IL-4–expressing bronchoalveolar T cells from asthmatic and healthy subjects preferentially express CCR3 and CCR4

Angela J. Morgan, MRCP,[a] Fiona A. Symon, PhD,[a] Mike A. Berry, MRCP,[a] Ian D. Pavord, FRCP, MD,[a] Christopher J. Corrigan, FRCP, PhD,[b] and Andrew J. Wardlaw, FRCP, PhD[a]

Leicester and London, United Kingdom

Background: The concept of the polarization of chemokine receptor expression by T_H1 and T_H2 cells provides an attractive mechanism for their differential recruitment to tissue, which could be subject to disease-specific therapeutic intervention. The paradigm that T_H1 cells preferentially express CXCR3 and CCR5 and T_H2 cells preferentially express CCR3, CCR4, and CCR8 has been well established in the setting of *in vitro* polarized cell lines; however, the situation *in vivo* appears less clear-cut.

Objective: We sought to investigate whether this pattern of polarization can be demonstrated in human lung tissue.

Methods: We used single-cell analysis to investigate the relationship between chemokine receptor expression and cytokine production on peripheral blood and bronchoalveolar lavage fluid T cells in patients with asthma, a putative T_H2 disease, as well as in healthy control subjects.

Results: We have found in both asthmatic and control subjects that IL-4–expressing blood and bronchoalveolar lavage fluid T cells are significantly more likely to express the T_H2 type 2 chemokine receptors CCR3 and CCR4, with 10-fold and 2-fold differences in expression, respectively, compared with IFN-γ–expressing cells.

Conclusion: We have provided evidence that polarization of T_H2-type chemokine receptors on IL-4–expressing cells can be demonstrated in an *in vivo* setting and therefore that these cells might indeed be susceptible to differential patterns of recruitment as a result of expression of the relevant chemokines at inflammatory sites. (J Allergy Clin Immunol 2005;116: 594-600.)

Key words: *Lung, human, T_H1/T_H2 cells, T cells, chemokines*

Abbreviations used
BAL: Bronchoalveolar lavage
BALF: Bronchoalveolar lavage fluid

Memory lymphocytes migrate around the body in a programmed fashion, preferentially returning to the organ where they became sensitized. This process, known as lymphocyte homing, increases the chance of a T cell encountering its cognate antigen.[1] Patterns of migration are controlled by organ-specific expression of adhesion molecules and chemokines, which attract subsets of lymphocytes bearing the relevant ligand. The most well-described and specific pathways are those to the skin (involving CLA, CCR4, and CCR10),[2,3] the small intestine (α4β7 and CCR9),[4] and the lymph nodes (L-selectin and CCR7).[5] To date, no specific chemokine receptors have been identified that control homing of cells to the lung; however, it is apparent that the phenotype of lung-homing cells is distinct from that of both gut- and skin-homing T cells.[6]

Another pattern of lymphocyte migration involves differential trafficking of T_H1 and T_H2 cells. T_H1 cells are characterized by their ability to secrete IFN-γ and IL-2, whereas T_H2 cells secrete the cytokines IL-4, IL-5, and IL-13. T_H1 and T_H2 cells in both mice and human subjects were initially characterized in terms of the differential cytokine release of T-cell clones.[7,8]

Subsequently, by using *in situ* hybridization techniques to characterize cytokine production, T_H1 and T_H2 cells were identified in inflammatory lesions.

Most notably, Robinson et al[9] reported that up to 50% of bronchoalveolar lavage (BAL) fluid T cells in asthma expressed mRNA for IL-4, although we[10] and others,[11] using single-cell cytokine staining, have found evidence for much lower numbers of IL-4–expressing T cells. More recently, investigators have generated *in vitro* T_H1- and T_H2-polarized cell lines from naive T cells by exploiting the cross-inhibitory effects of IFN-γ and IL-4 on T_H2 and T_H1 development, respectively. These cells show differential expression of chemokine receptors, with T_H1 cells showing a strong bias toward expression of CXCR3 and CCR5 and T_H2 cells showing a strong bias toward CCR3, CCR4, and CCR8. In contrast, analysis of the relationship between cytokine production and chemokine receptor expression in freshly isolated peripheral blood T cells showed only a limited bias toward the differential patterns described above.[12]

From [a]the Institute for Lung Health, Department of Infection, Immunity and Inflammation, Leicester University; and [b]GKT School of Medicine, Department of Asthma, Allergy and Respiratory Science, Guy's Hospital, London.

Supported by Asthma UK.

Disclosure of potential conflict of interest: All authors—none disclosed.

Received for publication September 15, 2004; revised March 13, 2005; accepted for publication March 23, 2005.

Available online July 18, 2005.

Reprint requests: Andrew J. Wardlaw, FRCP, PhD, Institute for Lung Health, Glenfield Hospital, Groby Rd, Leicester University, Leicester LE3 9QP, United Kingdom. E-mail: Aw24@le.ac.uk.

0091-6749/$30.00

doi:10.1016/j.jaci.2005.03.052

We have previously observed that the expression of CCR5, CXCR3, CCR3, and CCR4 on BAL fluid (BALF) T cells did not differ between asthmatic subjects and healthy volunteers.[6] However, because we found that only 5% to 10% of T cells were IL-4 producing, it remained possible that CCR3- and CCR4-expressing cells were preferentially contained within this subset. We have therefore undertaken a study to measure expression of the T_H1- and T_H2-associated chemokine receptors in BALF T cells expressing IL-4 and IFN-γ from patients with mild asthma and healthy control subjects.

METHODS

Subject selection

Sixteen asthmatic subjects and 9 healthy control subjects participated in the study (see Table I for clinical characteristics). Each subject underwent spirometry, skin prick testing to 4 common aeroallergens to determine atopic status, methacholine challenge testing to determine PC_{20} FEV_1 methacholine values, and chest radiography.

Asthmatic subjects were defined as having a compatible history, such as cough, episodic wheeze, or breathlessness relieved by β_2-agonists. All demonstrated a PC_{20} FEV_1 methacholine value of less than 8 mg/mL (performed at least 2 weeks before bronchoscopy to minimize the potential for airway inflammation caused by the procedure) or greater than 15% reversibility to an inhaled β_2-agonist. All asthmatic subjects were receiving intermittent treatment with only a β_2-agonist and were defined as having stage 1 disease (mild disease) on the basis of British Thoracic Society criteria.[13] None had received inhaled or oral corticosteroids within the 6 weeks before the study.

Control subjects had no history of respiratory disease, a PC_{20} FEV_1 methacholine value of greater than 16 mg/mL, and normal spirometry.

All subjects involved in the study had normal chest radiographic results.

Clinical investigations

The PC_{20} FEV_1 (concentration of methacholine required to cause a 20% decrease in FEV_1) was measured with a tidal breathing method as per European Respiratory Society guidelines on airway responsiveness testing.[14]

Skin prick testing for atopy was performed to *Dermatophagoides pteronyssinus*, cat fur, grass pollen, and dog fur, with histamine and saline as positive and negative controls, respectively. A wheal of more than 2 mm in diameter greater than that elicited by the negative control was considered a positive test result.

A 15-mL heparinized blood sample was obtained from all volunteers immediately before the bronchoscopy.

All patients underwent fiberoptic bronchoscopy (Olympus company, Tokyo, Japan) and lavage in accordance with standard procedure[15] as a day case. Asthmatic subjects were premedicated with 2.5 mg of nebulized salbutamol to reduce BAL-associated bronchospasm. All subjects received topical lignocaine applied to the inside of the nose and the back of the throat. If requested, subjects were lightly sedated with 2.5 mg of intravenous midazolam. Supplemental oxygen through nasal prongs was administered at a rate of 2 L/min. Pulse oximetry and blood pressure were monitored throughout the procedure. Four milliliters of 2% lignocaine was applied to the vocal cords, followed by aliquots of 2 mL to the trachea and the right and left main bronchi. The scope was wedged in the middle lobe bronchus, and a 20-mL bronchial wash was performed. For the BAL, 180 mL of warmed 0.9% saline was instilled into the middle lobe bronchus in aliquots of 60 mL. Gentle suction was applied to retrieve as much fluid as possible.

TABLE I. Clinical characteristics of asthmatic and control subjects

	Asthmatic subjects	Control subjects
N	16	9
Age, y	47 (22-69)	43 (22-61)
Sex, F/M	9/7	5/4
Atopic,* %	64	0
FEV_1, % predicted	93 (69-131)	107 (92-125)
PC_{20}, mg/mL	1.9	All >16

Values are presented as means (ranges).
*Determined by means of skin prick testing: at least one positive test result.

All subjects provided written informed consent. All clinical studies were reviewed and approved by the Leicestershire and Rutland Research and Ethics Committee.

Laboratory methods

Mononuclear cells were isolated from blood after density gradient centrifugation with Histopaque 1077 (Sigma, Dorset, United Kingdom) and washed with PBS (Invitrogen, Paisley, United Kingdom) and 0.5% BSA (Sigma) before further labeling of cells.

The BALF sample was filtered through a 48-μm mesh to remove debris. If necessary, a red blood cell depletion step was then carried out with a standard osmotic shock technique. Briefly, this involved the resuspension of the cell pellet in 5 mL of distilled water for exactly 30 seconds, followed by quenching with a large volume of PBS buffer before further centrifugation. A differential cell count for each BAL sample was obtained by using the Romanowsky stain and counted by an individual blinded to the subjects' clinical status. Differential counts were expressed as the percentage of nonsquamous cells.

Cell samples from both peripheral blood and BALF were incubated with 10% FCS to reduce nonspecific binding before incubation with one of 5 primary unlabelled monoclonal chemokine receptor antibodies. In each case, the entire BAL sample was used. The number of cells analyzed varied for each patient and chemokine receptor depending on the number of cells available; in some cases where there was insufficient cell recovery from the BALF, fewer than 5 chemokine receptors were studied. Chemokine receptor antibodies used were CCR3 and CCR4 (a kind gift of Millennium Pharmaceuticals), CCR5 (BD Pharmingen, Oxford, United Kingdom), and CXCR3 and CXCR6 (R&D, Oxford, United Kingdom); all were mouse mAbs and were used at a final concentration of between 5 and 10 μg/mL. Staining was visualized with an FITC-labeled rabbit anti-mouse antibody (DAKO, Glostrup, Denmark) at a dilution of 1:20.

Half of each chemokine receptor–labeled cell aliquot was then subjected to stimulation for 4 hours at 37°C in medium (RPMI plus 10% FCS) containing 5 ng/mL phorbol 12-myristate 13-acetate (Sigma), 250 ng/mL calcium ionophore (Sigma), and 10 μg/mL brefeldin (Sigma). The other half of the sample was not subjected to this stimulation procedure to obtain a resting sample.

Subsequently, after blocking with 10% normal mouse serum, staining for CD3 was performed with directly labeled antibody (CD3PerCP, BD Pharmingen). Cells were then fixed and permeabilized with 4% paraformaldehyde (Sigma)/0.1% saponin (Sigma) for 15 minutes at 4°C before direct labeled anticytokine antibody (either IFN-γ phycoerythrin or IL-4 phycoerythrin, both BD Pharmingen) was added to half of the cells of each resting or stimulated chemokine receptor–labeled sample. Isotype controls were used at each stage of the experiment.