The Differences in the Expression of CD45 Isoforms on Peripheral Blood Lymphocytes Derived from Patients with Seasonal or Perennial Atopic Allergy

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PAWLIK, I., MACKIEWICZ, U., LACKI, J.K., WIKTOROWICZ, K. and KONUS, J. The Differences in the Expression of CD45 Isoforms on Peripheral Blood Lymphocytes Derived from Patients with Seasonal or Perennial Atopic Allergy. Tohoku J. Exp. Med., 1997, 182 (1), 1–8 — In order to determine the role of memory/naive T cells in atopic allergy patients we analyzed peripheral blood mononuclear cells before and during the grass pollen season. The study comprised 28 patients with seasonal symptoms of atopic allergy and 18 with perennial symptoms. Flow cytometry was employed to detect the expression of CD3, CD4, CD4CD45RA, CD4CD45RO, CD8, CD16, and CD19 molecules on peripheral blood lymphocytes. Allergic patients showed a decreased proportion of memory (CD4+CD45RO+) T cells compared with healthy subject (p < 0.05). The proportion of naive (CD4+CD45RA+) helper T cells did not differ between allergic patients and controls. The percentage of CD4+CD45RO+ cells increased during natural antigen exposure (grass pollen season) in allergic patients with seasonal symptoms. The results show at least two important observations. A potential homing tendency to nasal, bronchial and conjunctival mucosa of memory T cells (CD45RO) in atopic allergy patients may explain their deficiency in peripheral blood. Secondly, the grass pollen season may switch their phenotype from naive into memory T cells causing the increase of CD45RO cells. These events do not occur in non-allergic individuals and may thus constitute new insight into the basic mechanism of atopic allergy. ——— atopic allergy; CD4+CD45RA+; CD4+CD45RO+

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Atopic allergy is a genetically determined disorder of immediate type hypersensitivity to environmental allergens, most commonly associated with hyperproduction of IgE (Corrigan and Kay 1990; Romagnani 1990; Kay and Durham 1991; Karlsson and Hellquist 1994). It is well known that 10–15% of the human population is afflicted by atopy. The level of responsiveness in an allergic

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patient is very likely dependent upon the individual immune system (Corrigan and Kay 1990; Romagnani 1990; Kay and Durham 1991; Karlsson and Hellquist 1994).

The T cell population can be divided into subsets having distinct phenotypic and functional properties based on their differential expression of various cell surface structures (Kirstensson et al. 1990; Akbar et al. 1991a, b; Deans et al. 1991; Alexander et al. 1992). The CD45 family is comprised of at least five isoforms, which range in size from 180 to 220 kDa, and are generated by the alternative splicing of three exons encoded by a single leukocyte common gene (Deans et al. 1989; Akbar 1991a, b; Alexander et al. 1992; Beverly 1992). It has been already suggested that CD45 plays a regulatory role in T cell activation (Akbar et al. 1991a, b; Deans et al. 1991; Alexander et al. 1992; Biselli et al. 1992; Croft 1994; Croft et al. 1994). The subsets of CD45 cells have distinct functional capabilities concerning lymphokine production and the ability to deliver help to B cells (Kirstensson et al. 1990; Akbar 1991a, b; Alexander et al. 1992; Roth 1994). CD4CD45RO cells represent preactivated cells containing the pool of memory cells, whereas the cells expressing CD4CD45RA consist of the not yet activated naive cells (Rothstein et al. 1991; Roth 1994). After activation, CD4CD45RA cells down regulate CD45RA expression and concomitantly gain high density expression of CD45RO and CD29 (Kirstensson et al. 1990; Rothstein et al. 1991; Biselli et al. 1992; Roth 1994). The conversion of cells from the CD45RA+ to the CD45RA−CD45RO+ phenotype, is believed by many to be irreversible and unidirectional (Rothstein et al. 1991; Roth 1994). On the other hand, it is documented that alternative splicing of the different CD45 isoforms is highly regulated after activation and that conversion from CD45RA+ to CD45RA− is neither unidirectional nor irreversible (Rothstein et al. 1991).

Although many of the effector systems in allergic reactions are directly B cell dependent, T lymphocytes are responsible for several regulatory mechanisms, e.g., regulation of B cell proliferation and IgE synthesis (Romagnani 1990). Upon activation CD4+ lymphocytes produce different cytokines (Corrigan et al. 1988; Corrigan and Kay 1990; Kay and Durham 1991; Del Prete 1992; Kay 1994). Unprimed naive T cells expressing CD45RA antigen synthesize IL-2, while primed memory T cells expressing CD45RO antigen are responsible for IL-4 and IL-5 production (Deans et al. 1989; Beverley 1992; Dianzani et al. 1992; Brofil et al. 1994; Croft et al. 1994). Recent reports show differences in CD4+ subpopulation expressing different isoforms of CD45 antigen between patients with Down Syndrome (Barrena et al. 1994), Chronic Fatigue Syndrome (Tirelli et al. 1994) and other diseases compared with healthy subjects (Morimoto et al. 1987; Akbar et al. 1993).

In order to get a better insight into regulatory mechanisms in atopic allergy, we investigated the expression of CD45RA and CD45RO antigens in peripheral blood CD4 cells from patients with perennial and seasonal atopic allergy before
and during the grass pollen season. Additionally, we followed the changes in the proportion of CD4CD45RA and CD4CD45RO cells derived from peripheral blood of atopic allergy patients after stimulation with PHA.

**Material and Methods**

*Patients.* The study was comprised of 46 patients (27 females, 19 males) with atopic allergy. The age range was 18-44 years with a mean of 30 years. Patients were recruited from the Outpatient Department of Clinical Immunology and Allergy, Institute of Internal Medicine, University School of Medicine in Poznan (Poland). Atopy was defined on the basis of history, one or more positive skin prick tests to a group of 16 common aeroallergens (in the presence of a positive histamine control and a negative vehicle control) and increased total IgE levels. Patients suffering from allergic rhinitis, conjunctivitis, or allergic asthma were included into the study. The study comprised 16 patients with seasonal, and 10 patients with perennial allergic disorders. No oral glucocorticosteroids and/or antihistaminics were taken by the patients. A group of twenty-one normal healthy volunteers (mean age 34 years, range 19 to 44 years) was also studied. In all cases atopic allergy and/or inflammatory disorders were excluded.

*Lymphocyte's phenotype determination.* Heparinized peripheral blood was collected during two sampling occasions, one before natural exposure to grass pollen allergens in winter, and one during the pollen season. Monoclonal antibodies directly conjugated with the fluorochroms fluorescein isothiocyanate (FITC), phycoerythrin (PE), or Cy-5-phycoerythrin (CYP) were employed to determine the cell phenotype. The TRIO set (Ortho, Rariton, NJ, USA) was used to identify the expression of CD3, CD4, CD8, CD16, and CD19 antigens. The set comprised the following antibodies: 1) control-FITC/control-PE/control-CYP, 2) CD4-FITC/CD8-PE/CD3-CYP, 3) CD3-FITC/CD19-PE/CD16-CYP. Anti-CD4-FITC/CD45RA-PE and anti-CD4-FITC/CD45RO-PE monoclonal antibodies (Ortho) were used to recognize naïve/memory T cells. One-hundred microliters of the blood were incubated in Micro Test tubes together with 10 μl of the antibodies. The incubation was performed in the dark, at room temperature for 30 min. After that 2 ml of lysing solution (Ortho) was added followed by mixing for 10 min. A two-colour or three-colour flow analysis was performed on a Cytron Absolute flow cytometer (Ortho) using ImmunoCount programme.

*Culture conditions.* Peripheral blood mononuclear cells (PBMC) from patients and healthy donors were isolated from heparinized venous blood by Ficoll-Hypaque density gradient centrifugation. After washing the cells were depleted of adherent cells by incubation on plastic surfaces for 30 min, at 37°C. Less than 1% of CD14+ monocytes were identified in the lymphocyte gate. Purity of resting T cells was greater than 97.5%. Specimens were processed within 3 hr from sampling. Lymphocytes (0.2 ml) were incubated in microplates (Falcon, Franklin Lakes, NJ, USA) in Eagle's medium supplemented with 10% FCS
(Gibco, Gaithersburg, MD, USA), 20 mg/ml gentamicin and 1 μg/ml PHA (HA, Wellcome). Cultures were incubated at 37°C in 5% CO₂ and stopped after 48, 72, 96, 120 hr for CD45RA and CD45RO evaluation. A two-colour flow analysis was performed on a Cytron Absolute flow cytometer (Ortho) using ImmunoCount programme.

**Statistical analysis.** All paired data were analyzed using the Wilcoxon rank sum test. Comparisons of unpaired data were made using the Mann-Whitney test.

**Results**

We did not observe any differences in total lymphocyte count between atopic allergy patients and healthy controls. The percentage of CD3 positive cells in atopic allergy patients did not differ significantly from the control group (73.4% ± 11.6% vs. 76.3% ± 14.5%). Similarly we did not notice any differences between the proportion of CD3⁺CD4⁺ and CD3⁺CD8⁺ cells in atopic patients and healthy controls (43.9% ± 13.5% vs. 41.1% ± 15.7% and 22.9% ± 8.9% vs. 24.8 ± 9.5%, respectively). No statistically significant differences were found in the proportion of circulating CD16 positive NK cells and CD19 positive B cells (16.0% ± 4.6% vs. 13.2% ± 4.0% and 9.4% ± 2.6% vs. 8.4 ± 2.8%, respectively).

The proportion of naive T cells (CD4⁺CD45RA⁺) did not differ between allergic patients and controls. However, allergic patients showed a decrease in the CD4⁺CD45RO⁺ memory cells compared with healthy subjects (Table 1). The deficiency was more profound in patients with perennial allergy compared with patients manifested seasonal symptoms of atopic allergy (Table 1).

Blood samples were taken twice in twenty individuals: before natural exposure to grass pollen allergens in winter, and one during the pollen season. The group included ten patients with seasonal symptoms of atopic allergy and 10 healthy controls. An absence of difference was noticed concerning the proportion of naive T cells (CD4⁺CD45RA⁺) independent of natural exposure to aeroallergens (Table 2). We observed an increase in the percentage of memory CD4⁺CD45RO⁺ cells during the grass pollen season (Table 2). However, the percentage of CD4⁺CD45RO⁺ cells did not reach the level observed in healthy controls.

| Table 1. The comparison of the expression of CD45RA and CD45RO antigens on peripheral blood CD4 cells derived from seasonal and perennial atopic patients and healthy controls |
|---------------------------------|-----------------|-----------------|
|                                 | CD4⁺CD45RA⁺     | CD4⁺CD45RO⁺     |
| Healthy controls                | n = 21          | 52.1% ± 14.7%   | 43.9% ± 9.8%    |
| Atopic allergy                  | n = 46          | 47.8% ± 15.3%   | *23.4% ± 19.2%  |
| Seasonal atopic allergy         | n = 28          | 46.5% ± 15.2%   | 29.6% ± 12.1%   |
| Perennial atopic allergy        | n = 18          | 48.4% ± 17.1%   | *13.9% ± 8.6%   |

* *p* < 0.05 vs. healthy controls.
Table 2. The comparison of the expression of CD45RA and CD45RO antigens on peripheral blood CD4 cells in 10 seasonal atopic before and during natural antigen exposure (grass pollen season)

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<th>Before grass pollen season</th>
<th>During grass pollen season</th>
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<tr>
<td>CD4⁺CD45RA⁺</td>
<td>51.9% ± 14.4%</td>
<td>42.3% ± 6.1%</td>
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<tr>
<td>CD4⁺CD45RO⁺</td>
<td>21.5% ± 13.1%</td>
<td>*34.8% ± 8.8%</td>
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Healthy controls (n = 10)

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<th>CD4⁺CD45RA⁺</th>
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<tr>
<td>51.7% ± 13.9%</td>
<td>52.5% ± 14.2%</td>
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<tr>
<td>42.8% ± 11.3%</td>
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* p < 0.05 vs. before grass pollen season.

Fig. 1. The kinetics of the appearance of CD45RA and CD45RO antigens on the membrane of CD4⁺ T cells derived from seasonal atopic allergy patients.

Fig. 1 summarizes kinetics of the appearance of CD45RA and CD45RO on the membrane of CD4⁺ T cells. CD45RA expression was downregulated upon activation by PHA. As depicted in the figure, the percentage of cells carrying CD45RA antigen remained at a plateau value for 72 hr and then declined to a very low level by hour 120. The intensity of CD45RO expression of CD4⁺ T cells increased with time in healthy subjects and patients with atopic allergy, upon incubation with PHA. The peak value for CD45RO expression was 120 hr. The cultures exhibited a similar behavior of CD45RO expression on CD4⁺ T cells in both groups. CD4 expression was not consistently modulated upon activation.
DISCUSSION

Our present work using flow cytometry revealed no differences in a proportion of CD3+ T cells, helper-inducer CD4+ cells, suppressor-cytotoxic CD8+ cells, CD19+ B cells, and CD16+ NK cells between patients with allergy and healthy subjects. The recent studies have also demonstrated that the absolute count of CD4 and CD8 T lymphocytes in the peripheral blood of the asthmatic patients did not differ from those in normal control subjects (Corrigan et al. 1988; Karlsson and Hellquist 1994). It was established that non-MHC restricted cells such as NK cells are apparently not involved in the allergic reaction (Karlsson and Hellquist 1994).

The physiologic significance of the expression of the CD45 isoforms is related to previous antigenic exposure. CD45RA and CD45RO are expressed on peripheral blood T lymphocytes in a reciprocal fashion: CD45RAhigh cells are CD45ROlow and vice versa (Deans et al. 1989; Kirstensson et al. 1990; Akbar et al. 1991a; Beverely 1992; Dianzani et al. 1992; Brofil et al. 1994; Karlsson and Hellquist 1994). Analysis of CD45 expression by T lymphocyte clones in vitro has shown that CD4+CD45RA+ lymphocytes after activation progressively loose surface expression of CD45RA and acquire CD45RO (Deans et al. 1989; Beverely 1992; Barrena et al. 1993; Croft et al. 1994; Karlsson and Hellquist 1994; Roth 1994). These changes may be partially reversible after antigen stimulation in a cyclic fashion (Rothstein et al. 1991; Roth 1994). In the present study, decreased percentages of CD45RO+CD4+ T lymphocytes were present in the peripheral blood of allergic patients as compared with the control group. No differences were observed in the percentages of CD45RA expressing cells. However, during grass pollen season the proportion of memory CD4+CD45RO+ cells increased, not reaching the values observed in healthy donors.

The predominance of memory CD4+CD45RO+ T cells in the nasal, bronchial and conjunctival mucosa, and the increase in activated T cells in patients with allergic diseases are findings that indicate the cell-mediated response plays an active role in the allergic reaction (Corrigan et al. 1988; Romagnani 1990; Del Prete 1992; Karlsson and Hellquist 1994; Kay 1994). Therefore, the migration of CD4+CD45RO+ cells to inflammatory sites may explain their deficiency in blood in atopic allergy patients. The grass pollen season may switch their phenotype from naive into memory T cells causing the increase in CD45RO cells.

Moreover, in the present report we show a dramatic reduction of CD45RA expression upon activation by PHA. CD45RA expression on CD4+ T lymphocytes was down-regulated starting by time 72 hr and no difference was observed between healthy subjects and patients with atopic allergy. It is generally accepted that down-regulation of CD45RA on CD4+ T lymphocytes upon activation correlates with maturation of CD4+ lymphocytes that switch from a "virgin" status (CD4+CD45RA+) to a more mature "helper" status (CD4+...
CD45RO$^+$) (Biselli et al. 1989; Kirstensson et al. 1990; Rothstein et al. 1991; Roth 1994). A complete loss of CD45RA after activation with PHA has been already shown (Biselli et al. 1989). Also Rothstein et al. (1991) have documented that after Con A stimulation of CD4$^+$CD45RA$^+$ cells, CD45RA expression was diminished but not lost.

The results of the present study imply that in atopic allergy the antigen presentation activates Th cells in peripheral blood and undergo phenotype shift from naive to memory Th cells. The activated Th memory cells in peripheral blood constitute potent sources of IL-4, IL-5 and an allergen-induced increase in Th-associated cytokines (e.g., IL-3; GM-CSF; IL-13). IL-4 is known to induce IgE synthesis mast cell proliferation and to increase vascular permeability (Romagnani 1990; Del Prete 1992). The increased insight into the immune function of memory and virgin cells may provide an explanation for mechanisms of atopic allergy and open strategies to alter it.

References


