The CC Chemokine Eotaxin (CCL11) Is a Partial Agonist of CC Chemokine Receptor 2b*

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Despite sharing considerable homology with the members of the monocyte chemoattractant protein (MCP) family, the CC chemokine eotaxin (CCL11) has previously been reported to signal exclusively via the receptor CC chemokine receptor 3 (CCR3). Using the monocyte cell line THP-1, we investigated the relative abilities of eotaxin and MCPs 1–4 to induce CCR2 signaling, employing assays of directed cell migration and intracellular calcium flux. Surprisingly, 1 μM concentrations of eotaxin were able to recruit THP-1 cells in chemotaxis assays, and this migration was sensitive to antagonism of CCR2 but not CCR3. Radiolabeled eotaxin binding assays performed on transfectants bearing CCR2b or CCR3 confirmed eotaxin binding to CCR2 with a Kd of 7.50 ± 3.30 nM, compared with a Kd of 1.68 ± 0.91 nM at CCR3. In addition, whereas 1 μM concentrations of eotaxin were able to recruit CCR2b transfectants, substimulatory concentrations of eotaxin inhibited MCP-1-induced chemotaxis of CCR2b transfectants and also inhibited MCP-1-induced intracellular calcium flux of THP-1 cells. Collectively, these findings suggest that eotaxin is a partial agonist of the CCR2b receptor. A greater understanding of the interaction of CCR2 with all of its ligands, both full and partial agonists, may aid the rational design of specific antagonists that hold great promise as future therapeutic treatments for a variety of inflammatory disorders.

Chemokines represent an expanding family of structurally highly related small proteins that play a crucial role in inflammation (1). Upwards of 40 chemokines have been described to date, and they can be divided into four different structural families, according to the position of their amino-terminal cysteine residues. Most chemokines belong to two main families: the CXC class, where one amino acid separates the first two cysteine residues, or the CC class, possessing two adjacent cysteine residues. In addition, a C class featuring a single cysteine residue, or the CC class, possessing two adjacent cysteine residues, or the CC class, possessing two adjacent cysteine residues, may aid the rational design of specific antagonists that hold great promise as future therapeutic treatments for a variety of inflammatory disorders.

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¶ Experimental Procedures

Materials—Analytical grade reagents were purchased from BDH Chemicals Ltd. or Sigma. Fura-2/AM was purchased from Molecular Probes, Inc. (Eugene, OR). IgG1 (clone MOPC 21) and IgG2a (clone UPC 10) negative control antibodies were obtained from Sigma. The mAbs

The abbreviations used are: CCR, CC chemokine receptor; MCP, monocyte chemoattractant protein; mAb, monoclonal antibody; FACS, fluorescence-activated cell sorting; PCR, polymerase chain reaction; bp, base pair(s).

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2D4 (anti-human CCR1), 1D9 (anti-human CCR2), 2D7 (anti-human CCR5), and 7B11 (anti-human CCR3) were kind gifts of Millennium Pharmaceuticals, Inc. (Boston, MA). Rabbit IgG was from Vector Laboratories, whereas fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulins (Fab',2 fragment) were purchased from DAKO (Ely, United Kingdom). Recombinant chemokines were purchased from Peprotech EC Ltd., and oligonucleotide primers were purchased from MWG-Biotech UK (Milton Keynes, United Kingdom). 125I-Radiolabeled antibodies were from Amersham Pharmacia Biotech and had a specific activity of ~2000 Ci/mmol.

Maintenance of Cells—THP-1 cells, the previously described 4DE4-CCR3 transfectants, and the murine pre-B cell line L1.2 were maintained as previously described (6). Stably transfected cells were cultured in the same medium with the addition of 1 mg/ml Geneticin (G418) to maintain selection.

Transient and Stable Transfection of L1.2 Cells—The vector pCDNA3 (Invitrogen) containing the cDNA encoding CCR2b was introduced into L1.2 cells by electroporation as previously described (21). For transient transfections, 10 mM sodium butyrate was added to the medium overnight, and cells were used the following day. To generate stable transfectants, cells were cultured for 48 h before supplementing with fresh medium containing G418 at 1 mg/ml final concentration to allow the positive selection of the transfected cells. Individual pools of cells were then screened for receptor expression by FACS analysis using appropriate antibodies, and clones subsequently obtained by limiting dilution.

**FACS Analysis of Chemokine Receptor Expression**—L1.2 transfectants bearing known chemokine receptors or THP-1 cells were assessed by FACS analysis as follows. 5 × 10^6 harvested cells were incubated at 4 °C for 30 min with FACS buffer (phosphate-buffered saline, 1% bovine serum albumin, 0.01% NaN3) containing specific antibodies directed against CCR1 (2D4; 10 μg/ml), CCR2 (LS132.1D9; 10 μg/ml), CCR3 (7B11; 3 μg/ml), or CCR5 (2D7; 10 μg/ml). An irrelevant IgG1 or IgG2a antibody was used as an appropriate isotype control. For THP-1 staining, an additional incubation step with rabbit IgG was added prior to incubation with the primary antibody to reduce background binding, presumably to Fc receptors expressed in abundance on these cells. Unbound antibody was removed by washing with 2 ml of FACS buffer, and the cells were then resuspended in the same buffer containing a 1:20 dilution of fluorescein isothiocyanate-conjugated goat anti-mouse Fab',2 secondary antibody. After a 30-min incubation, cells were washed again with 2 ml of FACS buffer and resuspended in 500 μl of the same buffer. Surface expression was then analyzed using a FACScalibur flow cytometer (Becton Dickinson, Mountain View, CA).

**Reverse Transcription-PCR Analysis**—Total RNA was extracted from 5 × 10^6 THP-1 cells using RNAzol™ (Biogenes, Poole, UK) according to the manufacturer’s instructions, after which 1 μg of total RNA was reverse transcribed using Superscript™ II, RNase H- Reverse Transcriptase, (Life Technologies, Inc., Paisley, UK). The cDNAs obtained were amplified by PCR using the following primer pairs specific for each receptor: CCR1, sense primer (5'-CGA CTA CAA GAA GGA GGA TGA CT-3') and antisense primer (5'-GGC TTC GTT GAG GAA AGT GGG CAT-3'); CCR2a, antisense primer (5'-GGC TTC TTG GAT GAC TCA CTG CGG-3'); CCR2b, antisense primer (5'-TAA ACC ACG GCA GAC TTC CTG-3'); CCR3, sense primer (5'-GTC AGG GGG CAT AAC TGG GTT-3') and antisense primer (5'-ACT GCA AAG AGT TCT TTC AAA CA-3').

Intron-spanning primers for glyceraldehyde-3-phosphate dehydrogenase were also employed as a positive control to assay for potential genomic DNA contamination. Reactions were carried out using the Tag-Beat™ Hot start polymerase kit (Promega, Madison, WI), and the resulting PCR products were analyzed by electrophoresis on a 3% MetaPhor gel (BioWhittaker Molecular Applications, Rockland, ME).

**Intracellular Calcium Measurements**—These were performed as previously described (22) using THP-1 cells that were loaded with the fluorescent dye Fura-2/AM (Molecular Probes, Inc., Sunnyvale, CA). Cells were stimulated with the appropriate chemokine, and real time data were recovered using a fluorimeter (LS-50B; PerkinElmer Life Sciences). Data were expressed as the relative ratio of fluorescence emitted at 510 nm after sequential stimulation at 340 and 380 nm.

**Assays of Chemotactic Responsiveness**—Assays of the chemotactic responsiveness of THP-1 cells or L1.2 transfectant cells were carried out as previously described (6) using ChemoTx™ microchemotaxis cham-
 bers with a 5-μm pore (Receptor Technologies, Adderbury, UK). In receptor blockade experiments, cells were preincubated with LS132.1D9 as a blocking CCR2 mAb (10 μg/ml) or 7B11 as a CCR3-blocking mAb (3 μg/ml) at room temperature for 10 min before chemotactic activity was assessed. Preincubation of cells with an irrelevant control antibody was also performed as a negative control.

Radioligand Binding Assays—125I-Eotaxin binding assays were performed on CCR2b and CCR3 transfectants as previously described (22). Nonspecific binding was typically 20–40% of the total counts. The data were fit to a curve, and the dissociation constant (Kd) for eotaxin at each receptor was calculated using the program LIGAND (23). The cold displacement binding assays involving 125I-labeled MCP-1 binding to CCR2b transfectants and THP-1 membranes were carried out as follows (24). The CCR2b transfectants were treated overnight with 2 mM sodium butyrate before harvesting by centrifugation. The cells (2.5 × 10^5) or membranes (20 μg/well) were incubated at room temperature for 90 min with either 0.1 nM (transfectants) or 0.2 nM (THP-1 membranes) 125I-labeled MCP-1 in 100 μl of binding buffer (50 mM HEPES, pH 7.2, 1 mM CaCl_2, 5 mM MgCl_2, 0.5% bovine serum albumin, and 0.02% sodium azide) in the presence of increasing concentrations of unlabeled chemokines. Bound and free tracer were separated by filtration using 96-well GF/B Unifilter plates (Packard Instrument Co.) presoaked in 0.3% polyethyleneimine. The filters were washed at 4°C with 300 μl of binding buffer supplemented with 0.5 M NaCl to reduce nonspecific binding. IC_{50} values (concentrations at which specific binding of the iodinated chemokine was inhibited by 50%) were calculated by a two-parameter logistic curve fit using KaleidaGraph software (Synergy Software, Inc. Reading, PA). K_i values were calculated with the Cheng-Prusoff (25) correction (K_i = IC_{50} tracer concentration/tracer K_d) using a K_d value for MCP-1 of 0.43 nM for the CCR2b transfectants and 0.13 nM for the THP-1 membranes.

RESULTS

The Antibody 1D9 Is Specific for CCR2—The monoclonal antibody 1D9 was produced against human CCR2 by immunizing mice with L1.2 transfectants expressing CCR2. Figure 1 illustrates its selectivity for CCR2 as verified by a panel of L1.2 transfectants bearing either CCR2b or the two most closely related receptors at the protein level, CCR1 (54.7% identity) and CCR5 (69.7% identity). Figure 1A shows that 1D9 clearly recognizes CCR2 stable transfectants but not CCR5 (Fig. 1D) or CCR1 (Fig. 1G) transfectants. Control staining of these latter two cell lines is shown in Fig. 1E and I, with the anti-CCR6 antibody 2D7 or the anti-CCR1 antibody 2D4. We have previously shown that 1D9 does not stain eosinophils and is also unable to block CCR3/eotaxin-mediated eosinophil shape change, ruling out any cross-reactivity with the more distantly related receptor CCR3 (52.3% identity with CCR2) (26).

THP-1 cells express CCR1, CCR2a/b, and CCR3 at both the protein and mRNA levels. A, THP-1 cells were examined for the expression of CCR1, CCR2, and CCR3 by flow cytometry, following incubation with the specific primary antibodies 4DE 10 μg/ml (CCR1), LS132.1D9 10 μg/ml (CCR2), and 7B11 3 μg/ml (CCR3) and subsequent detection with a goat anti-mouse fluorescein isothiocyanate-conjugated secondary mAb (open histograms). An IgG1 or an IgG2a antibody (solid histograms) was used as the relevant isotype control. B, total RNA isolated from THP-1 cells was assayed for the presence of CCR1, CCR2a, CCR2b, and CCR3 mRNA by reverse transcriptase-PCR. PCR products of the expected sizes were obtained. Lane 1, CCR1 (300 bp); lane 2, CCR2a (260 bp); lane 3, CCR2b (210 bp); lane 4, CCR3 (280 bp). Fully transcribed unspliced DNA was detected as a 3.4-kilobase pair band for CCR2 receptor (lane 2) (27). Lanes 5, 6, 7, and 8 are the respective negative controls (minus cDNA). Glyceraldehyde-3-phosphate dehydrogenase primers were used as a positive control and gave the expected band of 361 bp (lane 9), indicative of a lack of genomic DNA contamination. Data shown are from one experiment representative of two separate experiments.

2 G. LaRosa, manuscript in preparation.
The Monocytic Cell Line THP-1 Expresses CCR1, CCR2, and CCR3—MCP-1 was originally identified by its ability to recruit monocytes and is typically associated with diseases characterized by monocyte infiltration. We assessed the monocytic cell line THP-1 for the expression of CCR2 and related receptors. CCR1, CCR2, and CCR3 were detected at the protein level by FACS analysis by specific monoclonal antibodies (Fig. 2A).

Using reverse transcriptase-PCR, mRNA for all three species could also be detected (Fig. 2B). The expected bands of 300 and 280 bp were detected for CCR1 and CCR3, respectively, whereas both isoforms of CCR2 (CCR2a and CCR2b) could be detected (260 and 210 bp for CCR2a and CCR2b, respectively). A larger band presumably corresponding to the fully transcribed unspliced form of the CCR2 gene was also detected (27). Negative controls for each primer pair (minus cDNA template) showed a lack of genomic contamination (lanes 5–8), as did the intron-spanning glyceraldehyde-3-phosphate dehydrogenase primers (lane 9).

Chemokine-induced Intracellular Calcium Mobilization in THP-1 Cells—It is well documented that upon binding to their receptors, chemokines activate many intracellular signaling pathways, one or more of which may lead to an increase in intracellular calcium concentrations. THP-1 cells were loaded with the fluorescent dye Fura-2/AM and subsequently stimulated with different concentrations of the chemokines MCP-1 to -4 and eotaxin. Mobilization of intracellular calcium was detected by real time fluorescence measurement. Fig. 3A shows the response of cells stimulated with a 10 nM concentration of each chemokine administered at the time point denoted by an arrow. The data shown are from one experiment representative of five separate experiments. B, the responses to increasing concentrations of MCP-1 (unfilled circles), MCP-2 (filled circles), MCP-3 (unfilled squares), MCP-4 (filled squares), and eotaxin (unfilled triangles) are shown. Data shown are from one experiment representative of three separate experiments.

Fig. 3. THP-1 cells respond with an intracellular calcium flux to low nanomolar concentrations of MCPs 1–4 but not to eotaxin. THP-1 cells (1 × 10^7/ml) were loaded with the fluorescent dye Fura-2/AM, and agonist-dependent intracellular calcium release was subsequently measured. A shows the responses of the cells to a 10 nM final concentration of chemokine administered at the time point denoted by an arrow. The data shown are from one experiment representative of five separate experiments. B, the responses to increasing concentrations of MCP-1 (unfilled circles), MCP-2 (filled circles), MCP-3 (unfilled squares), MCP-4 (filled squares), and eotaxin (unfilled triangles) are shown. Data shown are from one experiment representative of three separate experiments.

![Graph A](image1.png)

![Graph B](image2.png)

![Graph C](image3.png)

![Graph D](image4.png)

**Fig. 3.** THP-1 cells respond with an intracellular calcium flux to low nanomolar concentrations of MCPs 1–4 but not to eotaxin. THP-1 cells (1 × 10^7/ml) were loaded with the fluorescent dye Fura-2/AM, and agonist-dependent intracellular calcium release was subsequently measured. A shows the responses of the cells to a 10 nM final concentration of chemokine administered at the time point denoted by an arrow. The data shown are from one experiment representative of five separate experiments. B, the responses to increasing concentrations of MCP-1 (unfilled circles), MCP-2 (filled circles), MCP-3 (unfilled squares), MCP-4 (filled squares), and eotaxin (unfilled triangles) are shown. Data shown are from one experiment representative of three separate experiments.

**Fig. 4.** Eotaxin is chemotactic for THP-1 cells at 1 μM concentrations and exerts its effects via CCR2. A, THP-1 cells were assessed for their ability to migrate in response to increasing concentrations of MCP-1 (unfilled circles), MCP-2 (filled circles), MCP-3 (unfilled squares), MCP-4 (filled squares), and eotaxin (unfilled triangles). Data shown are from one experiment, representative of three separate experiments. In B, THP-1 cells were preincubated at room temperature for 10 min with either buffer (open bars) IgG2a control antibody (solid bars), mAb LS132.1D9 (10 μg/ml) as a CCR2 antagonist (shaded bars), or the mAb 7B11 (3 μg/ml) as a CCR3 antagonist (checkered bars). Subsequently, the cells were assessed for their ability to migrate in response to 5 nM MCP-1, 0.5 nM MCP-3, 5 nM MIP-1α, or 100 nM eotaxin. Data are represented as a percentage of the control response to chemokine alone (open bars) and are depicted as the mean ± S.E. from four separate experiments. Statistical evaluation was performed using analysis of variance and Bonferroni’s post-test (*, p < 0.01; **, p < 0.001).

Eotaxin Is a Partial Agonist of CCR2b
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recruit THP-1 cells at low concentrations, was able to recruit calcium flux (Fig. 4, modest levels of chemotaxis were induced by MCP-2 and response obtained to a concentration of 1 nM. In contrast, only a conscious and more potent ligand, with a maximal chemotactic response to eotaxin, whose activity we and others have shown to be optimal in the low nanomolar range (6, 19, 28).

Eotaxin-induced Chemotaxis of THP-1 Cells Is Mediated by CCR2—Whereas MCP-1 binds exclusively to CCR2, the other members of the MCP family can also bind and signal through CCR3. As shown in Fig. 2, A and B, THP-1 cells possess both CCR2 and CCR3 at the protein and mRNA levels. To dissect the roles of each receptor in inducing chemotaxis to MCP-1, CCR3, and eotaxin, we specifically inhibited both receptors in the same experiment, where B/F represents the observed ratio of bound to free eotaxin, and bound represents the bound eotaxin (mol). Results shown are representative of three separate experiments for each cell line. The calculated $K_b$ ± S.D. for eotaxin binding to CCR2b was 7.50 ± 3.30 nM, and for binding to CCR3 it was 1.68 ± 0.91 nM. B and D, displacement of 0.1 nM 125I-eotaxin with either buffer or 100 nM concentrations of unlabeled MCP-1, -2, -3, or -4 or eotaxin. Data shown are expressed as the percentage of control binding in the presence of buffer alone and are mean values ± S.E. from four separate experiments.

Eotaxin Is a Partial Agonist of CCR2b—To confirm that eotaxin was able to signal through CCR2, we assessed the ability of cells transfected with CCR2b to migrate in response to eotaxin and MCP-1. The data are shown in Fig. 6A and complement our data obtained using THP-1 cells. Whereas MCP-1 was able to induce chemotaxis via CCR2b at subnanomolar concentrations, eotaxin was only efficacious at the relatively high concentration of 1 μM, despite being able to bind to CCR2 with a $K_d$ of 1.68 nM. Thus, eotaxin is a ligand for CCR2b, albeit with a lower affinity than the one it displays at CCR3.

Eotaxin Is a Partial Agonist of CCR2—To confirm that eotaxin was able to signal through CCR2, we assessed the ability of cells transfected with CCR2b to migrate in response to eotaxin and MCP-1. The data are shown in Fig. 6A and complement our data obtained using THP-1 cells. Whereas MCP-1 was able to induce chemotaxis via CCR2b at subnanomolar concentrations, eotaxin was only efficacious at the relatively high concentration of 1 μM, despite being able to bind to CCR2 with a low nanomolar affinity. In contrast, the ligands eotaxin-2 and eotaxin-3 were inactive in assays of chemotaxis at all concentrations tested. We subsequently sought to investigate the effect of substimulatory concentrations of eotaxin on MCP-1-induced chemotaxis in L1.2 cells expressing CCR2b. A 0.1 nM MCP-1 fixed concentration was chosen, since this gave adequate migration (Fig. 6A). Fig. 6B shows that low concentrations of eotaxin were sufficient to inhibit MCP-1-induced chemotaxis in a concentration-dependent manner. In keeping with these findings, eotaxin was able to
compete for the binding of 125I-radiolabeled MCP-1 to both CCR2 transfectants (Fig. 6C, Ki values of 0.15 nM for MCP-1 and 8.0 nM for eotaxin) and to THP-1 membranes (Fig. 6D, Ki values of 0.06 nM for MCP-1 and 12.0 nM for eotaxin).

Viewed as a whole, we conclude that eotaxin is a partial agonist of CCR2, being active in inducing chemotaxis at high concentrations yet inhibiting processes via the same receptor at low concentrations. To confirm our findings, we returned to the THP-1 cell line and investigated the ability of substimulatory concentrations of eotaxin to antagonize MCP-1-induced intracellular calcium fluxes. As can be seen in Fig. 7, complementary to our findings in CCR2b transfectants, substimulatory concentrations of eotaxin were able to antagonize CCR2b signaling induced by MCP-1.

DISCUSSION

Eotaxin is a CC chemokine that was originally identified as a eosinophil chemoattractant (15) and subsequently identified as a principal ligand for the chemokine receptor CCR3 (19, 28, 29). Using both the monocytic cell line THP-1 and CCR2b transfectants, we have demonstrated conclusively that eotaxin binds to and signals via CCR2 and not exclusively through CCR3 as was previously believed. Our findings are in contrast to those recently published by Ogilvie et al. (30), who described eotaxin as a natural antagonist of CCR2 and an agonist of CCR5. Using the THP-1 cell line, we were unable to demonstrate functional CCR5 expression either by FACS analysis using a CCR5-specific antibody or by intracellular calcium flux in response to the CCR5-specific ligand MIP-1β (data not shown). This, coupled with the data we obtained with CCR2 blocking antibodies, suggests to us that in the THP-1 cell line, the chemotactic activity of eotaxin is mediated by CCR2.

In addition, we have directly assessed the binding of eotaxin to CCR2 and demonstrated that it binds with low nanomolar affinity, around 5-fold lower than its affinity for CCR3. This lower affinity of eotaxin for CCR2 may, in part, explain the higher concentration of eotaxin needed to induce CCR2 as compared with CCR3 signaling. Chemotaxis induced by 0.1 nM MCP-1 was inhibited in a dose-dependent fashion by eotaxin with an IC50 of 0.15 nM (Fig. 6B). These concentrations of eotaxin are readily reached in vivo at sites of inflammation (31).
Eotaxin Is a Partial Agonist of CCR2b

Concentrations of 100 nM eotaxin were unable to completely block MCP-1-induced chemotaxis, suggesting either that this dose of eotaxin was able to induce some level of chemotaxis or that relatively few CCR2 molecules need to be occupied by MCP-1 to drive efficient chemotaxis of the transfectants. This latter explanation agrees with the recent finding by Janetopoulos et al. (32) that the steady state level of G-protein activation can achieve a level of saturation well before all receptors are occupied, suggesting that G-protein-coupled receptors act catalytically.

Current theories of G-protein-coupled receptor activation favor the ternary complex model (33) and its more recent variant (34). This model proposes that the binding of the agonist to G-protein-coupled receptor stabilizes the formation of a ternary complex of agonist (A), receptor (R), and G-proteins (G), the so-called AR*G complex. In this complex, R* is the active state that can couple to the G-proteins, in contrast to the uncoupled inactive R state of receptor. Binding of ligand to the uncoupled receptor R may be described in terms of the stabilization of a partly activated form of the receptor (R*) that can couple with G-proteins. In addition, different states of R* are believed to exist, and it is possible that different agonists may stabilize different conformations of R*, accounting for different efficacies observed in signal transduction (35). In the setting of CCR2 activation, eotaxin can be visualized as being less efficient at stabilizing the R* state of receptor than is MCP-1; hence, a higher concentration of eotaxin is required for effective chemotaxis. Thus, eotaxin is a partial agonist for CCR2 and exhibits only a weak affinity for the activation state of the receptor in contradicion to a full agonist, such as MCP-1, which has strong affinity for the activated R* state.

Eotaxin shares more than 60% identity at the amino acid level with all four members of the MCP family (Fig. 8), so its agonist activity at CCR2, albeit partial, may not be so surprising. Interestingly, the two other eotaxin species, eotaxin-2 and eotaxin-3, were inactive at CCR2 as measured by assays of chemotaxis, which again may be predicted, since they share less than 40% identity with either eotaxin or the MCPs. Many of the residues believed to be critical for the interaction of MCP-1 with CCR2, namely Pro8, Arg234, and Lys354, are conserved in eotaxin (36). Whereas the cores of the two proteins are relatively similar, the amino-terminal regions (amino acids 1–9) are quite different, resembling either an ordered or disordered β-strand structure in MCP-1 and eotaxin, respectively (37, 38). For a variety of chemokines, the amino terminus has been shown to be critical for receptor binding and activation. Indeed, the biological activity of eotaxin at CCR3 is dramatically reduced when the first two amino-terminal amino acids are removed by the activity of dipeptidyl-peptidase IV (CD26/DPP IV) (39). Work by Han et al. (40) has previously highlighted a crucial role in CCR2 activation for histidine 100 in the first extracellular loop of the receptor. They concluded that this basic residue of CCR2 did not contribute to ligand binding but was vital for receptor activation and subsequent signaling events and postulated that aspartate 3 of mature MCP-1 (also present in MCP-2 and -4) ion-bonded with histidine 100 of CCR2, resulting in a functional receptor-G-protein complex. It is tempting to speculate that the lack of negative charge within the amino terminus compromises the ability of eotaxin to induce formation of the AR*G complex and subsequently induce signaling. The introduction of a negative charge into the amino terminus of eotaxin by site-directed mutagenesis would directly test this hypothesis.

Eotaxin has previously been shown to have antagonist properties at CXCR3 (41), possessing a low affinity for the receptor. Since CCR3 is expressed on cells believed to be involved in Th2 responses and CXCR3 in cells characteristic of the Th1 response (42, 43), it was postulated by Weng et al. (41) that eotaxin may play a role in the impairment of Th1 responses in pathological conditions. Moreover, CCR2 has also been proposed to be characteristic of cells involved in the development of a Th1 response (44, 45), and mice null for CCR2 exhibit a Th2 bias (46–48). In view of the data we present here, we propose that similar to its antagonistic activity at CXCR3, low nanomolar concentrations of eotaxin, as found in inflammatory settings, may be an important modulator of inflammation and Th1/Th2 bias. Thus, eotaxin is a potential target for the treatment of allergic inflammatory disease independent of its actions at CCR3, the receptor previously supposed to be its sole receptor.

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