

Involvement of CCR3-Reactive Chemokines in Eosinophil Survival

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Key Words

Eotaxin · Apoptosis · GM-CSF · CC chemokine

Abstract

Background: Although eosinophils undergo apoptosis and are thus eliminated from sites of inflammation, they survive longer if survival factors, such as IL-3, IL-5 and GM-CSF, are present. However, it is often observed that some eosinophils survive even when incubated without any survival factors (spontaneous survival). The aim of the present study was to investigate what kind of factor(s) is associated with this spontaneous survival of eosinophils. **Methods:** Eosinophils were purified from bronchoalveolar lavage of antigen-exposed Balb/c mice and stained with propidium iodide to detect apoptotic ones. **Results:** We found that the spontaneous survival of eosinophils was reduced by treatment of the cells with anti-CC chemokine receptor-3 (CCR3) antibody (Ab). Moreover, eotaxin-1, eotaxin-2, and eotaxin-3 all prolonged the survival of eosinophils in a dose-dependent fashion. The survival-prolonging effect of eotaxin-1 was enhanced in the presence of eotaxin-3, indicating that these chemokines might work synergistically. **Conclusion:** We speculate that eosinophils survive longer under

the influence of CCR3-reactive chemokines, which aid the infiltration of these cells into the tissue and that eosinophils may survive even longer if they encounter survival factors at local inflammatory sites.

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Introduction

The eosinophil, a pro-inflammatory granulocyte, has been implicated in the body's defense against parasitic helminth infections, and it may play a major role in allergic diseases, such as bronchial asthma [1]. Eosinophils exert their effects by releasing cytotoxic proteins, lipid mediators, oxygen metabolites, and cytokines, all with the potential to induce pathophysiological changes [2]. A pulmonary segmental allergen challenge to sensitive individuals causes eosinophil recruitment into the airways and the associated release of biologically active proteins from intracellular granules together with an increase in vascular permeability [3, 4]. Indeed, even in patients with mild asthma, eosinophil infiltration of the respiratory mucosa and epithelium is a consistent finding, the number of infiltrating eosinophils correlating with the severity of the disease [5]. However, despite an increasingly sophisti-

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cated understanding of how eosinophilic airway inflammation is induced, the determinants of the severity and persistence of this response remain relatively obscure.

The development of eosinophilic lung inflammation requires the coordinated expression of hematopoietic cytokines, adhesion molecules, and chemokines [6]. Numerous chemokines have been identified as eosinophil chemoattractants: these include RANTES and MIP-1 α [7]. However, these chemokines do not selectively promote eosinophil recruitment. In contrast, eotaxin (of which there are three homologues: eotaxin-1, -2, and -3) does act as an eosinophil-selective chemoattractant. Eotaxin is a CC chemokine generated predominantly from inflamed epithelium, but also from macrophages, fibroblasts, and other minor sources [8]. CCR3, which is a CC chemokine receptor selectively expressed on eosinophils but not on neutrophils or monocytes, is considered a promiscuous receptor for it interacts with multiple ligands, including eotaxin-1, -2, -3, MCP-2, -3, -4, RANTES, and MIP-5 [9]. However, eotaxin-1, -2, and -3 recruit both eosinophils and a subset of antigen-experienced Th2-biased lymphocytes via a selective activation of the CCR3 receptor [10]. Eotaxin is highly expressed both in human asthmatics and in animal asthma models [11, 12], and intratracheal administration of eotaxin in mice leads to a rapid and substantial accumulation of eosinophils in bronchoalveolar lavage (BAL) fluid [8]. In guinea pigs, eotaxin caused an accumulation of eosinophils within the lung [13, 14], and Northern blot analysis revealed a consecutive expression of eotaxin mRNA in the airways that increased considerably following an allergen challenge [14, 15]. Furthermore, in clinical asthma, the degree of eotaxin expression is directly related to the degree to which lung function is impaired [16].

The intensity of eosinophilic inflammation is regulated, in part, by the readiness with which eosinophils undergo apoptosis (programmed cell death). Known eosinophil survival factors (such as IL-5, which signals via a β c-chain shared with GM-CSF and IL-3) are highly expressed in the inflamed lung [17–19]. On the other hand, some eosinophils survive after 72 h in culture even if survival factors are lacking [spontaneous survival, ref. 20]. If apoptosis were induced more quickly among eosinophils after their infiltration into a tissue, serious inflammation would not occur. We therefore thought it worthwhile to investigate which factors might be associated with the spontaneous survival of eosinophils. In this experiment, we purified eosinophils by a negative purification method that is a very rapid lectin-based one to obtain active eosinophils [21].

Materials and Methods

Animals

All experimental procedures conformed to international standards of animal welfare and were approved by the Laboratory Animal Committee of Kissei Pharmaceutical Co. Ltd.; they also conformed to current Japanese law. Male Balb/c mice (SPF; 5 weeks old) purchased from SLC Japan Inc. (Shizuoka, Japan) were maintained under a 12-hour light/12-hour dark cycle, and had free access to water and standard laboratory food until the day of the experiment.

Induction and Purification of Lung Eosinophils

We utilized a newly developed lectin-based isolation procedure to recover ultrapure eosinophils rapidly from the BAL fluid obtained aseptically from ovalbumin (OVA)-immunized and -challenged male Balb/c mice (20–25 g; 6–7 weeks) [21]. Briefly, mice were immunized and boosted with 10 μ g of OVA (grade V; Sigma) adsorbed onto Al(OH)₃ gel (Serva, France) and administered intraperitoneally on days 0 and 14. On day 21, the mice were challenged [using an aerosol of OVA (50 mg/ml) delivered for 20 min via a jet nebulizer (Pari Master, Starnberg, Germany)] to elicit pulmonary eosinophilic inflammation. On day 23, each mouse was anesthetized with urethane (0.3 ml, 14% w/v in water, i.p.), its trachea cannulated (SP 30; Duran Plastics, UK), and its lungs lavaged with four 0.3-ml aliquots of PBS at room temperature. The 48-hour time point was chosen as this induces a selective eosinophil infiltration, with <2% neutrophils or lymphocytes, thus allowing eosinophils to be purified. In contrast, earlier time points (e.g., 24 h) were associated with a mixed granulocyte infiltration. Furthermore, we could not obtain a sufficient number of eosinophils at later time points (e.g., 96 h).

Eosinophils were purified by lectin affinity negative selection, as reported previously [21]. In brief, Dynabeads coated with anti-CD4, -CD8, and -CD19 antibodies (DynaL, Oslo, Norway) were added to the BAL specimens to remove lymphocytes. Then, 10 μ g/ml biotinated *Griffonia simplicifolia* Lectin I (BSL-I; Vector Labs, California, USA) was incubated with pooled lavages for 60 min. Lectin-bound cells were allowed to adhere to streptavidin-conjugated magnetic beads (Dynabeads; Dynal) for 10 min, and were then selected by using a strong magnetic field (DynaL).

Eosinophil purity was determined by staining cytospin preparations (Shandon Scientific Ltd., UK) with Diff-Quik (Baxter Diagnostics, Switzerland), and differential cell counts were made according to standard morphological criteria. This procedure routinely yielded >98% pure eosinophils with $99.1 \pm 8.2\%$ viability ($92.8 \pm 9.8\%$ cells exhibited intact DNA; $n = 5$). In previous experiments, eosinophils were not activated during the purification procedure [21]. Furthermore, the viability obtained by this method was similar to that reported earlier, where eosinophils were purified from mouse BAL by a density gradient centrifugation method [22]. We used 5 mice for purification of eosinophils because this method gave a high yield of the cells from just a few mice. But, on the other hand, there is a possibility that our results may not reflect the mouse population in general; although reproducibility was observed.

Detection of Apoptosis in Eosinophils

Apoptotic eosinophils were measured by FACS as the reduction in DNA content secondary to chromatin fragmentation, with propidium iodide (PI) used as the DNA stain [21, 22]. Briefly, purified eosinophils (2×10^5 /well) were cultured in 96-well plates either in

medium (DMEM) alone or in the presence of a CC chemokine [rm-eotaxin-1, rm-eotaxin-2, rh-eotaxin-3, rm-RANTES, rm-MCP-3, or rm-MIP-1 α (all purchased from Pepro Tech, Rocky Hill, N.J., USA)] at the desired concentration. The number of cultured eosinophils did not change in the presence or absence of these cytokines. The cells were transferred to 5-ml polypropylene FACS tubes, and 0.2 ml of ice-cold PI staining buffer (comprising 0.5 mg PI, 0.1 ml of 10% sodium citrate, and 0.1 ml of 10% Triton X-100 in distilled water) was added to each tube [23]. The samples were gently vortexed, sealed, and stored overnight at 4°C in the dark. Data were collected as 10,000 PI-gated fluorescent events. Fluorescence analysis refers to all scatter-defined living cells and to all acquired cell fragments of PI single-stained samples to avoid not counting the degranulated eosinophils.

To determine which cytokines might enhance the survival of eosinophils cultured with eotaxin, a single cytokine antibody (Ab) [anti-mCCR3 (23321A), anti-mIL-3 (18010D), anti-mIL-4 (18190D), anti-mIL-5 (18050D), anti-mIL-9 (18450D), anti-mGM-CSF (18090D), anti-mIFN- γ (18180D) Ab (from BD Pharmingen, USA), anti-mIL-13 (43413), anti-hTGF- β (431835), anti-hEGF (43236), or anti-hIGF-1 (42291)], each from Genzyme Technne, USA, was added to the medium (final conc. 5 μ g/ml). Cell death by apoptosis was analyzed by flow cytometry following staining with PI, as described above.

Cytokine Assay

Eosinophils (5×10^5 cells/well in 0.2 ml of DMEM with 0.1% FCS) were cultured in 96-well plates, and then the supernatants were collected at 72 h and stored at -20°C until assayed. GM-CSF, IL-3, IL-5, and IFN- γ were measured by using ELISA kits (Endogen Inc., Woburn, Mass., USA), the sensitivities of detection being 10, 10, 20 and 37 pg/ml, respectively. Eotaxin-1 was measured with an ELISA kit obtained from R&D Systems (Minneapolis, Minn., USA), the sensitivity of detection being 15.6 pg/ml.

Statistical Analysis

Data were expressed as means $\pm$ SEM. Pairs of groups were compared using a Student's *t* test. The statistical analysis of the prolonging effect of eotaxin on eosinophil survival was performed using a one-way ANOVA followed by a post-hoc analysis of variance (Dunnett's test for multiple comparisons). *p* values <0.05 were considered significant.

Results

Role of CCR3 in the Survival of Eosinophils

We screened several antibodies that might reduce the survival of eosinophils purified 2 days after the OVA challenge, and we found that anti-CCR3 Ab reduced the survival of eosinophils cultured in medium alone (compare fig. 1b with isotype control, fig. 1a). Evidence of this inhibitory effect of anti-CCR3 Ab on spontaneous survival was first observed in 24-hour cultures, although it was not significant at this time (fig. 2). Half of the spontaneously surviving eosinophils had died as a result of apoptosis in the presence of anti-CCR3 Ab (5 μ g/ml) at

48 h ($31.5 \pm 5.3\%$, medium vs. $15.3 \pm 2.2\%$, α -CCR3) and also at 72 h ($16.3 \pm 2.7\%$, medium, vs. $8.4 \pm 2.2\%$, α -CCR3). An increased concentration of anti-CCR3 Ab (15 μ g/ml) completely inhibited the spontaneous survival of eosinophils at 72 h ($2.8 \pm 0.6\%$). Furthermore, eosinophils purified at later time points (4 days) showed less spontaneous survival, but it was strongly inhibited by anti-CCR3 Ab (table 1). Eotaxin-1 was not detectable in the medium after 72 h in culture (data not shown).

Eotaxin Promotes Eosinophil Survival

To test the effect of CC chemokines on eosinophil apoptosis, we initially added a single high concentration (10 μ g/ml) of each of the CC chemokines, i.e., eotaxin-1, eotaxin-2, eotaxin-3, RANTES, MCP-3, and MIP-1 α . After 72 h in culture, the cells were harvested, stained with PI, and analyzed by FACS. Eosinophil survival was $44.0 \pm 0.7\%$ (*n* = 5, *p* < 0.01) in the presence of 10 ng/ml IL-5 against only $15.2 \pm 0.8\%$ (*n* = 5) in medium alone (table 2). Among the CC chemokines, eotaxin-1, -2, and -3 exerted powerful survival effects on the eosinophils, whereas RANTES and MCP-3 had very weak effects. MIP-1 α slightly, but significantly, increased their survival (table 2). Interestingly, eosinophils purified 4 days after the antigen challenge showed a stronger survival response to eotaxin-1 than those obtained at 2 days (table 1).

We next established that eotaxin-1, -2, and -3 each dose dependently prolonged the survival of eosinophils in the absence of any additional survival factors. In fact, the effect of eotaxin-1 and -3 were significant over the dose range of 0.15–15 μ g/ml; whereas eotaxin-2 had significant effects at 1.5–15 μ g/ml (fig. 3). Although eotaxin-1 and -3 were equally effective, eotaxin-2 was less potent (but, see Discussion).

The presence of the anti-CCR3 Ab (5 μ g/ml) greatly reduced the survival-prolonging effect of 5 μ g/ml eotaxin-1 (compare fig. 1e with isotype control, fig. 1d). But this concentration of the Ab had no effect on 15 μ g/ml eotaxin-1-induced survival ($43.2 \pm 0.5\%$, control, vs. $42.8 \pm 0.5\%$, α -CCR3). An increased concentration of CCR3 mAb (15 μ g/ml) completely inhibited eotaxin-induced survival ($2.13 \pm 0.48\%$ at 1.5 μ g/ml eotaxin-1, 6.7 ± 0.76 at 5 μ g/ml eotaxin-1), while it partially inhibited 15 μ g/ml eotaxin-induced survival ($21.6 \pm 2.52\%$). The survival-prolonging effects of eotaxin-2 and eotaxin-3 were also inhibited by anti-CCR3 Ab (data not shown). Isotype control Ab did not alter the survival-promoting effects.

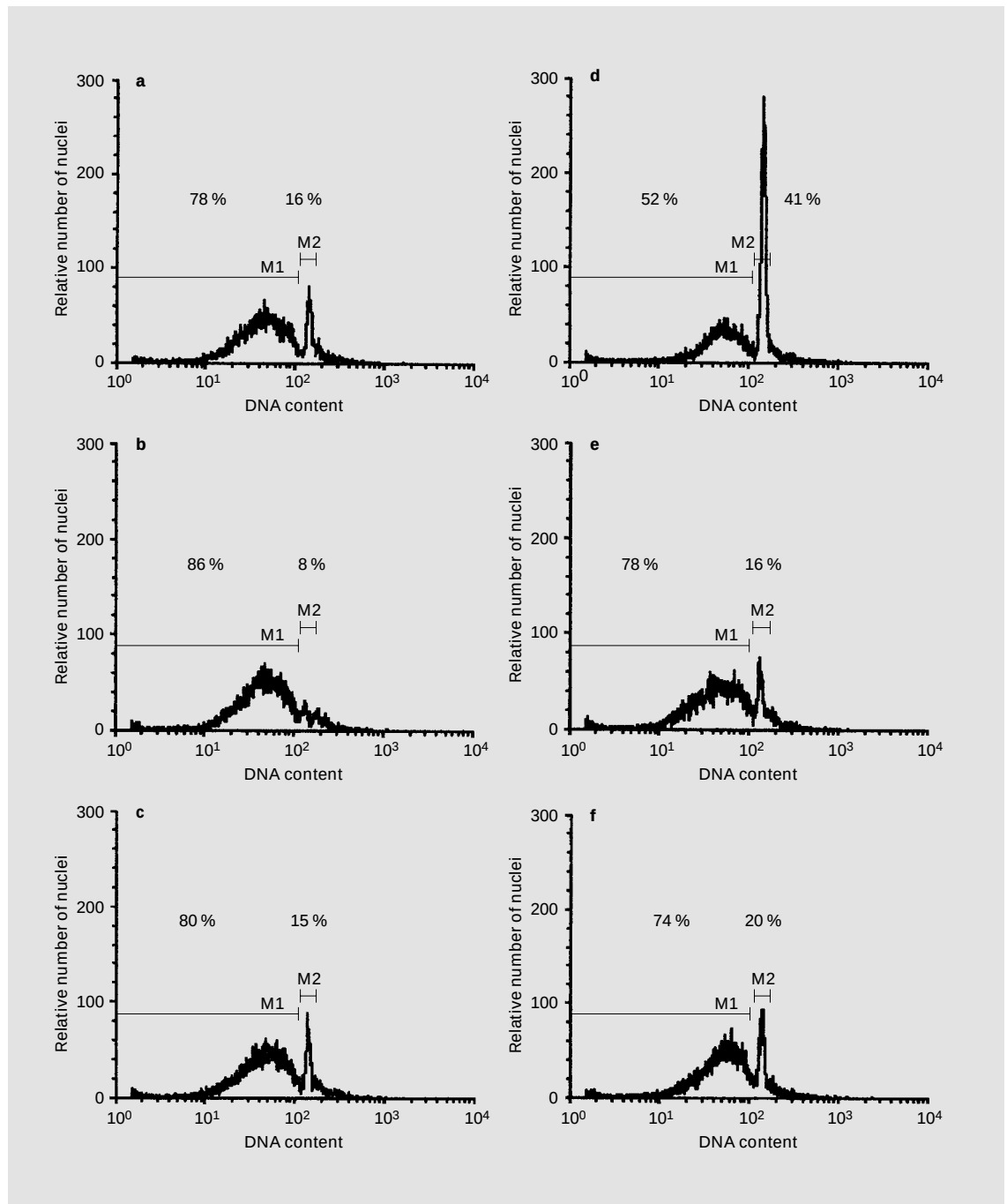


Fig. 1. Typical PI staining of eosinophils. Eosinophils were cultured for 72 h in the presence (d–f) or absence (a–c) of cotaxin-1 (5 μ g/ml). Anti-CCR3 (b, e), anti-GM-CSF (c, f), or isotype control Ab (a, d) were added to the medium (each at 5 μ g/ml). M1 and M2 represent apoptotic and nonapoptotic cells, respectively.

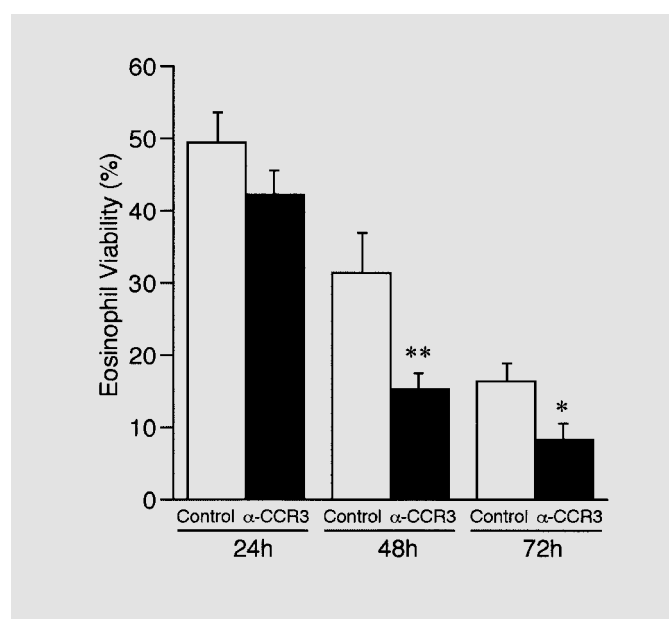


Fig. 2. Survival of eosinophils cultured in the absence of survival reagents is reduced by anti-CCR3 Ab. Eosinophils harvested at 2 days after antigenic challenge were cultured for 24, 48, or 72 h in the absence of survival reagents. Anti-CCR3 Ab or isotype control Ab was added to the medium, and the viability of the eosinophils was assessed by using PI staining. Each bar shows the mean $\pm$ SEM from five experiments. * $p < 0.05$, ** $p < 0.01$ vs. isotype control Ab.

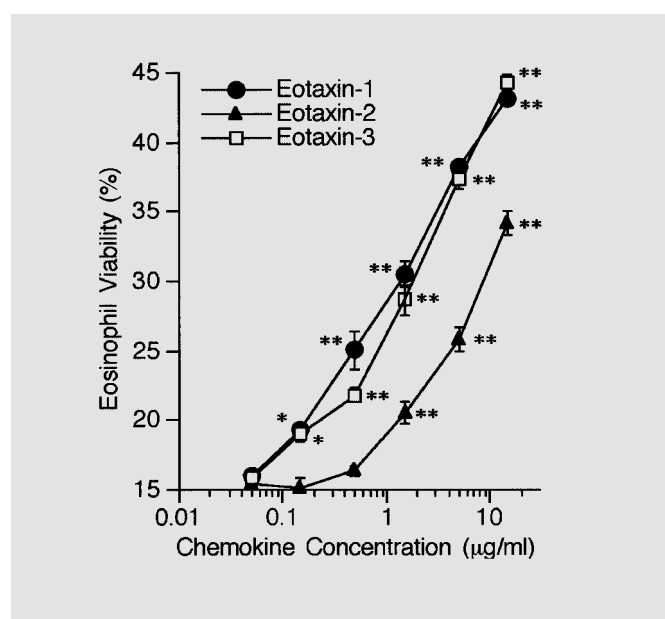


Fig. 3. Dose dependence of effects of eotaxins on eosinophil survival. Eosinophils were cultured for 72 h in the presence of eotaxin-1, -2, or -3. Viability of eosinophils was then assessed by using PI staining. Each datum point shows the mean $\pm$ SEM from 5 experiments. * $p < 0.05$, ** $p < 0.01$ vs. medium alone.

Table 1. Effect of harvesting time on eosinophil survival

Time	Spontaneous survival	Spontaneous survival + α-CCR3	Eotaxin (10 μg/ml)-induced survival
2 days	16.3 $\pm$ 2.7	8.4 $\pm$ 2.2	42.5 $\pm$ 0.5
4 days	9.2 $\pm$ 1.0	2.5 $\pm$ 0.5	50.8 $\pm$ 1.1

Eosinophils were harvested at the indicated time points and cultured in the presence or absence of eotaxin-1 or 5 μg/ml anti-CCR3 Ab (α-CCR3). Viability of eosinophils was assessed using PI staining after 72 h in culture. Data are presented as the mean $\pm$ SEM of 3–5 experiments.

Synergistic Effects of Eotaxin-1 and Eotaxin-3 on Eosinophil Survival

We next examined whether two of these CC chemokines might act synergistically to prolong the survival of eosinophils. When eosinophils were cultured with 0.5 μg/ml of either eotaxin-1 or eotaxin-3 alone, the survival was 21.9 ± 2.7 and $22.2 \pm 3.7\%$, respectively (fig. 4). These values were exceeded significantly when eotaxin-1 was combined with eotaxin-3 ($35.1 \pm 2.1\%$), indicating that

these CC chemokines can act synergistically to increase the survival of eosinophils.

Autocrine Eosinophil GM-CSF Mediates the Survival-Enhancing Effects of Eotaxin, but Not Spontaneous Survival Itself

To try to understand how eotaxin-1 might prolong the survival of eosinophils, we screened neutralizing antibodies against known survival factors (IL-3, IL-5, GM-

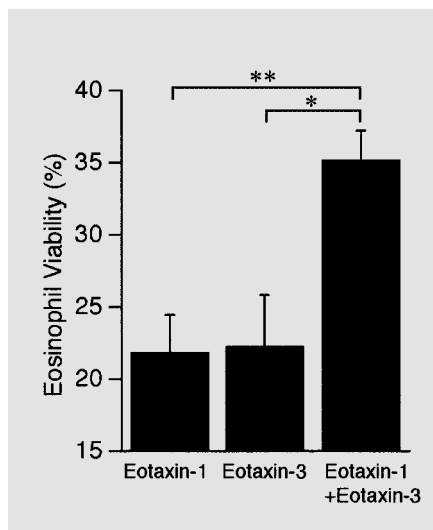


Fig. 4. Synergistic effects of eotaxin-1 and eotaxin-3 on eosinophil survival. Eosinophils were incubated with eotaxin-1 or eotaxin-3 (each, 0.5 μ g/ml) for 72 h. For examination of a possible synergistic effect of these chemokines, eosinophils were incubated with eotaxin-1 plus eotaxin-3. Viability was assessed by PI staining. Each bar shows the mean $\pm$ SEM from 5 experiments. * $p < 0.05$, ** $p < 0.01$.

Table 2. CC-chemokine-enhanced eosinophil survival

Chemokine	Conc., μ g/ml	Survival, %
Eotaxin-1	10	42.5 $\pm$ 0.5**
Eotaxin-2	10	32.7 $\pm$ 1.9**
Eotaxin-3	10	44.0 $\pm$ 3.1**
RANTES	10	15.8 $\pm$ 1.6
MCP-3	10	15.0 $\pm$ 0.6
MIP-1 α	10	20.4 $\pm$ 0.7**
IL-5	0.01	44.0 $\pm$ 0.7**
No cytokine	0	15.2 $\pm$ 0.8

Eosinophils were cultured in the presence or absence of 10 μ g/ml of one of the indicated CC chemokines. Viability of eosinophils was assessed by using PI staining after 72 h in culture. Data are presented as the mean $\pm$ SEM of 5 experiments. ** $p < 0.01$ vs. no cytokine.

CSF, and IFN- γ). After 72 h in culture, eosinophils were harvested and stained with PI. Anti-IL-3, -IL-5 or -IFN- γ Ab had no effect on the survival of eosinophils cultured with 5 μ g/ml eotaxin-1 (41.5, 40.0 and 39.6% viability, respectively; data not shown). In contrast, anti-GM-CSF abrogated the anti-apoptotic effect of eotaxin-1 (20.0% viability, fig. 1f). GM-CSF was detected in the culture

medium after the cells had been incubated for 72 h with eotaxin-1 (167.3 $\pm$ 17.3 pg/ml), but we could not detect IL-3, IL-5, or IFN- γ . However, anti-GM-CSF did not affect the spontaneous survival of eosinophils (fig. 1c), nor did IL-3, IL-5, or IFN- γ (data not shown), whereas anti-CCR3 Ab reduced it by one half (fig. 1b). Although we investigated other antibodies associated with asthma (anti-IL-9, -IL-13, -TGF- β , -EGF, and -IGF-1), no inhibition was observed with any of them.

Discussion

In this experiment, we obtained evidence strongly suggesting that the survival of eosinophils purified from BAL is prolonged via a mechanism that involves CCR3 in the absence of survival factors such as IL-3, IL-5, and GM-CSF (spontaneous survival). This spontaneous survival was inhibited by anti-CCR3 Ab, suggesting that CCR3 signals are important for the spontaneous survival of eosinophils. The effect of this pathway is modest in comparison with that of other factors such as IL-5 or GM-CSF, but eosinophils utilize this pathway when these cytokines are lacking. There are reports in the literature indicating that eosinophils themselves produce eotaxin [24], and so eosinophils could survive using eotaxin, or indeed other CCR3-reactive chemokines, that they themselves produce. Therefore, the above observations indicate that the eosinophils recruited into inflammatory tissue from the blood can survive and remain in the tissue even if they do not receive any survival signals. The translocation of eosinophils to the bronchial mucosa leads potentially to a priming of the subsequent immune responses and an augmentation of allergic pulmonary inflammation via the secretion of various cytokines [25, 26]. Hence, we speculate that eotaxin may be very important both in the initial stages of eosinophilic inflammation and for its exacerbation.

In our experiment, 16% of the eosinophils survived after 72 h in medium alone, but only 3% survived in the presence of anti-CCR3 Ab. This indicates that about 13% more eosinophils survived for this time when CCR3-reactive chemokines were present. Although these responses were modest, we tried to clarify which chemokine might be associated with this CCR3-mediated eosinophil survival-prolonging effect. We found that eotaxin-1, eotaxin-2, and eotaxin-3 all possessed the ability to prolong the survival of eosinophils. As was shown in figure 3, eosinophil survival was about 16% with 50 ng/ml eotaxin-1 or -3 (not significantly different from the value seen in the

absence of eotaxin), and about 19.5% (significantly greater than that without eotaxin) with 150 ng/ml of either of these eotaxins. In other words, to produce a significant enhancement of eosinophil survival after 72 h in culture, a concentration greater than 50 ng/ml eotaxin-1 (or eotaxin-3) must be present. BAL eotaxin levels were reported to be about 1.4 ng/ml [27] in a murine asthmatic model and about 58 ng/ml [7 nM, ref. 12] in a guinea pig model. These levels of eotaxin are at, or below, the concentrations needed to enhance eosinophil survival (according to our experiment). These facts suggest that in an asthmatic animal, these chemokines are unlikely to be present at a sufficient concentration to prolong eosinophil survival if each has to act alone. Consequently, we speculated that two or more CCR3-reactive chemokines might work synergistically to prolong the survival of eosinophils, and indeed we found evidence that eotaxin-1 and eotaxin-3 could prolong the survival of eosinophils by acting in a synergistic fashion, as shown in figure 4.

The eotaxin-induced survival of eosinophils was inhibited by anti-GM-CSF Ab, and GM-CSF was detected in the supernatant of the culture medium. These data suggest that GM-CSF is produced from eosinophils following their stimulation by eotaxin, and is then used by them to help prolong their survival. However, anti-GM-CSF Ab had no effect on the spontaneous survival of eosinophils, suggesting that the mechanism responsible for spontaneous survival itself may be different from that responsible for the eotaxin-induced enhancement of survival. The former mechanism is of interest to us, but none of the antibodies we tested (those against IL-3, IL-4, IL-5, IL-9, IL-13, IFN- γ , TGF- β , EGF, and IGF-1) affected eosinophil survival per se. This strongly suggests that these cytokines, which are closely related to asthma, are not involved in mediating spontaneous survival among eosinophils. In the case of spontaneous survival, we propose that eosinophils are in a critical situation such that the CCR3 stimulation directly produces the survival-prolonging signal without using the GM-CSF pathway. Eosinophils purified 4 days after the challenge were more sensitive to anti-CCR3 Ab stimulation than those obtained at 2 days, indicating that CCR3 pathway is enhanced in starved eosinophils. Therefore, we speculate that eosinophils use this pathway more if the lack of survival factors continues. We clearly need to carry out further studies on signal transduction in eosinophils to understand this mechanism.

We found differences in potency among eotaxin-1, -2, and -3, with eotaxin-2 being less potent at prolonging eosinophil survival than either eotaxin-1 or eotaxin-3.

Previously, Zimmermann et al. [28] reported differences in activity levels among different batches of eotaxin-2. We could not examine this point because we could obtain only one batch of eotaxin-2, but it is possible that we would have seen more powerful effects with eotaxin-2 had we used a different batch. Although it is unknown whether mouse eotaxin-3 exists, the response to human eotaxin-3 was inhibited by anti-CCR3 Ab just as were those responses to eotaxin-1 and eotaxin-2. Therefore, human eotaxin-3 would appear capable of prolonging eosinophil survival via the mouse CCR3 receptor, and so we can assume that mouse eotaxin-3 (if it exists) would have the same effect.

There have been reports claiming that eotaxin had no effect on eosinophil survival [29, 30]. Contrary to these observations, our results showed that the survival of eosinophils was prolonged by eotaxin. The reason for this discrepancy is probably related to the method of eosinophil purification. With our method, we could obtain eosinophils within a short time and at a high yield, as reported previously [21]. By contrast, purification of eosinophils using the gradient separation method is a time-consuming one and gives a low yield, as judged from previous reports [29, 30]. Therefore, we speculate that eotaxin-responsive eosinophils either died or were lost during the latter process. There is another report claiming that eotaxin does not operate as a survival factor [31]. In that study, the authors purified eosinophils by nongradient methods from the spleens of IL-5 transgenic mice, and reported that the viability of eosinophils was not affected by eotaxin at 1,000 ng/ml. We speculate that the signals in IL-5 transgenic mice may differ from those in wild mice and that the former's eosinophils may be insensitive to the survival signals from eotaxin.

In conclusion, we found that eotaxin and its related compounds enhanced mouse eosinophil survival via the CCR3 receptor. These results suggest that in the inflamed lung, CCR3-reactive chemokines regulate the intensity of eosinophilic inflammation not only by recruiting eosinophils, but also by enhancing their spontaneous survival.

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