

Short communication

Expression of CD23 antigen and its ligands in children with intrinsic and extrinsic asthma

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The role of the low-affinity IgE receptor CD23 in immune reactions has been further emphasized by recent discoveries of novel surface ligands for CD23: CD21, CD11b, and CD11c. We previously observed the difference between the expression of CD23 and CD21 antigens in children suffering from extrinsic asthma when compared to healthy controls. In the present study, we investigated the expression of CD23 and its ligand CD21 on CD20⁺ B cells in 44 asthmatic children (23 allergic and 21 nonallergic) using three-color immunofluorescence analysis. In addition, the expression of two other ligands for CD23, CD11b, and CD11c, on T cells (CD3⁺), a subpopulation of T cells (CD4⁺ and CD8⁺), natural killer cells (CD56⁺), and monocytes (CD14⁺) was tested by two-color immunofluorescence analysis in 12 allergic and 14 nonallergic children. We found that children with extrinsic asthma had higher levels of CD23⁺ B cells than those with intrinsic asthma. No difference was observed in the percentage of either CD23⁺CD21⁺ or CD23⁺CD21⁻ B cells. The CD11b antigen was expressed on each tested population, but only on CD4⁺ T cells was CD11b significantly increased in children with extrinsic asthma. CD11c was expressed mainly on monocytes, and no difference was observed between tested groups. The increased percentage of CD11b antigen on CD4⁺ T cells and the increased percentage of CD23 antigen on B cells in children with extrinsic asthma provide further evidence of the immunologic differences between intrinsic and extrinsic asthma.

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The increased percentage of CD23 marker, a low-affinity IgE receptor, in various allergic diseases in man has led to numerous studies on the importance of this molecule in the regulation of IgE synthesis (1, 2). Henchoz et al. suggested that a possible role of CD23 in the regulation of IgE is to enhance the T–B-cell interaction through binding to its surface counterstructure(s) (3). It was recently reported that, besides CD21 (4, 5), CD23 binds to CD11b and CD11c, the α -chain of the β 2 integrin complexes CD11b/CD18 and CD11c/CD18 on monocytes (6). Binding of CD23 with CD11b and CD11c regulates important activities of monocytes which could contribute to the release of inflammatory mediators by these cells.

Asthma has been conventionally divided into allergic (extrinsic) and nonallergic (intrinsic) asthma (7). The pathologic mechanisms underlying intrinsic asthma have not been clarified, but it is known that extrinsic asthma is caused by IgE-mediated mechanisms. In the present study, we investigated the expression of CD23 and its ligands, CD21, CD11b, and CD11c in children with intrinsic asthma and children with extrinsic asthma.

Material and methods

Forty-four asthmatic children (28 boys, 16 girls; aged 3–15 years, median 9 years) were included in

the study. Extrinsic asthma was diagnosed in 23 children (17 boys, six girls) who had:

- 1) typical history of bronchial asthma
- 2) positive skin tests to aeroallergens
- 3) elevated total IgE levels in serum higher than the geometric mean plus 2 SD of those in nonallergic children
- 4) RAST of classes 3–4
- 5) peripheral blood eosinophilia.

Twenty-one patients with intrinsic asthma (11 boys, 10 girls) had a history of bronchial asthma only. At the time of the study, the patients had stable bronchial asthma without evident manifestations of illness. Only patients with no previous immunotherapy and corticosteroid therapy were examined. Total and specific serum IgE levels were determined with PRIST and RAST kits (Institute of Immunology, Zagreb, Croatia), as previously described (8). Fifteen healthy children (seven boys, eight girls; aged 8–10 years, median 8.5 years) without evident allergic, immunologic, or hematologic disorders provided blood for control purposes. In addition, we used historical controls from sex- and age-matched groups for analysis of CD23 antigen (9). The blood samples were obtained after informed consent by parents.

Flow-cytometry analysis of surface antigens

The following murine antibodies to human lymphocyte cell-surface antigens were used: anti-CD3 (PE-conjugated), anti-CD4 (FITC-, PE-, and PerCP-conjugated), anti-CD8 (FITC- and PE-conjugated), anti-CD11b (PE-conjugated), anti-CD14 (PE-conjugated), anti-CD19 (PE-conjugated), anti-CD20 (PerCP-conjugated), anti-CD56 (PE-conjugated), anti-CD23 (PE-conjugated), anti-CD45 (FITC-conjugated; all purchased from Becton Dickinson, Heidelberg, Germany), anti-CD23 (FITC-conjugated, Dako, Glostrup, Denmark), anti-CD21 (FITC-conjugated, Serotec, Oxford, UK, and FITC-conjugated, Immunotech, Marseille, France), anti-CD11b (FITC-conjugated, Serotec), and anti-CD11c (FITC-conjugated, Serotec, and FITC-conjugated, Dako). In each experiment, FITC-conjugated IgG1, PE-conjugated IgG2a, and/or PerCP-conjugated IgG1 controls for detection of nonspecific binding were included.

Samples of heparinized peripheral blood were used and the standard procedure of double or triple direct immunofluorescence staining was performed. Briefly, 50 μ l of heparinized blood was incubated for 15 min in the dark at room temperature with 5 μ l of FITC and 5 μ l of PE-conjugated antibody. In experiments in which three-color-immunofluorescence staining was performed, 5 μ l

of PerCP-conjugated monoclonal antibody was added. Erythrocytes were lysed by adding 2 ml of 10% FACS Lysing solution (Becton Dickinson, San Jose, CA, USA) for 10 min in the dark at room temperature. After washing, the cells were resuspended in 0.5 ml of the staining solution. Control suspensions were prepared by the same procedure, with the fluorochrome-conjugated normal mouse immunoglobulins of the same isotype as the corresponding mAbs. Data analysis involved counting a minimum of 5000 cells for double-color immunofluorescence or 20 000 cells for three-color immunofluorescence (per sample) on a flow cytometer (FACScan, Becton Dickinson, Mountain View, CA, USA) with CELLQuest software (Becton Dickinson) on an Apple Macintosh[®] computer. Correlated analysis of forward and right-angle scatter was used to establish a lymphocyte and monocyte gate.

Statistical analysis

Results are shown as median (minimum–maximum values). Multiple comparison of tested groups was done by comparing Conover's inequality if H_0 was rejected by Kruskal-Wallis ANOVA corrected for ties. The correlation was evaluated with the Spearman rank correlation coefficient (r_s). $P < 0.05$ was considered significant.

Results

The expression of CD23 antigen on peripheral blood lymphocytes obtained from patients with stable asthma as well as from healthy controls was mainly associated with B cells (CD20⁺). Patients with extrinsic asthma ($n=23$) have a significantly higher percentage of CD23⁺ B cells ($P < 0.001$) than children with intrinsic asthma ($n=21$) and healthy, age-matched historical controls ($n=15$; $P < 0.01$) (Fig. 1). We found no correlation between the percentage of CD23⁺ B cells and total serum IgE levels in the children tested (data not shown).

In contrast to the percentage of CD23⁺ B cells, the percentage of CD21⁺ B cells in children with extrinsic asthma (83; 77–92) did not differ from the percentage of CD21⁺ B cells in children with intrinsic asthma (82; 84–95) and healthy controls (82; 80–95). Children with extrinsic asthma and children with intrinsic asthma had similar mean fluorescence intensity (MFI) of both CD21 (110; 105–116 and 112; 106–116, respectively) and CD23 (122; 115–144 and 120; 108–132, respectively). However, when values were compared to the values of MFI obtained in healthy controls, we found that both children with extrinsic asthma and with intrinsic asthma had increased MFI of CD21

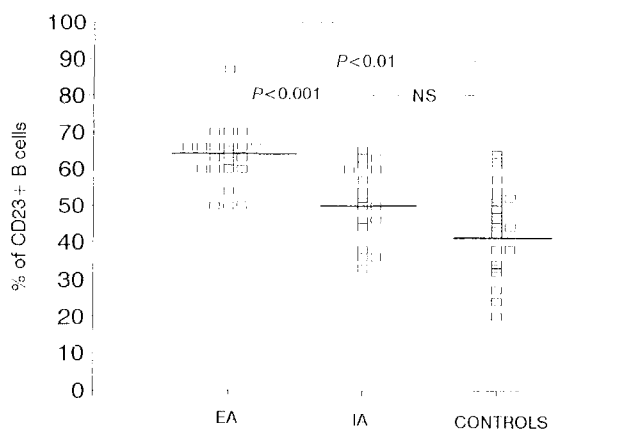


Fig. 1. Children with extrinsic asthma (EA; $n=23$) had increased percentage of CD23⁺ B cells in comparison to children with intrinsic asthma (IA; $n=21$) and healthy controls ($n=15$). Each rectangle represents result of one donor, and horizontal line represents median. NS: nonsignificant.

and CD23 antigens in comparison to healthy controls (90; 87–101 and 96; 89–106, $P<0.01$; data not shown).

The expression of CD11b and CD11c markers on major populations of immune cells obtained from peripheral blood of patients with extrinsic asthma and patients with intrinsic asthma was determined by double-color immunofluorescence analysis on CD3 (total T cells), CD4 and CD8 (T-cell subsets), CD19 (total B cells), and natural killer (NK) cells (CD56⁺). In addition, CD11b and CD11c antigens were determined on a monocyte population gated by cell size, granularity, and CD14 expression. CD11b⁺ and CD11c⁺ subsets were quantified as:

- 1) percentage of total lymphocytes
- 2) percentage of particular lymphocyte subsets (CD3⁺, CD4⁺, CD8⁺, CD19⁺, and CD56⁺)
- 3) percentage of monocytes (CD14⁺).

The accuracy of determination of the monocytic gate was tested by CD45/CD14 analysis. No difference between the patients with extrinsic asthma and patients with intrinsic asthma and controls for CD3, CD4, CD8, CD19, CD56, CD14, CD11b, and CD11c markers was found (data not shown). Although NK cells and monocytes were major cell subsets expressing CD11b and CD11c markers, no difference in expression of these markers was observed (data not shown). However, children with extrinsic asthma had an increased percentage of CD4⁺ T cells expressing CD11b antigen ($P<0.001$) if compared to children with intrinsic asthma and controls (Fig. 2). A representative panel of the expression of CD11b antigen (obtained with two different monoclonal antibodies) in a child with extrinsic asthma and in a child with intrinsic asthma

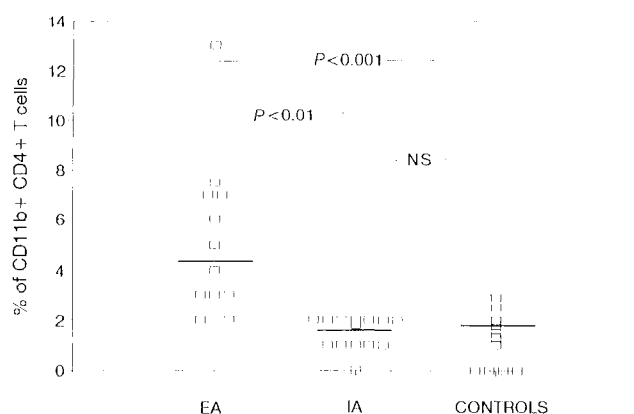


Fig. 2. Percentages of CD11b⁺ CD4⁺ T cells in children with extrinsic asthma (EA; $n=12$), those with intrinsic asthma (IA; $n=14$), and healthy controls ($n=15$). Each rectangle represents result of one donor, and horizontal line represents median. NS: nonsignificant.

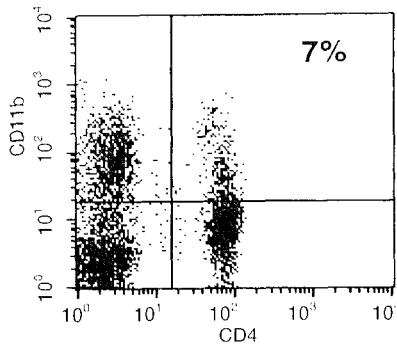
is shown in Fig. 3I and II, respectively. We also found a positive correlation between the percentage of CD4⁺ CD11b⁺ T cells from total CD4⁺ and total serum IgE levels ($r_s=0.6149$; $P<0.001$) in all the children tested (Fig. 4). Aberrant expression of CD23 and CD11b antigens on B cells and CD4⁺ T cells, respectively, in extrinsic asthma was further confirmed by comparison of values obtained in two patients with extrinsic asthma and four patients with intrinsic asthma where two different monoclonal antibodies recognizing different epitopes of CD23 (data not shown) and the CD11b molecule were used (Fig. 3). Expression of CD11c was mainly associated with monocytes in each of the three groups tested, but we found no difference in the expression of this marker on examined lymphocyte subsets and monocytes (data not shown).

Discussion

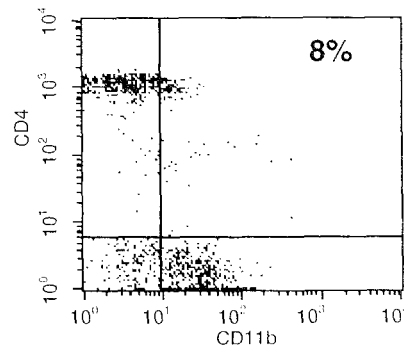
The present data suggest that CD11b, a novel surface ligand for CD23, when expressed on CD4⁺ T cells, participates in IgE-mediated mechanisms in allergic disease. In particular, we found an increased percentage of CD11b antigen on CD4⁺ T cells in children with allergic asthma. This finding could be considered disease specific, because children with chronic, nonallergic obstructive pulmonary disease, that is, intrinsic asthma, showed no increase in the percentage of CD11b⁺ CD4⁺ T cells. Sánchez-Guerrero et al. have already demonstrated that children with extrinsic asthma and allergic rhinitis had a significantly increased percentage of CD23⁺ B cells in comparison to children with intrinsic asthma (10), and proposed that the percentage of CD23 on B cells may be a good disease marker.

(I) extrinsic asthma

a

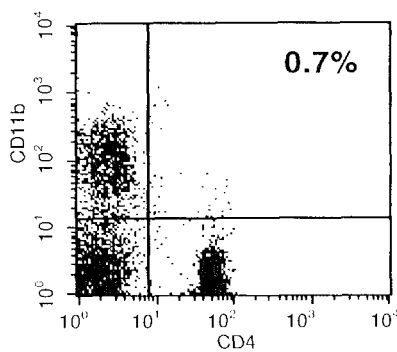


b



(II) intrinsic asthma

c



d

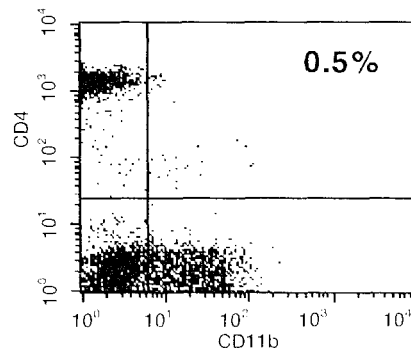


Fig. 3. Representative dot plot of two-color immunofluorescence analysis of CD4 and CD11b antigens on lymphocytes in child with extrinsic asthma (I) and child with intrinsic asthma (II). Two antibodies (a and b) which recognized different epitopes on CD11b antigen were used (see Material and methods). Numbers indicate percentage of CD4⁺ T cells expressing CD11b antigen above negative control background staining.

Most studies on the role of CD11b and CD11c markers have been performed on myeloid cells. These antigens bind fibrinogen, ICAM-a, coagulant factor X, and components of complement, and contribute to cell adhesion and phagocytosis (11). The tissue-specific distribution of CD11b antigen on T cells has been recently characterized by Hoshino et al. (12); however, the role of these cells in immune reactions is still unknown. Patients with systemic lupus erythematosus had an increased percentage of CD11b⁺ CD4⁺ T cells in peripheral blood (13). The CD4⁺ CD11b⁺ T-cell subpopulation can augment IgG synthesis (14) by producing B-cell growth factors (15), contributing by these mechanisms to the unregulated antibody production characteristic of systemic lupus erythematosus (13). Therefore, it would be of interest to determine

whether an increased percentage of CD11b⁺ CD4⁺ T cells, which we found to correlate positively with total serum IgE levels, contributes to this increased synthesis. An increase of CD11b molecules on CD4⁺ T cells in patients with allergic asthma may be a result of the direct influence of specific allergen(s) and/or cytokines produced by allergen-specific T cells as a consequence of specific or nonspecific stimulation. Our preliminary data from *in vitro* studies showed that the CD11b antigen is upregulated on CD4⁺ T cells upon stimulation with phytohemagglutinin and anti-CD3, but interleukin (IL)-4, a cytokine with a pivotal role in allergic reactions, alone had no effect on the expression of CD11b antigen on CD4⁺ T cells.

An increased percentage of CD23⁺ B cells in children with extrinsic asthma in comparison to

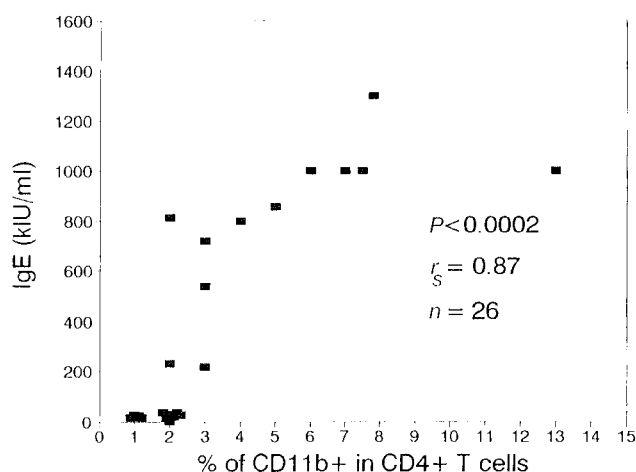


Fig. 4. Positive correlation between total IgE levels and percentage of CD11b⁺ CD4⁺ T cells in all children tested ($n=26$). Each rectangle represents result of one donor.

children with intrinsic asthma and control subjects is a result of excessive production of IL-4 and IL-13 by allergen-specific T cells (16). It would be of interest to investigate whether allergen-specific T cells express CD11b, and, if so, to determine the outcome of binding CD23 antigen on B cells and CD11b on T cells. In conclusion, an increased percentage of CD11b antigen on CD4⁺ T cells and an increased percentage of CD23 antigen on B cells in children with extrinsic asthma, but not in intrinsic asthma, are further evidence of distinct immunologic mechanisms in these diseases.

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