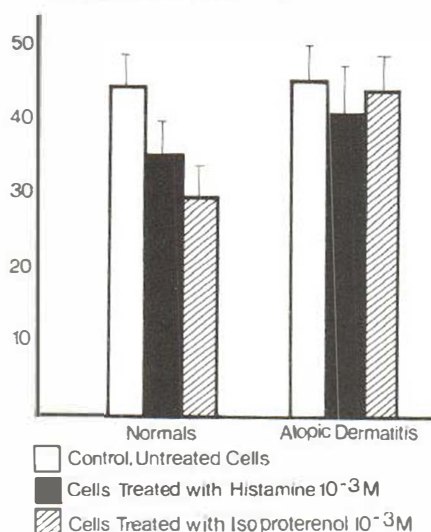


Fig. 1. Influence of histamine and isoproterenol on leukocyte adherence in normals (10) and in patients with severe atopic dermatitis (11). The top of each column indicates the percentage of adherent cells (Pa) \pm S.E.M.

Percent inhibition = $P_i = 100 - P_{ca}$

Total number of cells per ml counted whole blood = T

Total number of cells per ml counted on effluent blood = E

Total number of adherent cells $T_a = T - E$

Significance was determined by using Student's *t*-test.

RESULTS

1. Adherence of untreated leukocytes

The percentage of adherent cells (Pa) was calculated from the number of effluent cells and the total number of cells applied to the columns. The mean value from 11 severe AD patients was 46.7%, while the normal, from 10 controls, was 45.0%. Obviously, the ability to adhere to nylon fibers was not altered in untreated leukocytes from atopics, compared with normals. The total leukocyte counts in AD patients and normals averaged $4948/\mu\text{l} \pm 2612$ (SD) and $6711/\mu\text{l} \pm 1682$ (SD) (SD), respectively. This difference was not significant. Wright-stained differentials were carried out in all specimens. No significant variations in cell types were found between AD patients and normals. Some atopic patients showed increased eosinophil percentages (4–10%), but eosinophils behaved similarly to neutrophils as regards adherence characteristics. The percentage of adherent lymphocytes was very low

(range for Pa 1%–10.5%), indicating that very few lymphocytes adhere to the column. Mean Pa for granulocytes in our system was $90\% \pm 13$ (2 standard deviations). Mononuclear phagocyte adherence appears to be similar to granulocytes, though paucity of cells in effluents makes it difficult to obtain absolutely reliable quantitation.

2. Effect of isoproterenol and histamine on normal leukocyte adherence

Results obtained by incubating normal leukocytes with different concentrations of histamine, ranging from 10^{-3} M to 10^{-7} M, showed a maximum response, giving the most pronounced inhibition of leukocyte adherence at 10^{-3} M and a reasonable inhibition also at 10^{-4} M and 10^{-5} M. The influence on inhibition was nearly eliminated at 10^{-6} M and 10^{-7} M of histamine (Pa for histamine at different concentrations investigated in 10 normal persons: 10^{-3} M: 33.5%, 10^{-4} M: 35.1%, 10^{-5} M: 37.3%, 10^{-6} M: 42.1% and 10^{-7} M: 46.0%).

Isoproterenol was tested on normal leukocytes at 10^{-3} M, 10^{-4} M and 10^{-5} M. A maximum effect was recorded at 10^{-3} M (Pa for isoproterenol at different concentrations investigated in 10 normal persons: 10^{-3} M: 31.1%, 10^{-4} M: 38.2% and 10^{-5} M: 41.3%).

In our further study we used histamine 10^{-3} M and isoproterenol 10^{-3} M when comparing the response of atopics and normal subjects.

The effect of isoproterenol was somewhat more inhibitory than histamine but both drugs had a highly significant effect on adherence.

Theophylline at a concentration of 10^{-1} M did not further augment the effect of histamine. Differential counts of effluent leukocytes showed that all 3 classes of granulocytes (neutrophils, basophils and eosinophils) were equally affected by the drugs.

Table 1. Effect of isoproterenol and histamine on adherence of normal human leukocytes

Mean of percentage inhibition in 10 normal subjects. $P_i \pm$ S.E.M.

Agent	Percentage inhibition	<i>p</i> -value
Isoproterenol (10^{-3} M)	$33.6\% \pm 6.5$	<0.001
Histamine (10^{-3} M)	$20.8\% \pm 2.3$	<0.001
Histamine (10^{-3} M) + Theophyllin (10^{-4} M)	$20.6\% \pm 6.5$	<0.01

Table II. Effect of isoproterenol (10^{-3} M) on adherence of leukocytes from normals and patients with various atopic conditions

Mean percentage of control adherence. Pca \pm S.E.M.

Subjects	Percentage of control adherence	p-value
Normals (10)	66.4% \pm 4.4	0.001
Atopic dermatitis severe (11)	100.2% \pm 5.1	NS
mild (5)	105.0% \pm 2.3	NS
Hayfever (6)	92.3% \pm 3.8	NS

Monocyte adherence did not appear to be decreased.

3. Comparison of drug effects on the adherence of leukocytes from normal and atopic subjects

We found that leukocytes from patients with atopic dermatitis were relatively resistant to the effects of histamine and isoproterenol (Fig. 1). While normal leukocyte adherence was 21%–34% inhibited (Pi indicated in Table I), atopic cells showed only 9% and 4% inhibition by maximum 10^{-3} M concentrations of histamine and isoproterenol. These differences were significant ($P > 0.05$ in both cases).

4. Drug effects on leukocytes from patients with other atopic conditions

In this study, cells from patients with severe and mild atopic dermatitis and with hayfever were incubated with isoproterenol 10^{-3} M and then tested for adherence. Adherence for the atopic groups was not significantly below the control adherence level, though hayfever patients were marginal (Table II).

5. Effects of H-1 and H-2 histamine antagonists on histamine-induced inhibition of cell adherence

Cells were incubated for 30 min at 37°C in the presence of histamine alone or histamine plus either chlorpheniramine or metiamide to evaluate H-1 and H-2 receptor blockade, respectively. As seen in Table III, chlorpheniramine did not block histamine effects, while metiamide caused inhibition of both histamine concentrations. Neither metiamide nor chlorpheniramine at the 10^{-5} M concentration had any direct effect on cell adherence. Higher concen-

trations of metiamide could not be used because of low solubility in aqueous, physiologic solutions.

DISCUSSION

Atopic dermatitis is characterized by dry, pruritic, lichenified skin, abnormal cutaneous physiologic and pharmacologic responses and defects of humoral and cellular immunity (5). In spite of multiple features, there is no specific laboratory marker with which to identify the disease.

Szentivanyi suggested (1968) that the basic defect in atopic disease might be related to blockade of beta-adrenergic receptors of various cells (9). Abnormal immunologic responses might be a reflection of an imbalance in the cyclic nucleotide regulatory system.

Subsequent *in vitro* studies have supported Szentivanyi's concept. Epidermis and leukocytes from patients with atopic dermatitis show decreased responses to beta-adrenergic agents (2, 4). However, Parker et al. have shown a similarly diminished response of atopic leukocyte cAMP to PGE₁, suggesting a more generalized defect (8).

We have examined the leukocyte adherence response to histamine and to the beta-adrenergic agonist, isoproterenol. Both agents have receptors on leukocytes and both caused significant inhibition of leukocyte adherence, presumably by increasing intracellular cAMP (1).

We noted no baseline difference between normal and atopic leukocytes but there was a significantly smaller histamine and isoproterenol effect on the adherence of cells from patients with atopic dermatitis and hayfever.

Our findings of histamine inhibition of leukocyte adherence and the blocking of this effect by the H-2

Table III. Histamine antagonists: percentage inhibition of histamine effect on leukocyte adherence Pi \pm S.E.M.

Agent	Percentage inhibition
Histamine (10^{-6} M)	
+ Chlorpheniramine (10^{-5} M)	0%
+ Metiamide (10^{-5} M)	8.5% \pm 1.1
Histamine (10^{-7} M)	
+ Chlorpheniramine (10^{-5} M)	3.5% \pm 3.1
+ Metiamide (10^{-5} M)	32.5% \pm 4.6

histamine receptor antagonist, metiamide, suggest that this effect may be mediated by stimulation of cAMP. The lack of histamine effect in leukocytes from atopic dermatitis patients supports Parker's finding that atopic cells may have defects other than 'beta-adrenergic blockade' (8). These findings are in agreement with the report of decreased cAMP response to histamine in granulocytes from asthmatic individuals (3).

These preliminary studies suggest a potentially useful functional assay for diagnosing and monitoring patients with atopic disease. This *in vitro* system also provides for possible testing of agents which may be useful in the treatment of atopic dermatitis.

REFERENCES

1. Bryant, R. E. & Sutcliffe, M. C.: A Method for Quantitation of Human Leukocyte Adherence to Glass. *Proc Soc Exp Biol Med* 141: 196-202, 1972.
2. Busse, W. W. & Lee, T. P.: Decreased Adrenergic Responses in Lymphocyte and Granulocytes in Atopic Eczema. *J Allergy Clin Immunol* 58: 586, 1976.
3. Busse, W. W. & Sosman, J.: Decreased H₂ Histamine Responses of Granulocytes of Asthmatic Patients. *J Clin Invest* 59: 1080, 1977.
4. Carr, R. H., Busse, W. W. & Reed, C. E.: Failure of Catecholamines to Inhibit Epidermal Mitosis *In Vitro*. *J Allergy Clin Immunol* 55: 255-262, 1973.
5. Hanifin, J. M. & Lobitz, W. C. Jr: Newer Concepts of Atopic Dermatitis. *Arch Dermatol* 113: 663-670, 1977.
6. Hill, H. R., Estenson, R. D., Quie, P. G., Hogan, N. A. & Goldberg, N. D.: Modulation of Human Neutrophil Chemotactic Responses by Cyclic 3', 5'-Guanosine Monophosphate and Cyclic 3', 5'-Adenosine Monophosphate. *Metabolism* 24: 447, 1975.
7. MacGregor, R. R., Spagnudo, P. J. & Lentnek, A. L.: Inhibition of Granulocyte Adherence by Ethanol, Prednisone and Aspirin Measured with an Assay System. *N Engl J Med* 291: 642, 1974.
8. Parker, C. W., Kennedy, S. & Eisen, A. Z.: Leukocyte and Lymphocyte Cyclic AMP Responses in Atopic Eczema. *J Invest Dermatol* 68: 302, 1977.
9. Szentivanyi, A.: The Beta Adrenergic Theory of the Atopic Abnormality in Bronchial Asthma. *J Allergy Clin Immunol* 42: 203-232, 1968.

Received September 3, 1979

H. Thulin, M.D.
Department of Dermatology
Marselisborg Hospital
DK-8000 Århus C
Denmark