

Human Eosinophils Show Chemotaxis to Lymphoid Chemokines and Exhibit Antigen-Presenting-Cell-Like Properties upon Stimulation with IFN- γ , IL-3 and GM-CSF

Yun-Jae Jung^a So-Youn Woo^b Myoung Ho Jang^c Masayuki Miyasaka^d
Kyung-Ha Ryu^e Hae-Kyung Park^b Ju-Young Seoh^b

^aDepartment of Microbiology, Gachon Medical School, Incheon, and ^bDepartment of Microbiology, Ewha Womans University Graduate School of Medicine, Seoul, Republic of Korea; ^cLaboratory of Gastrointestinal Immunology, WPI Immunology Frontier Center, Osaka University, and ^dLaboratory of Immunodynamics, Department of Microbiology and Immunology, Osaka University Graduate School of Medicine, Osaka, Japan; ^eDepartment of Pediatrics, Ewha Womans University Graduate School of Medicine, Seoul, Republic of Korea

Key Words

Antigen presentation • CCL21 • Chemotaxis • EoL-1 cells • Eosinophils

Abstract

Background: Eosinophils are multifunctional leukocytes. Under physiological conditions, they circulate in the blood and through the tissues to serve their functions. In certain inflammatory states, they enter the T-cell areas of lymph nodes (LNs) that drain the inflamed tissue and communicate with T cells in LNs, but the underlying mechanism that regulates their trafficking to LNs is not yet fully explored. Here, we report that a human eosinophilic leukemia cell line, EoL-1, and human peripheral blood (PB) eosinophils become reactive to the lymphoid chemokines CCL21 and CCL25 upon stimulation. **Methods:** EoL-1 cells were differentiated with dibutyryl cyclic AMP (dEoL-1) and subsequently pulsed with IFN- γ , IL-3 and GM-CSF. The eosinophil fraction was purified from normal human adult PB and incubated for 1 day with the same cytokine combination. **Results:** Upon cytokine stimulation, dEoL-1 cells expressed chemokine receptors CCR7, CCR9 and CCR3 and developed chemotactic responsiveness to CCL21, CCL25 and CCL11, which bind to the respective receptors. Human PB eosinophils also showed

chemotactic responsiveness to CCL21 and CCL25 upon stimulation with IFN- γ , IL-3 and GM-CSF. In addition, the cytokine-stimulated dEoL-1 cells expressed costimulatory molecules, including CD40, CD80, CD86 and HLA-DR, and also expressed a tolerogenic and Th2-polarizing enzyme, indoleamine 2,3-dioxygenase. **Conclusions:** These in vitro observations raise the possibility that eosinophils may utilize lymphoid chemokines to enter LNs and serve antigen-presenting functions in the LN under certain inflammatory conditions.

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Introduction

Eosinophils are multifunctional leukocytes that have been implicated in the pathogenesis of numerous inflammatory processes, including helminth infections and allergic diseases. Although eosinophils have been considered as terminally differentiated cells that mainly act as the first-line defense against parasites in the tissues in

This study was supported by a grant (F01-2004-000-10147-0, Korea-Japan Joint Research Program) from the Korea Science and Engineering Foundation.

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1018–2438/08/1463–0227\$24.50/0

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Correspondence to: Dr. Ju-Young Seoh
Department of Microbiology
Ewha Womans University Graduate School of Medicine
Mok-6-dong 911-1, Yangchon Gu, Seoul 158-710 (Republic of Korea)
Tel. +82 2 2650 5738, Fax +82 2 2653 8891, E-Mail jyseoh@ewha.ac.kr

which they reside [1], it has been suggested that eosinophils are recruited from the circulation to inflammatory foci, where they modulate immune responses to diverse stimuli [2].

Recent reports have shown that eosinophils are also involved in numerous homeostatic processes in the thymus, mammary gland, uterus and gastrointestinal tract [2]. In addition, several lines of evidence indicate that they may exhibit characteristics seen in antigen-presenting cells (APCs) in certain states [3]. It has also been demonstrated that eosinophils migrate and accumulate in draining lymph nodes (LNs) of the lungs in case of an intranasal antigen challenge in sensitized mice [4]. The antigen presentation and allergen-induced recruitment of eosinophils to lung tissue have been suggested as evidence of their interaction with T lymphocytes [5]. More specifically, interferon- γ (IFN- γ)-treated eosinophils are known to interact with T cells and promote Th2 polarization through the expression of functionally active indoleamine 2,3-dioxygenase (IDO) in lymphoid tissue [6]. In regard to eosinophil chemotaxis, CCL11-CCR3 is the most selective eosinophil chemoattractant axis to be identified thus far [7, 8]. The molecular mechanisms underlying the migration of eosinophils to LNs, however, are not fully understood, and lymphoid chemokines could be the source of trafficking for those cells.

Although considerable progress has been made, eosinophil research has been hindered by the small number of cells that can be obtained from the peripheral blood (PB) of healthy donors and the inability to expand eosinophils in vitro [9]. A human eosinophilic cell line, EoL-1, has been established, which is considered to be a useful in vitro model with which to study human eosinophils [10, 11]. It has been demonstrated that EoL-1 cells can be induced to become eosinophilic granule-containing cells by a number of stimuli, including butyric acid [12] and dibutyryl cyclic AMP (dbcAMP) [13].

Here, we report that when EoL-1 cells were first differentiated with dbcAMP (dEoL-1) and then stimulated with a combination of IFN- γ , interleukin (IL)-3 and granulocyte macrophage-colony stimulating factor (GM-CSF), they responded to certain lymphoid chemokines such as CCL21 and CCL25 by expressing the respective cognate receptors, i.e. CCR7 and CCR9. Responsiveness to the lymphoid chemokines was also observed in cytokine-stimulated PB eosinophils. In addition, the cytokine-stimulated dEoL-1 cells expressed a few of the co-stimulatory molecules as well as a tolerogenic enzyme, IDO, which raised the possibility that eosinophils may modulate the nature of the T cell response.

Materials and Methods

Cell Culture

EoL-1 and HL-60 cells were maintained in RPMI 1640 (Gibco, Grand Island, N.Y., USA) supplemented with 10% fetal bovine serum (Gibco) in 5% CO₂ at 37°C. EoL-1 cells were induced to differentiate by the addition of 0.1 mM dbcAMP (Sigma, St. Louis, Mo., USA) for 9 days [13]. The cell concentration was adjusted to 5×10^5 cells/ml every 3 days. Then the cells were further stimulated for another day with IFN- γ , IL-3 and GM-CSF, and the concentration of each of the three cytokines was 10 ng/ml. HL-60 cells were differentiated with 0.8% dimethylformamide (Sigma) for 4 days (dHL-60) [14].

Isolation of PB Eosinophils

The eosinophil fraction was purified from normal adult human PB. In brief, venous blood anticoagulated with 10 U/ml heparin (Choongwae, Seoul, Republic of Korea) was diluted with Hanks' balanced salt solution (HBSS, pH 7.4; Gibco) at a 1:2 ratio. Diluted blood was overlaid with Histopaque (density 1.083 g/ml; Sigma) and centrifuged at 400 g at room temperature for 30 min. After removal of supernatant and mononuclear cells at the interface, the pellet was resuspended in HBSS. Six percent dextran 500 (Amersham Biosciences, Piscataway, N.J., USA) in 0.9% NaCl was added to yield a final concentration of 1%, and the cell suspension was allowed to stand at room temperature to let red blood cells settle. After 1 h, the supernatant was harvested and the remaining cells were washed with HBSS by centrifugation at 600 g for 10 min. Red blood cells were lysed by incubation in 0.17 M Tris-NH₄Cl for 2 min at 37°C. The remaining granulocyte fraction was incubated with mouse anti-human CD16 monoclonal antibody conjugated with magnetic particles (Miltenyi Biotec, Auburn, Calif., USA) for 30 min on ice, and were loaded onto the separation column (Miltenyi Biotec) positioned in the superparamagnetic field. Cells were eluted with phosphate-buffered saline (pH 7.4) with 2 mM EDTA and 0.5% bovine serum albumin. The purity of the CD16⁺ fraction was determined by flow cytometry (Becton Dickinson Bioscience, San Jose, Calif., USA) and was consistently >97%. The purity was also determined by morphologic observation, and isolated cells showed characteristics of eosinophils, such as deep-purple-colored cytoplasmic granules and a bilobed nucleus following staining with Diff-Quik solution (Sysmex, Kobe, Japan). The purified cells were used immediately or incubated for 1 day with IFN- γ , IL-3 and GM-CSF (all were 10 ng/ml) for the chemotactic assay.

Morphological Analysis

For morphological analysis, cultured EoL-1 cells were spun at 500 rpm for 5 min on glass slides (Cytospin 3, Shandon, Pittsburgh, Pa., USA). The slides were air-dried, stained with Diff-Quik solution and observed under a light microscope.

Electron-Microscopic Examination

For electron-microscopic studies, cultured EoL-1 cells were prefixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer. The cells were then post-fixed in 2% osmium tetroxide, dehydrated and embedded in epoxy resin. Ultrathin 60- to 70-nm sections were cut by a Reichert-Jung ultramicrotome (Leica Microsystems, Heidelberg, Germany). The thin sections were stained with 1–2% aqueous uranyl acetate, followed by 1% lead citrate, and were ob-

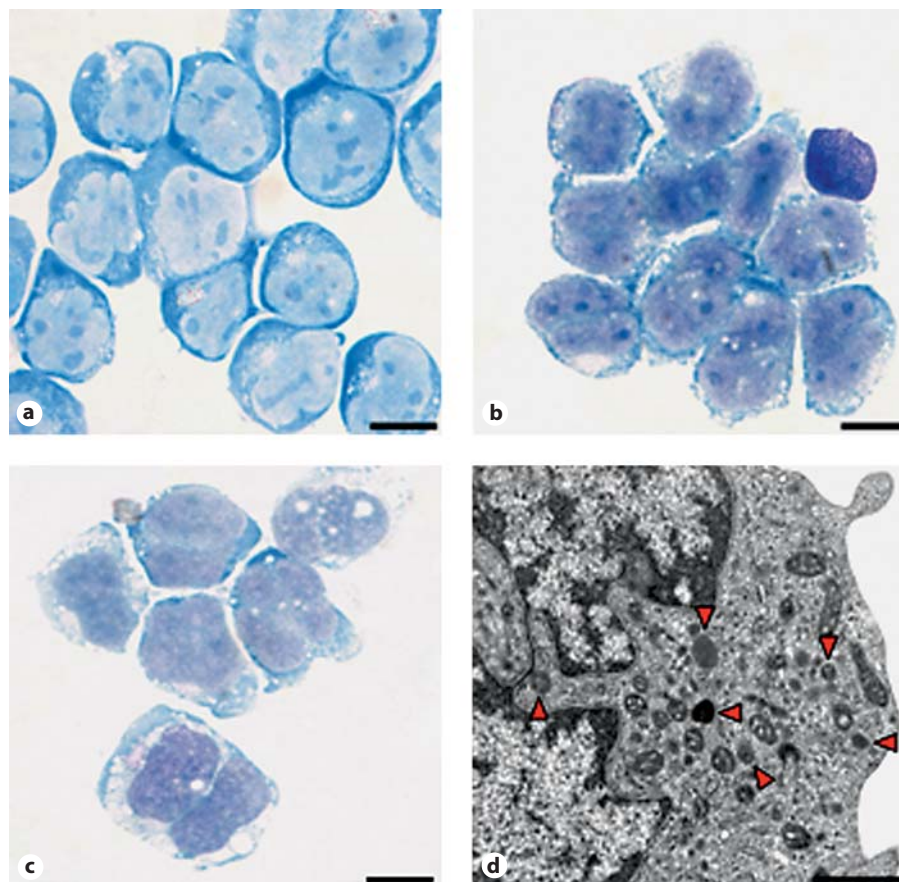


Fig. 1. Morphological differentiation observed in stimulated EoL-1 cells. EoL-1 cells (**a**) were treated with dbcAMP for 9 days (dEoL-1, **b**) and stimulated for an additional day with IFN- γ , IL-3 and GM-CSF (**c**, **d**). Diff-Quik staining of the unstimulated EoL-1 cells shows a high nuclear/cytoplasmic ratio and a lack of nuclear lobulation. dEoL-1 as well as cytokine-stimulated dEoL-1 show nuclear lobulation, formation of cytoplasmic vacuoles, and increased cellular aggregation. Although crystalloid inclusion had not yet developed, uniformly dense or round-shaped internal structures were frequently observed in the large granules (red arrowheads) in electron-microscopic examination (**d**). Scale bars = 10 (**a–c**) and 0.4 μ m (**d**).

served and photographed with an H-7650 transmission electron microscope (Hitachi, Tokyo, Japan) at an accelerating voltage of 75 kV.

Flow Cytometry

Aliquots of EoL-1 cells, before and after stimulation with dbcAMP and IFN- γ , IL-3 and GM-CSF, were stained with a panel of monoclonal antibodies for 30 min at 4°C: phycoerythrin (PE)-anti CD11c, fluorescein isothiocyanate (FITC) anti-CD40, PE anti-HLA-DR, PE anti-CD80, FITC anti-CD86 (BD Biosciences), PE anti-CCR3, PE anti-CCR7 and FITC anti-CCR9 (R & D, Minneapolis, Minn., USA). The level of expression was compared with that of unstimulated cells. The cells were fixed with 1% paraformaldehyde solution and analyzed with a FACSCalibur (BD Bioscience).

Reverse Transcription-Polymerase Chain Reaction

Total mRNA was extracted from the cultured cells. One microgram of mRNA was reverse-transcribed using a reverse transcription system (Promega, Madison, Wisc., USA), and the complementary DNA was amplified with *Taq* DNA polymerase (Takara, Shiga, Japan) as follows: an initial denaturation step (at 94°C for 3 min), 35 cycles of PCR (95°C for 30 s, 55°C for 30 s and 72°C for 15 s) and 72°C for 10 min using the GeneAmp PCR system 9600 (Perkin Elmer, Norwalk, Conn., USA). The following prim-

ers were used for amplification: human glyceraldehyde 3-phosphate dehydrogenase (GAPDH), 5'-GTCTTCTCCACCATGGAAGGCT-3' and 5'-CATGCCAGTGAGCTTCCCCTTCA-3'; CCR7, 5'-TCCTTCTCATCAGCAAGCTGTC-3' and 5'-GAGG-CAGCCCAGGTCCTTGAAG-3'; CCR3, 5'-ATGCTGGTGACAGAGTGAT-3' and 5'-AGGTGA GTGTGGAAGGCTTA-3', and CCR9, 5'-TTCCTCCCACCCTTGTAAGT-3', 5'-AAGCCTTT-TCTCCCTCCAAG-3'. The amplified DNA products were electrophoresed on 1% agarose gels and then stained with 0.5 μ g/ml of ethidium bromide.

Chemotaxis Assay Using a Time-Lapse Videomicroscopy

In vitro chemotactic activities of the cells were analyzed by a time-lapse videomicroscopic technique using special equipment (EZ-TAXIScan™; Effector Cell Institute, Tokyo, Japan) [15]. The chemokines, recombinant human CCL11, CCL25 and CCL21, were purchased from R & D Systems. Six microchannels (depth, 6 μ m) were created between a 10% FBS-coated coverslip and a silicon chip according to the instructions of the manufacturer. One microliter of cell suspension that had been resuspended at 1×10^6 cells/ml in RPMI 1640 with 0.5% endotoxin-free BSA was injected into each well at one end of the microchannel, and 1 μ l of chemokine (100 μ g/ml) was injected into the well at the other end. Before application, the cells were sometimes pretreated with pertussis toxin (100 ng/ml; Calbiochem, San Diego, Calif., USA)

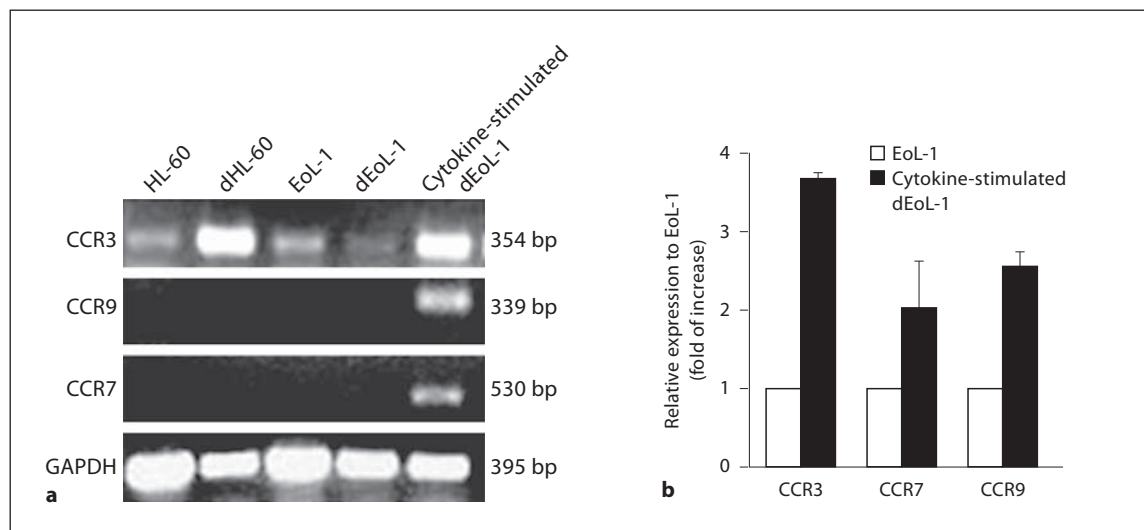


Fig. 2. The chemokine receptors CCR3, CCR7 and CCR9 were expressed only after stimulation with IFN- γ , IL-3 and GM-CSF in the dbcAMP-treated EoL-1 cells. **a** Expression of chemokine receptor mRNA. cDNAs were prepared from total RNAs obtained from undifferentiated HL-60 and EoL-1 cells, dHL-60, dEoL-1

and cytokine-stimulated dEoL-1. **b** Expression of chemokine receptor proteins on the cell surface. The expression levels are shown as the ratio of the mean fluorescence intensity of cytokine-stimulated dEoL-1 to the fluorescence intensity of undifferentiated cells. Error bar indicates SEM.

for 1 h at 37°C. After the addition of chemokines in the opposite compartment, the assay was allowed to proceed for 90–180 min at 37°C, and the migratory behavior of the cells was recorded by a CCD camera every 30 s. After the assay, the digital images were converted into movies for analysis, and the number of cells moving into the assay field was also analyzed using software (TAXI-Scan Analyzer) provided by the manufacturer.

Results

Eosinophilic Precursor EoL-1 Cells Expressed Lymphoid Chemokine Receptors CCR7 and CCR9 following Cytokine-Induced Differentiation

The EoL-1 cells represent the undifferentiated promyelocytic phase of eosinophils bearing a large nucleus with fine chromatin and prominent nucleoli (fig. 1a). After treatment with dbcAMP and stimulation with IFN- γ , IL-3 and GM-CSF, EoL-1 cells displayed morphological signs of differentiation; they showed nuclear lobulation, distinct vacuoles (fig. 1b, c) and large granules in the cytoplasm (fig. 1d), although the crystalloid inclusion typical of mature eosinophils had not yet developed in the granules. Consistent with these morphological observations, the cytokine-stimulated dEoL-1 cells strongly expressed CCR3, the receptor for the eosinophil-directing chemokine CCL11 (fig. 2a). Interest-

ingly, the stimulated dEoL-1 cells also expressed CCR7 and CCR9, the receptors for the lymphoid chemokines CCL21 and CCL25, respectively (fig. 2a). In contrast, neither unstimulated EoL-1 nor unstimulated or stimulated promyelocytic HL-60 cells that can also be induced to differentiate to eosinophils [16, 17] expressed CCR7 or CCR9. Flow-cytometric analysis confirmed the expression of CCR3, CCR7 and CCR9 in the cytokine-stimulated dEoL-1 cells (fig. 2b).

Cytokine-Stimulated dEoL-1 Cells Showed Directional Migration to the Lymphoid Chemokines

Next, we examined the functional abilities of the chemokine receptors induced on the cytokine-stimulated dEoL-1 cells using a time-lapse videomicroscopic chemotaxis assay [15]. dEoL-1 cells stimulated with IFN- γ , IL-3 and GM-CSF migrated directionally in response to CCL11, CCL21 or CCL25 (fig. 3a; online suppl. movie 1–3, www.karger.com/doi/10.1159/000115891), whereas neither undifferentiated EoL-1 cells nor those treated with dbcAMP alone showed this type of migration (data not shown). Furthermore, the PB eosinophils stimulated with the cytokines for 1 day also migrated directionally in response to CCL11, CCL21 or CCL25 (fig. 3b; online suppl. movie 4–6, www.karger.com/doi/10.1159/000115891), whereas unstimulated eosinophils

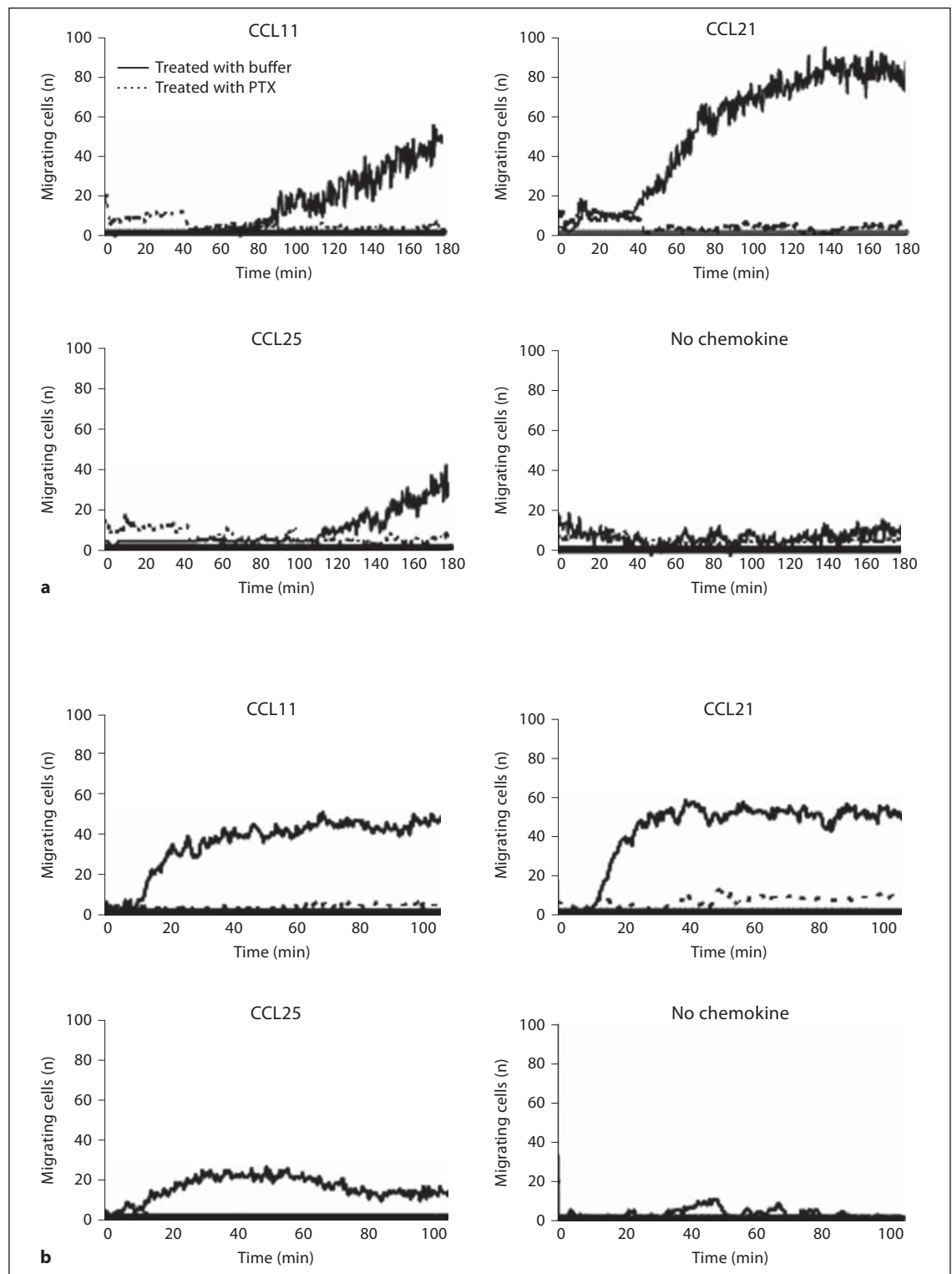


Fig. 3. Kinetic analysis of the migrating cells showed the chemotactic response to CCL21 and CCL25, as well as to CCL11, in the cytokine-stimulated dEoL-1 cells (**a**) and PB eosinophils (**b**). Cells migrating toward the chemokines in the assay field were automatically counted using software (TAXIScan analyzer). Note that

significant fractions of the cytokine-stimulated dEoL-1 cells and PB eosinophils had begun migrating after the additions of CCL11, CCL21 and CCL25. Pretreatment of cells with pertussis toxin (PTX) almost completely abolished the migratory response of both the stimulated dEoL-1 cells and the PB eosinophils.

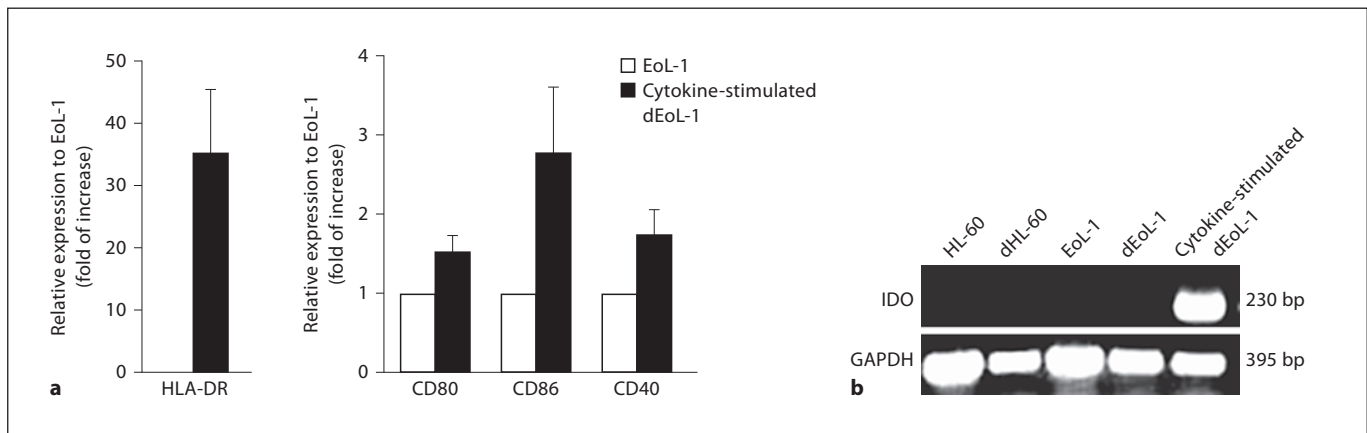


Fig. 4. a Antigen-presenting and costimulatory molecules were increased by treatment with dbcAMP and cytokines in EoL-1 cells. Antigen-presenting (HLA-DR) and costimulatory (CD80, CD86 and CD40) molecule expression was analyzed by flow cytometry. The expression levels are shown as the ratio of the mean

fluorescence intensity of cytokine-stimulated dEoL-1 to the fluorescence intensity of undifferentiated cells. **b** IDO was expressed at the mRNA level only after stimulation with IFN- γ , IL-3 and GM-CSF in the dbcAMP-treated EoL-1 cells.

showed directional migration in response to only CCL11, but not to CCL21 or CCL25 (data not shown). The proportion of the migrating cells was high, in the order of CCL21, CCL11 and CCL25, in both stimulated dEoL-1 cells and PB eosinophils, and the initiation of migration was faster in eosinophils than in EoL-1 cells (fig. 3). Pre-treatment with pertussis toxin almost completely abolished the directional migration of the cytokine-stimulated dEoL-1 cells and eosinophils, demonstrating G α_i -mediated signaling processes (fig. 3).

Cytokine-Stimulated dEoL-1 Cells Showed Surface Expression of APC-Related Costimulatory Molecules as well as IDO

Because it has previously been reported that eosinophils may function as APCs upon activation [5], we examined the expression of costimulatory molecules that are essential for antigen presentation in the cytokine-stimulated dEoL-1 cells. As shown in figure 4a, the cytokine-stimulated dEoL-1 cells showed a marked increase in the expression of HLA-DR. They also showed upregulated expression of CD80, CD86 and CD40. In addition, they showed prominent expression of a Th2-inducing enzyme, IDO, at the mRNA level, which was not seen in unstimulated EoL-1 cells or unstimulated HL-60 cells or HL-60 cells stimulated with dimethylformamide (fig. 4b).

Discussion

During the inflammatory response, eosinophils can present antigens to and communicate with T cells, and are recruited to regional LNs [18]. However, the manner in which they migrate to the area where APCs and T cells come together has not yet been elucidated. In the present study, we demonstrated that IFN- γ , IL-3- and GM-CSF-stimulated dEoL-1 cells and PB eosinophils are capable of responding to the lymphoid chemokines that recruit native T cells and dendritic cells to LNs. In addition, upon cytokine stimulation, the dEoL-1 cells showed expression of various costimulatory molecules and IDO.

Because eosinophils comprise only 1–4% of circulating leukocytes in normal humans and have a short life span in vitro, it has been difficult to study the mechanism underlying their migration to tissues in detail. The human eosinophilic leukemia cell line EoL-1 can be induced to differentiate into eosinophilic granule-containing cells and die by apoptosis when differentiated by dbcAMP [13, 19]. For this reason, EoL-1 cells have been used as models of eosinophil function in various studies, including those investigating chemotaxis, receptor expression, mediator release and apoptosis induction [12, 20, 21].

In this study, cytokine-stimulated eosinophils and dEoL-1 cells demonstrated chemotactic responsiveness to the lymphoid chemokines, CCL21 and CCL25 (fig. 3; online suppl. movie 2, 3, 5, 6, www.karger.com/doi/10.1159/000115891). The locomotive pattern of the

stimulated dEoL-1 cells was slightly different from that of stimulated eosinophils. Kinetic analysis showed an earlier start of migration toward the chemokines in stimulated PB eosinophils compared to that in cytokine-stimulated dEoL-1 cells (fig. 3). Although we currently cannot fully explain this observation, the later responsiveness of stimulated dEoL-1 cells may in part originate from the heterogeneity of the cell line in terms of the capacity to differentiate within the population [22]. The heterogeneity of differentiation of the cytokine-stimulated dEoL-1 could be estimated by examining the phenotype of the migrating cells. We found that the cytokine-stimulated dEoL-1 cells migrated 3–4 times more efficiently than unstimulated EoL-1 or dEoL-1 cells (online suppl. fig. S1A, www.karger.com/doi/10.1159/000115891) and that the cytokine-stimulated dEoL-1 cells, which migrated in response to CCL11, contained 11% of CCR3-expressing cells (online suppl. fig. S1B, www.karger.com/doi/10.1159/000115891).

CCL21 was first known to serve a homing function for T lymphocytes and also to direct mature dendritic cells into T-cell zones of LNs [23, 24]. CCL21 is expressed at high levels in the paracortical areas of LNs [25]. Findings of this study may provide a clue as to how eosinophils are recruited to regional LNs under inflammatory conditions, but further in vivo studies are needed.

Eosinophils are mainly tissue-dwelling cells that are preferentially distributed in the thymus and gastrointestinal tract under steady state conditions [2]. The tissue levels of eosinophils are orchestrated by chemokines; CCL11, in particular, is the most selective eosinophil chemoattractant to be identified so far [8, 9]. In CCL11-deficient mice, eosinophils in the jejunum are substantially reduced, but are not completely absent [26], which indicates that CCL11 is important, but it is not the sole chemokine that critically regulates the intestinal distribution of eosinophils. In addition, the widespread distribution of CCL11 in various tissues, including the skin, heart,

skeletal muscle, lung and intestine [27], does not explain the selective tissue distribution of eosinophils under steady-state conditions. In contrast, the expression of CCL25 is restricted only to the thymus and gastrointestinal tract, exactly coinciding with the tissue distribution of eosinophils. In the present study, the videomicroscopic assay demonstrated that both the eosinophils and the cytokine-stimulated dEoL-1 cells are capable of responding to CCL25 (fig. 3). This finding may provide evidence to support that CCL25 is involved in the selective tissue distribution of eosinophils in steady-state conditions, and this issue warrants further in vivo studies for confirmation.

Upon stimulation with dbcAMP and IFN- γ , IL-3 and GM-CSF, the dEoL-1 cells were capable of expressing APC-related molecules (fig. 4a). Collectively, these findings are consistent with the finding that eosinophils may function as APCs in inflammatory conditions in vivo [28, 29]. Furthermore, IDO, one of the key enzymes of eosinophils that are involved in the polarization of the Th2 response, has also been demonstrated to be expressed in stimulated dEoL-1 cells (fig. 4b).

Taken together, these findings allow us to speculate that tissue eosinophils, in a certain microenvironment where cytokines such as IFN- γ , IL-3 and GM-CSF are present, may traffic to the places where CCL21 is expressed. During the migration, they may functionally mature to express the APC-related molecules. Considering that eosinophils are armed with various kinds of mediators, including both Th1- and Th2-type cytokines, the pattern of the eosinophil response may play a pivotal role in the following adaptive immune response [2].

Therefore, the results of this study suggest that eosinophils, in a certain situation, may migrate to the sites where CCL21 and CCL25 are distributed, and may also be involved in the crosstalk with APCs and/or T cells.

References

- 1 Butterworth AE: The eosinophil and its role in immunity to helminth infection. *Curr Top Microbiol Immunol* 1977;77:127–168.
- 2 Rothenberg ME, Hogan SP: The eosinophil. *Annu Rev Immunol* 2006;24:147–174.
- 3 Oehler L, Majdic O, Pickl WF, Stockl J, Riedl E, Drach J, Rappersberger K, Geissler K, Knapp W: Neutrophil granulocyte-committed cells can be driven to acquire dendritic cell characteristics. *J Exp Med* 1998;187:1019–1028.
- 4 Duez C, Dakhama A, Tomkinson A, Marquillies P, Balhorn A, Tonnel AB, Bratton DL, Gelfand EW: Migration and accumulation of eosinophils toward regional lymph nodes after airway allergen challenge. *J Allergy Clin Immunol* 2004;114:820–825.
- 5 Shi HZ: Eosinophils function as antigen-presenting cells. *J Leukoc Biol* 2004;76:520–527.
- 6 Odemuyiwa SO, Ghahary A, Li Y, Puttagunta L, Lee JE, Musat-Marcu S, Moqbel R: Cutting edge: human eosinophils regulate T cell subset selection through indoleamine 2,3-dioxygenase. *J Immunol* 2004;173:5909–5913.
- 7 Jose PJ, Griffiths-Johnson DA, Collins PD, Walsh DT, Moqbel R, Totty NF, Truong O, Hsuan JJ, Williams TJ: Eotaxin: a potent eosinophil chemoattractant cytokine detected in a guinea pig model of allergic airways inflammation. *J Exp Med* 1994;179:881–887.

- 8 Ponath PD, Qin S, Ringler DJ, Clark-Lewis I, Wang J, Kassam N, Smith H, Shi X, Gonzalo JA, Newman W, Gutierrez-Ramos JC, Mackay CR: Cloning of the human eosinophil chemoattractant, eotaxin. Expression, receptor binding, and functional properties suggest a mechanism for the selective recruitment of eosinophils. *J Clin Invest* 1996; 97:604–612.
- 9 Zimmermann N, Daugherty BL, Stark JM, Rothenberg ME: Molecular analysis of CCR-3 events in eosinophilic cells. *J Immunol* 2000;164:1055–1064.
- 10 Saito H, Bourinbaier A, Ginsburg M, Minato K, Ceresi E, Yamada K, Machover D, Breard J, Mathe G: Establishment and characterization of a new human eosinophilic leukemia cell line. *Blood* 1985;66:1233–1240.
- 11 Mayumi M: Eol-1, a human eosinophilic cell line. *Leuk Lymphoma* 1992;7:243–250.
- 12 Saito H, Hayakawa T, Mita H, Akiyama K, Shida T: Effect of butyric acid on induction of differentiation into eosinophil-like cells in human eosinophilic leukemia cells, Eol-1 cell line: possible role of granulocyte-macrophage colony-stimulating factor as an autocrine differentiating factor. *Int Arch Allergy Immunol* 1993;100:240–247.
- 13 Wong CK, Ho CY, Lam CW, Zhang JP, Hjelm NM: Differentiation of a human eosinophilic leukemic cell line, Eol-1: characterization by the expression of cytokine receptors, adhesion molecules, CD95 and eosinophilic cationic protein (ECP). *Immunol Lett* 1999; 68:317–323.
- 14 Fontana JA, Wright DG, Schiffman E, Corcoran BA, Deisseroth AB: Development of chemotactic responsiveness in myeloid precursor cells: Studies with a human leukemia cell line. *Proc Natl Acad Sci USA* 1980;77:3664–3668.
- 15 Kanegasaki S, Nomura Y, Nitta N, Akiyama S, Tamatani T, Goshoh Y, Yoshida T, Sato T, Kikuchi Y: A novel optical assay system for the quantitative measurement of chemotaxis. *J Immunol Methods* 2003;282:1–11.
- 16 Breitman TR, Hemmi H, Imaizumi M: Induction by physiological agents of differentiation of the human leukemia cell line HL-60 to cells with functional characteristics. *Prog Clin Biol Res* 1986;226:215–233.
- 17 Howe RS, Fischkoff SA, Rossi RM, Lyttle CR: Chemotactic capabilities of HL-60 human myeloid leukemia cells differentiated to eosinophils. *Exp Hematol* 1990;18:299–303.
- 18 Shi HZ, Humbles A, Gerard C, Jin Z, Weller PF: Lymph node trafficking and antigen presentation by endobronchial eosinophils. *J Clin Invest* 2000;105:945–953.
- 19 Tai G, Eun-Young J, Yuji H, Masahiko K, Toshio H, Kenji K, Kenshi F, Mitsufumi M: Different effects of cyclic AMP and butyrate on eosinophilic differentiation, apoptosis and bcl-2 expression of a human eosinophilic leukemia cell line, Eol-1. *Hematol Oncol* 1996;14:181–192.
- 20 Uenoyama Y, Ohshima Y, Morita M, Akutagawa H, Nambu M, Kim KM, Mayumi M, Mikawa H: Dibutyl cyclic AMP induces formyl peptide receptor expression and chemotactic responses in a human eosinophilic cell line, Eol-1. *Exp Hematol* 1991;19: 823–828.
- 21 Al-Rabia MW, Blaylock MG, Sexton DW, Walsh GM: Membrane receptor-mediated apoptosis and caspase activation in the differentiated Eol-1 eosinophilic cell line. *J Leukoc Biol* 2004;75:1045–1055.
- 22 Freshney RI: *Culture of Animal Cells: A Manual of Basic Techniques*, ed 4. New York, Wiley-Liss, 2000.
- 23 Gunn MD, Kyuwa S, Tam C, Kakiuchi T, Matsuzawa A, Williams LT, Nakano H: Mice lacking expression of secondary lymphoid organ chemokine have defects in lymphocyte homing and dendritic cell localization. *J Exp Med* 1999;189:451–460.
- 24 Gunn MD, Tangemann K, Tam C, Cyster JG, Rosen SD, Williams LT: A chemokine expressed in lymphoid high endothelial venules promotes the adhesion and chemotaxis of naive T lymphocytes. *Proc Natl Acad Sci USA* 1998;95:258–263.
- 25 Cyster JG: Chemokines and the homing of dendritic cells to the T cell areas of lymphoid organs. *J Exp Med* 1999;189:447–450.
- 26 Matthews AN, Friend DS, Zimmermann N, Sarafi MN, Luster AD, Pearlman E, Wert SE, Rothenberg ME: Eotaxin is required for the baseline level of tissue eosinophils. *Proc Natl Acad Sci USA* 1998;95:6273–6278.
- 27 Rothenberg ME, Luster AD, Leder P: Murine eotaxin: an eosinophil chemoattractant inducible in endothelial cells and in interleukin 4-induced tumor suppression. *Proc Natl Acad Sci USA* 1995;92:8960–8964.
- 28 Lucey DR, Nicholson-Weller A, Weller PF: Mature human eosinophils have the capacity to express HLA-DR. *Proc Natl Acad Sci USA* 1989;86:1348–1351.
- 29 Weller PF, Rand TH, Barrett T, Elovic A, Wong DT, Finberg RW: Accessory cell function of human eosinophils. HLA-DR-dependent, MHC-restricted antigen-presentation and IL-1 alpha expression. *J Immunol* 1993; 150:2554–2562.