Hypodense Eosinophils: Characterization of Surface Molecule Expression

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ABSTRACT

Distinct eosinophil populations have been characterized on the basis of discontinuous Percoll density gradients. In peripheral blood, normal individuals show a low number of normodense and hypodense eosinophils, contrasting with the high amount of hypodense cells in patients who have allergies. To characterize these two eosinophil populations, we analyzed membrane expression of several antigens and cytokine receptors in normodense and hypodense eosinophils from patients who have allergies and controls. Hypodense eosinophils expressed higher levels of CD122, CD69, and CD4 in both patients with allergies and control individuals when compared to normodense eosinophils. The expression of CD125, CD124, CD25, CD132, and CD23 were similar in both cell types. (Allergy and Asthma Proc 23:117–124, 2002)

Because human eosinophils have a slightly higher density than other leukocytes, density gradient centrifugation commonly is used to isolate them.[1] Initial studies[2] revealed that human blood often contained a population of eosinophils that differs in density from normal eosinophils. Eosinophils of lower density were termed light density or hypodense cells, whereas those of normal density were termed normodense cells. Normodense eosinophils, from normal individuals, have a peak density of ~1.088 g/mL. In contrast, light-density eosinophils have a peak density of 1.070–1.085 g/mL[2]. The numbers of hypodense eosinophils directly correlate with the degree of peripheral blood eosinophilia.[3]

Morphologically, hypodense eosinophils from individuals with eosinophilia are different from normodense eosinophils of normal controls; individual granules are smaller and the total granule area of the cell is reduced also. A series of changes including cell swelling, a decrease in the cytoplasm volume occupied by granules, and an increase in granule lucency appear to contribute to the generation of hypodense eosinophils *in vitro*. A reduction in eosinophil granule size may be the cause of its lower density *in vivo*.[4] Hypodense eosinophils appear to be metabolically more active than the normodense ones. They contain less major basic protein per million cells than the normodense cells and the levels of eosinophil peroxidase may be reduced. They also show an increased oxygen consumption parallel with an augmented ability to generate superoxide anion, an increased production and releasability of LTC4 after incubation with immunoglobulin G (IgG)–coated beads or calcium ionophore A23187, and a potent cytotoxic activity against antibody-coated targets, when compared to normodense eosinophils.[5]

Comparative peripheral blood eosinophil studies have been limited by the difficulty in isolating sufficient numbers of pure eosinophils from healthy noneosinophilic subjects. Commonly used isolation methods are based on the physical

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differences among eosinophils and neutrophils, the usual contaminating leukocyte. Percoll density gradients were used initially to obtain pure normal density eosinophils[6]; however, a relatively small number of eosinophils is obtained from peripheral blood of normal subjects. Recently, protocols using negative selection of eosinophils with anti-CD16–coated magnetic beads have been a major advance in cell recovery and purity.[7] However, an important concern has been the fact that eosinophils isolated by these diverse protocols may have different functional responses *in vitro*.

Eosinophils possess a variety of surface markers and receptors involved in differentiation, tissue recruitment, and activation, as well as in the synthesis and secretion of protein and lipid mediators. Cell receptors can be divided into (1) those that are expressed constitutively on normal blood eosinophils and in which expression may be up-regulated during eosinophil activation and (2) those that are induced during priming or activation and expressed only on the activated cell. Although eosinophils express the low-affinity IgG receptor (FcγRII, CD32), they lack the FcγRIII receptor (CD16) present on neutrophils.[8] Eosinophils express β1- (CD29), β2- (CD18), and β7-integrins. Among the β1-integrins, very late activator antigen (VLA) 4 (CD49d/CD29), which binds the vascular cell adhesion molecule 1, plays an important role for eosinophil migration from the blood stream into the tissues.[9] In addition, eosinophils have a predominant and specific marker, interleukin (IL)-5 receptor (CD125), in which its expression is ~1000 receptors per cell.[10]

The expression of CD25 (α-chain of the IL-2 receptor) was shown in eosinophils without *in vitro* activation. CD122 (β-chain of the IL-2 receptor) was undetectable under the same conditions, although it was functionally evaluated.[11] The γ-chain of IL-2 receptor (CD132) was detected on granulocytes, with no reference to eosinophils in particular.[12]

The presence of the α-chain of the IL-4 receptor (CD124) was inferred by the biological activities of the cells stimulated with IL-4 and it seems to be increased in some inflammatory skin diseases such as atopic dermatitis.[13]

Constitutive expression of the low-affinity IgE receptor (FcεRII/CD23) has been established at the messenger RNA (mRNA) level on human eosinophils; however, flow cytometric analysis shows only a limited and heterogeneous expression of CD23 receptors.[14]

CD69 is an early activation cell marker for lymphocytes and its cross-linking on platelets triggers aggregation and mediator release. Unstimulated peripheral blood eosinophils do not express CD69, but cultured eosinophils with granulocyte-macrophage colony–stimulating factor (GM-CSF), IL-3, or IL-5 do express CD69.[15]

Inducible surface markers identified principally on *in vivo* or *in vitro* activated eosinophils include the T-cell–associated marker CD4, human leucocyte antigen-DR, the α- and β-chains of the IL-2 receptor (CD25 and CD122), Mac-2, FcεRII (CD23), and CD69.[15]

In this study, we investigated, using flow cytometry, surface marker expression on normo- and hypodense eosinophils obtained by Percoll density gradients from peripheral blood of normal individuals and patients who have allergies. Normodense and hypodense eosinophils were identified by the absence of CD16 expression and the positiveness of CD49d. This novel approach avoids the use of negative selection, which may further activate these cells and, consequently, modulate surface marker expression.

MATERIALS AND METHODS

Study Subjects

The patient group included 15 untreated individuals selected from the Allergic Disease Outpatient Clinical Unit of the Institute of Immunology. Eight patients had perennial allergic rhinitis (PAR) and the other seven patients had extrinsic bronchial asthma. The age range of the group was between 15 and 40 years. All patients were sensitive to at least one regional allergen (prick tests); none had received oral corticosteroid treatment for at least 3 months before the study, and parasitic infection was ruled out in all of them by serial stool tests. Increased eosinophils in the nasal cytology smears (>25% of total cells) were present in PAR patients. Reversible airflow obstruction in the bronchial asthma patients was established through spirometry; forced expiratory volume in 1 second was (FEV_1) <80% predicted and FEV_1 increases at least in 12% of the patients after using a short-acting inhaled β_2-agonist. The control group consisted of 15 sex- and age-matched healthy subjects who were nonatopic and were selected among voluntary blood donors of the Central University Hospital Blood Bank. The Institute Ethical Committee approved the study protocol.

Cell Separation

Eosinophils were purified from 50 mL of EDTA-anticoagulated peripheral blood samples obtained from patients with allergies and controls following the method described by Gärtner.[16] Briefly, five parts of peripheral blood were mixed with one part of 6% dextran (Sigma Chemical Co., St. Louis, MO) in 0.15 M of NaCl and kept at room temperature for 30 minutes. The leukocyte-rich suspension was collected and washed once with phosphate-buffered saline (PBS) gel (0.01 M of phosphate buffer, 2 mM of EDTA, 5 mM of glucose, and 0.1% gelatine) and centrifuged at 450 × *g* for 10 minutes at 4°C. The cells, adjusted at 25–30 × 10^6/mL, were resuspended in 1 mL of Percoll solution (Percoll: Pharmacia LKB Biotechnology, Inc., Uppsala, Sweden; density, 1.070 g/mL) layered over a discontinuous isotonic Percoll gradient, and centrifuged at 1600 × *g* for 30 minutes. Percoll densities (1.070, 1.080, 1.085, 1.090, and 1.100 g/mL) were obtained according to

Day's protocol.[17] Normodense eosinophils were collected from the 1.090- and 1.100-g/mL layers, and hypodense eosinophils were obtained from the 1.085- and 1.080-g/mL layers. Cells were washed in PBS gel and contaminating red cells were lysed with 15 mL of NH_4Cl solution (150 mM of NH_4Cl, 10 mM of $NaHCO_3$, and 1 mM of EDTA). After mixing for 7 minutes at room temperature, the suspension was centrifuged at 450 × *g* for 10 minutes at 4°C and washed twice in PBS gel.

Cell viability was estimated by trypan blue exclusion. The differential cell count was determined by examining 100 cells stained with eosin/methylene blue solution in methanol.

Reagents

The following panel of monoclonal antibodies included CD16-PC5, CD49d-fluorescein isothiocyanate (FITC), CD124-PE, CD25-RD1, CD122-FITC, CD23-RD1, CD4-FITC, CD69-PE, and mouse isotype control antibodies (IgG1 PC5, IgG1-FITC, and IgG1-RD1) and were purchased from Beckman Coulter Immunology (Hialeah, FL) and CD125-PE and CD132-PE were obtained from Pharmingen (San Diego, CA):

- CD16 (clone 3G8). Reacts with the low-affinity receptor for IgG (FcγRIII). 3G8 strongly stains neutrophils and NK cells.
- CD49d (cloneHP2/1). This is the integrin α4-chain that noncovalently pairs with CD29 (β1-chain) to form the VLA-4 or with the integrin β7-chain.
- CD124 (clone S456C9). Reacts with the human IL-4 receptor α-chain.
- CD25 (clone IL-2R1). CD25 (IL-2R1, Tac, or p55) antigen is the low-affinity receptor for IL-2 (IL-2Rα).
- CD122 (clone IL-2R (p75). Reacts with the human IL-2 receptor β-chain (p75).
- CD23 (clone B6). Low-affinity receptor for IgE (FcεRII).
- CD4 (clone T4). CD4 is a coreceptor in major histocompatibility complex class II restricted antigen-induced activation, and it is expressed in the T-helper lymphocytes.
- CD69 (clone TP1.55.3). Activation inducer molecule. This activation antigen is one of the earliest appearing cell surface glycoproteins after T- or B-lymphocyte activation but is absent in resting lymphocytes.
- CD125 (clone A14). Reacts with the α-chain of the IL-5 receptor (IL-5R), expressed on eosinophils and basophils.
- CD132 (clone TUGh4). Reacts with the common γ-subunit shared by IL-2, IL-4, IL-7, IL-9, and IL-15 receptors.

Flow Cytometry

Purified normodense and hypodense eosinophils were analyzed in an Epics Elite flow cytometer (Coulter Electronics, Hialeah, FL), after previous alignment with DNA fluorescent check beads. To discriminate between eosinophils and neutrophils, cells were stained with anti-CD16, and an electronic map was created over the $CD16^-$ population. We confirmed that these $CD16^-$ cells were eosinophils through the high expression of CD49d (>95%). In this CD16 histogram another gate was set (that contains only $CD49d^+$ cells) for FITC on RD1/PE determination. Background fluorescence was assessed using isotype controls labeled with FITC or RD1/PE. The specific positiveness was assessed using the Immuno-4 program (Coulter Electronics).

Flow cytometric analysis was performed on 5000 $CD16^-/CD49d^+$ cells. Cell surface molecules, CD125, CD25, CD122, CD132, CD124, CD23, CD69, and CD4 were determined by triple color flow cytometry on purified eosinophils preparations. Briefly, the cells were adjusted to 1×10^6/mL and incubated with monoclonal antibody (MAb) anti-CD16-PC5 plus the corresponding FITC or RD1 MAb's for 30 minutes at 4°C. Then, the cells were washed three times with PBS gel. MAb binding was quantitated by flow cytometry and expressed as the percentage of stained cells. Nonspecific binding was determined using the irrelevant mouse isotypes IgG1-PC5, IgG1-FITC, and IgG1-RD1.

Statistical Analysis

Measurements from control individuals' and patients' eosinophils preparations were pooled for data analysis. Data are presented as the mean ± SD (X ± SD) of the percentage of positive cells. Statistical analysis was performed using Student's *t*-test for paired or unpaired data. The limit for significance was taken as $p < 0.05$.

RESULTS

The purity of cells obtained with the Percoll gradients was higher than 95% for the normodense eosinophils and 7% for the hypodense eosinophils. Despite this fact, hypodense eosinophils can be identified using flow cytometry by the $CD16^-/CD49d^+$ map. The viability was higher than 95% in every case. The number of eosinophils obtained from 50 mL of peripheral blood in patients was significantly higher when compared with controls ($p < 0.001$). Although there was a significant prevalence of hypodense eosinophils in all studied individuals ($p < 0.01$), in the patient's group the values of hypodense eosinophils was increased when compared with controls ($p < 0.01$). The ratios hypodense/normodense eosinophils in absolute values for controls and patients were 2.5 and 8.5, respectively (Table I).

TABLE I

Absolute and Relative Values of Normodense and Hypodense Eosinophils in Controls and Patients

	Eosinophils				
	Hypodense		Normodense		Total*
	Cells × 10^6	%	Cells × 10^6	%	Cells × 10^6
Controls	3.3 ± 1.2	70.3 ± 7.1	1.3 ± 0.5#	29.7 ± 7#	4.49 ± 1.4
Patients	13.6 ± 9.5§	89.2 ± 5.2§	1.6 ± 1.6#	10.8 ± 5.2#§	14.8 ± 1.4

**Absolute number of eosinophils obtained from 50 mL of peripheral blood.*
#p < 0.01; normodense vs hypodense eosinophils; §p < 0.01; patients vs controls.

The high expression of CD49d and CD125 in $CD16^-$ cells obtained from the 1.090–1.100 g/mL and 1.085–1.080 g/mL of Percoll density layers confirmed the eosinophilic nature of these cells (normodense and hypodense eosinophils, respectively; Fig 1; Table II. We did not find significant differences in the expression of CD49d between hypodense and normodense eosinophils or between controls and patients. CD125 was significantly lower ($p < 0.05$) in control hypodense eosinophils as compared with normodense eosinophils of the same group.

The α- and γ-chains of the IL-2 receptor are constitutively expressed in both eosinophil types, with no differences between groups. The percentage of CD122 positiveness was significantly increased in hypodense eosinophils of both groups ($p < 0.001$ and $p < 0.05$, respectively), contrasting with a decrease in mean fluorescence intensity (MFI), significant only in patients ($p < 0.05$). There was a lack of expression of the α-chain of the IL-4 receptor (CD124) in all groups studied (Table II).

When comparing individuals, CD23 and CD69 expressions were lower in the patient's group in both eosinophil populations as compared with controls, although the differences were not significant (Table III). On the other hand, hypodense eosinophils tend to show lower values of CD23 in all samples studied (Table III).

A higher expression of the activation marker CD69 was observed in hypodense eosinophils of both groups. This difference was not significant (Table III). There were no differences in the values of CD4 between groups.

Three of the eight PAR patients included in the study, with severe symptoms, were studied before and after 1 month of treatment with nasal Beclomethasone and environmental control. Remission of the clinical symptoms was obtained in all three patients (Tables IV and V). In both normo- and hypodense eosinophils there was (1) a significant increase of the MFI of CD125 after treatment; (2) a decreased expression of CD132 ($p < 0.001$) although its MFI was increased (nonsignificant); the contrary was observed with CD122 expression; (3) a mild, but significant ($p < 0.05$) modulation of CD124 expression after treatment; and (4) CD4 and CD23 were mildly modulated. In normodense eosinophils, a significant ($p < 0.05$) decrease of the MFI for CD25 expression was obtained after treatment.

DISCUSSION

Cells of the immune system use surface molecules for selective trafficking, intercellular communication, and focused cellular responses to a broad inflammatory stimuli.[18] Peripheral blood eosinophils typically circulate as resting cells but undergo activation that precedes degranulation, inflammatory mediator production, and cytokine secretion. Eosinophil activation occurs either before or concurrent with their passing through tissues and then they are recruited to specific inflammatory foci. Therefore, whole-blood eosinophils represent a useful starting point to evaluate early cellular activation. Activated eosinophils have been associated with symptoms or pathology in eosinophilic conditions such as asthma, hypereosinophilic syndrome, and helminth parasite infections.[19] The mechanism of these activation processes is believed to relate in part to cytokines (particularly IL-3, IL-5, and GM-CSF) secreted by lymphocytes in the local immune reaction or by vascular endothelial cells (IL-3 and GM-CSF).[20] Hence, understanding the nature and mechanisms underlying eosinophil activation should greatly enhance our understanding of their role in the immune reactions in which they participate.

By using separation on discontinuous density gradients, previous works have indicated the presence of a subpopulation of human eosinophils in the lightest density gradients referred to as hypodense eosinophils. Several lines of evidence suggest that hypodense eosinophils might correspond to a particular step of *in vivo* activation with regard to its metabolic response, cytotoxic ability, and organ tissue distribution.[6] Flow cytometry is a powerful method for assessing surface molecule expression on selected cell populations. In this study, we combined the isolation of eosinophils by Percoll densities and flow cytometry to evaluate surface molecule expression by eosinophils of different densities in control and in patients who have allergies.

Our results clearly confirm that CD16/CD49d expression can effectively discriminate between eosinophils and neu-

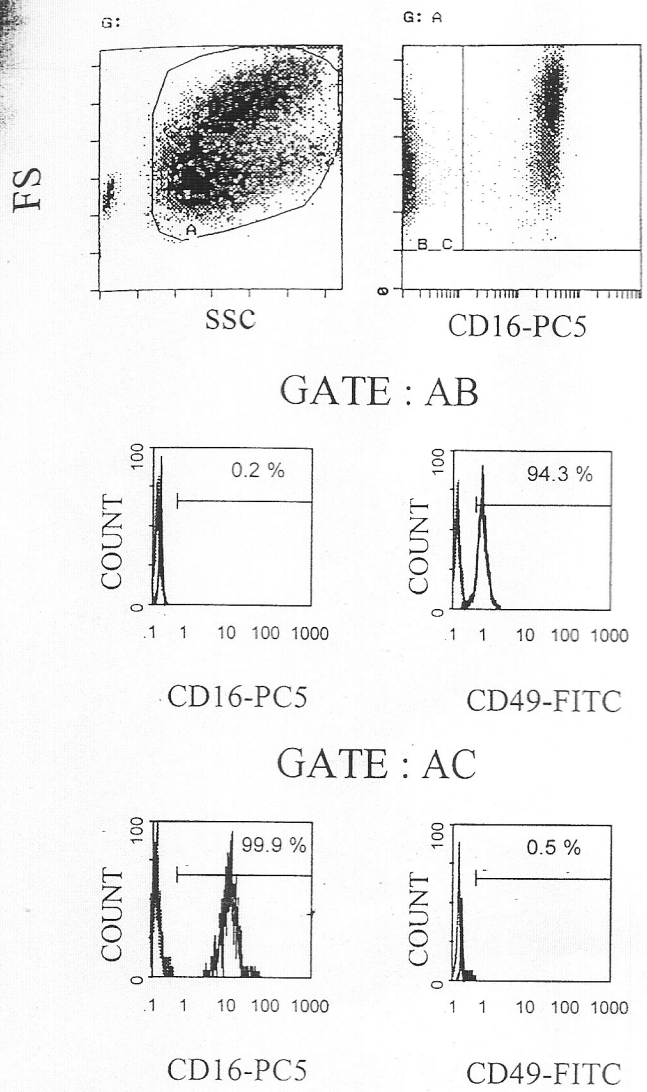

Figure 1. *Illustration of the adjustments made in order to ascertain eosinophil and neutrophil cell populations in a typical sample obtained from a control. In the top part of the figure, an electronic map, named A, was created in the cytogram (forward [FS] and side-scatter [SC]). CD16 was assessed in these cell populations and eosinophils were separated from neutrophils creating two electronic maps in the FS versus CD16-PC5 cytogram. The map nominated B, gated on map A, contains all the cells that lack CD16 expression, and the map C, also gated on map A, contains all the cells that were CD16$^+$ as shown in the figure. The other histograms show CD49d expression on both cell types. The cells that are CD16$^-$ express CD49d (eosinophils) and the cells that express CD16$^+$ lack CD49d expression (neutrophils).*

trophils. However, there are situations in which CD16 may be misleading. Some anti-CD16 antibodies are specific for only one form of the polymorphic Fc-receptor and the CD16 surface antigen can be shed from activated neutrophils under pathological conditions. Therefore, we evaluated CD49d, which is expressed on all eosinophils but not on neutrophils. Together with CD29, it forms the VLA-4 complex, which has been shown to be involved in adhesion to vascular adhesion molecule 1 on activated endothelial cells.[9] The high expression of CD49d in the cells gated with the electronic map created over the CD16$^-$ population confirms that we were looking at eosinophils.

As reported by others,[21] patients had a significant increase of eosinophils in peripheral blood. In both controls and patients there was a prevalence of the hypodense over the normodense eosinophils, but patients had a significant increase of hypodense eosinophils and a significant decrease of the normodense eosinophils when compared with controls.

IL-5 is a T-cell–derived soluble factor that supports the proliferation and differentiation of murine eosinophils as well as B-lineage cells.[22] An increased IL-5 production has been described in eosinophilia associated with malignant disease, helminth infection, allergic disease, and idiopathic hypereosinophilic syndrome.[10] IL-5 acts on various cells via a specific cell surface receptor and this was the reason to evaluate the IL-5 receptor on normo- and hypodense eosinophils from patients who have allergies and control donors. In all cases, we detected a high expression of IL-5R, with no significant differences in both normodense and hypodense eosinophils from the group of patients.

IL-2 receptors are classified into three types differing in their affinities to associate with IL-2: the high-, intermediate-, and low-affinity receptors, and there are at least three distinct subunits, the α-, β-, and γ-chains. The α-chain (IL-2Rα, CD25) is a 55-kDa cell surface glycoprotein (p55), comprising the low-affinity IL-2 receptor. The IL-2Rβ, (CD122), is a 75-kDa cell surface glycoprotein and is an essential subunit for functional IL-2 receptors. The third component of the IL-2 receptor, the γ-chain (CD132), is a 64-kDa molecule that is shared also with other cytokine receptors. Because IL-2Rα formed the low-affinity receptor by itself and IL-2Rβ or IL-2Rγ alone possessed undetectable affinity to IL-2 binding, an affinity conversion model has been proposed such that IL-2Rα first binds IL-2 after which IL-2Rβ and IL-2Rγ associate with the IL-2–bound IL-2Rα to constitute the high-affinity receptor. Expressions of IL-2Rα, IL-2Rβ, and IL-2Rγ on various populations of human peripheral blood cells were examined by two-color flow cytometry.[23] IL-2Rγ expression was seen on all of the cell populations including CD4$^-$ T, CD8$^+$ T, CD20$^+$ B, and CD56$^+$ NK cells and CD14$^+$ monocytes. Most of the granulocytes also expressed IL-2Rγ. IL-2Rα and IL-2Rβ were differentially expressed on these cell populations, although their expressions were enhanced by extracellular stimuli such as antigens and mitogens. As far as we know, this is the first report of the expression of the IL-2R chains in normo- and hypodense eosinophils. In both control donors and patients with allergies, we found a significant increase of the percentage of positive expression of CD122 in hypodense eosinophils when compared with normodense eosin-

TABLE II

Expression of IL-5, IL-2, and IL-4 Receptors on Eosinophils

	Controls		Patients	
	%	MFI	%	MFI
Normodense				
CD49	94.1 ± 2.7	1.8 ± 0.5	95.4 ± 3.0	1.7 ± 0.5
CD125	75.6 ± 10.7	0.3 ± 0.04	65.1 ± 15.6	0.3 ± 0.1
CD25	19.1 ± 9.9	1.6 ± 0.9	16.6 ± 11.3	1.6 ± 0.5
CD122	8.5 ± 2.9	1.4 ± 0.5	5.2 ± 0.9	1.9 ± 1.2
CD132	21.6 ± 12.2	0.6 ± 0.4	17.9 ± 11.5	0.4 ± 0.2
CD124	3.4 ± 1.2	0.3 ± 0.1	3.6 ± 0.9	0.2 ± 0.1
Hypodense				
CD49	90.4 ± 10.4	1.7 ± 0.8	94.4 ± 4.9	1.6 ± 0.5
CD125	62.5 ± 12.1*	0.3 ± 0.03*	71.4 ± 11.4	0.3 ± 0.03
CD25	14.3 ± 5.5	1.5 ± 1.2	13.6 ± 8.9	1.6 ± 1.03
CD122	30.3 ± 16.7**	1.2 ± 0.7	23.6 ± 6.5**	1.06 ± 0.9*
CD132	19.3 ± 11.4	0.6 ± 0.4	19.6 ± 13.4	0.4 ± 0.2
CD124	3.3 ± 0.6	0.3 ± 0.2	3.3 ± 0.6	0.3 ± 0.1

*Normodense and hypodense eosinophils isolated from patients and controls were triple stained with anti-CD16 plus the corresponding MAb's. Data are presented as $\bar{X} \pm SD$ of the percentage of positive expression and MFI in logarithmic units. *$p < 0.05$; **$p < 0.001$; hypodense eosinophils as compared with normodense eosinophils isolated from each group.*

ophils. We did not find any differences between the groups for CD25 and CD132. So, apparently, hypodense eosinophils are characterized by an increase in the percentage of positive CD122 expression, with no changes in the fluorescence intensity.

The high-affinity IL-4 receptor (CD124) is expressed constitutively on a wide variety of hematopoietic and non-hematopoietic cells such as fibroblasts, neuroblasts, keratinocytes, hepatocytes, and stromal cells.[24] We detected very low levels of expression of the IL-4 receptors in all the populations studied, with no differences between hypodense and normodense eosinophils and between control and patients.

CD69 and CD25 are best known as very early activation markers on lymphocytes.[15] However, we could not find an increase of these markers on patients' eosinophils. The expression of CD23 was decreased in patients with allergies as compared with normal donors, although the difference was not significant. CD23 is broadly expressed on many cell types; however, after initial up-regulation, this molecule undergoes autoproteolysis with subsequent shedding from the cell surface, which results in the production of a soluble form of CD23 (sCD23). Moreover, it is possible that the receptor is shed by IgE.[25]

The effect of topical steroids, in a specific disease such as severe perennial rhinitis, seems to have implications in the

TABLE III

Expression of CD23, CD69, and CD4 on Eosinophils

	Controls		Patients	
	%	MFI	%	MFI
Normodense				
CD23	3.7 ± 2.8	0.3 ± 0.1	2.9 ± 3.0	0.25 ± 0.06
CD69	8.5 ± 11.2	0.3 ± 0.1	6.7 ± 3.9	0.4 ± 0.4
CD4	11.1 ± 9.0	0.53 ± 0.3	11.2 ± 10	0.5 ± 0.2
Hypodense				
CD23	3.4 ± 2.3	0.3 ± 0.1	2.1 ± 2.4	0.31 ± 0.1
CD69	11.0 ± 5.5	0.4 ± 0.2	9.9 ± 4.6	0.3 ± 0.1
CD4	10.3 ± 5.8	0.8 ± 1.3	11.9 ± 7.1	0.4 ± 0.1

Normodense and hypodense eosinophils isolated from patients and controls were triple stained with anti-CD16b plus the corresponding MAb's. Data are presented as $\bar{X} \pm SD$ of the percentage of positive expression and MFI in logarithmic units.

TABLE IV

Surface Molecule Expression on Normodense Eosinophils of PAR Patients Before and After Treatment

	Pretreatment		Posttreatment	
	%	MFI	%	MFI
CD49	95 ± 4	2 ± 0.4	97 ± 3	1.7 ± 0.2
CD125	68 ± 23	0.2 ± 0.02	57 ± 18	0.6 ± 0.1*
CD25	14 ± 8	2 ± 0.5	10 ± 6	0.5 ± 0.1*
CD122	7 ± 4	2 ± 0.8	51 ± 27	2 ± 1
CD132	31 ± 9	0.5 ± 0.3	16 ± 6	5 ± 3
CD124	5 ± 3	0.2 ± 0.03	5 ± 4	0.6 ± 0.1*
CD4	18 ± 9	0.5 ± 0.2	20 ± 11	1 ± 0.5
CD69	9 ± 3	0.7 ± 0.8	14 ± 3	0.7 ± 0.03
CD23	3 ± 4	0.2 ± 0.01	1 ± 1	0.7 ± 0.2

Normodense and hypodense eosinophils isolated from patients and controls were triple stained with anti-CD16b plus the corresponding MAb's. Data are presented as $\bar{X} \pm SD$ of the percentage of positive expression and MFI.
$p > 0.05$; normodense eosinophils pretreatment as compared with normodense eosinophils posttreatment.

expression of IL-2 and IL-4 receptors. No major effects were observed in other markers that we used for normo- and hypodense eosinophils, probably because only a small number of patients were evaluated and the treatment used has eminent local and not systemic effects.

TABLE V

Surface Molecule Expression on Hypodense Eosinophils of PAR Patients Before and After Treatment

	Pretreatment		Posttreatment	
	%	MFI	%	MFI
CD49	97 ± 2	2 ± 0.5	97 ± 3	2 ± 1
CD125	72 ± 14	0.3 ± 0.01	58 ± 17	0.6 ± 0.1*
CD25	9 ± 5	3 ± 2	7 ± 3	0.5 ± 0.1
CD122	11 ± 3	2 ± 1	39 ± 27	1 ± 0.4
CD132	30 ± 20	0.6 ± 0.4	9 ± 2	16 ± 15
CD124	4 ± 0.4	0.3 ± 0.1	3 ± 2	0.7 ± 0.2*
CD4	20 ± 9	0.4 ± 0.1	12 ± 9	1 ± 0.4
CD69	10 ± 7	1 ± 1	14 ± 4	0.7 ± 0.03
CD23	4 ± 4	0.3 ± 0.01	0.4 ± 0.4	0.7 ± 0.3

Normodense and hypodense eosinophils isolated from patients and controls were triple stained with anti-CD16b plus the corresponding MAb's. Data are presented as $\bar{X} \pm SD$ of the percentage of positive expression and MFI.
$p > 0.05$; hypodense eosinophils pretreatment as compared with hypodense eosinophils posttreatment.

The immunostaining of eosinophils obtained with a discontinuous density gradient represents a powerful and efficient method for evaluating the level of activation of freshly isolated peripheral blood eosinophils. It also represents an efficient method for evaluating potentially important molecules under various eosinophilic conditions. In our experience, IL-2Rβ could help to differentiate normo- and hypodense eosinophils. These studies identified cell surface molecules involved in eosinophil isolation and provided targets for the study of eosinophils activation that will further strengthen our understanding of their role in immediate hypersensitivity immunology.

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