

Chemokine-receptor expression on T cells in lung compartments of challenged asthmatic patients

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Summary

Background The interaction of chemokines with their receptors strongly influences the migration of leucocytes.

Objective In order to assess the contribution of these molecules to the local recruitment of T cells in bronchial asthma, we analysed the expression of 14 chemokine receptors on lung-derived T cells.

Methods Chemokine-receptor expression by T cells derived from the peripheral blood, the bronchoalveolar lavage fluid and the bronchial mucosa was analysed by flow cytometry and immunohistochemistry. Expression profiles in healthy and mildly asthmatic individuals were compared, the latter prior and after segmental allergen provocation.

Results Compared with peripheral blood, alveolar T cells expressed significantly more CCR2, CCR5, CCR6, CXCR3 and CCR4. However, no differences were observed between healthy controls and unchallenged asthmatics. In patients developing significant inflammatory responses following specific allergen challenge, a marked increase in the percentage of CCR4⁺ and CCR7⁺, and reduced numbers of CXCR3-bearing alveolar T cells were detected. Following specific allergen challenge, chemokine-receptor expression profiles of T cells from the alveolar space and the mucosa or the submucosa were similar, excluding a particular subcompartmentalization of the chemokine/chemokine-receptor system.

Conclusion The expression of certain chemokine receptors by lung T cells suggests a contribution to the physiological recruitment of T cells to the lungs, both in healthy controls and unchallenged mild asthmatics. However, strong allergen-induced airway responses were associated with a specific chemokine-receptor profile, suggesting the involvement of certain chemokine receptors in the pathogenesis of allergic bronchial inflammation.

Keywords bronchial hyper-reactivity, chemokines, chemotaxis, leucocyte, segmental allergen challenge

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Introduction

T cells initiate and maintain the inflammatory reaction of allergic asthma [1, 2]. They recruit and activate effector cells, such as eosinophils, and induce the synthesis of IgE, thus controlling the development of the early and the late asthmatic reaction.

The migration of T cells from the blood into lung tissue is regulated by the interaction of T cell adhesion molecules with their counterparts on pulmonary endothelial cells [3].

Furthermore, the recruitment of T cells is influenced by the interaction of chemokines with their corresponding G-protein-coupled cell surface receptors [4, 5].

Several studies have demonstrated an increase in inflammatory chemokines in asthmatic subjects, suggesting an involvement of these molecules in the recruitment of leucocytes in allergen-mediated airway inflammation [6–18]. Only a limited number of studies have analysed the expression of the chemokine receptors. In atopic asthma, mRNA for CCR4 was found to be increased after segmental allergen challenge [17]. Further support for an involvement of CCR4 and CCR8 in the recruitment of T cells into inflamed lung tissues came from an immunohistochemical study in bronchial biopsies from either saline or allergen-challenged asthmatics [18].

We therefore analysed the expression of a panel of 14 chemokine receptors, including CCR4, on T cells in the bronchial

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Table 1. Clinical characteristics of patients with asthma

Patient	Sex	Age (years)	Duration of asthma (years)	FEV ₁ % pred	IgE (KU × L ⁻¹)	Allergen	Specific IgE (KU × L ⁻¹)	Allergen dose (mBu)	Medication
1	F	24	14	103	551	D. pt.	> 100	2650	ICS, β
2	F	25	5	107	221	Birch	18.4	12.9	ICS, β
3	F	44	10	102	139	Birch	18.5	775	β
4*	M	30	22	96.5	242	Birch	24.1	112.5	ICS, β
5*	F	23	2	100	264	D. pt.	> 100	190	ICS, β
6*	F	34	15	90	96	Birch	6.2	160	No

*Patients with a strong inflammatory reaction after segmental allergen challenge (see Fig. 4). % pred, percentage of predicted value; mBu, milli biological units; D. pt., *Dermatophagoides pteronyssinus*; M, male; F, female; ICS, inhaled corticosteroids; β, β₂-agonists; FEV₁, forced expiratory volume in 1 s.

mucosa and the bronchoalveolar lavage fluid (BALF) of asthmatic patients before and after segmental allergen challenge in order to elucidate whether (i) T cells from healthy individuals exhibit a lung-specific repertoire of chemokine receptors, (ii) whether the chemokine receptor pattern differs between healthy controls and mild asthmatics, (iii) whether an allergen challenge causes recruitment of T cells expressing specific chemokine receptors, and (iv) to determine in which compartment within the bronchial mucosa chemokine-receptor-bearing T cells can be localized after an allergen challenge.

Materials and methods

Subjects

We studied six non-smoking patients with mild intermittent or mild persistent asthma (further characteristics are listed in Table 1). Asthma was diagnosed as previously defined [19]. All subjects had a history of recurrent cough, wheeze, sputum production and reversible bronchoconstriction after allergen inhalation. Inhaled corticosteroids were withheld at least 7 days prior to allergen challenge. Three healthy subjects served as controls. None had any evidence of recent or current respiratory tract infections. All subjects gave informed consent and the study protocol was approved by the local Ethics Committee.

Inhaled allergen challenge

All patients underwent an inhaled challenge to determine the individual dose of allergen that caused a 20% decrease in forced expiratory volume in 1 s (FEV₁), as described elsewhere [20]. Patients were included in the study if a second decline in FEV₁ of > 15% from baseline was observed 5–9 h after the inhaled allergen challenge. A tenfold higher dose was then used for the subsequent segmental allergen challenge. Inhaled allergen challenge and segmental allergen challenge were separated by at least 3 weeks.

Segmental allergen challenge, bronchoalveolar lavage and bronchial biopsies

Bronchoscopy, segmental allergen challenge and BAL were performed as previously described [21].

Table 2. Chemokine receptor-specific antibodies used in the study

Chemokine receptor	mAb clone	Species	Isotype	Reference
CCR1	2D4	Mouse	IgG1	[48]
CCR2	1D9	Mouse	IgG2a	[49]
CCR3	7B11	Mouse	IgG2a	[50]
CCR4	1G1	Mouse	IgG1	[51]
CCR5	2D7	Mouse	IgG2a	[52]
CCR6	11A9	Mouse	IgG1	[53]
CCR7	7H12	Mouse	IgG2b	[54]
CXCR1	5A12	Mouse	IgG2b	[49]
CXCR2	6C6	Mouse	IgG1	[49]
CXCR3	1C6	Mouse	IgG1	[29]
CXCR4	12G5	Mouse	IgG2a	[55]
CXCR5	BLR1	Rat	IgG2a	[56]
CX3CR1	1E5	Mouse	IgG1	(S. Qin, unpublished data)

mAb, monoclonal antibodies.

Preparation of peripheral blood mononuclear cells and bronchoalveolar lavage cells

Mononuclear cells from the peripheral blood and the BAL were isolated as previously reported [20, 22].

Monoclonal antibodies used in this study

Chemokine-receptor-specific monoclonal antibodies (mAb) used in this study are listed in Table 2 [23]. The mAb were tested for their staining properties on tonsillar tissue sections using immunohistochemistry and in parallel on tonsillar cell suspensions using flow cytometry. The CCR7-specific mAb did not lead to recognizable signals in tissue sections but functioned well in flow cytometry. The CCR3-specific mAb was tested in flow cytometry on purified eosinophils. The CCR3- and the CXCR4-specific mAb showed non-specific staining with bronchial biopsy tissue; all other anti-chemokine-receptor mAb gave a consistent staining pattern on frozen bronchial tissue sections. In addition, a CD3-specific mAb OKT3 (IgG2a, ATCC) was used. The mAb 2A11 (mouse IgG1) [24], MOPC-21 (mouse IgG1, Sigma, St Louis, MO, USA) and 1D10 (rat IgG2a) [25] served as isotype controls.

Flow cytometry

Flow cytometry was performed as previously described [25]. After detection of the primary antibody with fluorescein-

conjugated Igs (Jackson, West Grove, PA, USA) free binding sites were blocked with mouse γ -globulins (Jackson) and cells incubated with the phycoerythrin-conjugated mAb OKT3. Cells were analysed on a Becton Dickinson FACS Calibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) using a 488 nm laser.

Immunohistology

Cryostat sections (8 μ m) from bronchial biopsies were stained using the APAAP technique as previously described [26]. Reagents were purchased from DAKO (Hamburg, Germany). New Fuchsin (Merck, Darmstadt, Germany) was used as substrate; nuclei were counterstained with haematoxylin (Merck). Analysis of serial sections stained with anti-CD3 antibodies combined with morphological analysis strongly suggested that the chemokine-receptor-bearing cells most probably were T cells.

Statistical analysis

Statistical analyses were performed using GraphPad Prism[®] version 3.00 for Windows, GraphPad Software (San Diego, CA, USA) and SPSS[®] Version 11.0 for Windows, SPSS Inc. (Chicago, IL, USA).

Results

Chemokine-receptor patterns on T cells from the pulmonary compartment in healthy individuals

To determine whether lung T cells exhibit a specific chemokine-receptor pattern, we compared the expression of 14 chemokine receptors on BAL-derived T cells with the staining pattern of peripheral blood T cells of three healthy subjects using flow cytometry. Figure 1a shows the expression profiles of all analysed chemokine receptors for one representative subject; the mean of chemokine-receptor-positive T cells from the three analysed subjects is summarized in Fig. 1b. Four different patterns of chemokine-receptor expression were identified on T lymphocytes: (1) CCR1, CCR3, CCR9, CXCR1, CXCR2 and CXCR5 were only weakly expressed or completely absent on T cells derived from either compartment; (2) CXCR4 was identified on the majority of T cells from both compartments; (3) peripheral blood T cells exhibited a higher surface expression of CX3CR1 and CCR7; and (4) alveolar T cells expressed significantly more CCR2, CCR5, CCR6 and CXCR3, and to a lesser degree, also CCR4. The expression of chemokine receptors on T cells from both compartments was further characterized as mean intensity fluorescence (MIF), a parameter for the antigen density on the cell surface. No significant differences in the density on receptor-positive T cells from both compartments were observed for CCR5 and CXCR3 (MIF (mean $\pm$ SD) of CCR5 on peripheral blood mononuclear cell (PBMC) 449.7 ± 157.8 ; in BALF 522.4 ± 70.6 ; MIF of CXCR3 on PBMC 194.0 ± 14.1 ; in BALF 179.0 ± 47.3). CXCR4 showed a higher density on alveolar T cells compared with T cells from the peripheral blood (MIF 220.1 ± 23.6 vs. 70.4 ± 6.7), whereas the levels of CCR2, CCR7, CX3CR1, and to a lesser degree, CCR4 and CCR6 were higher on T cells from the peripheral blood (CCR2:

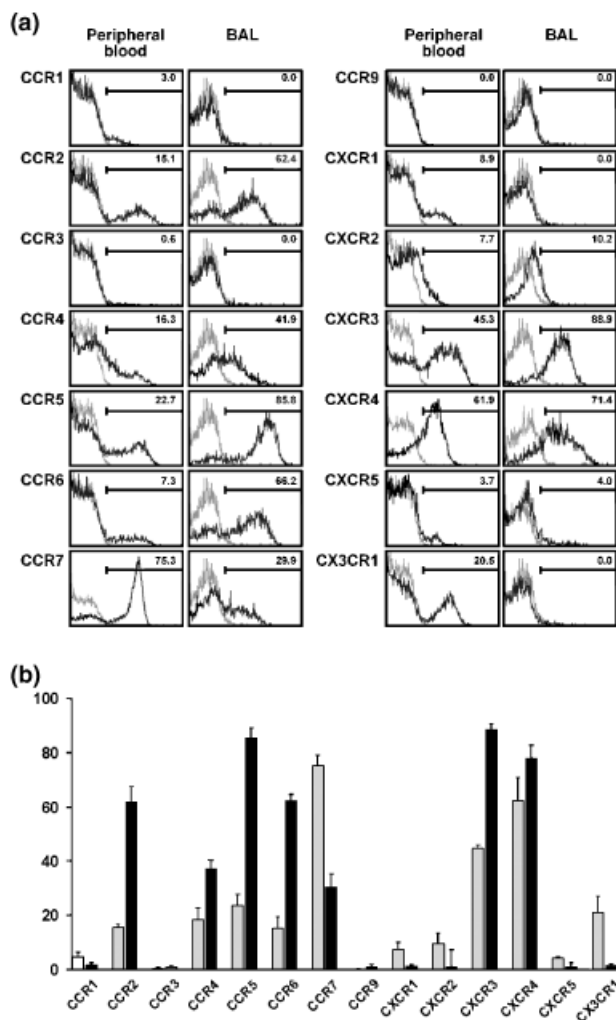


Fig. 1. Expression of chemokine receptors on peripheral blood and alveolar T cells in healthy subjects. (a) The grey profiles represent flow cytometry staining obtained with isotype controls, and the solid profiles represent the specific staining obtained with chemokine-specific antibodies. The percentages of chemokine-receptor-positive CD3⁺ cells in each population are indicated in the individual histograms. Representative data obtained from one healthy subject are shown. (b) Mean of chemokine-receptor-positive T cells in the peripheral blood (grey bars) and the bronchoalveolar lavage (BAL) (black bars) of the three analysed subjects.

448.3 ± 99.3 vs. 181.0 ± 25.8 ; CCR7: 222.4 ± 34.0 vs. 89.9 ± 9.0 ; CX3CR1: 155.5 ± 17.0 vs. 51.1 ± 4.1 ; CCR4: 109.9 ± 38.5 vs. 75.2 ± 6.4 ; CCR6: 285.7 ± 65.0 vs. 172.9 ± 58.4).

Chemokine-receptor expression pattern on peripheral blood and alveolar T cells in patients with asthma

Alveolar and peripheral blood T cells from six patients with mild asthma were analysed for chemokine-receptor expression. In these subjects, a consistent and statistically significantly higher expression of CCR2 ($P = 0.0009$), CCR5 ($P < 0.0001$), CCR6 ($P = 0.002$) and CXCR3 ($P < 0.0001$) was observed on BAL-derived T cells as compared with peripheral blood T cells from the same individuals (Fig. 2). There was also a trend towards a higher expression of CCR4 ($P = 0.016$) on alveolar T cells. In contrast, CCR7 ($P < 0.0001$) and CX3CR1 ($P = 0.001$) expression was higher on peripheral blood T cells.

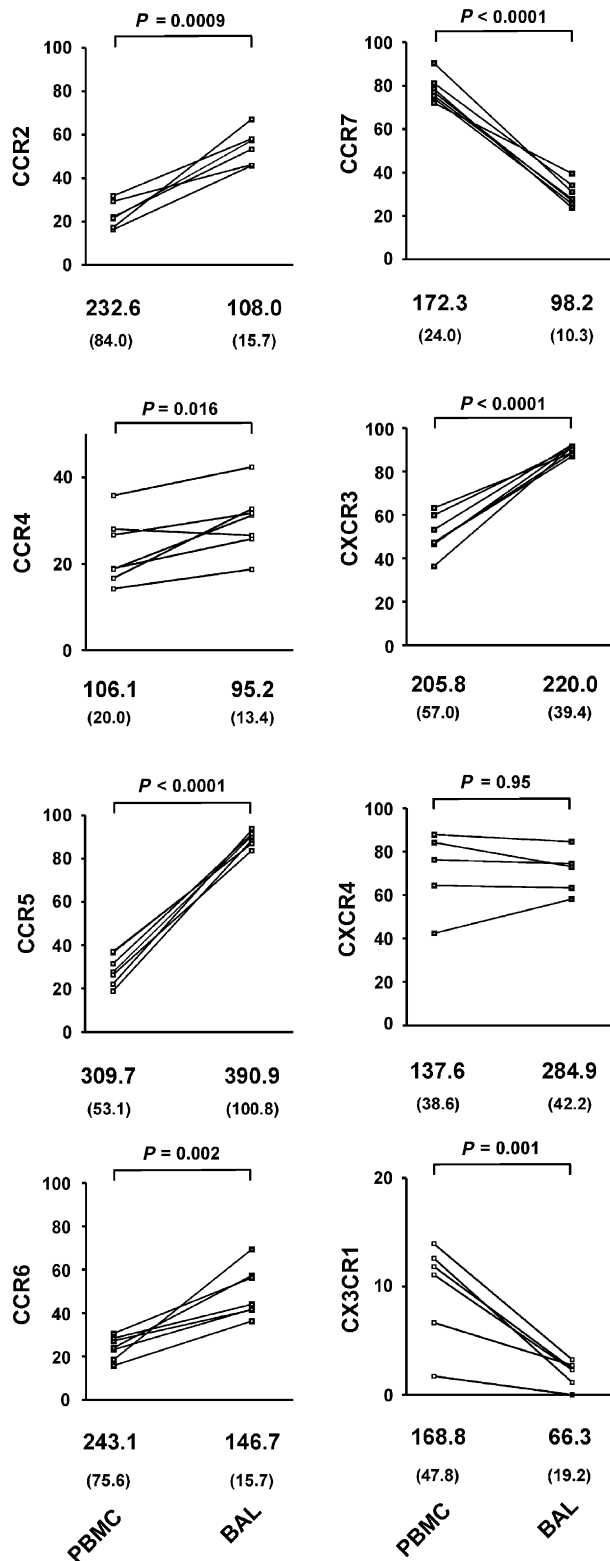


Fig. 2. Expression of chemokine receptors on peripheral blood and alveolar T cells in six patients with mild asthma. The percentages of chemokine-receptor-positive CD3⁺ cells within peripheral blood mononuclear cells and bronchoalveolar cells are indicated on the y-axis. Respective values obtained in each compartment in one given patient are connected by a solid line. The mean of the mean intensity fluorescence of receptor-positive T cells is given in bold letter, and the standard error of the mean is given in italics. Note the different scaling for CCR4 and CX3CR1.

Comparison of chemokine-receptor expression on alveolar and peripheral blood T cells from healthy individuals and asthmatic subjects

Analysis of the chemokine-receptor expression on alveolar T cells from healthy individuals and unchallenged asthmatics revealed no significant differences, although there was a trend for a reduced expression of CCR4 and CCR6 in asthmatic subjects (data not shown). In addition, comparison of chemokine-receptor expression on peripheral blood T cells from asthmatic and healthy subjects did not reveal notable differences (data not shown).

Expression of chemokine receptors on alveolar T cells after segmental allergen challenge

We investigated whether allergen-induced airway infiltration is accompanied by a change in chemokine-receptor expression of local T cells. Therefore, six asthmatic patients underwent segmental challenge with one of their specific allergens, followed by BAL of the allergen-challenged segment 42 h later. Chemokine-receptor expression on alveolar T cells was compared between allergen-exposed segments and saline-exposed control segments in each patient. The response of the patients to the allergen differed significantly. Three patients exhibited only a mild inflammatory response, as judged by a small influx of eosinophils into the alveolar space (Table 3). In contrast, the other three patients responded with a strong influx of eosinophils (Table 3). Allergen exposure did not significantly change the expression of CCR2, CCR5, CCR6, CXCR4, and CX3CR1 on T cells 42 h after segmental allergen provocation in any of the patients studied (Fig. 3). In the three patients with a mild inflammatory response (as judged by eosinophil infiltration), the expression of the other chemokine receptors also remained unchanged. However, in the three asthmatics responding with a strong local eosinophilia (represented by filled circles in Fig. 3), the percentage of CCR7⁺ and CCR4⁺ T cells increased substantially following allergen exposure, while there was a significant decrease in the percentage of CXCR3⁺ T cells. This observation was confirmed by a regression analysis of the correlation between the percentage of chemokine-receptor-positive T cells and eosinophilic infiltration comprising all analysed subjects (Fig. 4).

Localization of chemokine-receptor-bearing T cells in the bronchial mucosa of asthmatic subjects

In order to determine the localization of chemokine-receptor-bearing T cells in the bronchial mucosa, biopsies were taken

Table 3. Increase of eosinophils in the bronchoalveolar lavage (BAL) fluid after allergen challenge

Patients	% Eosinophils		Fold increase
	Saline challenge	Allergen challenge	
1	51.0	84.3	1.7 ×
2	0.8	7.7	9.6 ×
3	4.5	14.8	3.3 ×
4	1.7	59.0	35 ×
5	1.0	72.8	73 ×
6	1.5	54.0	36 ×

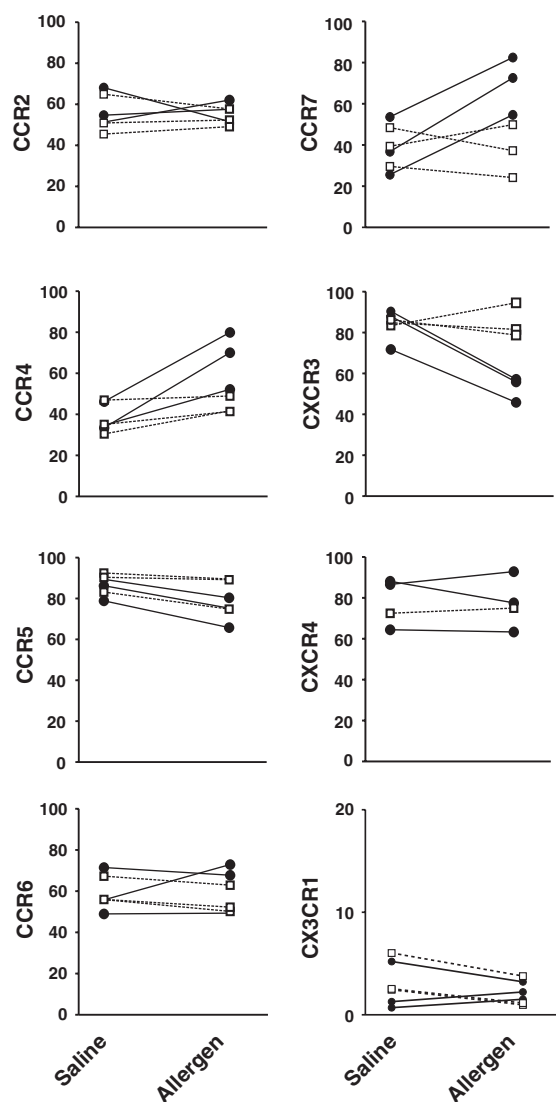


Fig. 3. Expression of chemokine receptors on bronchoalveolar T cells after allergen challenge. CD3⁺ cells were analysed for chemokine-receptor expression by flow cytometry; the percentage values of positive cells are indicated. The values obtained for each individual patient are connected with a line. Patients with only a mild eosinophilic response to the allergen are represented by open squares (□), and patients with a strong inflammatory response are represented by filled circles (●). Note the different scaling for CX3CR1.

after segmental allergen challenge in four of the patients participating in the BAL study (patients 1–4, compare Tables 1 and 3), and analysed by immunohistology. In all sections, CD3⁺ cells were located in intra- and subepithelial infiltrates (Fig. 5). These T cell infiltrates stained positive for all chemokine receptors (CCR2, CCR4, CCR5, CXCR3), which were also detected on BAL-derived T cells and to which suitable mAb were available. Chemokine receptors that were not detected by the flow cytometry studies also failed to give a positive signal in the immunohistochemistry. Overall, the percentages of positive cells were approximately in the same order of magnitude as counted by flow cytometry.

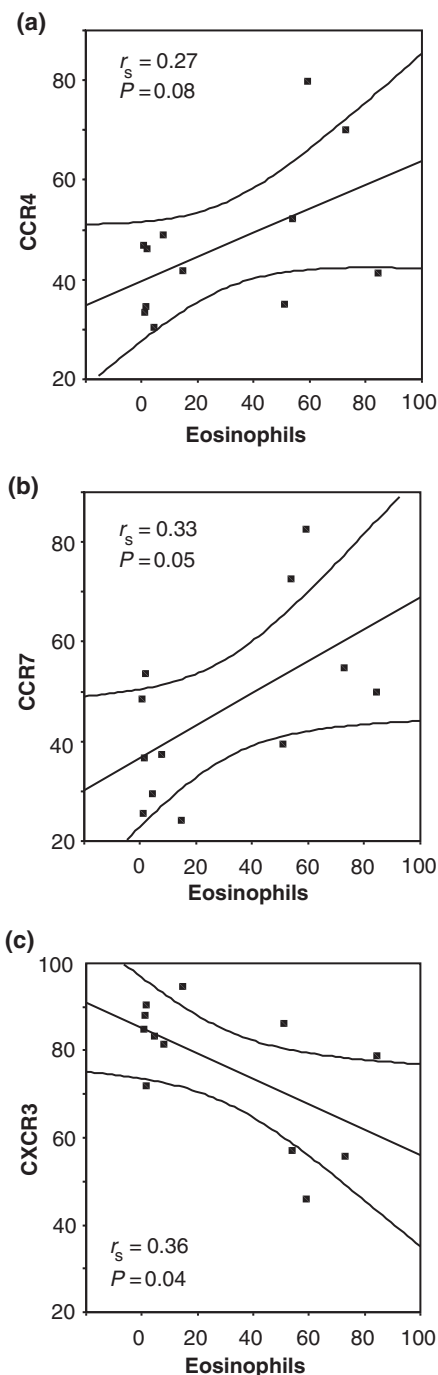


Fig. 4. Regression analysis of correlation between the percentage of chemokine-receptor-positive T cells and the degree of eosinophilic infiltration in the bronchoalveolar lavage. (a) CCR4, (b) CCR7 and (c) CXCR3. Per cent chemokine-receptor-positive T cells on the y-axis; per cent eosinophils on the x-axis. Linear regression: straight line; 95% confidence interval: curved line.

Discussion

The present study is the first to comprehensively analyse chemokine-receptor expression on T lymphocytes in peripheral blood and bronchoalveolar in healthy individuals and in patients suffering from mild allergic asthma, both at steady state as well as after allergen challenge.

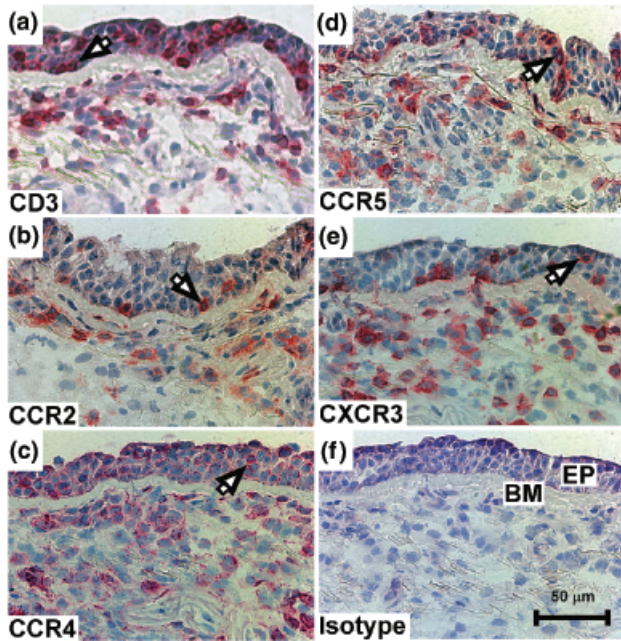


Fig. 5. Localization of chemokine-receptor-positive T cells in bronchial tissue after allergen challenge. Staining of (a) CD3, (b) CCR2, (c) CCR4, (d) CCR5, (e) CXCR3 and (f) isotype control. EP, epithelium; BM, basal membrane; in each microphotograph an arrow points to one representative intraepithially located CD3⁺ and chemokine-receptor-positive cell.

Campbell et al. [27] recently suggested a characteristic homing pathway of T cells to the lung mediated by the expression of adhesion molecules and chemokine receptors. Since understanding of organ-specific cell migration in healthy individuals is a prerequisite to assess changes in the disease state, we further studied the pattern of chemokine receptors by comparing the expression of these molecules on BAL-derived T cells with those from T lymphocytes in the peripheral blood. Our data do not support the hypothesis that isolated up-regulation of CCR1, CCR3, CCR9, CXCR1, CXCR2, CXCR5, CCR7 and CX3CR1 is involved in the directed migration of T cells into the healthy lung. A significantly higher percentage of BAL-derived T cells, however, expressed CCR2, CCR4, CCR5, CCR6 and CXCR3 (Figs 1a and b). Therefore, these receptors might play a role in the physiological recruitment of T cells into the lung. Regarding the lung specificity of chemokine-receptor expression, it is of interest to compare our data with a number of other studies carried out in different tissue compartments. T cells from a variety of inflamed and non-inflamed tissues were positive for CCR5 and CXCR3 [28, 29]. CCR4 has been reported on infiltrating lymphocytes in atopic and psoriatic skin [30–32], whereas CCR6 is found on a subset of memory T cells with a postulated homing potential to mucosal sites and the skin [33]. Little is known about CCR2 expression in other organs.

However, based on the available data, none of the analysed chemokine receptors is exclusively expressed in the lung. Nevertheless, the combined expression of CCR2, CCR4, CCR5, CCR6 and CXCR3 at present seems to be characteristic for T cells in the normal lung.

Binding of chemokines leads to a downmodulation of the receptors [34–40] and this phenomenon could be responsible for the decreased levels of CCR2, CCR4, CCR6 in the BAL

as expressed by the MIF. A recent study also demonstrated a downmodulation of CCR3 on eosinophils after segmental allergen challenge [41]. Interestingly, although CCR5 and CXCR3 were expressed on significantly more T cells from the BAL compared with the peripheral blood, no differences in the receptor density were observed. CXCR4, expressed by a high number of T cells from both the peripheral blood and the BAL, is expressed with a higher density on the latter. The reason for this phenomenon is unknown.

Our data, which are based on a relatively small patient population because of the logistic challenge of segmental allergen provocation, do not provide evidence for an 'inflammation pattern' of chemokine-receptor expression on lung T cells in asthmatics with mild disease at steady state (data not shown). Interestingly, the pattern of chemokine-receptor expression on lung and peripheral blood T cells, respectively, was consistent within this group, and was thus apparently not influenced by sex, age, the allergen or disease duration (Fig. 2 and Table 1).

Since allergen exposure has been shown to induce acute broncho-pulmonary inflammation characterized by an increase in CCR4⁺ T cells in asthmatic subjects [17, 18], we addressed the contribution of other chemokine receptors to the acute allergic reaction. We therefore used segmental allergen challenge and subsequent flow cytometric analysis of chemokine-receptor expression to investigate the changes in a large panel of chemokine receptors in order to appreciate their possible contribution to allergen-induced inflammation.

In three patients who had a significant inflammatory response characterized by a 35–70-fold increase in eosinophils in BAL (Table 3), the percentage of T cells in the BAL expressing CCR7 and CCR4 was also significantly increased (Fig. 3). At the same time the percentage of T cells expressing CXCR3 decreased, whereas the expression of CCR2, CCR5, CXCR4, CCR6, and CX3CR1 remained virtually unchanged. When correlating the degree of eosinophilia with the percentage of chemokine-receptor-positive T cells in the BAL (Fig. 4) a significant correlation for CCR7 and CXCR3 was observed. For CCR4 a clear tendency towards a positive correlation was noticed. To the best of our knowledge, similar results have not yet been reported. Our observations, although obtained in a relatively small group of patients, were very similar among the investigated individuals. In view of the data obtained from healthy subjects and those from mild asthmatics exposed to allergen, who did not respond with a marked inflammatory reaction, the relative and specific increase in T cells bearing CCR7 and CCR4 combined with a relative decrease of CXCR3 seems to represent an 'acute inflammation pattern' of chemokine receptors on T cells in the lung. Since increased concentrations of the thymus and activation-regulated chemokine, a ligand with a strong affinity to CCR4, have been measured in BAL fluid after segmental allergen challenge [42], the data are compatible with previous suggestions that CCR4 receptor/ligand interaction could contribute to the influx of T lymphocytes into the lung following acute allergen exposure.

In several studies it was suggested that CXCR3 is associated with the expression of T-helper type 1 (Th1) and not with Th2 cytokines [43, 44]. The observed decrease of CXCR3⁺ T cells after allergen challenge seems to support this hypothesis. The migration of T cells into secondary lymphatic

tissue and their proper localization within this microenvironment is mainly controlled by CCR7 and its ligands secondary lymphoid-tissue chemokine and EBI1-ligand chemokine [45]. After activation of naïve T cells, CCR7 is downmodulated over the course of several days (T. Kallinich, personal observations). It is therefore tempting to speculate that the increase of CCR7⁺ T cells after acute allergen challenge might reflect the rapid recruitment of T cells from the peripheral blood.

In a murine model of allergen-induced airway inflammation, it has previously been demonstrated that CCR3/eotaxin interaction was critically involved in the early recruitment of Th2 cells to the pulmonary compartment [46]. However, we could not corroborate these findings since CCR3 expression on T cells after allergen exposure of asthmatic subjects was not detected in our study. On the other hand, our findings are in agreement with a more recent study showing that less than 1% of T cells in bronchial biopsies of human asthmatics express CCR3 [47]. This discord suggests that CCR3 expression on T cells in murine hyper-reactivity models may not be representative for human allergen-induced airway inflammation.

Given the likely importance of locally released chemoattractants for the recruitment of T cells to the site of inflammation, it is of interest that there was no specific tissue distribution of individual T cell-associated chemokine receptors in the mucosa of mild asthmatics even after allergen challenge (Fig. 5).

Since our analysis of T cell chemokine-receptor expression was limited to cells obtained 42 h after segmental allergen challenge, we cannot fully exclude that the observed distribution might differ at other time points.

From our data, we conclude that the chemokine receptors CCR2, CCR4, CCR5, CCR6, CXCR3 and CXCR4 might contribute to the directed migration of T lymphocytes to the lung. Allergen challenge with a marked increase in bronchoalveolar eosinophils leads to a selective up-regulation of CCR4 and CCR7 and a reduction in CXCR3 expressions, suggesting that these receptors and their respective chemokines might be involved in the local regulation of T cell traffic 42 h after segmental allergen challenge. Further studies will be needed to clarify whether CCR4 and CCR7 up- and CXCR3 down-regulation as compared with a site-specific release of certain chemokine profiles determine the local T lymphocyte repertoire following allergen challenge.

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References

- 1 Azzawi M, Bradley B, Jeffery PK et al. Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. *Am Rev Respir Dis* 1990; 142:1407–13.
- 2 Gavett SH, Chen X, Finkelman F, Wills-Karp M. Depletion of murine CD4⁺ T lymphocytes prevents antigen-induced airway hyperreactivity and pulmonary eosinophilia. *Am J Respir Cell Mol Biol* 1994; 10:587–93.
- 3 Krug N, Tschernig T, Holgate S, Pabst R. How do lymphocytes get into the asthmatic airways? Lymphocyte traffic into and within the lung in asthma. *Clin Exp Allergy* 1998; 28:10–8.
- 4 Moser B, Loetscher P. Lymphocyte traffic control by chemokines. *Nat Immunol* 2001; 2:123–8.
- 5 Mackay CR. Chemokines: immunology's high impact factors. *Nat Immunol* 2001; 2:95–101.
- 6 Lamkhioued B, Garcia-Zepeda EA, Abi-Younes S et al. Monocyte chemoattractant protein (MCP)-4 expression in the airways of patients with asthma. Induction in epithelial cells and mononuclear cells by proinflammatory cytokines. *Am J Respir Crit Care Med* 2000; 162:723–32.
- 7 Miotto D, Christodoulopoulos P, Olivenstein R et al. Expression of IFN-gamma-inducible protein; monocyte chemotactic proteins 1, 3, and 4; and eotaxin in TH1- and TH2-mediated lung diseases. *J Allergy Clin Immunol* 2001; 107:664–70.
- 8 Powell N, Humbert M, Durham SR, Assoufi B, Kay AB, Corrigan CJ. Increased expression of mRNA encoding RANTES and MCP-3 in the bronchial mucosa in atopic asthma. *Eur Respir J* 1996; 9:2454–60.
- 9 Folkard SG, Westwick J, Millar AB. Production of interleukin-8, RANTES and MCP-1 in intrinsic and extrinsic asthmatics. *Eur Respir J* 1997; 10:2097–104.
- 10 Berin MC, Eckmann L, Broide DH, Kagnoff MF. Regulated production of the T helper 2-type T-cell chemoattractant TARC by human bronchial epithelial cells in vitro and in human lung xenografts. *Am J Respir Cell Mol Biol* 2001; 24:382–9.
- 11 Rozyk KJ, Plusa T, Kuna P, Pirozynska E. Monocyte chemotactic and activating factor/monocyte chemoattractant protein in bronchoalveolar lavage fluid from patients with atopic asthma and chronic bronchitis. Relationship to lung function tests, bronchial hyper-responsiveness and cytology of bronchoalveolar lavage fluid. *Immunol Lett* 1997; 58:47–52.
- 12 Tillie-Leblond I, Hammad H, Desurmont S et al. CC chemokines and interleukin-5 in bronchial lavage fluid from patients with status asthmaticus. Potential implication in eosinophil recruitment. *Am J Respir Crit Care Med* 2000; 162:586–92.
- 13 Berkman N, Ohnosa S, Chung FK, Breuer R. Eotaxin-3 but not eotaxin gene expression is upregulated in asthmatics 24 h after allergen challenge. *Am J Respir Cell Mol Biol* 2001; 24:682–7.
- 14 Holgate ST, Bodey KS, Janezic A, Frew AJ, Kaplan AP, Teran LM. Release of RANTES, MIP-1 alpha, and MCP-1 into asthmatic airways following endobronchial allergen challenge. *Am J Respir Crit Care Med* 1997; 156:1377–83.
- 15 Teran LM, Noso N, Carroll M, Davies DE, Holgate S, Schroder JM. Eosinophil recruitment following allergen challenge is associated with the release of the chemokine RANTES into asthmatic airways. *J Immunol* 1996; 157:1806–12.
- 16 Ying S, Meng Q, Zeibecoglou K et al. Eosinophil chemotactic chemokines (eotaxin, eotaxin-2, RANTES, monocyte chemoattractant protein-3 (MCP-3), and MCP-4), and C-C chemokine receptor 3 expression in bronchial biopsies from atopic and nonatopic (intrinsic) asthmatics. *J Immunol* 1999; 163:6321–9.
- 17 Nouri-Aria KT, Wilson D, Francis JN et al. CCR4 in human allergen-induced late responses in the skin and lung. *Eur J Immunol* 2002; 32:1933–8.
- 18 Panina-Bordignon P, Papi A, Mariani M et al. The C-C chemokine receptors CCR4 and CCR8 identify airway T cells of allergen-challenged atopic asthmatics. *J Clin Invest* 2001; 107:1357–64.
- 19 International consensus report on diagnosis and treatment of asthma. National Heart, Lung, and Blood Institute, National Institutes of Health. Bethesda, Maryland 20892. Publication no. 92-3091, March 1992. *Eur Respir J* 1992; 5:601–41.

- 20 Virchow JC Jr, Julius P, Matthys H, Kroegel C, Luttmann W. CD14 expression and soluble CD14 after segmental allergen provocation in atopic asthma. *Eur Respir J* 1998; 11:317–23.
- 21 Virchow JC Jr., Walker C, Hafner D et al. T cells and cytokines in bronchoalveolar lavage fluid after segmental allergen provocation in atopic asthma. *Am J Respir Crit Care Med* 1995; 151:960–8.
- 22 Luttmann W, Herzog V, Virchow JC Jr., Matthys H, Thierach KH, Kroegel C. Prostacyclin modulates granulocyte/macrophage colony-stimulating factor release by human blood mononuclear cells. *Pulm Pharmacol* 1996; 9:43–8.
- 23 Ponath PD, Kassam N, Qin S. Monoclonal antibodies to chemokine receptors. *Methods Mol Biol* 2000; 138:231–42.
- 24 Beier KC, Hutloff A, Dittrich AM et al. Induction, binding specificity and function of human ICOS. *Eur J Immunol* 2000; 30:3707–17.
- 25 Mages HW, Hutloff A, Heuck C et al. Molecular cloning and characterization of murine ICOS and identification of B7h as ICOS ligand. *Eur J Immunol* 2000; 30:1040–7.
- 26 Cordell JL, Falini B, Erber WN et al. Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). *J Histochem Cytochem* 1984; 32:219–29.
- 27 Campbell JJ, Brightling CE, Symon FA et al. Expression of chemokine receptors by lung T cells from normal and asthmatic subjects. *J Immunol* 2001; 166:2842–8.
- 28 Qin S, Rottman JB, Myers P et al. The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. *J Clin Invest* 1998; 101:746–54.
- 29 Kunkel EJ, Boisvert J, Murphy K et al. Expression of the chemokine receptors CCR4, CCR5, and CXCR3 by human tissue-infiltrating lymphocytes. *Am J Pathol* 2002; 160:347–55.
- 30 Campbell JJ, Haraldsen G, Pan J et al. The chemokine receptor CCR4 in vascular recognition by cutaneous but not intestinal memory T cells. *Nature* 1999; 400:776–80.
- 31 Vestergaard C, Bang K, Gesser B, Yoneyama H, Matsushima K, Larsen CG. A Th2 chemokine, TARC, produced by keratinocytes may recruit CLA+CCR4+lymphocytes into lesional atopic dermatitis skin. *J Invest Dermatol* 2000; 115:640–6.
- 32 Rottman JB, Smith TL, Ganley KG, Kikuchi T, Krueger JG. Potential role of the chemokine receptors CXCR3, CCR4, and the integrin α Ebeta7 in the pathogenesis of psoriasis vulgaris. *Lab Invest* 2001; 81:335–47.
- 33 Liao F, Rabin RL, Smith CS, Sharma G, Nutman TB, Farber JM. CC-chemokine receptor 6 is expressed on diverse memory subsets of T cells and determines responsiveness to macrophage inflammatory protein 3 α . *J Immunol* 1999; 162:186–94.
- 34 Franci C, Gosling J, Tsou CL, Coughlin SR, Charo IF. Phosphorylation by a G protein-coupled kinase inhibits signaling and promotes internalization of the monocyte chemoattractant protein-1 receptor. Critical role of carboxyl-tail serines/threonines in receptor function. *J Immunol* 1996; 157:5606–12.
- 35 Zimmermann N, Rothenberg ME. Receptor internalization is required for eotaxin-induced responses in human eosinophils. *J Allergy Clin Immunol* 2003; 111:97–105.
- 36 Inngjerdigen M, Damaj B, Maghazachi AA. Human NK cells express CC chemokine receptors 4 and 8 and respond to thymus and activation-regulated chemokine, macrophage-derived chemokine, and I-309. *J Immunol* 2000; 164:4048–54.
- 37 Mack M, Luckow B, Nelson PJ et al. Aminooxypentane-RANTES induces CCR5 internalization but inhibits recycling: a novel inhibitory mechanism of HIV infectivity. *J Exp Med* 1998; 187:1215–24.
- 38 Bardi G, Lipp M, Baggiolini M, Loetscher P. The T cell chemokine receptor CCR7 is internalized on stimulation with ELC, but not with SLC. *Eur J Immunol* 2001; 31:3291–7.
- 39 Loetscher P, Pellegrino A, Gong JH et al. The ligands of CXC chemokine receptor 3, I-TAC, Mig, and IP10, are natural antagonists for CCR3. *J Biol Chem* 2001; 276:2986–91.
- 40 Forster R, Kremmer E, Schubel A et al. Intracellular and surface expression of the HIV-1 coreceptor CXCR4/fusin on various leukocyte subsets: rapid internalization and recycling upon activation. *J Immunol* 1998; 160:1522–31.
- 41 Liu LY, Jarjour NN, Busse WW, Kelly EA. Chemokine receptor expression on human eosinophils from peripheral blood and bronchoalveolar lavage fluid after segmental antigen challenge. *J Allergy Clin Immunol* 2003; 112:556–62.
- 42 Sekiya T, Miyamasu M, Imanishi M et al. Inducible expression of a Th2-type CC chemokine thymus- and activation-regulated chemokine by human bronchial epithelial cells. *J Immunol* 2000; 165:2205–13.
- 43 Bonocchi R, Bianchi G, Bordignon PP et al. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. *J Exp Med* 1998; 187:129–34.
- 44 Sallusto F, Lenig D, Mackay CR, Lanzavecchia A. Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. *J Exp Med* 1998; 187:875–83.
- 45 Forster R, Schubel A, Breitfeld D et al. CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell* 1999; 99:23–33.
- 46 Lloyd CM, Delaney T, Nguyen T et al. CC chemokine receptor (CCR)3/eotaxin is followed by CCR4/monocyte-derived chemokine in mediating pulmonary T helper lymphocyte type 2 recruitment after serial antigen challenge in vivo. *J Exp Med* 2000; 191:265–74.
- 47 Bocchino V, Bertorelli G, Bertrand CP et al. Eotaxin and CCR3 are up-regulated in exacerbations of chronic bronchitis. *Allergy* 2002; 57:17–22.
- 48 Sallusto F, Schaerli P, Loetscher P et al. Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation. *Eur J Immunol* 1998; 28:2760–9.
- 49 Qin S, LaRosa G, Campbell JJ et al. Expression of monocyte chemoattractant protein-1 and interleukin-8 receptors on subsets of T cells: correlation with transendothelial chemotactic potential. *Eur J Immunol* 1996; 26:640–7.
- 50 Heath H, Qin S, Rao P et al. Chemokine receptor usage by human eosinophils. The importance of CCR3 demonstrated using an antagonistic monoclonal antibody. *J Clin Invest* 1997; 99: 178–84.
- 51 Andrew DP, Ruffing N, Kim CH et al. C–C chemokine receptor 4 expression defines a major subset of circulating nonintestinal memory T cells of both Th1 and Th2 potential. *J Immunol* 2001; 166:103–11.
- 52 Wu L, LaRosa G, Kassam N et al. Interaction of chemokine receptor CCR5 with its ligands: multiple domains for HIV-1 gp120 binding and a single domain for chemokine binding. *J Exp Med* 1997; 186:1373–81.
- 53 Sallusto F, Kremmer E, Palermo B et al. Switch in chemokine receptor expression upon TCR stimulation reveals novel homing potential for recently activated T cells. *Eur J Immunol* 1999; 29:2037–45.
- 54 Campbell JJ, Murphy KE, Kunkel EJ et al. CCR7 expression and memory T cell diversity in humans. *J Immunol* 2001; 166:877–84.
- 55 Bleul CC, Wu L, Hoxie JA, Springer TA, Mackay CR. The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes. *Proc Natl Acad Sci USA* 1997; 94:1925–30.
- 56 Forster R, Mattis AE, Kremmer E, Wolf E, Brem G, Lipp M. A putative chemokine receptor, BLR1, directs B cell migration to defined lymphoid organs and specific anatomic compartments of the spleen. *Cell* 1996; 87:1037–47.