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CHARACTERIZATION OF β-ADRENORECEPTORS ON INTACT CIRCULATING LYMPHOCYTES FROM PATIENTS WITH ATOPIC DERMATITIS

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Abstract. [^{125}I]hydroxy benzyl pindolol (HYP) was used in a binding assay to compare the number and affinity of β-adrenergic receptors on circulating lymphocytes from 8 patients with severe atopic dermatitis (AD) and 8 age-matched controls. The number of receptors per lymphocyte (R_T) found in the atopic group (856 ± 132) was not statistically different from that of the controls (702 ± 107). By contrast, the dissociation constant (K_D) was significantly higher in patients with AD ($K_D = 20.8 \pm 3.3 \times 10^{-11}$ M) than in controls ($K_D = 12.6 \pm 3.45 \times 10^{-11}$ M). The results thus indicate that lymphocyte β-adrenergic receptors from patients with AD have a lower affinity for HYP than those of controls.

Key words: Atopic dermatitis; Peripheral blood lymphocytes; Beta-adrenoreceptors; [^{125}I]hydroxybenzyl pindolol

Although the pathophysiology of atopic dermatitis (AD) still remains uncertain, it is tempting to explain the combined cutaneous and immunological defects seen in this disease by a single cellular alteration. Several years ago, Szentivanyi proposed that the primary defect in atopy lies in an impaired cellular response to β-adrenergic agonists (26). Most of the data related to this hypothesis were obtained in pharmacological assays measuring either a metabolic, a physiological or a cellular response to β-adrenergic agonists. At the lymphocyte level, the presence of specific β-adrenergic receptors has been demonstrated in assays measuring either the synthesis of cAMP or the activity of the enzyme adenylate cyclase induced by various agonists in the presence or absence of antagonists (25). Methodological progress allowed the direct characterization of the β-adrenergic receptor on lymphocytic crude membrane preparations (27)—and even on intact living cells such as glioma cells (23), muscle cells (16) and human lymphocytes (20). In the present work we have used [^{125}I]hydroxybenzyl pindolol (HYP) to determine the number and the affinity of beta receptors present on peripheral blood lymphocytes (PBL) from patients with AD.

MATERIAL AND METHOD

Patients

Eight patients with atopic dermatitis (AD) (6 women, 2 men) were compared with 8 healthy subjects (1 woman, 7 men). The two groups were matched for age (mean: 28 years; range: 19–50). All the patients included in this study suffered from severe AD (more than 25% of body area involved with pruritus, excoriation and erythema or lichenification). The diagnosis of AD was based on the criteria proposed by Hanifin and Lobitz (10): pruritus, typical flexural lichenification and a chronic course.

The patients had elevated serum IgE levels (mean value: 765 UI/ml) as well as specific IgE antibodies to common allergens. All these subjects had a past history of asthma or allergic rhinitis. None had received systemic corticosteroids, beta adrenergic drugs or other anti-asthmatic treatment during the last 3 years.

The experiments were conducted in order to test simultaneously and under identical conditions equal numbers of patients and controls.

Lymphocyte preparations

The lymphocytes were isolated from heparinized blood by Ficoll-Hypaque centrifugation, macrophages and polymorphonuclears were removed by iron-powder treatment. The lymphocyte preparations contained less than 1% peroxidase-containing cells and almost no platelets. Viability of the cells was higher than 95%.

Method

Binding experiments were performed exactly as described previously (20). Briefly, $2–4 \times 10^6$ lymphoid cells were incubated for 60 min at 30°C in Hanks' Balanced Salt Solution (supplemented with 5×10^{-5} M phentolamine, 10 mM $MgCl_2$ and 50 mM Tris, pH 7.4) with varying amounts of [^{125}I]hydroxybenzyl pindolol ((±) ^{125}I-HYP). After 60 min, the mixture was diluted with 5 ml of 50 mM Tris HCl, pH 7.4, containing 10 mM $MgCl_2$ and was immediately filtered on a G F C Whatman fibreglass filter. The filter was washed with 25 ml of the same buffer at 37°C, and counted in a Packard gamma counter (efficiency 65%). The radioactivity bound in the presence of 10^{-7} M cold HYP or 10^{-7} M (−) propranolol was considered as the "non-specific binding".

The specific binding to lymphocytes was defined as the difference between the total and the non-specific binding. The number of receptors and the dissociation constant (K_D)

Table I. *Receptors on peripheral blood lymphocytes in patients with atopic dermatitis and in controls*

R_T = number of β-receptors per circulating lymphocyte in normal subjects and in patients with atopic dermatitis; K_D = the dissociation constant for [^{125}I]-HYP binding. Mean ± S.E.M.

	Patients	Controls
N	8	8
R_T	856 ± 132	702 ± 107
K_D	$20.8 \pm 3.31 \times 10^{-11}$**	$12.6 \pm 3.45 \times 10^{-11}$

** $t = 6.23$, $p < 0.01$.

were calculated by using a Scatchard-plot calculated from the saturation curve. All measurements were done at equilibrium, which is reached after 30 min incubation and is stable for at least 90 min (20). Previous studies (20) have shown that under these experimental conditions, the lymphocyte binding sites displayed the saturability and stereospecificity expected of true beta adrenergic receptors. The displacements observed with three agonists (isoproterenol, epinephrine, norepinephrine) further showed an order of potency compatible with a beta-2-type of receptor.

RESULTS

As shown in Table I, peripheral blood lymphocytes from normal subjects displayed 702 ± 107 receptors per cell with a K_D of $12.6 \pm 3.4 \times 10^{-11}$ M. In the atopic group, the number of receptors per lymphocyte was comparable (856 ± 132) whereas the K_D was significantly higher ($20.8 \pm 3.3 \times 10^{-11}$ M; $p < 0.01$).

DISCUSSION

Logsdon et al. (12), Parker & Smith (18) and Alston (1) reported that lymphocytes from asthmatic patients had a selective, depressed response to beta adrenergic agonists. The interpretation of their findings is difficult, because most of the patients were under sympathicomimetic treatment, which is known to depress the leukocyte response to adrenergic agents (5, 16). Parker & Eisen (17), working on patients with AD who were not receiving bronchodilator therapy, have shown that maximum concentration of isoproterenol increased cAMP concentrations five-fold in leukocytes of normal subjects, but only two-fold in leukocytes from AD patients. Similar findings have been made by Reed (21). In the present study, we compared the number and the affinity of beta adrenergic receptors on intact circulating lymphocytes from patients with AD and from healthy controls of same age.

We found that PBL from healthy young adults display 700 receptors per cell; these results are very close to those of Sano (22) and to our earlier findings in intact tonsil lymphocytes (20). The data clearly show that the β-receptors on lymphocytes from patients with severe AD are not reduced in number but have a significantly lower affinity for [^{125}I]HYP. A direct conclusion from these results would be that the aforementioned findings of Parker and Eisen (17) and Reed (21) cannot be explained by a reduction in the number of leukocyte adrenoreceptors. A first objection to this interpretation could be that the patient's lymphocytes contain abnormal proportions of B and T cells (8, 13, 2, 14, 3, 4, 11). This argument would be particularly relevant in the light of our recent findings that T lymphocytes have many lower beta receptors than B lymphocytes (20). However, we (6) and others (9) could not find any reduction in the T cell content of PBL from patients with AD. A second possibility to be considered is the influence of topical corticosteroid treatment on the beta receptors. Indeed, all the patients included in this study had been treated for several days with two applications per day of corticosteroid ointments on more than 25% of the body surface. The absorption of steroid hormone is certainly not negligible under such clinical conditions. The possibility that this treatment might interfere with the number of beta receptors is strongly suggested by recent in vitro and in vivo findings that hydrocortisone induces the synthesis of new beta receptors (7, 15) without altering the K_D.

Thus it is still possible that untreated atopic patients have a reduced number of lymphocyte beta receptors and that this anomaly is corrected by local corticosteroid treatment.

Our findings of a significant difference in the K_D for [^{125}I]HYP between atopics and controls cannot be explained by either an eventual decrease in T cell number or the use of topical steroids. Indeed peripheral T and B lymphocytes do not differ in their K_D for [^{125}I]HYP (20) and hydrocortisone has no effect on this parameter (7, 15).

The present results showing a normal number but a reduced affinity of β-receptors in lymphocytes from atopic patients should be compared with those of Singh et al. (24). These authors, working on the maturation of mice thymocytes, observed a progressive reduction during the maturation process of

the thymocyte cAMP synthesis in response to isoproterenol. This could not be accounted for by a modification in the number of receptors but was paralleled by a progressive reduction in the affinity for [^{3}H]alprenolol, used to label the receptor sites.

We suggest on the basis of our findings in this study that there is a qualitative anomaly of the β-receptors in the lymphocytes from atopic patients, although we cannot exclude the possibility of a quantitative defect. Further experiments are necessary before a definite conclusion can be reached. For example, the K_D of various agonists should be compared in displacement experiments on cells from atopics and controls. Another basic question to be answered is the conceivable effect of the inflammatory mediators released in the patients on the function and expression of β-receptors.

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DISCUSSION

Dobson (Buffalo). Q: Are the changes in lymphocytes secondary to the disease or do they represent the primary change?

A: The changes in atopic dermatitis are very similar to the changes that have been found in the lymphocytes in psoriasis, and where there seems to be a clear relationship between the severity of the disease and the impairment of the lymphocytes functions. The suggestion has been made that

the changes in lymphocytes are therefore secondary to the inflammatory disease, rather than the primary cause. We studied patients in a very mild phase and an active phase and found no differences between the two groups. We have followed up the patients for one year and examined them in different phases of activity of their dermatitis and there are no differences in the data with regard to isoproterenol response and PHA responsiveness. Family studies should be performed to investigate if this defect is genetic, or due to inflammation.

Strannegård (Gothenburg): We have some data regarding the primary or secondary nature of the lymphocyte aberrations seen in atopics. A prospective study on newborn infants has been performed and a significantly lower number of T-lymphocytes was found in one-month-old children with atopic parents as compared with newborns with nonatopic parents. This argues strongly for the primary nature of the lymphocyte deficiency in atopy.

Voorhees (Ann Arbor): The basic question is not whether the defect is primary or secondary, but whether it is important or not. Most common diseases are an interaction of multiple genes with their environments and a way of approaching this on the molecular level is really not available in modern science at the moment.