Increased responsiveness of murine eosinophils to MIP-1β (CCL4) and TCA-3 (CCL1) is mediated by their specific receptors, CCR5 and CCR8

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Abstract: In the present study, we investigated the regulation of chemokine-mediated responses and receptor expression on eosinophils from mice. MIP-1α (CCL3) and eotaxin (CCL11) induced a significant and only partially overlapping intracellular calcium flux in antigen-elicited and peripheral blood eosinophils, and MCP-1 (CCL2), MDC (CCL22), MIP-1β (CCL4), and TCA-3 (CCL1) did not. To demonstrate functional use of the specific receptors, we examined chemotactic responses. Peripheral blood eosinophils migrated toward MIP-1α (CCL3) and eotaxin (CCL11) but not MCP-1 (CCL2), MDC (CCL22), MIP-1β (CCL4), and TCA-3 (CCL1). Antigen-elicited eosinophils migrated toward MIP-1α (CCL3) and eotaxin (CCL11), but also migrated in response to MIP-1β (CCL4) and TCA-3 (CCL1), suggesting the up-regulation of additional chemokine receptors on antigen-elicited eosinophils. The up-regulation of the additional chemokine-receptor responses appeared to be in part because of cytokine activation, because TNF-α and/or IL-4 were able to up-regulate CCR1, -3, -5, and -8 mRNA expression in eosinophils as well as migration responses to the appropriate ligands. Using antibodies specific for CCR5 and CCR8, the chemotactic response to MIP-1β and TCA-3, respectively, was reduced significantly. Finally, the expression of these new receptors appears to have an effect on activation and degranulation because MIP-1β (CCL4) and TCA-3 (CCL1) induce significant levels of LTC4 from elicited eosinophils. These results suggest that eosinophils may up-regulate and use additional chemokine receptors during progression of inflammatory, allergic responses for migration and activation. *J. Leukoc. Biol.* 71: 1019–1025; 2002.

Key Words: *allergy · chemokine receptors · chemotaxis · eosinophil recruitment*

INTRODUCTION

Eosinophils play a central role in numerous inflammatory diseases including allergic disorders, parasitic infections, and malignancies [1, 2]. When activated, eosinophils are able to release many mediators such as eosinophil peroxidase, lysozyme, lipid mediators, cytokines, and chemokines. As effector cells, eosinophils must be activated to be able to carry out their function. Once activated at a site of inflammation/immune response, these mediators can alter tissue function significantly and may lead to long-term pathophysiologic changes. After appropriate activation, eosinophils have increased expression of surface receptors involved in eosinophil recruitment and activation. It is the expression of these chemoattractant receptors that may play a key role in determining the increased tissue localization and activation during disease.

Chemokines mediate a range of proinflammatory effects on leukocytes, including chemotaxis, activation, and degranulation [3–5]. Historically, RANTES was the first CC-chemokine shown to induce eosinophil chemotaxis [6, 7]. In addition to RANTES, other CC-family chemokine members have been shown to be potent eosinophil chemoattractants; among them, eotaxin and monocyte chemoattractant protein (MCP)-4 appear to be the most effective as a result of their ability to bind to CCR3 [8–11]. However, other CC-chemokines, such as macrophage-inflammatory protein-1α (MIP-1α) and macrophage-derived chemokine (MDC) that do not bind to CCR3, also appear to alter eosinophil accumulation in vivo [12–15]. A family of G-protein-coupled seven-transmembrane receptors mediate chemokine action on leukocytes [16]. There are 10 known receptors that mediate the actions of CC-chemokines CCR1–CCR10. The expression of these receptors on different leukocyte subsets influences their migration and activation at inflammatory sites. In particular, peripheral eosinophils have been shown to express high levels of CCR3, and CCR1 is expressed at much lower levels—only at 1–5% of the level of CCR3. The expression of other CC-chemokine receptors on eosinophils is still to be elucidated. The data in the present study suggest that once eosinophils are activated properly or recruited to a site of T-helper cell type 2 (Th2)-induced in-

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flammation, additional chemokine receptors are up-regulated and can function to alter eosinophil migration and/or activation.

MATERIALS AND METHODS

Animals and antibodies

Dr. Fred Lewis at the Biomedical Research Laboratory (Frederick, MD) supplied Swiss Webster mice, infected heavily with *Schistosoma mansoni* helminth parasite. These mice displayed severe infection and very significant eosinophilia with >50% of circulating granulocytes as eosinophils. The interleukin (IL)-5 transgenic mice were provided by Dr. Sergio Lira (Schering-Plough, Kenilworth, NJ). Antibodies specific for CCR5 were purchased from R&D Systems (Rochester, MN), and CCR8-specific monoclonal antibodies were a gift from Dr. Carlos Martinez (Madrid, Spain).

Antigen-elicited peritoneal eosinophil purification

Eosinophils were elicited by injection of thioglycolate plus soluble egg antigen (SEA) into the peritoneum of *S. mansoni*-infected mice or with only thioglycolate in IL-5 transgenic mice. SEA was prepared in our laboratory by grinding isolated eggs from heavily infected *S. mansoni*-infected mice as described previously [12]. The injection of SEA into infected mice induces a pool of circulating eosinophils recruited into the peritoneum in an antigen-specific manner, whereas thioglycolate in IL-5 transgenic mice elicits cells in a nonspecific manner. The heavily infected schistosome mice and IL-5 transgenic mice have a similar level of circulating eosinophils (~50% of granulocytes). Using these two mice, we could elicit eosinophils that were from an antigen-induced Th2-associated environment or those from a nonspecific inflammatory environment that were enhanced for survival. After 48 h, the mice were lavaged peritoneally, and the cells were collected. The initial population that is isolated from the peritoneum was ~50% eosinophils with only 2–5% neutrophils and 35–45% mononuclear cells (lymphocytes and macrophages). Adherent cell populations were removed from the population by plastic adherence in tissue-culture dishes for 1 h. The nonadherent cells were washed and resuspended in phosphate-buffered saline (PBS)/bovine serum albumin (BSA; 90 μl PBS/BSA per 10^7 cells), and eosinophils were purified by negative immunomagnetic bead-coupled antibodies to exclude contaminating immune cells using the magnetic cell-sorter (MACS) system. The antibodies used were anti-Thy1 (for T cells), anti-B220 (for B cells), and anti-class II (for antigen-presenting cells). After the plate adherence and MACS separation, the population of cells contained >97% eosinophils with contaminating neutrophils (~1%) and mononuclear cells (1–2%).

Blood peripheral eosinophil

Eosinophils were purified from peripheral blood from *S. mansoni*-infected mice using Ficoll-Paque® (Pharmacia Biotech, Uppsala, Sweden). Erythrocytes were removed by hypotonic lysis. Routinely, >50% of the granulocytes were eosinophils. These cell populations were used only for chemotaxis; they could not be purified further because unlike human cells, there is no specific antibody for mouse neutrophils, and they could not be separated further by density gradients. However, they were stained differentially in chemotaxis assays to indicate the enumeration of only eosinophils that migrated to a specific stimulus.

Measurement [Ca^{++}] intracellular

Peritoneal-elicited eosinophils purified by immunomagnetic negative selection and peripheral blood eosinophil purified by Ficoll were suspended in 5 ml Hanks' balanced saline solution/0.1% BSA containing 2.5 μM Fura-2-AM and incubated at 37°C for 30 min. Measurement of changes in intracellular calcium from eosinophils was described previously [17]. Calculation of intracellular-free calcium was derived from the fluorescence spectra (excitation wavelengths, 340 and 380 nm; emission wavelengths, 510 nm) in accordance with established methods.

Eosinophil chemotaxis assay

Chemotaxis assay was based on the methods described previously [13]. Eosinophils were suspended at 3×10^6 cells/ml in Dulbecco's PBS plus 0.5% BSA and were placed in the top wells of the microchemotaxis chamber. Bottom wells were filled with CC-chemokines (R&D Systems) in the following concentrations: eotaxin, MIP-1α, MIP-1β, and trichloroacetic acid (TCA)-3 (10 and 100 ng/ml) or assay medium as negative control. Eosinophil migration was quantified by counting the number of eosinophil migrations completely through the filter in 10 high-powered fields (HPF) in triplicate samples. The data are expressed as the average number of countable, adherent cells per HPF (±SEM). The eosinophils were identified using differential staining (Diff-Quik) that clearly stained the granules eosin red, and the neutrophils and eosinophils could be delineated clearly and easily. Only eosinophils were counted in these assays.

Measurement of eosinophil LTC4 release

To analyze LTC4 release from the eosinophils suspended in phenol red-free Dulbecco's modified Eagle's medium containing L-serine (25 mM) to inhibit mediator degradation [18], cells were allowed to incubate for 4 h with a particular chemokine. The supernatant from the activated cells (2×10^6 eosinophils) was harvested, and LTC4 release was measured using a specific enzyme-linked immunosorbent assay (Calbiochem, Ann Arbor, MI). The assay is sensitive to 1 pg/ml LTC4.

Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

Total cellular RNA was extracted from 3×10^6 eosinophils using TRIzol reagent (Life Techonologies, Invitrogen Corp., Carlsbad, CA), and RT-PCR was conducted using standard methodology. Sequences of the primers for the amplification were: CCR1 (sense 5′-gaccagcatctacctgttca-3′ and antisense 5′-gcagaaacaaatacactcag-3′); CCR2 (sense 5′-cacgaagtatccaagagc-3′ and antisense 5′-catgctcttcagctttttac-3′); CCR3 (sense 5′-tgggcaacatgatggttgtg-3′ and antisense 5′-gctgtcttgagactcatgga-3′); CCR4 (sense 5′-cctgcctcctctctactcct-3′ and antisense 5′-acgtgtggttgtgctctgtg-3′); CCR5 (sense 5′-gctgaagagcgtgactgata-3′ and antisense 5′-gaggactgcatgtataatga-3′); CCR8 (sense 5′-gagcatcacagatatctacct-3′ and antisense 5′-ctcaaagactgctcttcat-3′). The PCR mixture was incubated in a thermocycler using the following temperature profile: denaturation step at 94°C for 4 min followed by 35 cycles (CCR1, CCR4, CCR5, and CCR8) and 30 cycles (CCR2 and CCR3) of denaturation at 94°C for 45 s, annealing at 55°C for 45 s, and extension at 72°C for 45 s. The final extension step was 72°C for 10 min. PCR samples were run on a 2% agarose gel stained with 10 mg/ml ethidium bromide, and the PCR products were visualized with UV light and were photographed.

Statistics

Data were analyzed by analysis of variance, and significance was determined with P values < 0.05.

RESULTS

Calcium flux responses are similar between peripheral and elicited eosinophils

A rapid, transient calcium flux is observed typically when leukocytes are stimulated with chemokine, and it serves as a convenient measure of chemokine-receptor activation. To investigate the signaling properties of chemokine receptors present in mouse eosinophils, we determined the ability of particular CC-chemokines specific for certain receptors to increase intracellular Ca^{++}. Activation of isolated, antigen-elicited eosinophils (see Materials and Methods) with MIP-1α (**Fig. 1A**) and eotaxin (Fig. 1B) induced a significant elevation in the intracellular calcium level in these cells. Eotaxin and MIP-1α desensitized the calcium response to themselves (data not shown), but only partially to one another, suggesting binding to shared and unshared receptors (Fig. 1, C and D). MCP-1, MDC, thymus and activation-regulated chemokine (TARC),

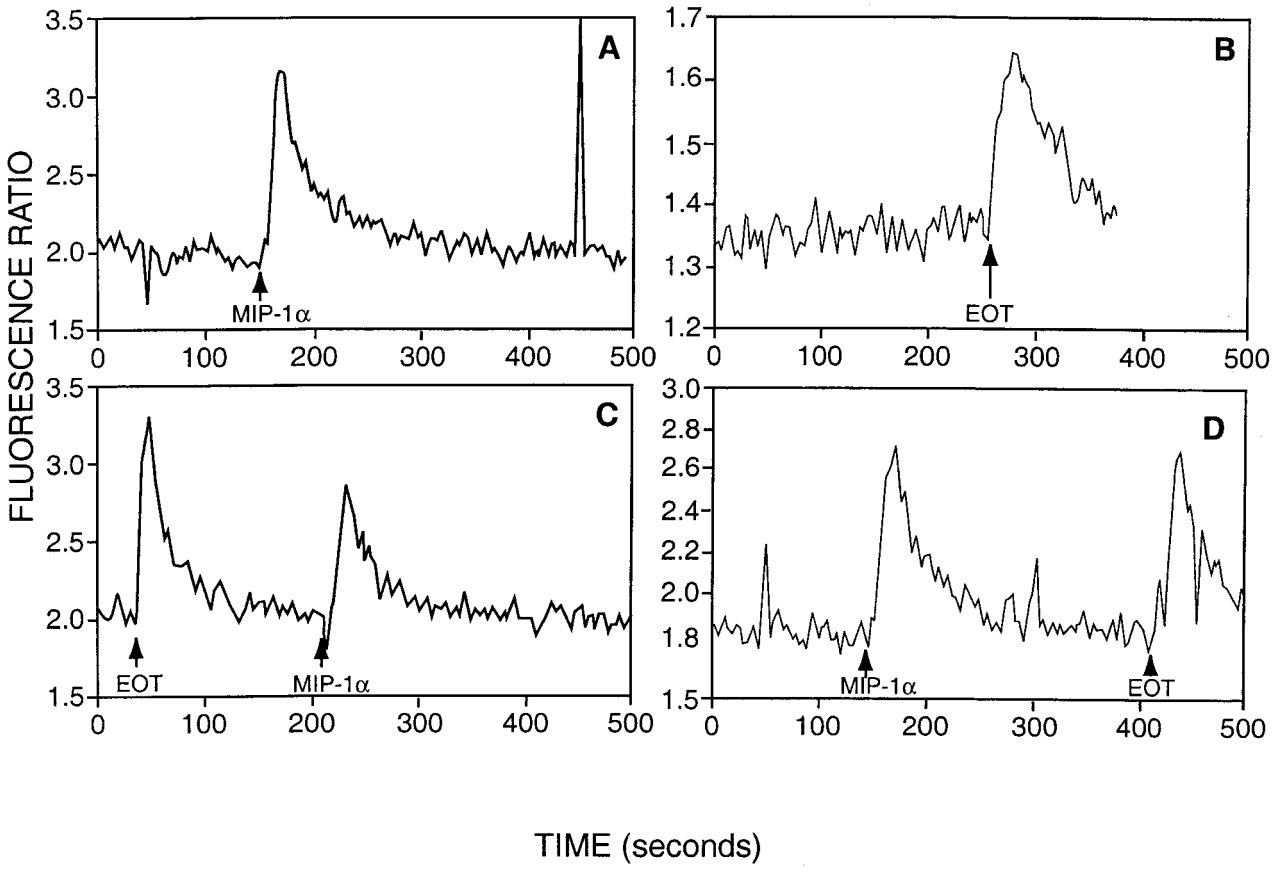

Fig. 1. Elevation of calcium intracellular in eosinophils from *S. mansoni*-infected mice induced by MIP-1α and eotaxin (EOT). Antigen-elicited eosinophils were loaded with Fura-2 and exposed to MIP-1α (A, C, and D, 30 ng/ml) and eotaxin (B–D, 30 ng/ml). Changes in fluorescence after activation were monitored using a fluorimeter. Results are representative of at least three experiments.

MIP-1β, or TCA-3, specific ligands for CCR2, CCR4, CCR5, and CCR8, did not increase the intracellular Ca^{++} level in these cells (data not shown) nor did any of these latter chemokines desensitize MIP-1α or eotaxin-induced responses (data not shown). In peripheral blood eosinophils, eotaxin and MIP-1α also induced a significant elevation in the intracellular calcium level, with a similar desensitization profile as elicited eosinophils, again suggesting that they bind to distinct, unshared receptors. MCP-1, TARC, MDC, or MIP-1β failed to induce a positive response in these cells (data not shown). These results suggest that antigen elicited and peripheral eosinophils have similar, functional CC-chemokine receptors. Similar data have been previously published indicating that these same CC-chemokines were able to induce changes in $[Ca^{2+}]$ in human eosinophils [19].

Chemokine-induced eosinophil migration is altered in antigen-elicited eosinophils

To determine the ability of various CC-chemokines to induce chemotaxis in antigen-elicited versus peripheral blood eosinophils, we then used a classic two-chamber assay. Chemotactic activity for peripheral blood eosinophils was observed with MIP-1α and eotaxin, but not MIP-1β and TCA-3 (**Fig. 2A**). These results suggest that peripheral blood eosinophils express functional CCR1 and CCR3 as described previously [20]. In contrast, antigen-elicited eosinophils migrated toward MIP-1α, eotaxin, MIP-1β, and TCA-3 (Fig. 2B). There was no migration to MCP-1 in peripheral or elicited eosinophils (unpublished results). These results suggest that antigen-elicited eosinophils express CCR1, CCR3, CCR5, and CCR8 functionally. It is interesting that MIP-1β and TCA-3 induced no evidence of calcium flux in eosinophils, but they appear to have functional receptors as evidenced by their migration responses.

Characterization of altered eosinophil responsiveness

The differences in chemotactic responses found in these studies between peripheral and antigen-elicited eosinophils suggest an increased expression during migration and/or specific activation of the transmigrated eosinophils. To address the first question of whether migration alone was responsible for increasing CCR expression, we used IL-5 transgenic mice. Eosinophils were elicited in a nonspecific manner using thioglycolate. Thus, low levels of immune-specific cytokines, such as IL-4 (<10 pg/ml), were produced at the site of elicitation. The comparison of eosinophil chemotaxis in these studies demonstrated that the thioglycolate (nonspecific)-elicited eosinophils from IL-5 trangenic mice responded in the same manner as peripheral cells (**Fig. 3**). That is, they migrated only to MIP-1α and eotaxin with no migration toward any concentration of MIP-1β or TCA-3 (1–100 ng/ml; data not showns). Thus, elicitation alone did not appear to be enough to up-regulate the

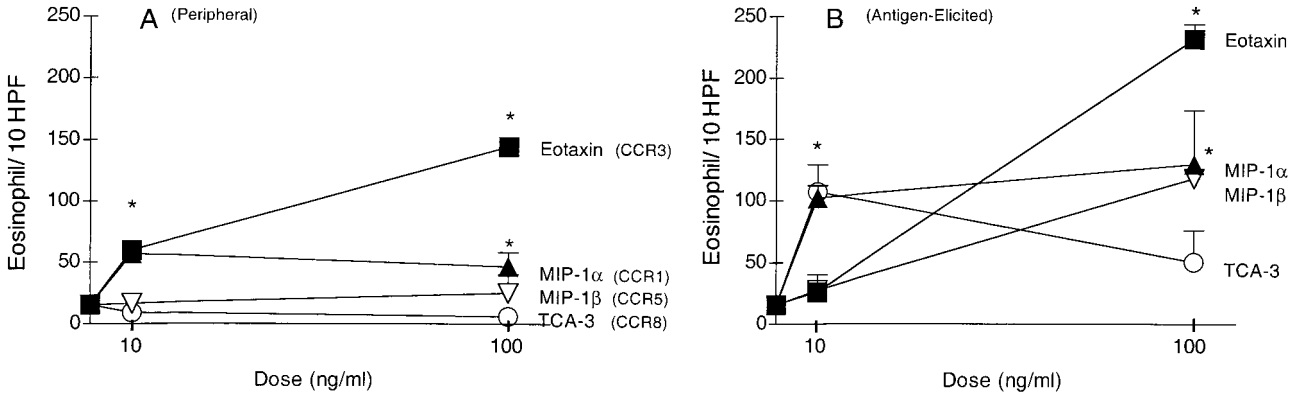

Fig. 2. Chemotactic activity of MIP-1α, eotaxin, MIP-1β, and TCA-3 on peripheral blood eosinophils (A) and antigen-elicited eosinophils (B). Cells (3×10^6/ml) were subjected to chemotaxis in a Boyden chamber, and the number of cells that migrated through the membrane was counted. The results represent the mean ± SEM of duplicate cultures with 10 HPF counted in each. Similar data were obtained in four other experiments (*, $P<0.001$ when compared with control).

additional chemokine responses. Cytokines, such as tumor necrosis factor α (TNF-α) and IL-4, have been shown to contribute to the regulation of eosinophil activation in vivo and in vitro [21–23]. The method used to elicit the eosinophils in *S. mansoni*-infected mice includes using specific schistosoma SEA. This antigen induces significant levels of inflammatory and Th2-type cytokines, especially TNF-α and IL-4 [24]. In our experiments synchronous activation of eosinophils from IL-5 transgenic animals with TNF-α or IL-4 were able to up-regulate CCR1, CCR3, CCR5, and CCR8 mRNA expression 6 h after cytokine stimulation in vitro (**Fig. 4**). The migratory responses of the elicited eosinophils were also examined after incubation with IL-4 or TNF-α (**Fig. 5**). The migration to MIP-1α, eotaxin, and MIP-1β was increased significantly after overnight incubation with TNF-α or IL-4. However, although CCR8 mRNA was up-regulated by TNF-α and IL-4, the chemotactic responses to TCA-3 were increased with TNF but decreased after IL-4. This latter observation will be examined further in future experiments. Taken together, these data suggest that TNF-α and IL-4 are able to up-regulate CC-chemokine receptor mRNA expression and are representative factors that are involved in facilitating increased chemokine-mediated migratory responses in eosinophils.

To determine whether increased MIP-1β and TCA-3 chemotactic activity was induced by CCR5 and CCR8, respectively, antigen (SEA)-elicited eosinophils from schistosome-infected mice were treated with antichemokine receptor antibodies for 15 min before subjecting them to chemotactic analysis. The data in **Figure 6** illustrates that the antireceptor antibodies blocked the chemotactic responses to their respective ligands significantly. It is interesting that chemotaxis to MIP-1α was not altered by the anti-CCR8 antibody treatment but was altered slightly by the anti-CCR5 treatment. These latter data suggest that at least part of the response to MIP-1α may be a result of CCR5 expression in antigen-elicited eosinophils. In separate experiments, eotaxin-induced chemotaxis was inhibited by anti-CCR3, but was not altered by anti-CCR5 or anti-CCR8 treatment (data not shown). Thus, these data verify that blocking specific chemokine receptors inhibits movement to the appropriate ligands.

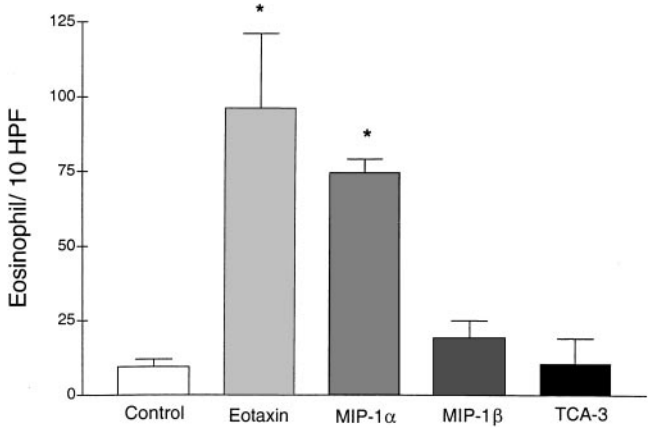

Fig. 3. Elicited eosinophils from IL-5 transgenic mice do not migrate to MIP-1β or TCA-3. Transgenic mice overexpressing IL-5 were injected intraperitoneally with thioglycolate to elicit the peripheral eosinophils nonspecifically. The isolated eosinophils were then subjected to eosinophil chemotaxis assays to chemokine levels that induced peak responses previously in Figure 2 (MIP-1α, 10 ng/ml; eotaxin, 100 ng/ml; MIP-1β, 100 ng/ml; and TCA-3, 10 ng/ml). The results represent the mean ± SEM of duplicate cultures with 10 HPF counted in each. Similar data were obtained in three other experiments (*, $P<0.001$ when compared with control).

Chemokine-mediated LTC4 release

Chemokines not only participate in leukocyte migration, but also induce degranulation and activation of effector cell pop-

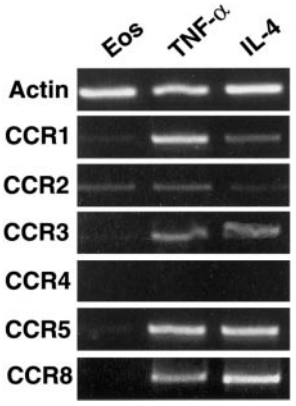

Fig. 4. Incubation of elicited eosinophils (Eos) from IL-5 transgenic animals with TNF-α (20 ng/ml) or IL-4 (20 ng/ml) for 6 h up-regulates CCR1, CCR3, CCR5, and CCR8 mRNA expression. Total mRNA (4 μg) purified from 3×10^6 cells was used in RT-PCR analysis. Data are representative of results repeated in three separate experiments.

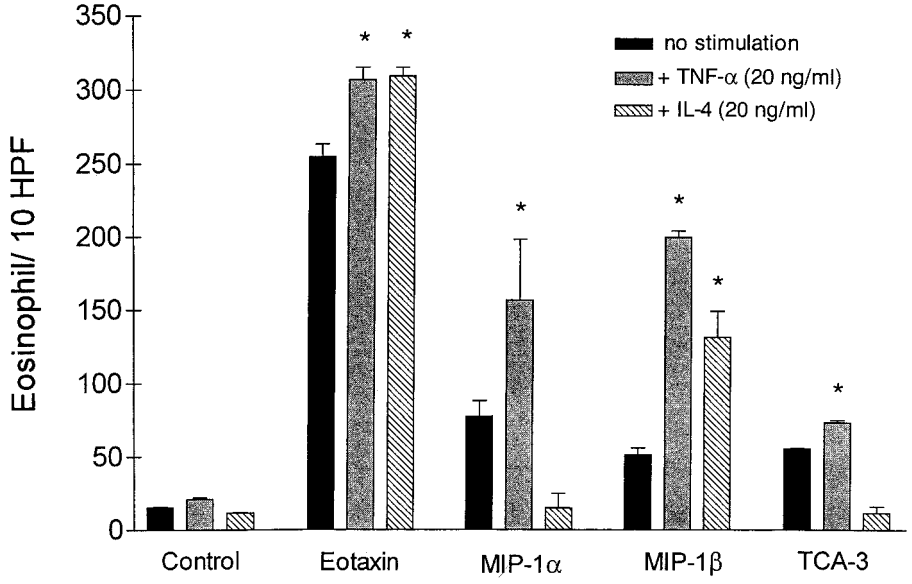

Fig. 5. Preincubation of elicited eosinophils with IL-4 or TNF-α increases migratory function of the cells. Eosinophils elicited in schistosome-infected mice were incubated overnight with or without TNF-α or IL-4 (20 ng/ml). After washing in fresh media, eosinophils were subjected to chemotactic assays with MIP-1α (10 ng/ml), eotaxin (100 ng/ml), MIP-1β (100 ng/ml), and TCA-3 (10 ng/ml). Data represent mean ± SEM of three repeats for each group. *, $P < 0.05$.

ulations. The ability of MIP-1β and TCA-3 to induce eosinophil degranulation and activation was assessed using in vitro cultures of elicited eosinophils from the *S. mansoni*-infected mice. Eosinophils were stimulated for 4 h in culture with various levels of the chemokines, and the supernatants were assessed for levels of LTC4 (**Fig. 7**). The data in Figure 7 suggest that eotaxin, MIP-1α, TCA-3, and MIP-1β induce significant LTC4 release and appear to be as effective as eotaxin and MIP-1α. These results are intriguing considering their differential signaling as observed with Ca flux above. Thus, the ability of elicited and cytokine-stimulated eosinophils to respond to additional chemokines may have the significant relevance for eosinophil activation and degranulation at the site of the response.

DISCUSSION

The presence of specific receptors CCR1 and CCR3 has been described previously in peripheral blood eosinophils from IL-5 transgenic mice [20], in eosinophilic cell lines [25], and in human peripheral eosinophils [26]. In the present studies, the

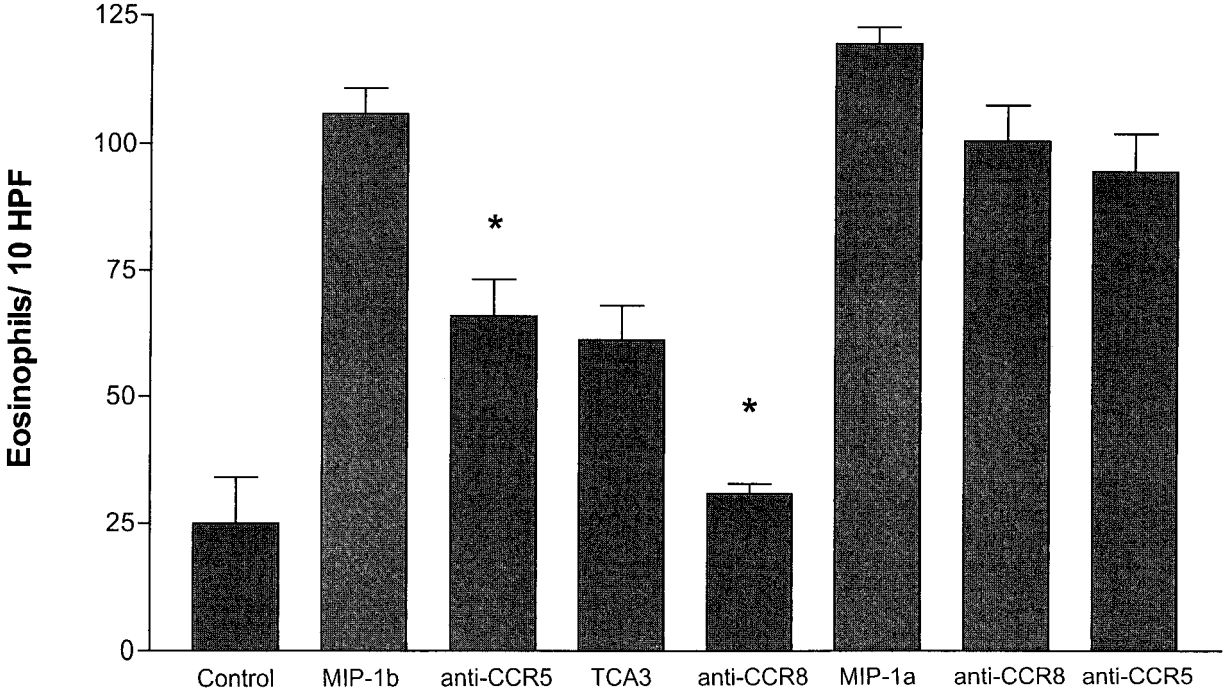

Fig. 6. Inhibition of MIP-1β and TCA-3-mediated chemotaxis by anti-CCR5 and anti-CCR8, respectively. Antigen-elicited eosinophils were treated with 10 μg anti-CCR5 or anti-CCR8 antibody for 15 min on ice. Subsequently, the eosinophils were subjected to chemotactic analysis as described. Data represent mean ± SEM from two repeat studies. TCA-3 and MIP-1β were assessed with different sets of eosinophils and therefore, have their own controls. Background eosinophil numbers ranged from 17 to 35 eosinophils/HPF (1000× original magnification).

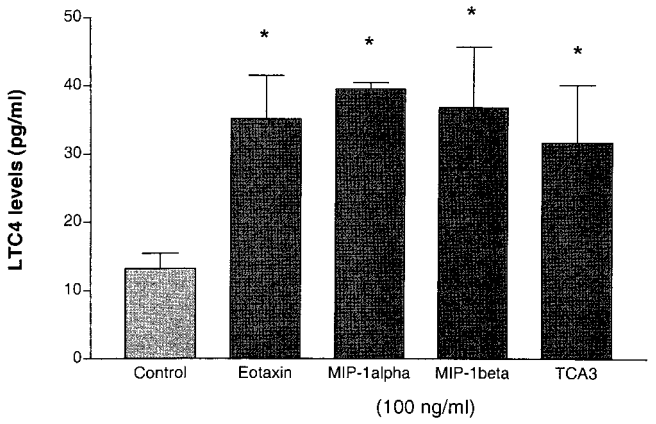

Fig. 7. Activation of eosinophils by MIP-1β and TCA-3. Isolated eosinophils from mice infected heavily with *S. mansoni* were subjected to activation with eotaxin, MIP-1α, MIP-1β, or TCA-3 (100 ng/ml). Culture supernatants were harvested at 4 h post-activation and assayed for LTC4 levels. Data represent mean ± SE of three repeat activations. *, $P < 0.05$.

increased reactivity to additional chemokines that do not bind CCR1 and CCR3 suggests that a broader profile of chemokines may be relevant for tissue eosinophilia in allergic disease progression. The expression of CCR5 and CCR8 on eosinophils appeared to depend on the activation state of the cells. Peripheral eosinophils or elicited eosinophils from IL-5 transgenic mice (which were not elicited by an antigen-specific response) displayed no evidence of MIP-1β or TCA-3-mediated responses. However, cytokine activation was a primary stimulus that could induce the de novo expression of the specific receptors CCR5 and CCR8 and increased migration to their specific ligands MIP-1β and TCA-3. Eosinophils elicited to a site of a Th2-type immune reaction in heavily infected *S. mansoni* mice displayed responses to the CCR5 and CCR8 ligands, MIP-1β, and TCA-3. These interactions were verified by blocking the CCR5 and CCR8 specifically during functional chemotactic responses, respectively. Thus, the immune environment appears to regulate the chemokine receptor display and chemokine responsiveness with eosinophils.

The dissociation of calcium flux response from chemotactic activity observed with peritoneal-elicited eosinophils has also been observed in T cells and human eosinophils, where chemotactic responses could be observed without detectable calcium mobilization [15, 27]. Although they represent two different parameters to analyze receptor function, the activation pathways between Ca^{++} flux and chemotaxis or degranulation may be different. One possible explanation may be that different subunits of the G-protein signaling pathway are involved, with the α subunit inducing Ca^{++} flux and β/γ subunits together responsible for chemotaxis [28–31]. Alternatively, chemokine receptors can couple non-Gi protein pathways [32, 33]. Functionally, the up-regulation of additional chemokine receptors in eosinophils that have migrated into inflamed tissue may have significant contributions to additional eosinophil functions, including migration, activation, and degranulation during disease. In fact, the expression of these new receptors appears to have a significant role in activation and degranulation of the eosinophils for LTC4 generation and release. These findings may serve as an important model, whereby one set of chemokines, such as eotaxin and MIP-1α, may be a primary stimulus for eosinophil migration, and other chemokines may be responsible for eosinophil activation and degranulation at the site of the immune response. Although the role of chemokines in asthma is not yet completely understood, multiple chemokines and their receptors appear to participate in eosinophil migration and activation during allergic airways disease [7, 34–37]. Therefore, understanding which chemokine receptors are being expressed during eosinophil activation will be an important approach for targeting therapy to prevent the migration and activation of eosinophils in allergic disease.

Many chemokines have now been shown to cause degranulation and release of multiple mediators from eosinophils [4, 38–40]. In these studies, the up-regulation of CCR5 and CCR8 by cytokines, such as IL-4 and TNF-α, suggests that these receptors are most highly expressed after the eosinophil migrates into the inflamed tissues. This is supported by the data using elicited eosinophils from IL-5 transgenic mice that were not exposed to antigen-induced immune cytokines and did not migrate to MIP-1β or TCA-3. The receptors could only be detected in eosinophils from IL-5 transgenic mice after IL-4 and TNF-α stimulation. This regulation of chemokine-receptor expression likely occurs on multiple leukocyte populations during immune responses and may function to regulate leukocyte movement and activation into the inflamed environment. It is interesting that a recent study from our laboratory has indicated that CCR8 −/− mice have a deficit in eosinophils recruited to the airway during allergen or parasitic responses [41]. In those earlier studies, no CCR8 was evident on peripheral eosinophils, but no attempt was made at examining recruited eosinophils. Thus, the lack of CCR8 expression on activated and recruited eosinophils in those studies may have had an impact on the inflammatory response.

In conclusion, elicited eosinophils from antigen-specific responses express receptors for multiple CC-chemokines that appear to be up-regulated by at least IL-4 and TNF-α. It may be important for eosinophils to use multiple chemokines as they move from vessel to inflamed tissue in order to be activated optimally and/or regulated. Furthermore, the data suggest that the environment and the activation state of eosinophils likely contribute to chemokine-receptor expression. That eosinophils may use additional receptor-mediated responses for eosinophil activation once they have migrated into tissue allows a greater ability to regulate their function. This would have a significant impact in patients with allergic disorders and may influence the determination of therapeutic targets for disease research.

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REFERENCES

1. Boyce, J. A. (1997) The pathobiology of eosinophilic inflammation. Allergy Asthma Proc. 18, 293–300.
2. Hirai, K., Miyamasu, M., Takaishi, T., Morita, Y. (1997) Regulation of the function of eosinophils and basophils. Crit. Rev. Immunol. 17, 325–352.
3. Kita, H., Gleich, G. J. (1996) Chemokines active on eosinophils: potential roles in allergic inflammation. J. Exp. Med. 183, 2421–2426.
4. Martin, L. B., Kita, H., Leiferman, K. M., Gleich, G. J. (1996) Eosinophils in allergy: role in disease, degranulation, and cytokines. Int. Arch. Allergy Immunol. 109, 207–215.
5. Gleich, G. J., Adolphson, C. R., Leiferman, K. M. (1993) The biology of the eosinophilic leukocyte. Annu. Rev. Med. 44, 85–101.
6. Teran, L. M., Noso, N., Carroll, M., Davies, E. E., Holgate, S., Schroder, J. M. (1996) Eosinophil recruitment following allergen challenge is associated with the release of the chemokine RANTES into asthmatic airways. J. Immunol. 157, 1806–1812.
7. Lukacs, N. W., Strieter, R. M., Standiford, T. J., Kunkel, S. L. (1996) Characterization of chemokine function in animal models of diseases. Methods 10, 158–165.
8. Stellato, C., Collins, P., Ponath, P. D., Soler, D., Newman, W., La Rosa, G., Li, H., White, J., Schwiebert, L. M., Bickel, C., Liu, M., Bochner, B. S., Williams, T., Schleimer, R. P. (1997) Production of the novel C-C chemokine MCP-4 by airway cells and comparison of its biological activity to other C-C chemokines. J. Clin. Investig. 99, 926–936.
9. Heath, H., Qin, S., Rao, P., Wu, L., LaRosa, G., Kassam, N., Ponath, P. D., Mackay, C. R. (1997) Chemokine receptor usage by human eosinophils. The importance of CCR3 demonstrated using an antagonistic monoclonal antibody. J. Clin. Investig. 99, 178–184.
10. Ying, S., Meng, Q., Zeibecoglou, K., Robinson, D. S., Macfarlane, A., Humbert, M., Kay, A. B. (1999) Eosinophil chemotactic chemokines (eotaxin, eotaxin-2, RANTES, monocyte chemoattractant protein-3 (MCP-3), and MCP-4), and C-C chemokine receptor 3 expression in bronchial biopsies from atopic and nonatopic (intrinsic) asthmatics. J. Immunol. 163, 6321–6329.
11. Ying, S., Robinson, D. S., Meng, Q., Rottman, J., Kennedy, R., Ringler, D. J., Mackay, C. R., Daugherty, B. L., Springer, M. S., Durham, S. R., Williams, T. J., Kay, A. B. (1997) Enhanced expression of eotaxin and CCR3 mRNA and protein in atopic asthma. Association with airway hyperresponsiveness and predominant co-localization of eotaxin mRNA to bronchial epithelial and endothelial cells. Eur. J. Immunol. 27, 3507–3516.
12. Lukacs, N. W., Strieter, R. M., Shaklee, C. L., Chensue, S. W., Kunkel, S. L. (1995) Macrophage inflammatory protein-1 alpha influences eosinophil recruitment in antigen-specific airway inflammation. Eur. J. Immunol. 25, 245–251.
13. Lukacs, N. W., Standiford, T. J., Chensue, S. W., Kunkel, R. G., Strieter, R. M., Kunkel, S. L. (1996) C-C chemokine-induced eosinophil chemotaxis during allergic airway inflammation. J. Leukoc. Biol. 60, 573–578.
14. Gonzalo, J. A., Pan, Y., Lloyd, C. M., Jia, G. Q., Yu, G., Dussault, B., Powers, C. A., Proudfoot, A. E., Coyle, A. J., Gearing, D., Gutierrez-Ramos, J. C. (1999) Mouse monocyte-derived chemokine is involved in airway hyperreactivity and lung inflammation. J. Immunol. 163, 403–411.
15. Bochner, B. S., Bickel, C. A., Taylor, M. L., MacGlashan Jr., D. W., Gray, P. W., Raport, C. J., Godiska, R. (1999) Macrophage-derived chemokine induces human eosinophil chemotaxis in a CC chemokine receptor 3- and CC chemokine receptor 4-independent manner. J. Allergy Clin. Immunol. 103, 527–532.
16. Zlotnik, A., Morales, J., Hedrick, J. A. (1999) Recent advances in chemokines and chemokine receptors. Crit. Rev. Immunol. 19, 1–47.
17. Sabroe, I., Williams, T. J., Hebert, C. A., Collins, P. D. (1997) Chemoattractant cross-desensitization of the human neutrophil IL-8 receptor involves receptor internalization and differential receptor subtype regulation. J. Immunol. 158, 1361–1369.
18. Owen Jr., W. F., Soberman, R. J., Yoshimoto, T., Sheffer, A. L., Lewis, R. A., Austen, K. F. (1987) Synthesis and release of leukotriene C4 by human eosinophils. J. Immunol. 138, 532–538.
19. Ponath, P. D., Qin, S., Ringler, D. J., Clark-Lewis, I., Wang, J., Kassam, N., Smith, H., Shi, X., Gonzalo, J. A., Newman, W., Gutierrez-Ramos, J. C., Mackay, C. R. (1996) Cloning of the human eosinophil chemoattractant, eotaxin. Expression, receptor binding, and functional properties suggest a mechanism for the selective recruitment of eosinophils. J. Clin. Investig. 97, 604–612.
20. Post, T. W., Bozic, C. R., Rothenberg, M. E., Luster, A. D., Gerard, N., Gerard, C. (1995) Molecular characterization of two murine eosinophil beta chemokine receptors. J. Immunol. 155, 5299–5305.
21. Sato, M., Takizawa, H., Kohyama, T., Ohtoshi, T., Takafuji, S., Kawasaki, S., Tohma, S., Ishii, A., Shoji, S., Ito, K. (1997) Eosinophil adhesion to human bronchial epithelial cells: regulation by cytokines. Int. Arch. Allergy Immunol. 113, 203–205.
22. Lukacs, N. W., Strieter, R. M., Chensue, S. W., Kunkel, S. L. (1994) Interleukin-4-dependent pulmonary eosinophil infiltration in a murine model of asthma. Am. J. Respir. Cell Mol. Biol. 10, 526–532.
23. Schleimer, R. P., Sterbinsky, S. A., Kaiser, J., Bickel, C. A., Klunk, D. A., Tomioka, K., Newman, W., Luscinskas, F. W., Gimbrone Jr., M. A., McIntyre, B. W., et al. (1992) IL-4 induces adherence of human eosinophils and basophils but not neutrophils to endothelium. Association with expression of VCAM-1. J. Immunol. 148, 1086–1092.
24. Boros, D. L. (1994) The role of cytokines in the formation of the schistosome egg granuloma. Immunobiology 191, 441–450.
25. Tiffany, H. L., Alkhatib, G., Combadiere, C., Berger, E. A., Murphy, P. M. (1998) CC chemokine receptors 1 and 3 are differentially regulated by IL-5 during maturation of eosinophilic HL-60 cells. J. Immunol. 160, 1385–1392.
26. Elsner, J., Petering, H., Hochstetter, R., Kimmig, D., Wells, T. N., Kapp, A., Proudfoot, A. E. (1997) The CC chemokine antagonist Met-RANTES inhibits eosinophil effector functions through the chemokine receptors CCR1 and CCR3. Eur. J. Immunol. 27, 2892–2898.
27. Murphy, P. M. (1996) Chemokine receptors: structure, function and role in microbial pathogenesis. Cytokine Growth Factor Rev. 7, 47–64.
28. Jin, T., Zhang, N., Long, Y., Parent, C. A., Devreotes, P. N. (2000) Localization of the G protein betagamma complex in living cells during chemotaxis. Science 287, 1034–1036.
29. Dekker, L. V., Segal, A. W. (2000) Perspectives: signal transduction. Signals to move cells. Science 287, 982–983, 985.
30. Servant, G., Weiner, O. D., Herzmark, P., Balla, T., Sedat, J. W., Bourne, H. R. (2000) Polarization of chemoattractant receptor signaling during neutrophil chemotaxis. Science 287, 1037–1040.
31. Neptune, E. R., Iiri, T., Bourne, H. R. (1999) Galphai is not required for chemotaxis mediated by Gi-coupled receptors. J. Biol. Chem. 274, 2824–2828.
32. Al-Aoukaty, A., Rolstad, B., Giaid, A., Maghazachi, A. A. (1998) MIP-3alpha, MIP-3beta and fractalkine induce the locomotion and the mobilization of intracellular calcium, and activate the heterotrimeric G proteins in human natural killer cells. Immunology 95, 618–624.
33. Sebok, K., Woodside, D., al-Aoukaty, A., Ho, A. D., Gluck, S., Maghazachi, A. A. (1993) IL-8 induces the locomotion of human IL-2-activated natural killer cells. Involvement of a guanine nucleotide binding (Go) protein. J. Immunol. 150, 1524–1534.
34. Taub, D. D. (1996) Chemokine-leukocyte interactions. The voodoo that they do so well. Cytokine Growth Factor Rev. 7, 355–376.
35. Campbell, E., Kunkel, S. L., Strieter, R. M., Lukacs, N. W. (1998) Temporal role of chemokines in a murine model of cockroach allergen-induced airway hyperreactivity and eosinophilia. J. Immunol. 161, 7047–7053.
36. Griffiths-Johnson, D. A., Collins, P. D., Jose, P. J., Williams, T. J. (1997) Animal models of asthma: role of chemokines. Methods Enzymol. 288, 241–266.
37. Luster, A. D., Rothenberg, M. E. (1997) Role of the monocyte chemoattractant protein and eotaxin subfamily of chemokines in allergic inflammation. J. Leukoc. Biol. 62, 620–633.
38. Beck, L. A., Dalke, S., Leiferman, K. M., Bickel, C. A., Hamilton, R., Rosen, H., Bochner, B. S., Schleimer, R. P. (1997) Cutaneous injection of RANTES causes eosinophil recruitment: comparison of nonallergic and allergic human subjects. J. Immunol. 159, 2962–2972.
39. Kita, H., Abu-Ghazaleh, R. I., Sur, S., Gleich, G. J. (1995) Eosinophil major basic protein induces degranulation and IL-8 production by human eosinophils. J. Immunol. 154, 4749–4758.
40. Rothenberg, M. E., Ownbey, R., Mehlhop, P. D., Loiselle, P. M., van de Rijn, M., Bonventre, J. V., Oettgen, H. C., Leder, P., Luster, A. D. (1996) Eotaxin triggers eosinophil-selective chemotaxis and calcium flux via a distinct receptor and induces pulmonary eosinophilia in the presence of interleukin 5 in mice. Mol. Med. 2, 334–348.
41. Chensue, S. W., Lukacs, N. W., Yang, T., Shang, X., Frait, K. A., Kunkel, S. L., Wiekowski, M. T., Hedrick, J. A., Cook, D. N., Zingoi, A., Narula, S. K., Napolitano, M., Lira, S. A. (2001) Aberrant Th2 cell function and impaired eosinophil recruitment in CCR8 knockout mice. J. Exp. Med. 193, 573–584.