

# Chemokine receptor expression profile of eosinophils at inflamed tissue sites: Decreased CCR3 and increased CXCR4 expression by lung eosinophils

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**Background:** To date, most studies dealing with eosinophil chemokine receptors have used eosinophils isolated from peripheral blood. During the movement of eosinophils from the peripheral blood to inflamed tissue sites, microenvironmental signals might alter their expression of chemokine receptors. However, little is known about the profile of expression of chemokine receptors by eosinophils at inflamed tissue sites in human beings.

**Objective:** The purpose of this study was to determine whether eosinophils that have migrated into inflamed tissues exhibit a profile of chemokine receptor expression that is qualitatively and/or quantitatively different from that of eosinophils in peripheral locations.

**Methods:** We studied simultaneously the expression and function of chemokine receptors in eosinophils in both bronchoalveolar lavage fluid (BALF) and peripheral blood specimens of 7 patients with eosinophilic lung diseases.

**Results:** De novo expression of CCR2, CCR4, and CCR5 was not detected at either the protein or the mRNA level. However, surface expression of CCR3 was decreased and CXCR4 was conversely increased with statistical significance in BALF eosinophils. Moreover, the changes in CCR3 and CXCR4 expression were reflected in the altered migratory response to their ligands. On the other hand, the levels of CXCR1, CXCR2, CXCR3, and CCR1 were virtually unchanged in BALF eosinophils, and these receptors did not have functional significance.

**Conclusion:** Eosinophils at inflamed tissue sites exhibited an expression profile qualitatively similar to that in peripheral

locations, except for decreased CCR3 and increased CXCR4 expression. Our results suggest that CCR3 is primarily and CXCR4 is cooperatively involved in eosinophil accumulation at inflamed tissue sites. (*J Allergy Clin Immunol* 2001;108:563-9.)

**Key words:** Eosinophils, CXCR4, CCR3, stromal cell–derived factor 1 $\alpha$ , CXCL12, eotaxin, CCL11, chemotaxis, bronchoalveolar lavage, eosinophilic pneumonia

Evidence to date has indicated that signals via chemokine receptors play an essential role in the pathogenesis of eosinophilic inflammation. CC chemokine receptor (CCR) 3 is strongly expressed on eosinophils<sup>1</sup> and is responsible for both migration<sup>2-4</sup> and degranulation.<sup>5-7</sup> Eotaxin/CC chemokine ligand (CCL) 11 represents the most potent chemoattractant<sup>8</sup> and secretagog<sup>7</sup> for eosinophils. In addition, we have recently demonstrated that CXC chemokine receptor (CXCR) 4 expression is inducible under certain circumstances and that a CXCR4-specific ligand, stromal cell–derived factor 1/CXC chemokine ligand (CXCL) 12, induces strong migration comparable to that induced by eotaxin/CCL11.<sup>9</sup> More recently, expression of functional CXCR3 was also reported,<sup>10</sup> though another report failed to observe the link of this receptor to the migratory response.<sup>11</sup> CCR1 expression is observed to a lesser extent than CCR3 expression,<sup>1</sup> and its ligand, macrophage inflammatory protein 1 $\alpha$  (MIP-1 $\alpha$ )/CCL3, induces eosinophil chemotaxis in a small and selected subset of atopic patients.<sup>3,12</sup> On the other hand, conflicting observations have been generated concerning the expression and function of CXCR1 and CXCR2.<sup>4,13-15</sup>

The expression profiles of eosinophil chemokine receptors have become increasingly understood, but most studies have used eosinophils isolated from peripheral blood (PB). As eosinophils move from the vascular compartment to inflamed tissue sites, microenvironmental signals might affect the receptor expression; eventually, eosinophils that have migrated into inflamed tissue sites might exhibit a pattern of receptor expression that is distinct from that of circulating eosinophils. In fact, Lukacs et al<sup>16</sup> described qualitative alteration of the expression of chemokine receptors—ie, de novo appearance of CCR2, CCR4, and CCR5—in murine eosinophils that had migrated into the peritoneal cavity, though they did not present detailed data in their report.

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TABLE I. Patient characteristics and differential counts of leukocytes

| Subject no. | Admitting diagnosis | Age (y) | Sex    |      | Differential count (%) |      |      |      |      |            |      | Final diagnosis |
|-------------|---------------------|---------|--------|------|------------------------|------|------|------|------|------------|------|-----------------|
|             |                     |         |        |      | Eos                    | Neu  | Lym  | Baso | Mono | Macrophage | Mast |                 |
| 1           | AEP s/o             | 47      | Male   | BALF | 4.0 (39.8)             | 0.4  | 4.9  | 0.1  | —    | 89.9       | 0.7  | AEP             |
|             |                     |         |        | PB   | 15.4                   | 43.0 | 23.8 | 1.4  | 15.6 | —          | —    |                 |
| 2           | ABPA s/o            | 62      | Male   | BALF | 18.6 (66.0)            | 22.0 | 35.6 | 1.0  | —    | 22.0       | 0.7  | PIE with asthma |
|             |                     |         |        | PB   | 6.0                    | 75.5 | 15.0 | 0.5  | 3.0  | —          | —    |                 |
| 3           | ABPA s/o            | 61      | Male   | BALF | 82.6 (>99)             | 0.1  | 4.1  | 0.4  | —    | 12.4       | 0.4  | PIE with asthma |
|             |                     |         |        | PB   | 19.0                   | 63.0 | 16.0 | 0.0  | 2.0  | —          | —    |                 |
| 4           | ABPA s/o            | 43      | Male   | BALF | 62.2 (>99)             | 0.2  | 5.2  | 1.2  | —    | 30.2       | 1.0  | ABPA            |
|             |                     |         |        | PB   | 19.0                   | 56.0 | 19.0 | 3.0  | 2.0  | —          | —    |                 |
| 5           | CEP s/o             | 71      | Female | BALF | 67.3 (>99)             | 1.0  | 4.3  | 5.9  | —    | 21.2       | 0.3  | CEP             |
|             |                     |         |        | PB   | 23.8                   | 51.4 | 20.0 | 0.8  | 4.0  | —          | —    |                 |
| 6           | Tuberculosis s/o    | 32      | Male   | BALF | 63.0 (>99)             | 0.6  | 4.4  | 0.0  | —    | 31.6       | 0.4  | CEP             |
|             |                     |         |        | PB   | 40.0                   | 44.0 | 12.0 | 0.0  | 4.0  | —          | —    |                 |
| 7           | CEP s/o             | 73      | Male   | BALF | 43.8 (>99)             | 0.0  | 5.4  | 0.4  | —    | 50.0       | 0.4  | CEP             |
|             |                     |         |        | PB   | 61.0                   | 19.5 | 10.5 | 2.0  | 7.0  | —          | —    |                 |

For BALF eosinophils, the data in parentheses show the purity (%) after purification.

*Eos*, Eosinophils; *Neu*, neutrophils; *Lym*, lymphocytes; *Baso*, basophils; *Mono*, monocytes; *Mast*, mast cells; *BALF*, bronchoalveolar lavage fluid; *PB*, peripheral blood; *AEP*, acute eosinophilic pneumonia; *ABPA*, allergic bronchopulmonary aspergillosis; *PIE*, pulmonary infiltration with eosinophilia; *CEP*, chronic eosinophilic pneumonia.

#### Abbreviations used

BALF: Bronchoalveolar lavage fluid  
MESF: Molecules of equivalent soluble fluorochrome units  
MIP-1 $\alpha$ : Macrophage inflammatory protein 1 $\alpha$   
PB: Peripheral blood

A better understanding of the chemokine receptor expression pattern of eosinophils at inflamed tissue sites is crucial for establishing a therapeutic strategy for eosinophilic inflammation via intervention in chemokine actions. In the present study, we explored chemokine receptor expression in eosinophils obtained from the bronchoalveolar lavage fluid (BALF) of patients with eosinophilic lung diseases. To clarify the differences in expression patterns, the relative levels of surface expression were simultaneously compared for BALF and PB eosinophils from the same patients.

## METHODS

### Subjects

Seven patients with eosinophilic lung disease (1 with acute eosinophilic pneumonia, 2 with pulmonary infiltration with eosinophilia with bronchial asthma, 1 with allergic bronchopulmonary aspergillosis, and 3 with chronic eosinophilic pneumonia) were recruited from among inpatients of the Department of Pulmonology, International Medical Center of Japan, and the Department of Medicine, University of Tokyo Hospital. The patient characteristics and differential counts of leukocytes are presented in Table I. None of the patients had received systemic corticosteroids. Bronchoscopic examination and blood collection were performed simultaneously after written informed consent was obtained. The interval between bronchoalveolar lavage and blood collection was never greater than 30 minutes. Both samples were processed simultaneously, as described below. PB samples from 7 nonatopic volunteers were used as normal controls.

### Purification of eosinophils

Bronchoalveolar lavage was performed through use of a flexible fiberoptic bronchoscope after administration of topical anesthesia. The bronchoscope was inserted into the involved lung segment, and a total 150 mL of normal saline solution was instilled. Harvested BALF was filtered through sterile nylon mesh and centrifuged at 280g for 8 minutes to obtain the cell preparation. BALF eosinophils were isolated by Percoll density centrifugation. In brief, the collected BALF cells were resuspended in 3 mL of Pipes A buffer<sup>8</sup> containing 4 mmol/L EDTA and overlaid on 5 mL of 1.080 g/mL Percoll solution (Pharmacia, Uppsala, Sweden). After centrifugation at 700g for 15 minutes at room temperature, the cells in the bottom fraction were collected. This separation achieved a purity of >99% from highly eosinophilic BALF (subjects 3-7) and a lower purity (subject 1, 39.8%; subject 2, 66.0%) from mildly eosinophilic BALF.

Eosinophils were separated from PB by density gradient centrifugation; this was followed by negative selection through use of anti-CD16 magnetic beads, as described previously.<sup>9</sup> The purity was consistently >99%.

Because the separation procedures are different for PB and BALF eosinophils, we compared the receptor expression between normal PB eosinophils separated by Percoll only and normal PB eosinophils separated by a combination of Percoll and anti-CD16 magnetic beads. We found no significant differences in the expression ( $n = 5$ ) regarding CCR2, CCR3, CCR4, CCR5, CXCR1, CXCR3, or CXCR4. However, the treatment with anti-CD16 magnetic beads significantly reduced CXCR2 expression. Furthermore, though the results did not reach statistical significance, we found a tendency toward decreased CCR1 expression in eosinophils treated with Percoll and anti-CD16 magnetic beads (data not shown).

### Flow cytometric analyses

The following mAbs were used in this study: anti-CCR1 (53504.111, DAKO, Kyoto, Japan), anti-CCR2 (48607.121, DAKO), anti-CCR3 (444, donated by Dr H. Kawasaki, University of Tokyo),<sup>17</sup> anti-CCR4 (KM2160),<sup>18</sup> anti-CCR5 (2D7, Pharmingen, San Diego, Calif), anti-CXCR1 (42705.111, DAKO), anti-CXCR2 (48311.211, R&D Systems, Minneapolis, Minn), anti-CXCR3 (49801.111, Genzyme, Cambridge, Mass), and anti-CXCR4 (12G5,

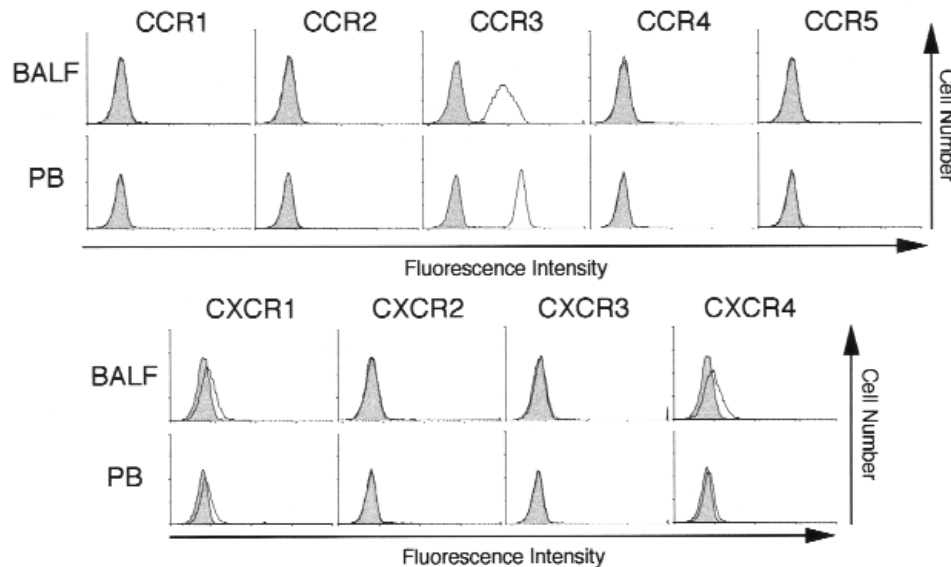


**FIG 1.** Chemokine receptor expression by BALF and PB eosinophils. BALF and PB eosinophils were treated with the indicated anti-chemokine receptor mAb (solid line) or with isotype control mouse IgG (shaded area). Representative (subject 3) results are shown.

Pharmingen). Flow cytometric analysis was performed as described previously.<sup>9</sup> In brief, purified eosinophils were stained with 10  $\mu$ g/mL anti-chemokine receptor mAb; this was followed by staining with FITC-labeled goat F (ab')<sub>2</sub> against mouse IgG (Jackson ImmunoResearch, West Grove, Pa). An isotype-matched mouse IgG was used as a negative control. Eosinophils were identified on the basis of their different forward/side scatter and fluorescence properties, as previously described.<sup>19</sup> Stained cells were analyzed through use of an EPICS XL SYSTEM II (Coulter, Miami, Fla). The median values of fluorescence intensity were converted to numbers of molecules of equivalent soluble fluorochrome units (MESF), as described previously.<sup>9</sup> Surface receptor levels, expressed in MESF units, were calculated through use of the following formula:

$$\Delta\text{MESF} = (\text{MESF of cells stained with anti-chemokine receptor mAb}) - (\text{MESF of cells stained with control IgG}).$$

Because we could distinguish the curve of antireceptor mAbs from that of control IgG, when the difference between their MESF values ( $\Delta\text{MESF}$ ) was  $>1000$  (Fig 1; for CXCR4 in PB,  $\Delta\text{MESF} = 1180$ ), we considered it to be positive expression.

### Chemotaxis assay of eosinophils

When sufficient numbers of purified eosinophils were obtained (subjects 3 to 5), chemotaxis assays were performed as previously described.<sup>8</sup> Each result was expressed as the chemotactic index, the ratio between the extent of migration toward chemokines and that toward the medium control. The chemokines used in the assay were exactly the same as those described previously.<sup>8</sup>

### RT-PCR analysis of chemokine receptors

The expression of transcripts of 15 chemokine receptors in BALF eosinophils was investigated by RT-PCR in 2 subjects from whom sufficient numbers of purified eosinophils were obtained (subjects 3 and 5). Total RNA was extracted from highly purified BALF eosinophils through use of a QIAGEN Total RNA Isolation Kit (Invitrogen, NV Leek, The Netherlands) and treated with DNase I to remove contaminating DNA. Reverse transcription for the first strand cDNA and hot-start PCR ampli-

fication were performed as previously described.<sup>9</sup> The PCR products were electrophoresed through a 2% agarose gel and visualized with ethidium bromide.

### Statistical analysis

Unless otherwise stated, all data are expressed as means  $\pm$  SEMs. To determine the significance of the expression between groups, net values of MESF ( $\Delta\text{MESF}$ ) were compared through use of a paired *t* test.

## RESULTS

To date, constitutive and/or inducible expression of CCR1,<sup>1</sup> CCR3,<sup>1-3</sup> CXCR1,<sup>4</sup> CXCR2,<sup>4</sup> CXCR3,<sup>10</sup> and CXCR4<sup>9</sup> has been reported in human eosinophils, whereas expression of CCR2, CCR4, and CCR5 has not been identified in human eosinophils. In the present study, we quantitated the surface expression of these 9 receptors in both PB and BALF eosinophils by flow cytometry (Table II and Fig 1). Like PB eosinophils in normal individuals, PB eosinophils in patients expressed CCR3 strongly and CXCR1 weakly, though the level of CCR3 was significantly increased in the patients in comparison with the normal subjects ( $P < .05$ ). On the other hand, expressions of CCR2, CCR4, CCR5, CXCR2, and CXCR3 were virtually undetectable in PB eosinophils of either patients or normal individuals (Table II). A small proportion of patients as well as normal individuals expressed significant levels of CCR1 and CXCR4.

BALF eosinophils also expressed CCR3 strongly and CXCR1 weakly. It should be mentioned that the level of CCR3 expression was significantly decreased in comparison with PB eosinophils from the same patients ( $P < .05$ ). No significant change in the level of CXCR1 was observed. Importantly, though weak but significant CXCR4 expression was detected in only 2 cases of

TABLE II. Surface expression of chemokine receptors

|                                   |      | Expression of chemokine receptors ( $\Delta$ MESF $\times$ 1000) |                     |                         |                     |                      |                     |                     |                     |                      |
|-----------------------------------|------|------------------------------------------------------------------|---------------------|-------------------------|---------------------|----------------------|---------------------|---------------------|---------------------|----------------------|
| Subject no.                       |      | CCR1                                                             | CCR2                | CCR3                    | CCR4                | CCR5                 | CXCR1               | CXCR2               | CXCR3               | CXCR4                |
| 1                                 | BALF | 0.53                                                             | 0.60                | 66.25                   | 0.08                | −0.23                | 0.44                | 0.09                | ND                  | 2.32                 |
|                                   | PB   | 0.33                                                             | 0.42                | 328.03                  | −0.14               | −0.16                | 1.93                | 0.24                | ND                  | 0.05                 |
| 2                                 | BALF | 0.03                                                             | 0.00                | 326.56                  | 0.37                | 0.63                 | 2.08                | 0.03                | ND                  | 8.81                 |
|                                   | PB   | 0.15                                                             | 0.10                | 427.00                  | 0.78                | 0.14                 | 1.54                | −0.04               | ND                  | 1.41                 |
| 3                                 | BALF | 0.20                                                             | −0.08               | 136.64                  | 0.05                | 0.04                 | 2.61                | 0.38                | 0.63                | 3.18                 |
|                                   | PB   | 0.03                                                             | −0.03               | 399.75                  | 0.14                | 0.13                 | 1.88                | 0.20                | −0.04               | 1.18                 |
| 4                                 | BALF | −0.03                                                            | 0.21                | 85.16                   | 0.99                | 0.47                 | 1.77                | 0.66                | 2.42                | 0.88                 |
|                                   | PB   | 0.40                                                             | −0.04               | 327.00                  | 0.41                | −0.33                | 1.83                | −0.14               | 0.01                | −0.33                |
| 5                                 | BALF | 0.20                                                             | 0.36                | 217.24                  | 0.18                | −0.02                | 1.48                | 0.09                | 0.33                | 2.44                 |
|                                   | PB   | 0.14                                                             | 0.08                | 282.70                  | 0.27                | −0.14                | 2.35                | −0.16               | 0.41                | −0.12                |
| 6                                 | BALF | 0.00                                                             | −0.22               | 166.71                  | −0.07               | 0.20                 | 1.38                | 0.35                | 0.08                | 5.86                 |
|                                   | PB   | 0.25                                                             | 0.26                | 312.20                  | 0.65                | −0.72                | 5.01                | 0.67                | 0.04                | 0.39                 |
| 7                                 | BALF | 0.71                                                             | 0.26                | 125.17                  | 0.09                | 0.07                 | 2.30                | 1.01                | 0.29                | 1.34                 |
|                                   | PB   | 7.75                                                             | 0.47                | 236.99                  | −0.04               | 0.04                 | 6.03                | 0.95                | −0.10               | 0.07                 |
| Eosinophilic lung disease (n = 7) | BALF | 0.2 $\pm$ 0.1 (0/7)                                              | 0.2 $\pm$ 0.1 (0/7) | 160.5 $\pm$ 31.0* (7/7) | 0.2 $\pm$ 0.1 (0/7) | 0.2 $\pm$ 0.1 (0/7)  | 1.7 $\pm$ 0.3 (6/7) | 0.4 $\pm$ 0.1 (1/7) | 0.7 $\pm$ 0.4 (1/5) | 3.6 $\pm$ 1.1† (6/7) |
|                                   | PB   | 1.3 $\pm$ 1.0 (1/7)                                              | 0.2 $\pm$ 0.1 (0/7) | 330.5 $\pm$ 22.8 (7/7)  | 0.3 $\pm$ 0.1 (0/7) | −0.1 $\pm$ 0.1 (0/7) | 2.9 $\pm$ 0.6 (7/7) | 0.2 $\pm$ 0.1 (0/7) | 0.1 $\pm$ 0.1 (0/5) | 0.4 $\pm$ 0.2 (2/7)  |
| Normal (n = 7)                    |      | 0.9 $\pm$ 0.4 (3/7)                                              | 0.1 $\pm$ 0.1 (0/7) | 252.1 $\pm$ 11.1† (7/7) | 0.1 $\pm$ 0.1 (0/7) | 0.0 $\pm$ 0.1 (0/7)  | 1.8 $\pm$ 0.2 (7/7) | 0.0 $\pm$ 0.0 (0/7) | 0.2 $\pm$ 0.2 (1/7) | 0.7 $\pm$ 0.2 (2/7)  |

BALF and PB eosinophils were obtained simultaneously, and the levels of surface expression of chemokine receptors were determined by flow cytometric analysis. The data are expressed as the calculated  $\Delta$ MESF values ( $\times$  1000). The bottom rows show the surface expression of receptors (mean  $\pm$  SEM of  $\Delta$ MESF value  $\times$  1000) as analyzed in 7 patients and normal controls (PB eosinophils only). Data shown in the parentheses are the rates of positivity, determined as described in the Methods section ( $\Delta$ MESF  $>$  1000).

BALF, Bronchoalveolar lavage fluid; PB, peripheral blood.

\* $P < .01$ .

† $P < .05$  vs corresponding value of PB eosinophils in eosinophilic lung disease.

patients' PB, we found significant CXCR4 expression in BALF eosinophils in almost all cases (positive: 6/7). When the  $\Delta$ MESF values were compared with those in PB eosinophils, CXCR4 expression in BALF eosinophils was increased with statistical significance ( $P < .05$ ). Significant expression of CCR1 was not found in any patient (positive: 0/7). The expression of CXCR3 was slightly increased in 1 case (subject 4), but 4 other subjects showed no significant difference. CXCR2 expression remained hardly detectable in BALF eosinophils (positive: 1/7). Furthermore, there was absolutely no de novo appearance of nonreported receptors—ie, CCR2 (positive: 0/7), CCR4 (positive: 0/7), or CCR5 (positive: 0/7).

The expression of transcripts of 15 chemokine receptors in BALF eosinophils was investigated by RT-PCR in 2 subjects (Fig 2). Strong expression of transcripts of CCR3 and CXCR4 and weak expression of CCR1 transcripts were detected in both donors. One donor weakly expressed CXCR2 transcripts. The mRNA expression pattern of chemokine receptors by BALF eosinophils did not differ fundamentally from that by PB eosinophils, which we previously reported for normal individuals.<sup>9</sup> Again, de novo appearance of nonreported receptors—ie, CCR2, CCR4, and CCR5—was not observed at all.

To determine whether changes in receptor expression had functional significance, we compared the extent of eosinophil migratory responses between BALF and PB eosinophils (Fig 3). In parallel with the quantitative

changes in the receptors, BALF eosinophils showed decreased migration toward the CCR3 ligand, eotaxin/CCL11, and increased migration toward the CXCR4 ligand, stromal cell–derived factor 1/CXCL12. On the other hand, no significant migratory response was initiated in either PB or BALF eosinophils when they were stimulated with MIP-1 $\alpha$ /CCL3, monocyte chemoattractant protein 1/CCL2, thymus and activation-regulated chemokine/CCL17, IL-8/CXCL8, growth-regulated oncogene- $\alpha$ /CXCL1, and IFN- $\gamma$ -inducible protein 10/CXCL10.

## DISCUSSION

The aim of this study was to determine whether eosinophils that have migrated into inflamed tissues exhibit a profile of chemokine receptor expression that is qualitatively and/or quantitatively different from that of eosinophils in peripheral locations. For this purpose, we analyzed BALF eosinophils from patients with eosinophilic lung diseases. By simultaneously analyzing PB eosinophils from the same patients, we were able to consistently detect subtle changes in the expression without our findings' being affected by donor-to-donor variation.

Previous published data have demonstrated the constitutive and/or inducible expression of CCR1,<sup>1</sup> CCR3,<sup>1-3</sup> CXCR1,<sup>4</sup> CXCR2,<sup>4</sup> CXCR3,<sup>10</sup> and CXCR4<sup>9</sup> by human PB eosinophils. In the present study, we found that the profile of chemokine receptor expression by eosinophils

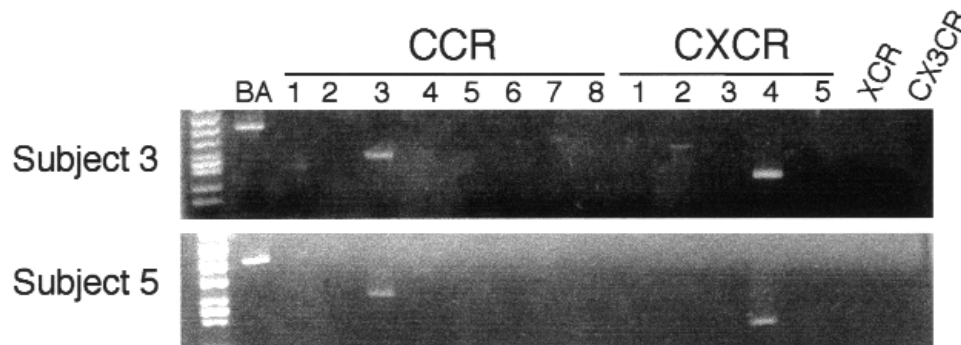


**FIG 2.** Expression of mRNA for chemokine receptors by BALF eosinophils. RT-PCR (subject 3, 27 cycles; subject 5, 30 cycles) was performed on BALF eosinophils (subject 3,  $2.0 \times 10^6$ ; subject 5,  $0.9 \times 10^6$ ; purity, >99%). The PCR products were electrophoresed and then visualized with ethidium bromide. BA,  $\beta$ -actin. XCR, XC chemokine receptor. CX3CR, CX3C chemokine receptor.

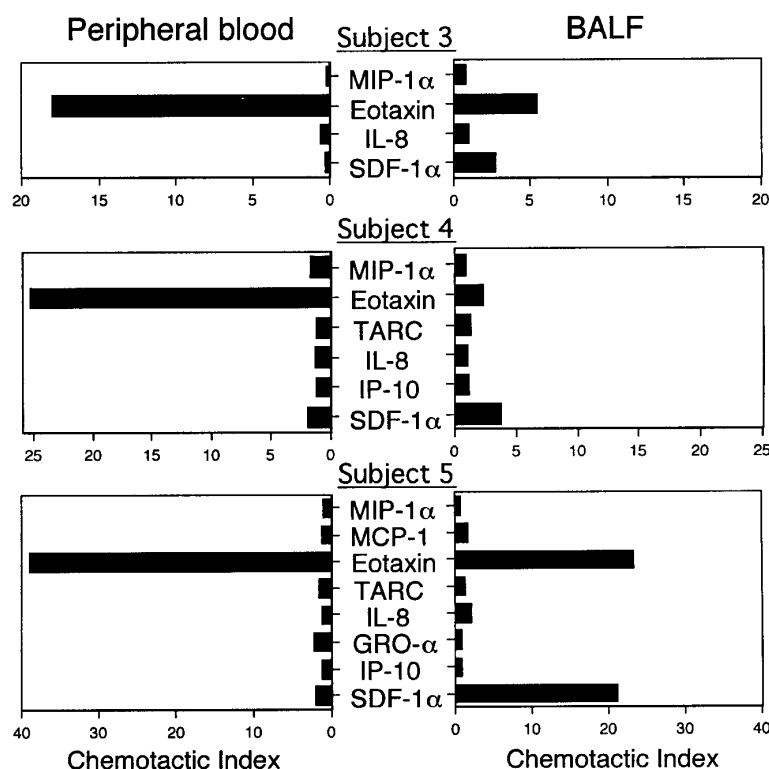


**FIG 3.** Migratory responses of PB and BALF eosinophils to chemokines. The migration of BALF and PB eosinophils was analyzed in subjects 3, 4, and 5. The percentage of BALF eosinophils and PB eosinophils that migrated toward the medium control were 2.1% and 0.9% (subject 3), 0.8% and 0.4% (subject 4), and 0.7% and 0.4% (subject 5), respectively (indicated as percents of total input cells).

in inflamed tissues was qualitatively similar to that by PB eosinophils. De novo expression of other receptors, including CCR2, CCR4, and CCR5, was not detected in the BALF eosinophils. However, we found quantitative changes in the BALF eosinophils in comparison with the PB eosinophils: expression of CCR3 was decreased and CXCR4 was conversely increased, with statistical significance, in BALF eosinophils. Moreover, functional stud-

ies revealed that the changes of the BALF eosinophils in CCR3 and CXCR4 expression were reflected in altered migratory responses to their ligands.

CCR3 was most dominantly expressed on both BALF and PB eosinophils, suggesting a primary as well as a causal role for this receptor in eosinophil recruitment throughout the locomotion process from the circulation to inflamed tissue sites. Decreased CCR3 expression by the

BALF eosinophils in comparison with PB eosinophils might be due, at least in part, to internalization of the surface receptors caused by binding with ligands such as eotaxin.

Our results also suggest a possible role of eosinophil CXCR4 at inflamed tissue sites. We previously demonstrated that expression of functional CXCR4 can be induced in eosinophils under certain conditions.<sup>9</sup> A significant increase in CXCR4 expression in BALF eosinophils concomitant with functional relevance might provide a basis for a role of CXCR4 in eosinophil influx into inflamed tissue sites. This possibility is further supported by a recent report demonstrating that systemic blockade of CXCR4 with antibody attenuated eosinophil influx into the airways of sensitized mice.<sup>20</sup>

BALF eosinophils showed neither an increase in the level of expression nor a functional significance for either CXCR1 or CXCR3. These results suggest that these receptors play only minor roles, if any, in eosinophil accumulation at tissue sites.

Only negligible amounts of CCR1 were detected on PB eosinophils from most patients and normal subjects. Surface expression of CCR1 seems to vary among species and individuals. Mouse eosinophils were shown to express CCR1 strongly and to respond to MIP-1 $\alpha$ .<sup>21</sup> On the other hand, the expression of CCR1 in human eosinophils has been debatable. Results of Scatchard analysis demonstrated that eosinophils from allergic and asthmatic donors express CCR1 corresponding to up to 5% of CCR3.<sup>1</sup> Sabroe et al<sup>22</sup> also demonstrated a low level of expression of CCR1. Importantly, they demonstrated the existence of high- and low-responders to CCR1 ligand MIP-1 $\alpha$  in atopic and nonatopic individuals. Thus it seems most likely that a small and selected subset of human beings express CCR1 moderately. In fact, we also found apparent expression of CCR1 in PB eosinophils from one of our 7 patients (subject 7;  $\Delta$ MESF, 7751). Furthermore, as noted in the Methods section, CCR1 expression in PB eosinophils might be underestimated because of the separating procedure. Thus the modes of expression of CCR1 in high-responders at inflamed tissue sites are rather inconclusive in this study, and this intriguing question merits further investigation.

Although not fully proven, the pathogenesis of diseases used in this study is potentially heterogeneous. Nevertheless, BALF eosinophils in our study invariably exhibited similar patterns of chemokine receptor expression. Although a disease-associated difference in the expression was not specifically investigated in this investigation, it is unlikely that the differences in the pathogenesis of eosinophilic lung diseases can be explained by differences in the expression of chemokine receptors on the eosinophils.

In summary, we have for the first time examined the profiles of chemokine receptor expression by eosinophils at inflamed tissue sites in human beings. Eosinophils at inflamed tissue sites exhibited expression patterns qualitatively similar to those of circulating eosinophils, and no additional expression was detected. However, tissue eosinophils exhibited decreased CCR3 and increased

CXCR4 expression in comparison with PB eosinophils. Our results suggest that CCR3 plays a primary role in eosinophil accumulation at inflamed sites, whereas CXCR4 possibly plays an additional role, acting preferentially on locally sequestered eosinophils. With regard to confirmation of our present observations, the use of BALF after antigen challenge is an intriguing subject meriting further investigation.

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