Production and Regulation of Eotaxin-2/CCL24 in a Differentiated Human Leukemic Cell Line, HT93

Naomi Yoshida, a Eriko Aizu-Yokota, a Yoshiko Sonoda, a Yasuhiro Moriwaki, b Kenji Kishi, c and Tadashi Kasahara, a, ∗

a Department of Biochemistry, Kyoritsu University of Pharmacy; b Department of Pharmacology, Kyoritsu University of Pharmacy; 1–5–30 Shibakoen, Minato-ku, Tokyo 105–8512, Japan; and c Shibata Hospital Department of Medicine; Shibata, Nigata 957–8588, Japan. Received January 9, 2007; accepted July 30, 2007; published online August 1, 2007

When a human leukemic cell line, HT93 was incubated with all-trans retinoic acid (ATRA), IL-5, or both, this cell line was differentiated into eosinophilic lineage, in that an eosinophilic specific granule proteins, major basic protein (MBP) and eosinophil peroxidase (EPO) appeared. Both CD11b and CC chemokine receptor, CCR3 expression were upregulated, while CD71 expression was downregulated by ATRA or ATRA+IL-5. Concomitantly, marked production of eotaxin-2/CCL24 was observed, but no production of eotaxin-1/CCL11 and eotaxin-3/CCL26 was detected. Since only 20 to 30% cells incubated with ATRA became positive for CCR3, CCR3+ population was enriched by a magnetic activated cell sorter (MACS). Enriched CCR3+ population produced higher eotaxin-2/CCL24 than the CCR3− population, indicating that differentiated eosinophils are capable of producing eotaxin-2/CCL24. During the ATRA-induced differentiation, expression of a transcriptional factor, GATA-1 was significantly increased. Introduction of siRNA against GATA-1 markedly reduced the ATRA-induced differentiation markers including CD11b and CCR3, as well as reduced eotaxin-2/CCL24 production. Finally, ATRA-induced differentiation and eotaxin-2/CCL24 production were greatly enhanced in the GATA-1-overexpressed clones. These results indicate that the ability to produce eotaxin-2/CCL24 is acquired during the differentiation into eosinophilic lineage which is dependent on GATA-1 expression.

Key words eotaxin-2/CCL24; eosinophil differentiation; all-trans retinoic acid; CCR3; GATA-1 siRNA

Eotaxin, a member of the CC chemokine family, consists of eotaxin-1/CCL11, eotaxin-2/CCL24 and eotaxin-3/CCL26. Eotaxin coordinates the recruitment of allergic inflammatory cells, in particular eosinophils, to the sites of allergic inflammation.1,2) Eotaxins are produced mainly from inflammed epithelium, but also from macrophages, dermal or lung fibroblasts, and other minor sources,3,4) and act via the binding to chemokine receptor, CCR3 (now designated CD193).3,5) While CCR3, a member of the CC receptor family, is expressed mainly on eosinophils, basophils as well as Th2 cells, multiple ligands are able to interact with CCR3, including eotaxin-1/CCL11, eotaxin-2/CCL24, eotaxin-3/CCL26, MCP-2/CCL8, MCP-3/CCL7, MCP-4/CCL13, RANTES/CCL5, and MIP-5/CCL15.6,7) Eotaxin-2/CCL24 was first described in 1997 by random sequencing of expressed sequence tags (ESTs) in a cDNA library from activated human monocytes.5) The biological activity of eotaxin-2/CCL24 was directed against eosinophils and basophils similar to other eosinophil members; eotaxin-2/CCL24 is an effective chemotactic factor for human eosinophils, with an EC 50 of 1—10 nM. Certain cloned molecular forms of eotaxin-2/CCL24 appear to have slightly greater chemotactic potency than eotaxin-1/CCL11.5,6)

Recent investigations on atopic and nonatopic individuals indicate that eotaxin-2/CCL24 is produced in skin and lung following exposure to antigen.8) Further, IL-4 was found to be the major stimulus for eotaxin-2/CCL24 production from nasal polyps, and in addition, IL-13 and IFN-γ were also involved,8) while differential production of eotaxin subfamilies has not been elucidated.

Here, we demonstrate that a human leukemic cell line, HT93, is capable of producing eotaxin-2/CCL24 exclusively, when differentiated into an eosinophilic lineage.9) Transcriptional regulation is a key step in the commitment and differentiation of hematopoietic cells.10,11) While detailed analysis of eosinophil-specific promoters has implicated the involvement of GATA-1, CCAAT/enhancer binding protein (C/EBP), and RFX in eosinophil-specific gene expression,13—15) GATA-1 particularly, promotes eosinophil commitment of primary myeloid progenitor cells and supports their terminal differentiation.16,17) Thus far, C/EBPα is known to be essential for eosinophil development, but GATA-binding proteins also play an important role in eosinophil gene regulation, differentiation, and maturation.15,18) We hypothesized that a transcriptional factor GATA may participate in eotaxin-2/CCL24 production. Given that an increase in GATA-1 but not GATA-2 was observed during all-trans retinoic acid (ATRA)-induced differentiation, we attempted to examine the effects of downregulation and overexpression of GATA-1 on the eotaxin-2/CCL24 production by HT93 cells.

MATERIALS AND METHODS

Cell Culture HT93 cell line established from human myeloid leukemia10,19) was used throughout this study. The cells were cultured in RPMI 1640 (Nissui Seiyaku, Tokyo) with 7% fetal bovine serum (FBS; Gibco/Invitrogen Co., BRL, NY, U.S.A.) at 37°C in CO2-incubator. For the eosinophilic differentiation, cells were incubated with all trans-retinoic acid (ATRA; 1 μM), IL-5 (10 ng/ml), ATRA+IL-5, or butyric acid (BA; 0.5 mM) for 4 d, since this condition was considered to be optimal in the preliminary experiments.

Flow Cytometric Analysis Cells were stained with monoclonal antibodies including anti-human CD11b (IgG1, Nichirei Co., Tokyo), anti-human CD71 (IgG1, Exbio, Czech Republic), anti-human CCR3 (IgG3, R&D Systems, Minneapolis, U.S.A.) and FITC-conjugated rabbit anti-mouse Ig (Dako, Denmark) at the optimal dilutions. Expression of cell
surface antigens was analyzed with a FACS Calibur (Becton Dickinson Instrument Systems, NJ, U.S.A.). In the two color analysis, cells were stained first with mouse anti-human CD11b and FITC-rabbit anti-mouse Ig, then followed by the PE-conjugated mouse anti-human CCR3 (Becton Dickinson Pharmingen, NJ, U.S.A.). As a control, FITC-conjugated rabbit Ig or PE-conjugated mouse Ig was used in place of each corresponding antibody.

**Measurement of Eotaxin-2/CCL24 by ELISA**  
Eotaxin-2/CCL24 in culture medium was measured using a specific ELISA as described elsewhere. In brief, ELA plates (Nalge Nunc International Co., Denmark) were coated with a monoclonal mouse anti-human eotaxin-2/CCL24 antibody (2 μg/ml, 100 μl/well; R&D Systems Inc.) in a coating buffer (Na₂CO₃, 10 mM, NaHCO₃, 30 mM, NaCl, 3 mM). The antibody was allowed to bind overnight at 4 °C, and was then blocked with 1% BSA-PBS (150 μl/well) for 2 h at 37 °C. After 2 h incubation with either culture medium or standard at 37 °C, the plates were washed and anti-eotaxin-2 polyclonal antibody (1:2000; Dako). Absorbance at 492 nm was measured using a Labsystems Multiskan MS (Dainippon Pharmaceutical Co., Ltd., Tokyo).

**Selection of CCR3 Positive Cells**  
The cells (1 × 10⁶) were placed in 2 ml of RPMI with 5% FBS. Monoclonal anti-human CCR3 antibody (R&D Systems, U.S.A.), at a ratio of 5 μg monoclonal antibody per 10⁶ target cells, was added to the BioMag™ goat anti-mouse IgG beads (Polyscience, Inc., Germany), incubated for 20 min at 4 °C. We added 1 ml of beads/antibody complex to 2 ml of cells (total beads-to-cell ratio is 50 : 1) and gently swirled the cell/beads mixture. They were incubated for 1 h at 4 °C, and were magnetically separated by autoMACS (Miltenyi Biotec K.K., Tokyo) into CCR3⁺ and CCR3⁻ fractions. CCR3⁺ fraction contained more than 60% CCR3⁺ cells, while CCR3⁻ population contained less than 5% positive cells.

**RT-PCR**  
Total RNA was isolated using an RNeasy Mini Kit (Qiagen K.K., Tokyo). One microgram of total RNA was reverse-transcribed using a reverse transcriptase (Takara Co., Rockville, MD, U.S.A.), and three clones #5, #8, and #20 bodies against mouse, rat or rabbit Ig from Dako. The cells (1 × 10⁶) were transfected with pEF-GATA-1 and 2.5 μg plasmid. Transfection of GATA-1 Expression Vector  
HT93 cells were suspended in complete RPMI medium containing 50 μg of pEF-GATA-1 and 2.5 μg pEF-neo prepared as described. After electroporation with a single pulse (330 V, 250 μF) using Gene Pulser (Bio Rad, CA, U.S.A.), the cells were transferred into 7% FBS RPMI medium. Selection of transfected cells was done using Geneticin® (G418, Gibco, Rockville, MD, U.S.A.), and three clones #5, #8, and #20 were used in this study.

### Table 1. Primer Sequences for RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense or antisense</th>
<th>Sequence</th>
<th>Annealing</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR3</td>
<td>Sense</td>
<td>5′-TGTGTTGCACTCAGCAGAT-3′</td>
<td>65 29</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5′-TCATGCAGACGGTTGAGG-3′</td>
<td></td>
</tr>
<tr>
<td>EPO</td>
<td>Sense</td>
<td>5′-CAGGTTTCAAGGAGATCT-3′</td>
<td>60 25</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5′-TTTCTCTGTCAGGGTGTT-3′</td>
<td></td>
</tr>
<tr>
<td>MBP</td>
<td>Sense</td>
<td>5′-AGTGGTGCGGCATCTCGG-3′</td>
<td>60 25</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5′-GCCAACGCTAGTACACAC-3′</td>
<td></td>
</tr>
<tr>
<td>Eotaxin-1</td>
<td>Sense</td>
<td>5′-TCTAGGGCCTTGTTTCTT-3′</td>
<td>55 30</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5′-GAAGGATCTCAGTGGTCTG-3′</td>
<td></td>
</tr>
<tr>
<td>Eotaxin-2</td>
<td>Sense</td>
<td>5′-AATACACTTCCCTACGGGCTCT-3′</td>
<td>63 30</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5′-TGACCTCTGGACAGACACCAA-3′</td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td>Sense</td>
<td>5′-CGAGATCCTCTCAAAATCA-3′</td>
<td>60 30</td>
</tr>
<tr>
<td></td>
<td>Antisense</td>
<td>5′-TGTTGTCATGCTCCCTTACA-3′</td>
<td></td>
</tr>
</tbody>
</table>

Santa Cruz Biotechnology, Inc. Transfection of 20—25 nucleotide siRNA was carried out using Oligofectamine (Invitrogen). The cells (1 × 10⁶) were transfected with 10—100 nM siRNA with Oligofectamine in serum-free medium and incubated for 24 h at 37 °C in a CO₂ incubator. After incubation, the cells were stimulated with ATRA for 4 d and examined for eotaxin-2/CCL24 production.

**Transfection of GATA-1 Expression Vector**  
HT93 cells were suspended in complete RPMI medium containing 50 μg of pEF-GATA-1 and 2.5 μg pEF-neo prepared as described. After electroporation with a single pulse (330 V, 250 μF) using Gene Pulser (Bio Rad, CA, U.S.A.), the cells were transferred into 7% FBS RPMI medium. Selection of transfected cells was done using Geneticin® (G418, Gibco, Rockville, MD, U.S.A.), and three clones #5, #8, and #20 were used in this study.

### RESULTS

**Differentiation of HT93 Cells to an Eosinophilic Lineage by ATRA and ATRA+IL-5**  
HT93 cells were established originally from a patient with acute promyelocytic leukemia and induced to differentiate to an eosinophil-like state by ATRA and ATRA+IL-5 (10 ng/ml) for 4 d resulted in granulocyte-like differentiation with both lobulative nuclei and bubbles with eosinophilic granules.

After stimulation of cells for 4 d with ATRA, ATRA+IL-5, both CD11b and CCR3 expression were upregulated and CD71 (transferrin receptor) expression was downregulated (Fig. 1A). IL-5 alone had no significant effect on the expression of these markers. Expression of an eosinophil granule-specific protein, major basic protein (MBP) was markedly induced at day 2 by ATRA or ATRA+IL-5 and eosinophil peroxidase (EPO) was slightly induced as well (Fig. 1B). In addition, expression of the eotaxin-2/CCL24 receptor, CCR3 mRNA was induced markedly by ATRA, or ATRA+IL-5. These results indicate that treatment of HT93 cells with ATRA or ATRA+IL-5 is able to induce eosinophilic differentiation. While the effect of IL-5 was not evident in the above markers, IL-5 promoted the viability of ATRA-in-
duced eosinophil-like cells to some extent (10—20%, data not shown).

**Production of Eotaxin-2/CCL24** We were interested in determining whether eotaxin family genes, namely eotaxin-1/CCL11, -2/CCL24 or -3/CCL26 mRNA were induced by the stimulation of HT93 cells with the above stimuli, since eotaxin-1/CCL11 is a highly potent eosinophil chemoattractant and is present in mature human eosinophils. Notable mRNA expression of eotaxin-2/CCL24, but no mRNA expression of eotaxin-1/CCL11 and eotaxin-3/CCL26 was observed in the cells treated with ATRA or ATRA/H11001 IL-5 on day 2 (Fig. 2A), with no mRNA expression on day 1 and day 3 as well (data not shown). This observation was confirmed also at the protein levels, i.e., significant levels of eotaxin-2/CCL24 were produced by ATRA or ATRA + IL-5 (Fig. 2B), and no production of eotaxin-1/CCL11 and eotaxin-3/CCL26 was detected by these stimulants at least for 4 d. The differentiated cells could produce more eotaxin-2/CCL24 by the stimulation with IL-4 (Fig. 2C), suggesting that mature eosinophils are capable of responding to IL-4 and producing eotaxin-2/CCL24. It should be noted that human fetal lung-derived fibroblasts (HFL-1 cells) express marked mRNA expression of eotaxin-1/CCL11 and eotaxin-3/CCL26, but not eotaxin-2/CCL24 mRNA expression, when stimulated with 10 ng/ml TNF-\( \alpha \) and 20 U/ml IL-4 (Fig. 2A).

**Correlation of CCR3 Expression and Eotaxin-2/CCL24 Production** Since CCR3 expression was upregulated by the stimulation with ATRA and ATRA + IL-5, we assumed that cells possessing CCR3 are capable of producing eotaxin-2/CCL24. Thus, we examined the correlation of CCR3 expression and eotaxin-2/CCL24 production. Since only 20 to 30% cells became positive for CCR3 by the ATRA-treatment (Fig. 3A), HT93 cells stimulated with ATRA for 3 d were separated into CCR3\( ^{-} \) and CCR3\( ^{+} \)fractions using autoMACS. When CCR3\( ^{-} \) and CCR3\( ^{+} \)cells were separated by autoMACS, CCR3\( ^{+} \)fraction contained more than 60% CCR3\( ^{+} \)cells, while CCR3\( ^{-} \)fraction contained less than 5% (Fig. 3B). The expression of CD11b and CCR3 markers were clearly associated as shown in the later (Fig. 4B). These two populations were incubated for an additional 1 d and eotaxin-2/CCL24 production was determined. As shown in Fig. 3C, CCR3\( ^{+} \)population exhibited marked eotaxin-2/CCL24 production compared to CCR3\( ^{-} \)population, supporting the notion that CCR3\( ^{+} \)cells are capable of producing eotaxin-2/CCL24. Of note is that CCR3\( ^{+} \)cells express more GATA-1 (Fig. 3D), suggesting that CCR3 expression is regulated by a transcription factor GATA-1.

**The Role of GATA-1 in the Production of Eotaxin-**
Given this remarkable eotaxin-2/CCL24 production by the ATRA-induced differentiation, we focused on the transcriptional factors which are presumed to bind to the promoter region of eotaxin-2/CCL24 gene. Among the binding sites of various transcriptional factors identified in the eotaxin-2/CCL24 promoter region, GATA binding sites are particularly abundant [see Discussion]. In addition, a transcriptional factor, GATA-1 participates in the differentiation of eosinophils, and we focused on the GATA-1. Since enriched CCR3 cells exhibited higher eotaxin-2/CCL24 production than CCR3 cells, we examined GATA-1 expression on these two populations. As shown in Fig. 3D, CCR3 cells expressed GATA-1 protein more strongly than CCR3 cells, indicating the parallel correlation of eotaxin-2/CCL24 production with GATA-1 expression.

In addition, when siRNA against GATA-1 was transfected into HT93 (ori) cells and the cells were stimulated with ATRA, GATA-1 protein expression was suppressed by the transfection of GATA-1 siRNA at doses of 10 nM and 30 nM, reducing from 21% (13.0% to 18.0%) down to 3.0% (0.5% to 2.5%) in case of CD11b, and 32.1% (18.0% to 14.1%) down to 3.9% (2.5% to 1.4%) in case of CCR3. Under these conditions, production of eotaxin-2/CCL24 was significantly suppressed particularly at 30 nM siRNA (from 700 pg/ml down to 110 pg/ml; Fig. 4C). Namely, we demonstrated that GATA-1 is prerequisite for the differentiation and eotaxin-2/CCL24 production. In the above experiments, we could not discriminate whether GATA-1 affected on the differentiation or on the eotaxin-2/CCL24 production. In order to examine the differential role of GATA-1, we introduced GATA-1 siRNA into the fully-differentiated cells, i.e., the cells differentiated with ATRA for 4 d. In this study, we used CCR3+ population enriched from the ATRA-treated cells,
and GATA-1 siRNA was transfected on day 4. As shown in Fig. 4D, eotaxin-2/CCL24 production was significantly suppressed by the GATA-1 siRNA, suggesting that GATA-1 is playing not only in eosinophil differentiation, but also on the eotaxin-2/CCL24 production as well. The same cells were stimulated with complement component, C5a (100 ng/ml) in the presence of cytochalasin B (5 μg/ml) as shown in the ref. 22) and the eotaxin-2/CCL24 production was estimated. The production was minimally enhanced (approx. 1.3 fold) above unstimulated control with no statistic significance.

The Role of GATA-1 in the Production of Eotaxin-2/CCL24: Experiments on the Overexpression of GATA-1

We finally examined whether overexpression of GATA-1 induces the augmentation of eotaxin-2/CCL24 production. GATA-1 was transfected into HT93 (ori) cells and three clones expressing GATA-1 including clones #5, #8 and #20, were obtained (Fig. 5A). GATA-1 expression was prominent in the clone #8 and #20 (Fig. 5A) and these clones also exhibited marked expression of both CD11b and CCR3 (Fig. 5B), confirming the differentiation into eosinophilic lineage.
In contrast to the enhanced expression of GATA-1, expression of GATA-2 was unchanged by the increasing doses of ATRA as shown in the clone #8 (Fig. 5C). Concomitantly, these clones (#8 and #20) produced eotaxin-2/CCL24 up to three- to five-fold greater than that HT93 (ori) cells stimulated with ATRA (Fig. 5D). These results confirmed that GATA-1 expression is associated strongly with the eosinophilic differentiation and the eotaxin-2/CCL24 production.

**DISCUSSION**

In this study, HT93 cells were used on the basis of reports that they can be induced to differentiate to eosinophil lineages. This cell line was differentiated into eosinophil-like or monocyte-like states by the treatment with ATRA, IL-5 or both; or butyric acid. Expression of CD11b was induced, while CD71, transferrin receptor which expresses on the proliferating cells, was downregulated by these stimuli. It should be noted that expression of CCR3 and eosinophil granule proteins was markedly induced by ATRA. These results indicate that treatment with ATRA or ATRA+IL-5 induces HT93 cells to eosinophil-like differentiation. Our notable finding is that HT93 cells are capable to express CCR3 as well as the eotaxin-2/CCL24 production simultaneously, when treated with ATRA. We also demonstrated that CCR3+ cells are responsible to produce eotaxin-2/CCL24. It does not necessarily mean that CCR3+ eosinophils release eotaxin-2/CCL24 at the inflammatory sites, since no direct data on the eotaxin-2/CCL24 production was presented at our hands. In this regards, however, we should mention that normal eosinophils released eotaxin (e.g., eotaxin-1/CCL11, but not eotaxin-2/CCL24) when stimulated with two secretagogues, C5a or ionomycin in the presence of cytochalasin B. HT93 cells differentiated to an eosinophil-like state by ATRA may serve as a useful in vitro model of eosinophils. While the effect of IL-5 was not evident in the expression of eosinophil markers, IL-5 promoted the viability of the differentiated eosinophils as described previously, but no significant supporting of cell viability was evident, probably because ATRA did not induce this cell line to cell death during 4 d-incubation period.

So far, two human cell lines, HL60clone15 and Eol-1 have been reported to differentiate into the eosinophil lineage by the treatment with n-butyrate. While n-butyrate is a most powerful inducer of the eosinophilic differentiation of these cell lines and has been intensively studied, the mechanism by which HL60clone15 differentiates into eosinophilic lineage remains to be elucidated. Recently, it has been proposed that since n-butyrate continuously inhibits histone deacetylation and the histone deacetylase inhibitors such as trichostatin effectively induce eosinophil differentiation, continuous acetylation of histon H3 and H4 is necessary for the differentiation into eosinophils. Whether this mechanism is also involved in the differentiation by ATRA is the HT93 cells remains to be solved and should be tested in the near future. Another important issue is whether normal human eosinophils or those of allergic patients are capable of producing eotaxins. It has been pointed out that normal eosinophils release eotaxin (eotaxin-1/CCL11) when stimulated with two secretagogues, C5a or ionomycin in the presence of cytochalasin B. We tested this notion, but the differentiated HT93 cells are capable of producing but minimal amount of eotaxin-2/CCL24 production under the same condition, probably because the cells were already prestimulated with ATRA and retained only low eotaxin-2/CCL24 producing capacity.

We have previously detected eotaxin-1/CCL11 or eotaxin-3/CCL26 production in primary human fetal lung fibroblasts (HFL-1) stimulated with IL-4, TNF-α or both. However, ATRA-induced differentiated HT93 cells produced eotaxin-2/CCL24, exclusively. In addition, eotaxin-1/CCL11 or -3/CCL26 production was not detected by the treatment with IL-4, TNF-α or both in the HT93 cells (data not shown). The reasons of the differences between fibroblasts and leukemic cells in their ability to produce different eotaxins are yet unknown, but the nuclear factors acting eotaxin promoters are different. It should be tested which cells and tissues are favorable to produce eotaxin-1/CCL11, -2/CCL24 or -3/CCL26 and why the different cells and tissues produce different set of eotaxins is of interesting topics to be explored. Since all of the three eotaxins have potent eosinophil chemoattractant and are involved in the allergic inflammation, the kinetics of the eotaxin species are different. For instance, up-regulation of eotaxin-1/CCL11 is at early stages of allergic reaction, while eotaxin-2/CCL24 production is observed at later stages. In addition, stimulants inducing eotaxin production are different among the eotaxin species. Eotaxin-1/CCL11 and eotaxin-3/CCL26 are produced mainly from dermal or lung fibroblasts, endothelial cells or macrophages, while eotaxin-2/CCL24 is not produced by the fibroblasts or endothelial cells as shown in Fig. 1A. Of note is that eotaxin-2/CCL11 generation is differentially regulated by lipopolysaccharide (LPS) and IL-4 in monocytes and macrophages. Eotaxin-2/CCL11 production is upregulated by LPS or proinflammatory cytokines including IL-1β, while eotaxin-1/CCL11 production is enhanced by the Th2 cytokines, IL-4 and IL-13, which in contrast down-regulated eotaxin-2/CCL24 production. Our observation is novel in that leukemic cells when differentiated into eosinophilic lineage can produce eotaxin-2/CCL24 but not eotaxin-1/CCL11 or eotaxin-3/CCL26. Whether eosinophils recruited in the allergic inflammatory sites are actually able to produce eotaxin-2/CCL24 should be evaluated, but we postulate such possibility in this study.

Eotaxins bind exclusively to the CCR3 which expresses selectively on the eosinophils, mast cells, basophils as well as a part of Th2 cells. CCR3 expression on eosinophils, in particular, may account for the striking accumulation of eosinophils at the sites which eotaxin is produced. As for CCR3 ligands, such as eotaxins (CCL11, CCL24 and CCL26) and CCL13/MCP-4, CL5/RANTES and CCL28/MEC are produced at the allergic inflammatory sites. We propose that the eosinophils recruited at the inflammatory sites could contribute to enhance allergic inflammation not only as cells producing MBP or ECP, but also as cells producing eotaxins.

The role of specific transcription factors during hematopoietic stem cell differentiation has been reported. GATA-binding sites have been found in the promoters of EPO or MBP genes. In the promoter region of eotaxin-2/CCL24 gene, GATA binding sites are particularly abun-
dant, since putative GATA-binding sites are estimated to be 9 for GATA-1, 3 for GATA-2 and 1 for GATA-3 at least in the upper 1kb promoter region of eotaxin-2/CCL24 gene (see GenBank; AC005102). In addition, GATA-1 expression is particularly increased during ATRA-induced differentiation as indicated in our study. In order to clarify the role of GATA-1 in the eotaxin-2/CCL24 gene expression, we introduced siRNA against GATA-1 in HT93 cells or established GATA-1 overexpressing clones. Production of eotaxin-2/CCL24 was effectively suppressed by siRNA, but was increased in GATA-1 overexpressing cells. It is hard to discriminate whether GATA-1 affects directly or sequentially on the differentiation and on the eotaxin-2/CCL24 production. At least from our data, we assumed that GATA-1 is prerequisite not only for the differentiation into eosinophilic lineage but also on the production of eotaxin-2/CCL24, since introduction of GATA-1 siRNA into the fully-differentiated cells significantly suppressed eotaxin-2/CCL24 production. Namely, these results suggested that GATA-1 is prerequisite for the production of eotaxin-2/CCL24 as well as the differentiation into eosinophilic lineage. Interesting finding is recently reported that a transcription factor GATA-1 binds to untranslated exon-1 of CCR3 gene and this region works as a promoter, indicating that GATA-1 works not only for the induction of eosinophilic lineage but also for the induction of CCR3.

In conclusion, we have shown that eosinophil-like differentiated cells acquire the ability to release eotaxin-2/CCL24, dependently on GATA-1. On these bases we speculate that eosinophils may be involved in accelerating inflammation via eotaxin-2/CCR3 signaling. The effect of eotaxin-2/CCL24 on eosinophils and other CCR3-expressing cells, i.e., basophils, mast cells and Th2 lymphocytes, in allergic inflammatory sites requires clarification. Further, an interesting finding is recently reported that mice with defects in eosinophil development (Adbl-GATA) and eosinophil recruitment (mice deficient in CCR3 knockout) and mice deficient in eotaxin-2/eotaxin-1/2 double knockout abolished allergen-induced eosinophil recruitment almost completely and impaired total allergic airway inflammation. Namely, multiple lines of independent evidences indicate that eosinophils via CCR3 have a central role in chronic allergic airway diseases.

Acknowledgements We thank to Dr. Yuji Yamaguchi, Fukuoka Urazoe Clinic, Fukuoka-shi, for giving us the expression vector of GATA-1. We also thank to Ms. Maki Hasegawa and Akiko Rokudai for their technical assistance. This work was done in part by the grants (No. 16390024) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES