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HISTAMINE RECEPTOR-BEARING MONONUCLEAR CELLS IN ATOPIC DERMATITIS

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Abstract. The number of mononuclear cells bearing membrane receptors for histamine was investigated in peripheral blood from children with atopic dermatitis (AD) by means of the Rosette Histamine Assay, using erythrocytes coated with histamine. Histamine Rosettes (HR) varied from 5.70 to 11.85% in healthy adults; from 3.25 to 7.75% in control children and from 2 to 6.55% in children with AD. Histidine and tryptamine inhibited HR formation slightly, whereas free histamine, histamine H1 and H2 receptor antagonists and the histamine H2 receptor agonist (dimaprit) reduced HR formation much more strongly; no significant difference in inhibition of HR formation by these test agents was found between the three groups of subjects, although the reduction in HR formation by histamine was not constant in children with AD.

Key words: Histamine; Histamine Rosettes (HR); Children with atopic dermatitis (AD)

Over the past few years, numerous workers have shown that monocyte and lymphocyte sub-populations particularly suppressor T cells (7, 8, 12), exhibited membrane receptors for histamine (1, 4, 8). Furthermore, the regulatory roles of histamine via suppressor cells in immune reactions are most probable (5, 7) and significant differences have been observed between atopic and control subjects (10, 12). Using a rosette technique with erythrocytes coated with histamine, we have studied the mononuclear cells (lymphocytes and monocytes) in the peripheral blood of children with AD. Modification of the spontaneous histamine-rosette formation by free histamine, histamine H1 and H2 receptor antagonists and agonists was also studied.

MATERIAL AND METHODS

Technique. The technique used was a slight modification of the method described by Saxon et al. (8). Briefly: peripheral blood mononuclear cells obtained using Ficoll-Hypaque density separation were mixed with sheep red blood cells (SRBC) coated with rabbit-serum-albumin (RSA) or a histamine-rabbit-serum-albumin (H-RSA) conjugate prepared *ad modum* Kedar & Bonavida (3). Then cell suspensions were centrifuged and placed at 0/4°C for 30 min. After staining, (Crystal Violet, 0.5%), cells were examined under the microscope; a rosette was defined as a stained cell which bound three or more erythrocytes. The percentages of HR were expressed as follows: % rosettes (H-RSA−SRBC)−% rosettes (RSA−SRBC).

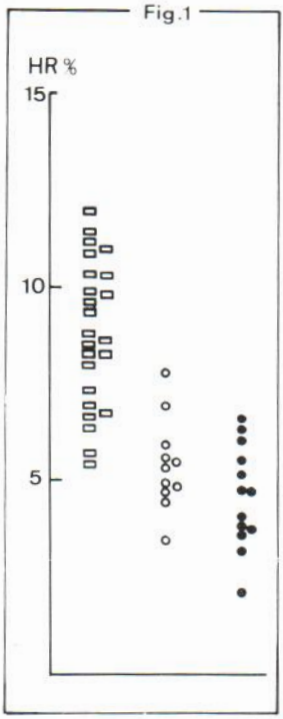
Inhibition of rosette formation was studied as follows: aliquots of the mononuclear cell suspension were incubated with test agents for 30 min. Then the samples were centrifuged, supernatants discarded and cell pellets treated as described above. Test agents were: histidine (100 γ/ml), tryptamine (100 γ/ml) histamine (100 γ/ml), methiamide (50 γ/ml), neoantergan (10 γ/ml) and dimaprit (100 γ/ml).

Subjects. Eight healthy adult volunteers (18/68 years) were studied twice at least, at 1 to 3 months intervals; 24 experiments were performed; 11 non-atopic children (2/15 years) were studied once only; none had lymphoproliferative disease or neoplasia; 11 children (2/14 years) with AD who had not received any treatment for at least one month; 2 were studied twice.

RESULTS

1. Number of mononuclear cells bearing histamine-membrane receptors: Fig. 1 shows that HR levels are significantly lower (U test) in children than in adults ($\alpha < 0.01$). HR are slightly lower in children with AD, but this was not significant as compared with control children. Spontaneous variations in the number of HR were observed: they were slight in most cases in normal adults as well as in 2 children with AD (Table I).

2. Incubations with test agents: Fig. 2 shows that histamine inhibited HR formation significantly more than did histidine and tryptamine (U test, $\alpha < 0.01$). Fig. 2 also shows that there was a significant inhibition of HR formation, not only with histamine, but also with methiamide, neoantergan and dimaprit (Wilcoxon test, $\alpha < 0.01$). The inhibitions induced by histamine and the other test agents were not significantly different in adults and control children as compared with children with AD. However, the inhibition of HR formation by histamine was not constant in children with AD (Fig. 2).



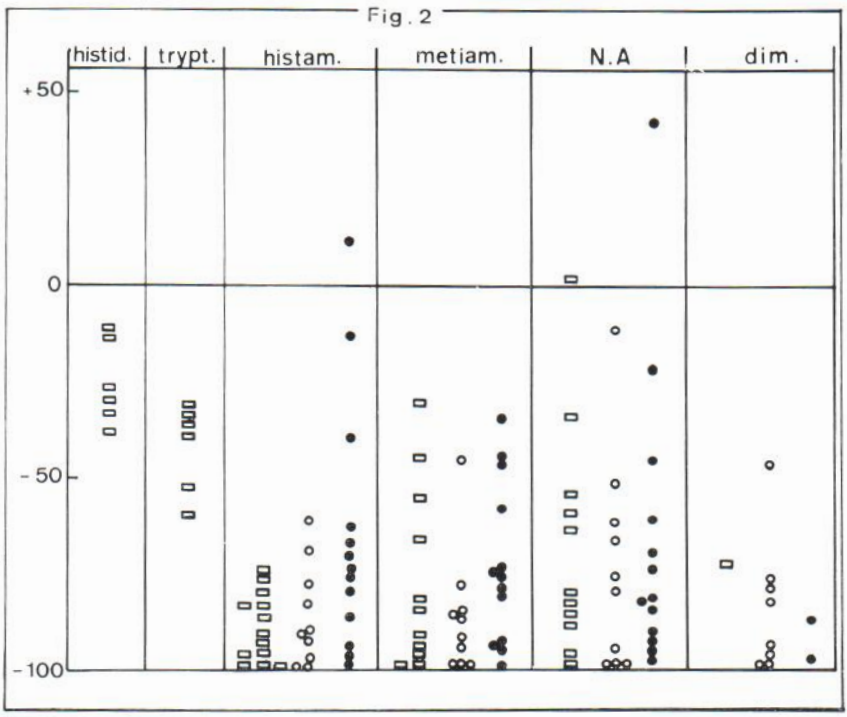


Fig. 1. HR (%) in control adults (□), control children (○) and children with atopic dermatitis (●).

Fig. 2. Inhibition (%) of HR formation by previous incubations with test agents: histidine (Histid.) 100 γ/ml; tryptamine (Trypt.) 100 γ/ml; histamine (Histam.) 100 γ/ml; methiamide (Methaim.) 50 γ/ml; neoantergan (NA) 10 γ/ml, and dimaprit (Dim.) 100 γ/ml, in normal adults (□), control children (○) and children with AD (●). Inhibition (%) was calculated as $[(a-b)/a] \times 100$, where a and b represent HR (%) in preparations preincubated with medium alone or with test agents respectively.

DISCUSSION

The rosette-histamine assay is a highly specific method for the detection of histamine membrane receptor bearing mononuclear cells, in that free histamine, histamine H1 and H2 receptor antagonists and agonists significantly inhibited HR formation, whereas the reduction in HR formation by substances closely related chemically, such as histidine, and other amines (tryptamine) was very slight. Similar results have recently been obtained with guinea-pig macrophages (2); Melmon et al. (4) have previously demonstrated the inhibition by histamine and histamine antagonists of histamine receptor bearing cell adherence to columns of Sepharose beads coated with a H-RSA conjugate.

Table I. *Spontaneous variations in HR (%) formation in healthy adults (cases 1 to 7) and 2 children with AD (cases 8, 9)*

	Tests				
Cases	I	II	III	IV	V
1	8.50	10.70			
2	11.40	5.40			
3	11	8	6.70		
4	6.90	7.40	10.20	8	
5	8.60	9.40	9.60	11.80	6.70
6	10.90	8.30	5.70	8.70	
7	8.30	8.50			
8	6	4.70			
9	5.40	4.80			

The levels of spontaneous HR formation that we have found are lower than those observed by other authors (8). These discrepancies probably result from the choice of control subjects (8) or from technical factors (1). Wide variations in the number of blood mononuclear cells bearing histamine membrane receptors have been observed by these authors; the reasons are so far unclear, but membrane environment (4) and technical considerations seem critical. These factors might also explain the spontaneous individual variations (Table I). In

children, the numbers of histamine rosettes were lower than in adults; this has been suggested previously (1).

The numbers of HR are slightly lower in children with AD than in control children, although the difference is not significant. Suggestive evidence of a suppressor T cell deficiency, which are thought to be histamine-membrane receptor bearing cells, has been obtained in recent studies (11). However, this sub-population represents about 12% of peripheral blood T lymphocytes (8), and decreased numbers of these cells might well not result in decreased numbers of total HR-forming mononuclear cells. Furthermore, this defect seems possibly qualitative as well as quantitative, although the results are sometimes contradictory (6, 9).

In vitro hyperreactivity to histamine has been described in atopic subjects (10, 12). We have found no statistical difference in the inhibition of HR formation by various test agents (histamine, histamine H1 and H2 receptor antagonists and dimaprit) in children with AD as compared with controls. However, in some children with AD, histamine (100 γ/ml) did not inhibit—and even stimulated—HR formation. This was never found in the other groups studied. Preliminary results with dose–response curves suggest an analogous decreased sensitivity of the inhibition of HR formation in children with AD.

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DISCUSSION

Jones (Atlanta). Q: You were unable to competitively block the histamine receptors by addition of histamine in some of the atopic children. How do you interpret that?

A: There are several possible interpretations. One is that the histamine receptors are already saturated in vivo by histamine.

Jones (Atlanta). Q: We have noticed in some of our studies that the histamine receptor may be present at some time in the lifetime of the cell or in a person but not present at other times. Do you have any comments on that?

A: By doing several determinations in the same patients at intervals of several weeks we found variations of something like 30%.