

Downregulation of CXCR6 and CXCR3 in Lymphocytes from Birch-allergic Patients

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Received 28 January 2008; Accepted in revised form 14 May 2008

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Abstract

Preferential expression of chemokine receptors on Th1 or Th2 T-helper cells has mostly been studied in cell lines generated *in vitro* or in animal models; however, results are less well characterized in humans. We determined T-cell responses through chemokine receptor expression on lymphocytes, and cytokine secretion in plasma from birch-allergic and healthy subjects. The expression of CCR2, CCR3, CCR4, CCR5, CCR7, CXCR3, CXCR4, CXCR6, IL-12 and IL-18R receptors was studied on CD4⁺ and CD8⁺ cells from birch-allergic ($n = 14$) and healthy ($n = 14$) subjects by flow cytometry. The concentration of IL-4, IL-5, IL-10, IL-12, IFN- γ and TNF- α cytokines was measured in plasma from the same individuals using a cytometric bead array human cytokines kit. The similar expression of CCR4 in T cells from atopic and healthy individuals argues against the use of the receptor as an *in vivo* marker of Th2 immune responses. Reduced percentages of CD4⁺ cells expressing IL-18R, CXCR6 and CXCR3 were found in the same group of samples. TNF- α , IFN- γ , IL-10, IL-5, IL-4 and IL-12 cytokines were elevated in samples from allergic individuals. Reduced expression of Th1-associated chemokine receptors together with higher levels of Th1, Th2 and anti-inflammatory cytokines in samples from allergic patients indicate that immune responses in peripheral blood in atopic diseases are complex and cannot be simplified to the Th1/Th2 paradigm. Not only the clinical picture of atopic diseases but also the clinical state at different time points of the disease might influence the results of studies including immunological markers associated with Th1- or Th2-type immune responses.

Introduction

CD4 cells are classified into Th1 and Th2 subsets based on their profiles of cytokine production [1, 2]. Th1 cells mainly contribute to the development of cell-mediated immunity, whereas Th2 cells regulate immune responses, facilitate IgE production and are involved in allergic diseases [3, 4]. Identification of Th1- [5, 6] and Th2- [7, 8] associated transcription factors have led to a better understanding of the process of the committing Th precursor cells to a particular subset. More recently it has been shown that pro-inflammatory Th-17 cells represent a novel subset of CD4⁺ T cells that originate under different polarizing conditions than Th1 or Th2 [9]. T cells with suppressor or regulatory function have been characterized in the CD4⁺ T cells [10].

While a definition of Th1/Th2 cells rests on their ability to produce IFN- γ but not IL-4 [11], or IL-4 but

not IFN- γ [11], respectively, recent studies have shown that some chemokine receptors are associated with Th1 or Th2 cells. Following antigenic challenge, lymphocytes are recruited into local tissues by adhesion molecules, chemokines and their receptors [12]. Chemokines not only regulate lymphocyte traffic [13, 14] but have also been linked to lymphocyte differentiation and effector functions [15, 16].

It has been reported that CCR5, CXCR3 and CXCR6 are predominantly expressed on Th1 cells [17–20], whereas CCR3 and CCR4 are predominantly expressed on Th2 cells [21–24]. The preferential expression of chemokine receptors on functional T-helper cells has mostly been analysed using *in vitro* generated Th1 and Th2 cell lines or clones stimulated with mitogens and/or Th-polarizing cytokines [17, 19, 23]. In a study including the analysis of the chemokine receptors CCR1, CCR2, CCR3, CCR4, CCR5, CCR6, CCR7, CCR9,

CXCR3, CXCR5 and CXCR6, it was shown that Th1, Th2, Th0 and non-polarized CD4⁺ T cells in blood and tissues can express any of these receptors [25]. It seems that certain combinations of these receptors define T-cell populations that are markedly enriched in major subsets of polarized and non-polarized T cells [26]. It has been suggested that lower expression of IL-12R β_2 mRNA after IL-2 stimulation indicates a reduced capacity to upregulate the receptor in atopic children [27]. Polarized Th1 immune responses in patients with common variable immunodeficiency [28] and patients with rheumatoid arthritis [29] have been associated with increased expression of IL-12R and IL-18R receptors. The expression of chemokine receptors on T cells has mostly been investigated using CD4⁺ cells, while the situation of CD8⁺ cells is less well characterized in humans.

In this article, we studied T-cell responses to a disease-specific allergen in patients with birch allergy. Thus, the expression of CCR2, CCR3, CCR4, CCR5, CCR7, CXCR3, CXCR4, CXCR6, IL-12 β R and IL-18R receptors in CD4⁺ and CD8⁺ T cells from healthy and birch-allergic asthmatic individuals was analysed. We also performed analyses of TNF- α , IFN- γ , IL-10, IL-5, IL-4 and IL-12 cytokines in plasma from the same individuals. Our data show a lack of association between the expression of CCR4 and the atopic status of the asthmatic individuals; however, the inverse correlation between IgE levels and the CXCR6 chemokine receptor confirms the value of CXCR6 as a good marker of Th1 cells. The higher levels of Th1, Th2 and anti-inflammatory cytokines in plasma from atopics reinforce the idea that immune responses in peripheral blood in atopic diseases cannot be simplified to the Th1/Th2 paradigm.

Materials and methods

Study population. Allergic patients referred to the Allergy Centre at Linköping University Hospital were recruited ($n = 14$) between October 2002 and March 2003. Healthy individuals were also invited ($n = 14$) as controls. Clinical examination, a lung function test and skin prick tests against inhalant allergens including birch, timothy, dog, cat, horse and mite allergens were performed on the allergic individuals. A skin prick test with a mean diameter of >3 mm was defined as positive. In the control group, total and specific IgE antibodies were measured in blood (Allergy Screen, ALK, Denmark). The allergy screen test consisted of a panel of 19 inhalant allergens, including timothy, birch, oak, olive, ragweed, mugwort, goosefoot, Bermuda grass, ribwort, cat, horse, dog, cockroach, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Alternaria tenia*, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*. Results of the test were regarded as either positive or negative. In the healthy controls low levels of total IgE antibodies were detected but none of

them had detectable specific IgE to common inhalant allergens by Allergy Screen. All allergic patients presented seasonal symptoms of allergic rhino-conjunctivitis (ARC) and all were sensitized to birch. All allergic individuals were also sensitized to other allergens, particularly furred pets and mites. Thus, seven of the birch-allergic individuals were also sensitized to cat. Nine of the 14 patients with ARC presented a clinical history of bronchial asthma, and inhaled corticosteroids on a daily basis up to 1000 μ g/day (Table 1). ARC was defined as sneezing, itching nose and eyes and redness in the eyes upon exposure to allergens. Bronchial asthma was defined as a clinical history of bronchial hyperactivity verified by a physician and FV1 variability $>15\%$ at least once during the last 12 months.

Table 1 Demographic description of the study population.

	Allergic ($n = 14$)	Non-allergic ($n = 14$)	P
Male/female	11/3	3/11	$<0.01^{\dagger}$
Age	16.6 (10.1–30)	22.6 (16.9–25.8)	0.07 [§]
Clinical symptoms			
ARC	5	0	
ARC + AB	6	0	
ARC + AB + AE	3	0	
Medication			
Nasal steroids	8/14	0	
Nasal antihistamines	1/14	0	
Local treatment in the eye	9/14	0	
Oral antihistamines	11/14	0	
Inhaled steroids	9/14	0	
Inhaled steroids (μ g/day)	400 (0–1000)	0	
Long-acting β_2 -agonists	6/14	0	
Oral leukotriene receptor	3/14	0	
Antagonists			
Systemic steroids	4/14*	0	
Total IgE (kU/l)	176 (45–3200)	20 (2–68)	$<0.001^{\dagger}$
Birch-specific IgE (kU/l)	24 (<0.35 –139)	<0.35	$<0.001^{\dagger}$
SPT against birch (mm)	5.5 (3.5–11.5)	– [†]	
Other sensitizations (SPT)			
Other pollens	12/14	– [†]	
Furred pets	9/14	–	
Mites	1/14	–	
Pollens and pets	10/14	–	
Pulmonary function			
FVC (% of normal)	96.5 (68–112)	– [†]	
FVE ₁ (% of normal)	92.0 (68–128)	–	

ARC, allergic rhinoconjunctivitis; AB, asthma bronchiale; AE, atopic eczema.

*Systemic steroids at least once the past year, no current systemic steroids.

[†]Skin prick test and pulmonary function were not performed in healthy individuals.

[‡]Fisher's exact test.

[§]Mann–Whitney.

Values are given as median (range).

Lymphocyte preparation and cell culture. Peripheral blood mononuclear cells (PBMC) from all the participants in the study were isolated, within 24 h of collection, from heparinized venous blood collected into cell preparation tubes (Becton-Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). Cells at interface were harvested and washed three times with RPMI-1640 (Gibco, Auckland, New Zealand) containing 5% human serum. After washing, PMNC were diluted to a concentration of 1×10^6 cells/ml in RPMI-1640 + 2% human serum supplemented with 2 mM L-glutamine and gentamicin (Sigma Chemical Co., St Louis, MO, USA). The cell suspension was added to a six-well plate and incubated for 1 h at 37 °C with 5% CO₂ (Forma CO₂-Incubator model 3862; Forma Scientific Inc., Marietta, OH, USA). After incubation, the supernatant non-adherent cells containing enriched lymphocytes were harvested into tubes, and washed with medium. After washing, the cells were diluted to a concentration of 1×10^6 cells/ml medium. One to two million cells were removed to a separate tube for fresh staining, i.e. *ex vivo* isolated cells. The remaining cells were stimulated for 24 h in 24-well culture plates. For the stimulation, 10 000 SU/ml of birch and cat allergens (Aquagen, ALK-Abelló, Denmark) and 1 mg/ml LPS (Sigma Chemical Co.) were added to 1×10^6 cells/ml. Wells with only non-stimulated cells were included as controls. After stimulation, lymphocytes were harvested, washed with PBS containing BSA and stained for flow cytometry analysis.

Flow cytometric detection of chemokine receptors. Cells were collected, washed in PBS containing 0.1% BSA and incubated for 30 min at 4 °C in darkness with the respective antibodies: fluorescein isothiocyanate (FITC)-conjugated anti-CXCR4, CXCR3, CCR3, CCR7 (R&D Systems, Minneapolis, MN, USA) and CCR5 (PharMingen, San Diego, CA, USA); R-phycoerythrin (PE)-conjugated anti-IL-18R, CCR2 and CXCR6 (R&D Systems) and IL-12 β 2R and CCR4 (PharMingen); peridin chlorophyll (PerCP)-conjugated anti-CD8 and allophycocyanin (APC)-conjugated anti-CD4 (BD Biosciences, San Jose, CA, USA).

Isotype controls were included to estimate the amount of nonspecific binding. Thus, cells were stained under identical conditions with FITC, PE, PerCP and APC fluorochrome-conjugated mouse IgG. Unstained cells were included as a control for autofluorescence. Four tubes containing cells stained with single antibodies marked with the four different fluorochromes were used for compensation to adjust for spectrally adjacent dye pairs.

Four-colour flow cytometry was performed with a Becton-Dickinson FACSCalibur (San Diego, CA, USA). Approximately 10 000 cells were acquired from each tube. The data were analysed using the CellQuest program (Becton-Dickinson Immunocytometry Systems). The cells were gated on forward and side scatter. Results

were expressed as percentages of cells expressing each surface molecule.

Cytokine analyses. The concentration of IL-2, IL-4, IL-5, IL-10, IFN- γ and TNF- α cytokines was measured in plasma samples from the same individuals using the cytometric bead array Human Cytokines Kit (BD Biosciences/Pharmingen) according to the manufacturer's instructions. The standard curve ranged from 0 to 2500 pg/ml, and values over 1.0 pg/ml were regarded as positive.

Statistical analysis. As percentages of cells expressing the chemokine receptors were not normally distributed, paired analyses were performed using the Wilcoxon signed rank test, unpaired analysis with the Mann-Whitney *U*-test and correlations were analysed using Spearman's rank order correlation coefficient test. $P < 5\%$ was considered statistically significant. Calculations were made using the statistical package SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, USA).

Ethical aspects. The study was approved by the Regional Ethics Committee for Human Research, Linköping University Hospital, Sweden.

Results

Comparison of chemokine receptor expression in allergic and healthy individuals

The percentages of *ex vivo* isolated CD4⁺ and CD8⁺ cells expressing CXCR6 were higher in healthy subjects than in allergic subjects (Fig. 1A). Incubation with medium alone did not affect the percentages of CD4⁺ and CD8⁺ T cells expressing CXCR6 in either group. A tendency to an increased expression of CXCR6 after 24 h stimulation compared to non-stimulated *ex vivo* CD8⁺ cells was observed in the same group of samples (Fig. 1B). A negative correlation was found between total IgE antibodies and the expression of CXCR6 receptor on CD4⁺ ($r = -0.457$, $P = 0.05$) and CD8⁺ ($r = -0.485$, $P = 0.05$) lymphocytes in all the samples (Fig. 5A,B).

Higher percentages of *ex vivo* isolated CD4⁺ cells expressed IL-18R in samples from healthy controls than in those from allergic subjects (Fig. 1C). Similarly, the percentage of CD4⁺ cells expressing IL-18R was higher in non-stimulated cells of the same group (Fig. 1C). A higher percentage of CD8⁺ IL-18R expressing cells were observed only after LPS stimulation (Fig. 1D).

No difference in the percentage of CD4⁺ or CD8⁺ cells expressing CXCR3 was seen between the groups in *ex vivo* isolated cells or samples cultured with medium alone. However, stimulation with either antigen resulted in increasing percentages of CD4⁺ CXCR3⁺ cells in samples from healthy subjects compared to allergic individuals (Fig. 1E), whereas the percentages of CD8⁺ CXCR3⁺ cells were not affected (Fig. 1F).

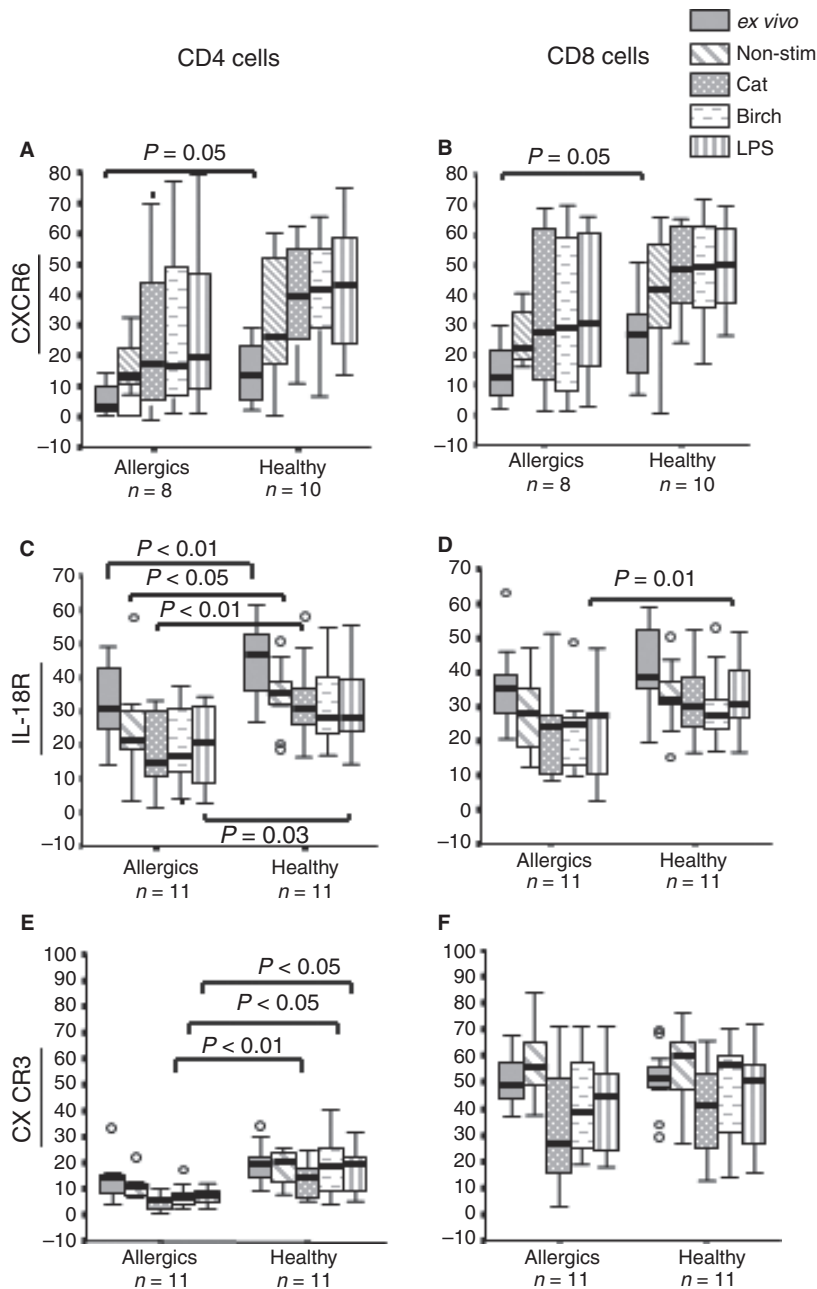


Figure 1 Expression of chemokine receptors CXCR6 (A, B), IL-18R (C, D) and CXCR3 (E, F) on CD4⁺ and CD8⁺ cells. The expression of the receptors was analysed on *ex vivo* isolated cells, and in samples cultured with medium alone, and with birch, cat and LPS for 24 h. The data are expressed as box-plot summaries of the median percentages (%) of cells. The level of significance is shown above the plots.

The percentages of CD4⁺ and CD8⁺ T cells expressing CCR4, CCR5, CCR7 and CXCR4 were similar for *ex vivo* isolated cells and for cat, birch and LPS-stimulated cells from allergic and healthy individuals (data not shown). The total IgE levels inversely correlated with CCR5 expression on CD4⁺ cells ($r = -0.416$, $P = 0.05$) (Fig. 5C). The expression of CCR3, CCR2 and IL-12R was very low, and no statistical analyses were performed on the data.

Effect of the culture conditions on receptor expression

Culture of the cells for 24 h with medium alone reduced the expression of some of the receptors included in the

study. This was the case for CD4⁺ and CD8⁺ cells expressing CCR4 (Figs. 2A,B and 3A,B) and IL-18 (Figs. 2C,D and 3C,D) in samples from both allergic and healthy subjects compared to *ex vivo* samples from the same individuals.

In contrast, the expression of CXCR6 (Figs. 2E,F and 3E,F) and CXCR4 (Figs. 2G,H and 3G,H) increased on CD4⁺ and CD8⁺ cells cultured in medium alone, both in the allergic and healthy groups, compared to *ex vivo* samples from the same subjects. Further, in samples from healthy individuals, CD8⁺ CXCR3⁺ expressing cells increased after incubation with medium alone compared to *ex vivo* CD8⁺ cells (Fig. 3I,J), but this effect was not

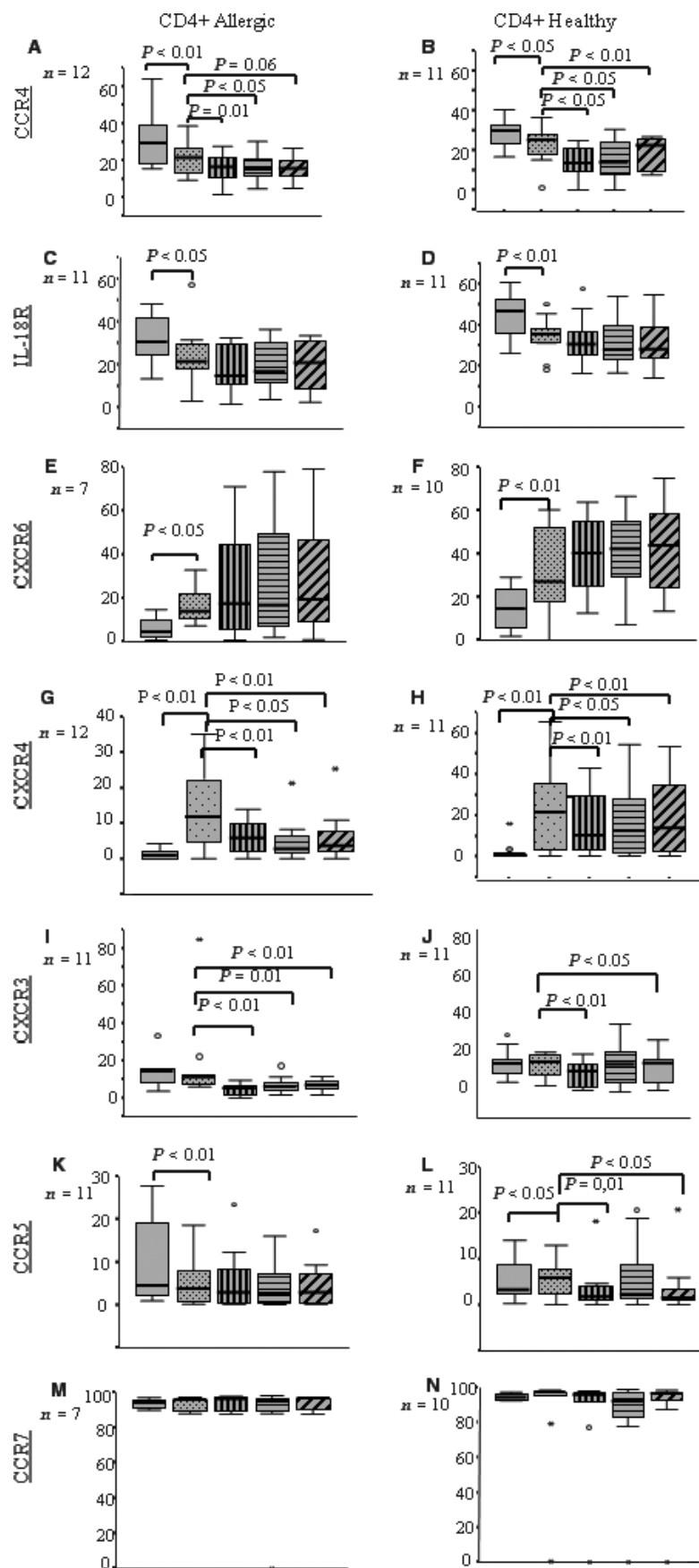


Figure 2 Expression of CCR4, IL-18R, CXCR6, CXCR4, CCR5, CXCR3 and CCR7 receptors on CD4⁺ cells from allergic (A, C, E, G, I, K, M) and healthy (B, D, F, H, J, L, N) individuals. The data are expressed as box-plot summaries of the median percentages (%) of *ex vivo* isolated cells, and after 24 h on non-stimulated cells and birch, cat and LPS-stimulated cells. The level of significance is shown above the plots.

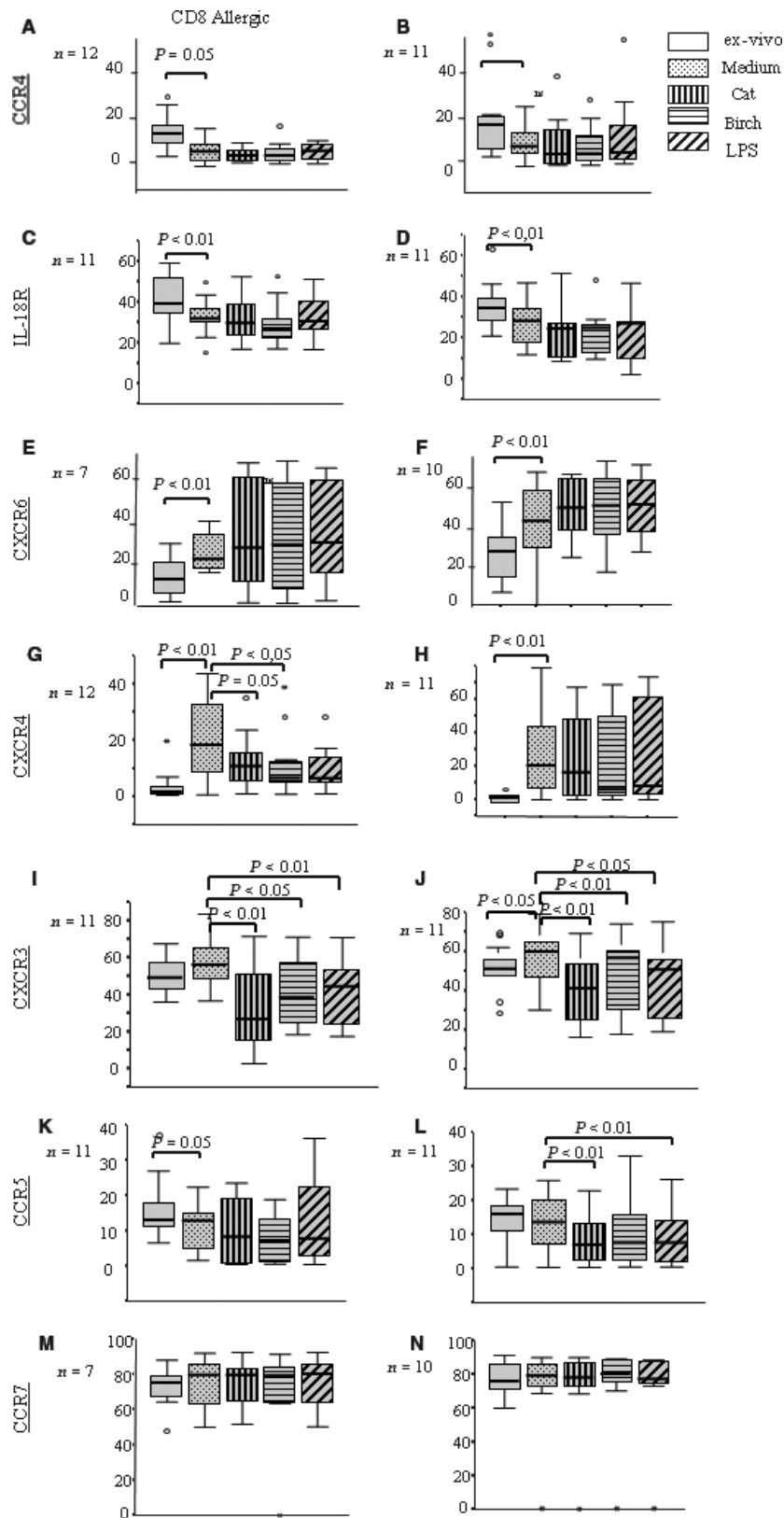


Figure 3 Expression of CCR4, IL-18R, CXCR6, CXCR4, CCR5, CXCR3 and CCR7 receptors on CD8⁺ cells from allergic (A, C, E, G, I, K, M) and healthy (B, D, F, H, J, L, N) individuals. The data are expressed as box-plot summaries of the median percentages (%) of *ex vivo* isolated cells, and after 24 h on non-stimulated cells and birch, cat and LPS-stimulated cells. The level of significance is shown above the plots.

observed in samples from the allergic group (Fig. 2I,J). Incubation for 24 h reduced CD4⁺CCR5⁺ and CD8⁺CCR5⁺ expressing cells in samples from allergic

subjects (Fig. 2K,L), while CD4⁺CCR5⁺ were increased in cells from healthy individuals cultured with medium alone compared to *ex vivo* cells (Fig. 3K,L).

Stimulation with antigen

Stimulation with either antigen resulted in a further reduction in CCR4 of CD4⁺ cells (Figs. 2A and 3A), and in CXCR3 on both CD4⁺ and CD8⁺ cells from allergic and healthy individuals (Figs. 2I,J and 3I,J). Similarly, CXCR4 expression was also reduced on both CD4⁺ and CD8⁺ cells from allergic patients, but stimulation only decreased CXCR4 on CD4⁺ cells from the healthy group (Fig. 2G,H). In the samples from the healthy group, even the expression of CCR5 decreased after cat and LPS stimulation, but not birch stimulation, both on CD4⁺ and CD8⁺ cells (Fig. 3K,L). In contrast, in the group of allergic subjects stimulation did not further affect CCR5 on either cell.

Differential expression of chemokine receptors on CD4⁺ and CD8⁺ cells

The expression of the chemokine receptors was studied on CD4⁺ and CD8⁺ lymphocytes from allergic and healthy individuals. CCR5, CXCR3 and CXCR6 receptors were preferentially expressed on *ex vivo* CD4⁺ cells and CD8⁺ after 24 h of culture in both groups. In contrast, CCR4, CXCR4 and CCR7 were preferentially expressed on CD4⁺ cells. A higher percentage of *ex vivo* isolated CD8⁺ cells than CD4⁺ cells expressed IL-18R in the samples from allergic subjects, whereas a similar expression of the receptor was observed in CD4⁺ and CD8⁺ cells from healthy individuals (data not shown).

Cytokine secretion in plasma

Higher levels of TNF- α , IFN- γ , IL-10, IL-5, IL-4 and IL-2 were detected in the samples from allergic subjects than in those from healthy individuals (Fig. 4). A strong correlation was found among the different cytokines (data not shown). No correlations were detected between the cytokine levels in plasma and chemokine receptor expression in CD4⁺ and CD8⁺ T cells (data not shown). The total IgE levels in plasma correlated only with TNF- α ($r = 0.653$, $P = 0.01$) (Fig. 5D).

Discussion

In the present study we examined the expression of chemokine receptors that have been previously associated with either Th1 or Th2 cell types on CD4⁺ and CD8⁺ cells from allergic patients and healthy individuals. We did observe a reduced expression of the Th1-associated chemokine receptors CXCR6 and IL-18R in *ex vivo* isolated cells from allergic patients, in parallel with increased levels of cytokines in plasma from the same individuals. Indeed, chemokine receptors have largely been analysed using *in vitro* generated Th1 and Th2 cell

lines or clones that have been stimulated with mitogenes and growth-promoting and Th-polarizing cytokines. Therefore, the expression of certain receptors as the result of the culture conditions rather than certain T-cell phenotypes could be one explanation for the controversial difference in results between *in vitro* and *in vivo* studies.

In our study, circulating CD4⁺ cells expressing CXCR6 were more common in healthy individuals than in patients with birch allergy. CXCR6 is considered as a Th1-cell associated chemokine receptor [20, 30]. Its expression defines unique subsets of highly polarized type 1 memory/effector T cells *in vivo*, with the capacity to release a large amount of IFN- γ upon activation [20, 31]. Thus, the poor expression of CXCR6 in allergic patients could reflect a reduction in Th1 activity. Supporting this view, we detected an inverse correlation between the percentages of *ex vivo* CD4⁺ and CD8⁺ cells expressing CXCR6 and total IgE levels in plasma. Further, a reduced percentage of CD4⁺ cells expressing IL-18R on *ex vivo* isolated CD4⁺ T cells from allergic individuals in our study could reinforce the idea of a poor induction of Th1 response in allergy. It has been shown that IL-18R is preferentially expressed on murine [32] and human [33] Th1-polarized cells compared with Th2-polarized cells, suggesting that this receptor is a suitable marker of Th1 cells. Reduced CXCR6 expression shortly after T-cell activation could be dependent on early intracellular signal events associated with the T-cell activation process [31]. Thus, the lower percentage of T cells from the birch-allergic patients expressing CXCR6 might be a consequence of an earlier T-cell activation process in allergic individuals, leading to faster downregulation of CXCR6. Indeed, it has been suggested that the reduction in CXCR6 following allergen challenge in T cells from bronchoalveolar fluids could be due to the internalization of the receptors following encounter with their ligands [34]. Alternatively, lower detection of CXCR6-bearing cells could indicate accumulation of these cells in the inflammatory sites. It seems that CXCR6 could have a particular role in maintaining T-cell activation in the lung [35]. CCR5, another Th1-associated chemokine receptor, was similarly expressed in samples from allergic and healthy individuals. However, while the percentage of CD4⁺ CCR5⁺ cells was downregulated after stimulation in samples from the healthy individuals, no further reduction in cells from allergic patients was observed. Chemokine binding to CCR5 leads to cellular activation and internalization of the receptor, and recall antigen activation of PBMC resulted in release of CCR5 ligands such as MIP-1 α and MIP-2 β [36]. Thus, it is possible that early release of CCR5 ligands as a result of allergic inflammation in allergic, but not in healthy, individuals is responsible for the unresponsiveness after stimulation in our study. Another possible explanation is the effect of inhaled steroids among the patients. Low doses were used

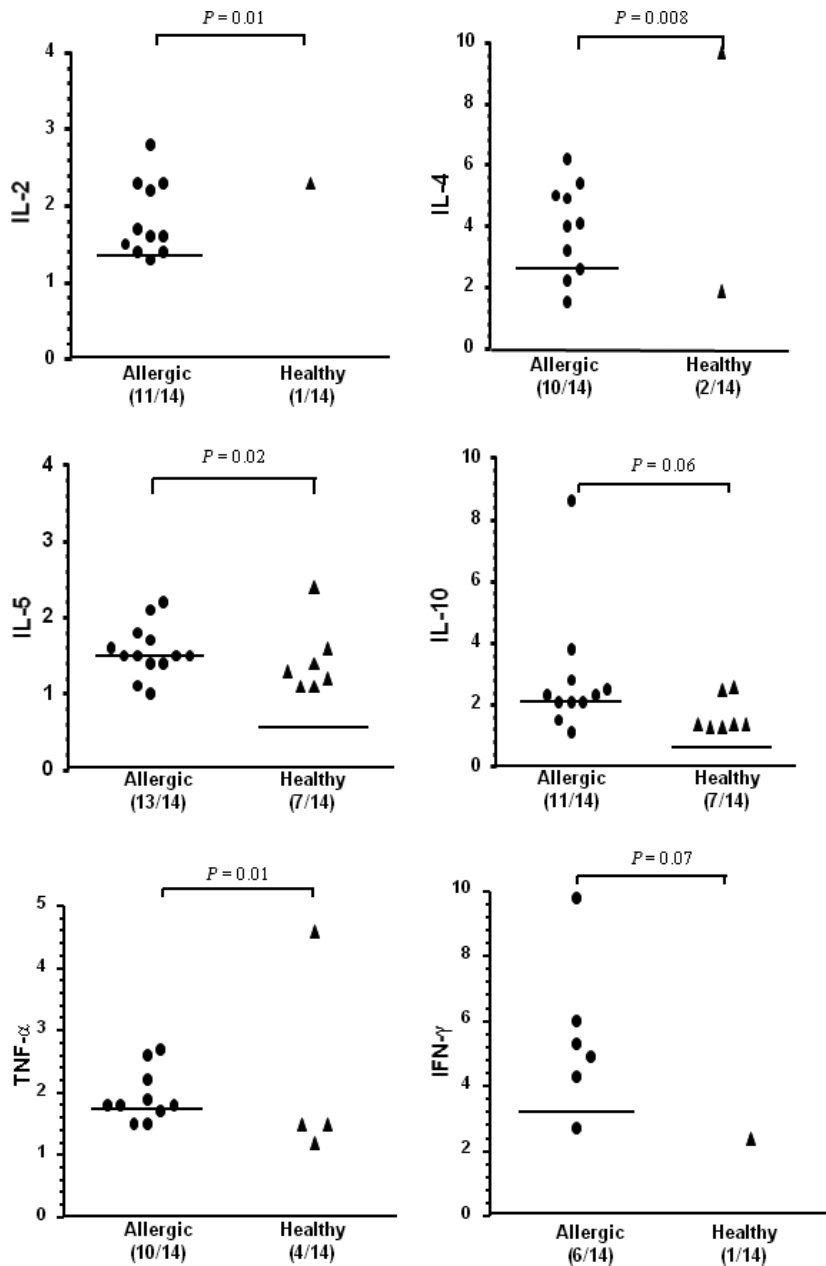


Figure 4 Comparison of the levels of spontaneous cytokine secretion in plasma from allergic (A = 14) and healthy (H = 14) individuals. Individual levels are represented as dot plots. Below the figures, numbers in parentheses indicate the amount of samples with detectable levels/total number of analysed samples. Differences in IL-4, IL-5, IL-10, IL-12, IFN- γ and TNF- α cytokine concentrations are shown. Horizontal lines indicate median values. The concentrations are expressed as pg/ml. Significances are indicated as P -values above the plots.

generally, with only one child taking 1000 $\mu\text{g}/\text{day}$. Low doses are not expected to have systemic anti-inflammatory effects; they rather decrease the local allergic inflammation in the lungs by decreasing inflammatory signals. Furthermore, we did not find any differences in receptor expression between those children using inhaled steroids and those who did not. The subgroup of patients not taking inhaled steroids was rather small.

Antigenic stimulation of cells from allergic patients led to a reduction in the percentage of CD4⁺ cells expressing CXCR3 compared with similar cells from healthy individuals. Antigenic reactivation of T cells, resulting in downregulation of CXCR3, has been observed on resting/effector cells stimulated with specific

antigen [37]. Interestingly, the decreased expression of CXCR3 after antigen stimulation was restricted to CD4 cells, and was not seen in cytotoxic CD8 cells.

Selective expression of CCR4 as a marker for type 2 immune responses has been the topic of many studies reporting controversial results [38, 39]. Based on this observation, we investigated the expression of this receptor in atopic asthmatic individuals compared with healthy controls. The percentage of cells expressing CCR4 receptor did not differ between allergic and healthy subjects in our study. CCR4 can be expressed by non-Th2 T cell lines generated in the presence of TGF- β [40] or even by some Th1 cells *in vitro* [23]. Its regulation is independent of the cytokine profile, as

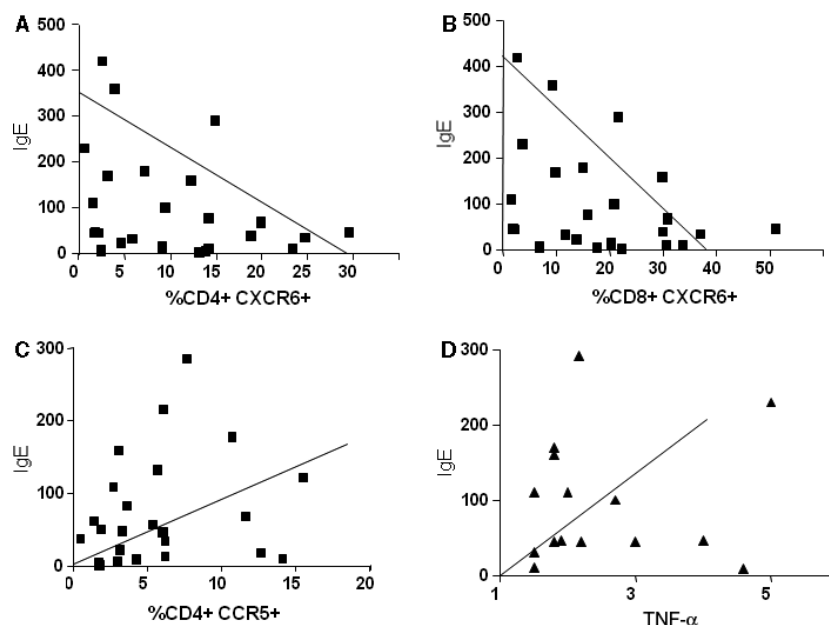


Figure 5 Correlation between total IgE levels in plasma samples and the percentages of *ex vivo* CD4⁺ cells expressing CXCR6⁺ (A), CD8⁺ cells expressing CXCR6⁺ (B) and CD4⁺ cells expressing CCR5⁺ (C). The total IgE and TNF- α level in plasma correlated in all the samples (D). Levels of correlations are indicated in the figures.

cells expressing CCR4 comprise cells with both Th1 and Th2 potential [41]. A recent study in NOD mice has linked CCR4-bearing T cells with type 1 diabetes, demonstrating that CCR4⁺ T cells facilitated the migration of pathogenic T-cell population to the islet lesion [42]. Similarly, CCR4 expression in skin lymphocytes isolated at the site of an epidermal delayed-type hypersensitivity and contact hypersensitivity inflamed skin – accepted models for Th1-mediated inflammation – also argue against the notion that CCR4 is a marker for Th2 [43].

Expression of CCR7 was commonly detected in CD4⁺ cells from both groups of patients in our study, whereas a somewhat lower percentage of CD8⁺ belonged to the CCR7⁺ T-cell population. The proven role of CCR7 in the entry of T cells via endothelial venules favoured the idea that this receptor has a constitutive function in the recirculation of naïve T cells [44] and is downregulated, together with CXCR4, following T-cell priming and differentiation [40]. More recently, it has been demonstrated that the majority of human cytokine-producing T cells lie within those cells that are CCR7⁺ [26], and that the majority of CD4⁺ cells producing effector cytokines upon stimulation retain the capacity to recirculate through secondary lymphoid tissues via the CCR7 receptor [45]. It has been reported that CXCR4, like CCR7, was mainly expressed on naïve T cells and only on a fraction of freshly isolated memory cells [46]. In our study, the percentages of CD4⁺ and CD8⁺ cells expressing CXCR4 receptor were very low in freshly separated cells and similar between both groups, and increased in non-stimulated cells after 24 h. This agrees with another study showing that freshly isolated PBMC from house dust mite atopic and non-atopic individuals expressed low levels of

CXCR4, which was enhanced following *in vitro* culture of T cells in medium alone [47].

It is well established that allergen-specific CD4⁺ T cells from allergic patients generally belong to the Th2-cell subset, which produces relatively high quantities of IL-4, IL-5, IL-10 and IL-13, but very low levels of IFN- γ after activation [48]. In our study, not only were plasma levels of both Th1 cytokines and Th2 cytokines higher in samples from atopic patients, but also the levels of the anti-inflammatory cytokine IL-10, which inhibits both Th1 and Th2 responses and IgE synthesis [49, 50]; in addition, the levels of TNF- α pro-inflammatory cytokine, associated with both Th1- and Th2-type immune responses [51], were higher in the samples from atopic individuals. The single association between TNF- α and total IgE levels detected by us agrees with a report showing the role of TNF- α as an important promoter of IgE production in mice [51].

Almost all patients included in our study presented more than one atopic disease and were sensitized to allergens other than birch. This might influence our results. However, immunological reactions are very specific, and it is for this reason that we used birch allergy as a model for chemokine receptor regulation. Birch is a predominant allergen in Sweden, and almost 60–70% of asthmatic patients are sensitized to it. It could be argued that other clinical pictures may influence the result, but statistical assessment of this issue was not feasible in this study.

In a study including monocyte-derived dendritic cells, we showed that both maturation and the specific Th1 function of these cells seem to be affected in allergic individuals [52]. Thus, our observation of a reduced expression of CXCR6 and IL-18R chemokine receptors in cells from allergic patients could indicate that the balance

of Th1- and Th2-type immune responses required for normal immune responses seems to be affected in allergic individuals. Indeed, higher levels of Th1, Th2 and anti-inflammatory cytokines in the samples from allergic subjects also indicate that immune responses in peripheral blood in atopic diseases are complex and cannot be simplified to the Th1/Th2 paradigm. The predominant expression of some chemokine receptors on CD8⁺ cells compared with CD4⁺ cells could have potential importance in the role of those receptors. It seems that the study of samples at different points of atopic diseases, including mild and acute asthma, could vary the results of studies including immunological markers associated with Th1- or Th2-type immune responses.

Acknowledgment

The authors would like to thank Dr Outi Vaarala for valuable scientific contribution. This work was supported by the Swedish Allergy and Cancer Foundation and the Medical Research Foundation of the County of Östergötland, Sweden.

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