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# CD21–CD23 ligand pair expression in children with allergic asthma

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## SUMMARY

The CD23 antigen, a low affinity receptor for IgE, was recently shown to interact with another ligand, CD21, and the pairing of these molecules is important in T cell–B cell interaction and control of IgE production. Here, we analysed the expression of CD21 and CD23 on $CD4^+$ and $CD20^+$ lymphocytes in 25 allergic children and 12 age-matched non-allergic controls. Both the percentage ($P < 0{\cdot}01$) and the absolute number ($P < 0{\cdot}001$) of $CD23^+$ cells were increased in allergic children. There was no difference of $CD21^+$ cells. Double positive $CD4^+CD23^+$ cells (2·5%) were only detected in one patient, in others all CD23 being expressed on B cells. The CD21 antigen was expressed only on B cells. Furthermore, allergic children had an increased mean fluorescence intensity of both the CD21 ($P < 0{\cdot}001$) and the CD23 ($P < 0{\cdot}001$) receptor. To analyse the possible difference in B cell subsets expressing CD21 and CD23 antigens, three-colour fluorescence analysis was performed. In allergic children the subset of $CD20^+CD21^-$ cells expressed more CD23 than in controls ($P < 0{\cdot}001$). These results may mean an impaired expression and possibly regulation of CD21–CD23 interaction in allergic conditions.

**Keywords** allergic children IgE regulation CD21 CD23

## INTRODUCTION

Low affinity receptor for IgE, FcεRII/CD23 and its soluble components, IgE binding factors (sCD23), play a role in IgE regulation [1,2]. We have previously observed that asthmatic children allergic to *Dermatophagoides pteronyssinus* have an elevated expression of FcεRII/CD23 on B lymphocytes [3]. Aubry *et al.* showed that certain anti-CD23 MoAbs inhibit conjugate formation *in vitro*, and that this inhibition is restricted mainly to $CD4^+$ T cells which form conjugates with B cells [4]. This confirmed the existence of the other, surface ligand for CD23, which was identified as CD21 [5,6]. Human complement receptor 2 (CR2; CD21) is a glycoprotein of mol. wt 145 kD, found on B lymphocytes, some T lymphocytes, follicular dendritic cells and pharyngeal epithelial cells. It is the receptor for Epstein–Barr virus (EBV), C3dg and iC3b proteins of the complement system and interferon-alpha (IFN-α) [7–9]. Furthermore, it was shown that occupancy of CD21 on B cells by some anti-CD21 MoAbs increased IL-4 induced IgE production, as did treatment with recombinant sCD23 [6]. It was hypothesized that ligand pair CD21–CD23 is involved in the T–B cell interaction, with unique capability to control IgE synthesis in an isotype-specific manner [6,10].

So far, there are no data of impaired function and/or regulation of CD21–CD23 interaction in allergic conditions. Therefore, we investigated the expression of CD21 and CD23 antigens on lymphocytes in children with allergic asthma. Using three-colour single-laser flow cytometry, the simultaneous expression of CD21 and CD23 on the same cell was investigated.

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## PATIENTS AND METHODS

*Patients*

Twenty-five children (18 boys, seven girls, aged 4–16 years, median 11 years) with allergic asthma were examined. Allergic asthma was diagnosed on the basis of the following criteria: (i) typical history of bronchial asthma; (ii) positive skin tests to *Dermatophagoides pteronyssinus* (Dpt; $n = 20$) and Dpt and standardized pollen allergen extracts ($n = 5$); (iii) elevated total IgE levels in serum, higher than the geometric mean + 2 s.d. of those in non-allergic children; (iv) RAST of class 3–4. At the time of the study the patients had stable bronchial asthma without evident allergic manifestations. Only patients with no previous immunotherapy and no corticosteroid therapy were examined.

Twelve healthy children (seven boys, five girls, aged 4–12 years, median 10 years) without evident allergic, immunological or haematological disorders provided blood for control purposes.

The blood samples were obtained from children after informed consent by their parents.

Total and specific serum IgE levels were determined using PRIST and RAST kits (Institute of Immunology, Zagreb, Croatia), as previously described [3].

*Monoclonal antibodies*

The following murine antibodies to human lymphocyte cell-surface antigens were used: anti-CD4 (FITC, PE and peridinin chlorophyll protein (PerCP)-conjugated), anti-CD20 (FITC, PE and PerCP-conjugated), PE-conjugated anti-CD23 (all from Becton Dickinson, Heidelberg, Germany) and FITC-conjugated anti-CD21 (CR2; Immunotech, Marseille, France). In each experiment, FITC-conjugated IgG1, PE-conjugated IgG2a and/or PerCP conjugated IgG1 controls for determination of non-specific binding were included.

*Flow cytometry analysis*

Heparinized samples of the peripheral blood were obtained, and the standard procedure of double, direct immunofluorescence staining of whole blood was performed. Briefly, 50 $\mu$l of heparinized blood were incubated in the dark at 4°C with 10 $\mu$l of FITC and 10 $\mu$l of PE-conjugated antibody for 30 min. In experiments in which three-colour immunofluorescence staining was performed, 10 $\mu$l of PerCP-conjugated MoAb were added. Erythrocytes were lysed by adding 2 ml of 10% FACS Lysing solution (Becton Dickinson, San Jose, CA) for 10 min, at room temperature in the dark. 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 MoAbs.

Cell fluorescence was analysed by FACScan flow cytometer (Becton Dickinson). Correlated analysis of forward and right angle scatter was used to establish a lymphocyte gate, counting a minimum of 5000 cells for double-colour immunofluorescence, or 20000 cells for three-colour immunofluorescence (per sample). The collected data were analysed using FACScan Research Software and Lysis software, and presented as percentages and mean fluorescence intensity. From the percentages of peripheral blood mononuclear cells (PBMC) and leucocyte count, the absolute number of lymphocytes was calculated.

*Statistical analysis*

The Mann–Whitney *U*-test was applied. The correlation was evaluated using the Spearman rank correlation coefficient ($r_s$). $P < 0{\cdot}05$ was considered significant.

## RESULTS

The allergic children included in our study had significantly higher total serum IgE levels (561 ± 331 U/ml) than control group (58 ± 21 U/ml). We confirmed our previous observation that peripheral blood of patients with allergic asthma contained a significantly greater percentage ($P < 0{\cdot}01$) and absolute number ($P < 0{\cdot}001$) of CD23$^+$ cells than that of normal children (Fig. 1). However, no such difference was observed when CD21 was analysed (data not shown). Antigens CD21 and CD23 were mainly expressed on B lymphocytes in both allergic children and controls, while no CD4$^+$CD21$^+$ were observed (data not shown). Only one child had 2·5% CD4$^+$CD23$^+$ cells. Although no significant difference in the percentage and absolute number of B cells expressing CD21 was found, the mean fluorescence intensity (MFI) of both CD21 and CD23 was significantly increased in allergic children ($P < 0{\cdot}001$), as shown in Fig. 2. The differences in the MFI could not be attributed to the differences in size of the cells, since the mean forward scatters for

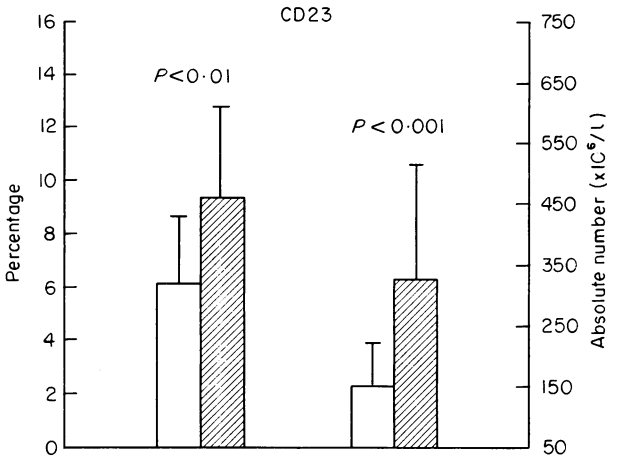


**Fig. 1.** Percentages and absolute numbers of CD23$^+$ lymphocytes in patients with allergic asthma (■; $n = 25$) and non-allergic children (□; $n = 12$). Top of each column is the mean and the vertical line the s.d. of each group. Statistical difference ($P$) was calculated by the Mann–Whitney *U*-test.

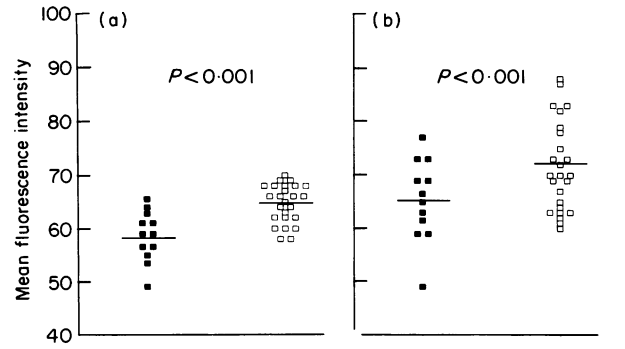


**Fig. 2.** Mean fluorescence intensity of CD21 (a) and CD23 (b) on lymphocytes of non-allergic (■) and allergic children (□). Each square represents the result of one donor. The horizontal bars represent the mean value. Statistical significance ($P$) was calculated by the Mann–Whitney *U*-test.

**Table 1.** Results of simultaneous three-colour analysis of the distribution of CD23 on CD20/CD21 subsets in control group and allergic children

| | Expression of CD23 (%) | |
|---|---|---|
| | Allergic children ($n = 4$) | Control ($n = 5$) |
| CD20$^+$CD21$^+$ | 68 ± 6 | 64 ± 9 |
| CD20$^+$CD21$^-$ | 31 ± 6* | 17 ± 4 |

* $P < 0{\cdot}001$ (Mann–Whitney *U*-test).

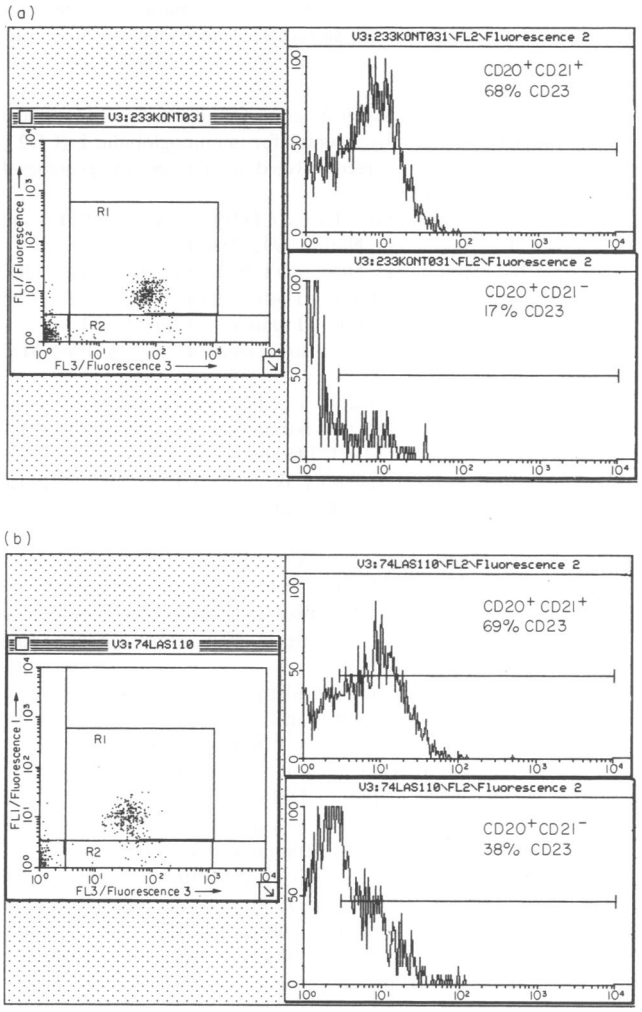


**Fig. 3.** A representative panel of the expression of surface markers on B cell subsets in a non-allergic child (a) and an allergic patient (b). Peripheral blood mononuclear cells (PBMC) were stained simultaneously with CD20–peridinin chlorophyll protein (PerCP) (FL3), CD21–FITC (FL1) and CD23–PE (FL2)-conjugated MoAbs. Regions were set around $CD20^+CD21^+$ (R1) and $CD20^+CD21^-$ (R2) cells on a CD20 (PerCP) and CD21 (FITC) dot plot. The fluorescence intensity of CD23 on these subsets were then presented as histograms. The numbers in histograms indicate the percentage of $CD23^+$ cells above negative control background staining.

lymphocytes (controls $35 \pm 3{\cdot}7$; allergic patients $33 \pm 3$) were not significantly different.

We found a positive correlation between the percentage of $CD23^+$ lymphocytes and total serum IgE levels ($r_s = 0{\cdot}45$; $P < 0{\cdot}01$) in all the tested children. No correlation was found when the percentage of CD21 cells and serum IgE levels were analysed ($r_s = 0{\cdot}16$; $P > 0{\cdot}05$).

Since our results have indicated a possible difference in surface expression of CD21 and CD23 on B cell subsets in allergic children, we tested this by three-colour analysis in four allergic children and five controls (Table 1). PBMC were stained simultaneously with FITC, PE and PerCP- conjugated MoAbs and analysed with FACScan single flow cytometry. We found that CD21 and CD23 were expressed mostly on the surface of the same $CD20^+$ cell. Furthermore, analysis of $CD20^+CD21^-$ cell subsets revealed that, in allergic children, these cells express significantly more CD23 antigen than in controls (Fig. 3). No significant difference was observed in other subsets of B cells which were $CD21^+CD23^+$.

## DISCUSSION

Results of the present study confirmed our previous observation that allergic children have increased CD23 expression on lymphocytes, which positively correlated with the serum IgE levels [3]. The role of CD23 in an antigen-specific IgE response was already confirmed by *in vitro* [11] and *in vivo* [10] experiments.

Bonnefoy *et al.* reported that the recently described ligand pair CD21–CD23 is involved in the control of IgE synthesis in an isotype-specific manner [10], since IL-4-induced IgE production was further increased when mimicking the effect of CD23 ligation to CD21 with certain anti-CD21 MoAbs [6]. Aubry *et al* also showed that certain anti-CD23 MoAbs inhibit the conjugate formation *in vitro* and that this inhibition is restricted mainly to $CD4^+$ T cells which form conjugates with B cells [4].

These results prompted us to analyse the possible impaired expression of CD21 and CD23 on $CD4^+$ and $CD20^+$ cells in conditions characterized by elevated serum IgE levels such as allergic bronchial asthma. We found that CD23 was expressed on $CD20^+$ cells, and only in one patient did we also find CD23 on $CD4^+$ cells (2·5%). Several groups reported that a small proportion of freshly isolated T lymphocytes from allergic persons expressed CD23, but with very low density [2,12], and that CD23 was *in vitro* induced by IL-4 and/or allergen on $CD4^+$ T cells only in allergic patients [13]. Fisher *et al.* suggested that, with the technical approach of using separated cells and biotinylated MoAbs, low density cell surface antigens could be detected and quantified [9]. Such an approach could be used to determine the expression of CD23 antigen on T lymphocytes, but it requires a larger blood volume and is unsuitable in children.

Analysis of CD21 antigen expression revealed that the antigen is expressed on B lymphocytes in all examined children included in our study. Although expression of CD21 has recently been described on peripheral T cells in normal healthy donors and in patients with systemic lupus erythematosus [9,14], we did not find CD21 on T cells of any of the examined children. We observed no significant difference in the percentage and absolute number of $CD21^+$ B cells between allergic children and controls. However, we did observe a significant increase in the MFI of both CD21 and CD23 in allergic children which indicates an increase of density of these antigens. Defranco postulated that the interaction between B and T lymphocytes depended on a number of adhesion molecules which interact and lead to cell activation [15]. The increased MFI of both CD21 and CD23 in allergic children included in our study are in accordance with the newly proposed adhesive function of this ligand pair [2].

The induction of human IgE synthesis requires IL-4 and T–B cell interaction [16]. The over-expression of CD23 in allergic persons seems to be the result of the excessive production of IL-4 by allergen-specific Th2 clones [17]. The primary, and most of the secondary, antigen-specific or polyclonal IgE antibody responses depend upon the production of IL-4 [1].

However, the secondary response was not completely inhibited by anti-IL-4, and is thought to be due to the formation of a memory pool of IgE-producing cells whose activation should be IL-4-independent [18]. Sherr *et al.* have reported that *in vitro* binding of CD23 on B cells with anti-IgE MoAb or IgE-anti IgE immune complexes suppresses ongoing human IgE synthesis [19]. Since engagement of CD21 on B cells by some anti-CD21 MoAbs increased IL-4-induced IgE production in a similar way as recombinant sCD23 [5], the pairing of CD21 and CD23 molecules may be important for persistent IgE response.

The existence of a ligand pair for CD23 on the subsets of B cells indicates that CD23 could also mediate homotypic B cell adhesion. In order to analyse the possible difference between the expression of CD21 and CD23 on a subset of $CD20^+$ cells in non-allergic and allergic children, we performed three-colour flow cytometry using a simple single-laser analyser and MoAb conjugated with FITC, PE and the recently described fluorochrome peridinin chlorophyll protein, PerCP [20]. We found that CD21 and CD23 were mostly expressed on the surface of the same B cell. Furthermore, we observed the up-regulation of CD23 antigen on a small subset of $CD21^-$ B cells only in allergic children. The known modulators of up-regulation of CD23 on B cells [2] seem to act on this particular B cell subset.

The expression of CD21 and CD23 is changed during B cell activation, maturation and differentiation [7]. Very recently, it was reported that CD21 is the ligand-binding subunit of CD19–CD21–TAPA-1 complex, which is important for promoting B cell activation [21]. Therefore, the biological significance of CD21–CD23 interaction in conditions characterized by increased IgE levels needs to be further investigated.

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