Functional expression of chemokine receptor 2 by normal human eosinophils

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Background: Within the granulocytes, the CC chemokines preferentially activate basophils and eosinophils on binding to chemokine receptors (CCRs). In vivo administration of neutralizing anti–monocyte chemoattractant protein 1 (MCP-1) antibodies can block accumulation of eosinophils in the lungs of antigen-challenged animals.
Objective: We studied a panel of chemokines for chemotactic activity in normal human eosinophils from healthy donors with a special focus on MCP-1, identified the respective receptor required for the biological response of eosinophils, and investigated mediators used for signal transduction.
Methods: Cells were enriched by magnetic cell sorting. Receptor expression in eosinophils was shown by RT-PCR and fluorescence-activated cell sorting. The biological response was tested in chemotaxis and calcium mobilization assays.
Results: Eosinophils have detectable mRNA for CCR2, and the receptor protein is expressed on cell surfaces. MCP-1 induces chemotaxis and calcium mobilization in eosinophils. The chemotactic activity of MCP-1 revealed a double-peaked dose-response curve; one of the peaks is abolished by addition of a blocking antibody to CCR2, but it is insensitive to blocking of CCR1 or CCR3. Specific enzyme inhibitors ruled out signaling characteristics of CCR2 in eosinophils.
Conclusion: Normal human eosinophils express functional CCR2 on cell surfaces. (J Allergy Clin Immunol 2001;108:581-7.)

Key words: *Eosinophils, eosinophilic infiltration, chemokines, chemotaxis, receptor, signal transduction*

Abbreviations used

C5a:	Complement fragment 5a
CC:	CC chemokine
CCR:	CC chemokine receptor
FACS:	Fluorescence-activated cell sorting
GFX:	Bisindolylmaleimide I
IBMX:	3-isobutyl-1-methylxanthine
MACS:	Magnetic cell sorting
MCP:	Monocyte chemoattractant protein
MIP-1α:	Monocyte inflammatory protein 1α
PAF:	Platelet-activating factor
PKC:	Protein kinase C
WTN:	Wortmannin

Chemokines are proinflammatory cytokines. They have been classified on the basis of the presence of 4 conserved cysteines, the first 2 of which can be adjacent (CC) or separated by 1 (CXC) or 3 (CX_3C) residues or can even lack one of the cysteines (C).[1-3] They exert their effects by interaction with seven-transmembrane, G protein–coupled receptors present in the membrane of the target cell. All of these receptors have a sequence of approximately 350 amino acids and a molecular weight of 40 kDa. The extracellular domain consists of the N-terminus and 3 extracellular loops that act in concert to bind the chemokine ligand. The intracellular region is composed of 3 loops and the C-terminus, which also collaborate to transduce the chemokine signal.

Identification of β-chemokine receptors (CCRs) has progressed rapidly, and at least 10 CCRs have been described to date.[1] By guiding leukocytes from the blood to inflammation sites, chemokines, as well as their respective receptors, play an important role. This recruitment is regulated in vivo by mechanisms that allow selective leukocyte-endothelial cell recognition.[4,5]

On the basis of a murine model of lung eosinophilia, it has been demonstrated that different chemokines have distinct functions in the attraction of leukocyte subpopulations.[6] Thus, the early accumulation of macrophages correlates with expression of monocyte chemoattractant protein 1 (MCP-1), whereas expression of RANTES and expression of eotaxin are concomitant with the presence of T cells and the presence of eosinophils, respectively. The early inflammatory process can be blocked by in vivo administration of neutralizing antichemokine antibodies to MCP-1, thus preventing accumulation of eosinophils, macrophages, or lymphocytes in the lung interstitium.[7]

The chemokine and leukocyte specificity of the CCRs overlap extensively.[8-10] Receptor usage by eosinophils has generated considerable interest, inasmuch as these cells are selectively recruited to specific inflammatory sites.[11,12] The known CCRs on eosinophils are CCR1 and CCR3, which can bind RANTES, monocyte inflammatory protein

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1α (MIP-1α), MCP-2, MCP-3, MCP-4, MCP-5, and eotaxins.[8-10] Although deletion of the NH_2-terminal glutamine residue converts MCP-1 into a potent eosinophil chemoattractant,[13] the complete molecule is thought to have no effect on eosinophil function; these cells should therefore lack expression of CCR2 or CCR4, both receptors for MCP-1.[14-16] However, mRNA for CCR2 was recently detected in eosinophils from an allergic patient, though functional expression of the receptor in eosinophils from healthy donors has not been reported thus far.[17]

METHODS

Reagents

RPMI 1640 was obtained from Biological Industries (Kibbutz Beit Haemek, Israel) and BSA from Aventis Behring (Marburg, Germany); complement fragment 5a (C5a), platelet-activating factor (PAF), Gro-α, 3-isobutyl-1-methylxanthine (IBMX), wortmannin (WTN), staurosporine, tyrphostin-23, and ionomycin were obtained from Sigma Chemical (St Louis, Mo). Bisindolylmaleimide I (GFX) was obtained from Boehringer Ingelheim (Vienna, Austria); RANTES, MCP-1, MCP-2, MCP-3, MCP-4, MIP-1α, eotaxin, and eotaxin 2 were obtained from R&D Systems (Minneapolis, Minn).

The mouse antihuman CCR2-specific blocking mAb (MCP-1R04; isotype IgG2a) was characterized previously. It recognizes the third extracellular loop (amino acids 273-292) of CCR2.[18] The respective control antibody (mouse IgG2a; UPC-10) was obtained from Sigma Chemical.

The biotinylated goat antimouse IgG and streptavidin-PE were obtained from Becton-Dickinson (San Jose, Calif), magnetic cell sorting (MACS) microbeads from Miltenyi Biotec (Bergisch Gladbach, Germany), and nitrocellulose filters from Sartorius AG (Göttingen, Germany). Fluorochrome fura-2-AM was obtained from Molecular Probes (Eugene, Ore), RNAClean from Thermo Hybaid US (Franklin, Mass), and primers for RT-PCR from MWG Biotech AG (Ebersberg, Germany). Taq polymerase was obtained from Applied Biosystems (Foster City, Calif); reverse transcriptase and a 1-kb DNA ladder were obtained from Gibco BRL Life Technologies (Vienna, Austria).

Enrichment of human eosinophils and isolation of monocytes

To enrich normal human eosinophils by $CD16^+$ cell depletion, we used MACS CD16 microbeads according to the manufacturer's protocol. In brief, granulocytes were obtained from the peripheral blood of healthy volunteers by dextran sedimentation and centrifugation through Ficoll-Hypaque (Pharmacia, Uppsala, Sweden). This step was repeated to remove most mononuclear cells; it was followed by hypotonic lysis of contaminating erythrocytes through use of sodium chloride solution.[19] After washing, cells were resuspended in 50 μL/5×10^7 cells ice-cold MACS buffer (PBS with 5 mmol/L EDTA and 0.5% BSA); an equal volume of MACS colloidal superparamagnetic microbeads conjugated with monoclonal antihuman CD16 mAb was added and incubated (for 30 minutes at 6°C). Recommended volumes of ice-cold MACS buffer were added to the cell/microbead mixture, and the cell suspension was loaded onto the separation column. The eluate containing $CD16^-$ eosinophils was collected, washed, and resuspended in RPMI 1640/0.5% BSA; the separation procedure was then repeated to increase purity. The purity of the sorted eosinophils was >98%, as determined by morphologic and fluorescence-activated cell sorting (FACS) analysis. Contaminating cells were <1% lymphocytes, <1% neutrophils and basophils, and monocytes/macrophages in negligible numbers. Monocytes were positive-selected from peripheral mononuclear cells through use of MACS microbeads conjugated to antihuman CD14 mAb.

RT-PCR

mRNA for CCR2 in freshly isolated eosinophils and monocytes was detected by RT-PCR. Total RNA was isolated from 8×10^6 cells through use of RNAClean. A reverse transcriptase reaction was performed on 1 μg of RNA through use of random hexamers. Ten μL of the reverse transcriptase reaction mixture was then subjected to 55 cycles of PCR in a 50-μL reaction mixture containing 10 pmol/μL of sense and antisense primer pairs in a thermocycler (Perkin-Elmer, Foster City, Calif), as follows: 95°C denaturation (30 seconds), 57°C annealing (60 seconds), and 72°C extension (30 seconds). Primers were designed to amplify a 693-bp coding sequence of CCR2 (sense, AGCCACAAGCTGAACAGAGA; antisense, CGAGTAGCAGATGACCATGA). The PCR products were then subjected to agarose gel electrophoresis.

FACS analysis of CCR2 surface expression

Analyses for the cell surface expression of CCR2 were performed on eosinophils and monocytes. A total of 5×10^5 cells were washed twice in PBS containing 0.5% BSA and incubated with 150 μg/mL human IgG/PBS/BSA 0.5% for 20 minutes at 4°C. After pelleting, cells were incubated alternatively with 10 μg/mL MCP-1R04 antibody or the respective isotype-matched control (IgG2a) for 30 minutes at 4°C. After washing, 10 μg/mL biotinylated goat antimouse IgG was incubated for another 30 minutes. Cells were washed twice; subsequently, monocytes and eosinophils were incubated with a 1:25 dilution of streptavidin-PE, washed twice, and immediately analyzed on a FACScan (Becton-Dickinson) with Cellquest software (Becton-Dickinson).

Chemotaxis assay

Chemotaxis assays were performed through use of a modified 48-well Boyden microchemotaxis chamber (Neuroprobe, Bethesda, Md), in which a cellulose nitrate filter with a pore size of 5 μm separates the upper and lower chambers.[19,20] Cells were resuspended in RPMI 1640/0.5% BSA to a final concentration of 1×10^6 cells/mL, and 50 μL of the cell suspension was placed in the upper chamber. Eosinophils migrated toward various concentration gradients of soluble chemoattractants in the lower chamber for 60 minutes. To rule out CCR2-dependent effects, cells were incubated with antihuman CCR1-, CCR2-, or CCR3-specific mAbs (for 30 minutes at 37°C) or with control antibody in a humidified atmosphere before migration toward chemoattractants.

To further study the effects of intracellular enzyme blockade, eosinophils were incubated with staurosporine (10 ng/mL), tyrphostin-23 (10 ng/mL), WTN (10 nmol/L), IBMX (1 μmol/L), or GFX (500 nmol/L) for 30 minutes at 37°C in a humidified atmosphere with 5% CO_2 before migration. After the migration periods, filters were dehydrated, fixed, and hematoxylin-eosin–stained. Cell migration depth into filters was quantified by microscopy, the distance (μm) from the filter surface to the leading front of cells being measured. Eosinophil random migration was <60 μm in all experiments. Data are given in terms of the "chemotaxis index"; this is the ratio between the distance of directed and undirected eosinophil migration into nitrocellulose filters.

Measurement of intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$)

To load cells with fura-2, cell layers on fibronectin-coated coverslips were incubated for 20 minutes with 2.5 μmol/L fura-2-AM. Measurements were made through use of an inverted phase-contrast

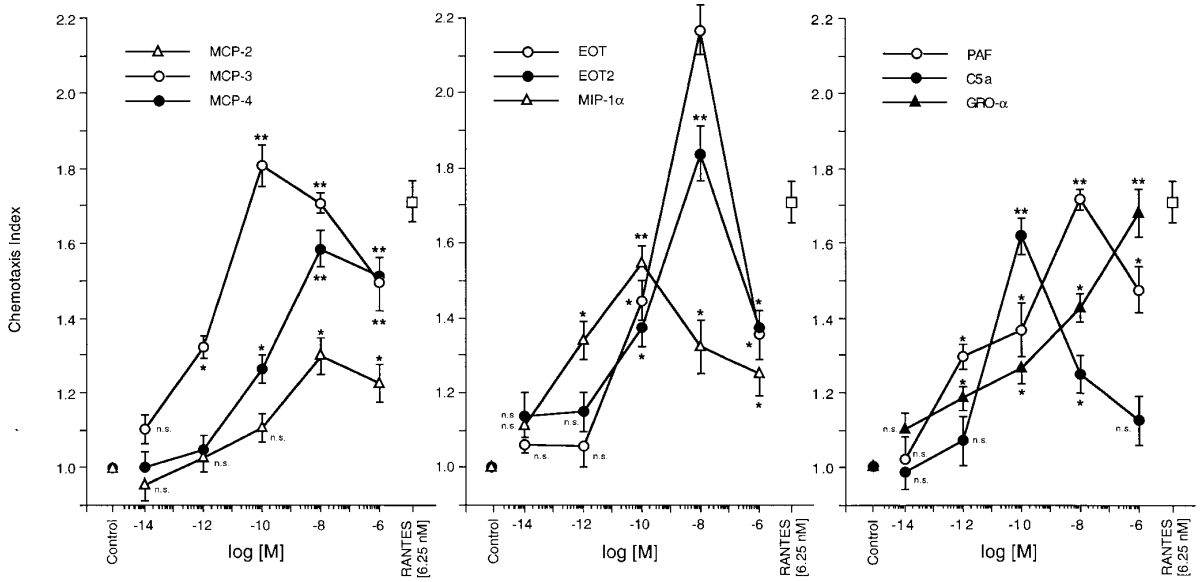

FIG 1. Dose response of eosinophils to various chemoattractants. Human eosinophils migrated into nitrocellulose filters toward soluble chemoattractants in the lower well of a Boyden microchemotaxis chamber. After fixing and staining, migration depth was quantified microscopically in the leading front assay. Data are expressed as means ± SEMs of the chemotaxis index (n = 5; see Materials and Methods section). Statistics: Mann-Whitney *U* test after Kruskal Wallis ANOVA ($P < .001$). *n.s.,* Not significant. $*P < .05$; $**P < .01$ vs medium control.

microscope (IX-70, Olympus, Austria) equipped for epifluorescence and photometry. Light from a fast monochromatic light source (Spectra Master, Olympus) with the monochromatic wavelength at 340 nm and 380 nm was deflected by a dichroic mirror (DM 400, Olympus) into the objective (UplanFl 60x/1.25 Oil, Olympus). Emitted fluorescence was directed through a 420-nm barrier filter to a charge-coupled device camera (OLYMPix KAI-0310 S/N, Olympus). Data acquisition and analysis were performed through use of Merlin Software (Life Sciences Resources, Cambridge, United Kingdom). $[Ca^{2+}]_i$ in nmol/L was calculated according to the ratio in situ calibration technique[21] through use of 10 μmol/L ionomycin.

Statistical analyses

Data are expressed as means and SEMs of the chemotaxis index or as nmol/L $[Ca^{2+}]_i$. Means were compared through use of the Kruskal-Wallis ANOVA and the Mann-Whitney *U* test. A difference of $P < .05$ was considered significant. Statistical analyses were performed through use of StatView software (Abacus Concepts, Berkeley, Calif).

RESULTS

Eosinophils chemotaxis toward several chemoattractants

Freshly prepared human eosinophils migrated toward MCP-2, MCP-3, MCP-4, eotaxin, eotaxin 2, MIP-1α, PAF, C5a, and Gro-α (each 0.1 fmol/L to 1 μmol/L) for 60 minutes at 37°C in a humidified atmosphere. Except for MCP-2, which was only weakly active, and Gro-α, all substances tested showed bell-shaped dose-response curves. Eotaxin at 10 nmol/L was most potent in inducing eosinophil migration. Maximal effects for the other chemokines were seen at concentrations between 0.1 and 10 nmol/L (Fig 1).

CCR2 expression in eosinophils and monocytes

CCR2 mRNA was analyzed in eosinophils from 2 separate healthy, nonatopic, and nonhypereosinophilic donors by RT-PCR. Monocytes were used as a positive control. mRNA for CCR2 was detected both in eosinophils preparations and in monocytes. As expected, size determination of the PCR product by agarose gel electrophoresis revealed a single band of approximately 700 bp (Fig 2).

Cell surface expression of CCR2 on eosinophils and monocytes

CCR2 cell surface expression was determined by FACS analyses of eosinophils and monocytes and revealed that the receptor protein is present in both cell populations. Eosinophils and monocytes stained intensely with anti-CCR2 mAb (MCP-1R04; Fig 3).

MCP-1-induced chemotaxis of eosinophils

Chemotaxis experiments were performed to confirm the biological activity of human MCP-1 on eosinophils. Freshly prepared and untouched cells were allowed to migrate toward various concentrations of MCP-1 (0.1 fmol/L to 0.1 μmol/L). Increasing concentrations produced a double-peaked dose-response curve. The first maximum was seen at 1 nmol/L of MCP-1, whereas further increases in the concentration of MCP-1 (10 nmol/L) declined the curve, which finally climaxed at 0.1 μmol/L. Addition of an antihuman CCR2-specific mAb (50 μg/mL) to cells prevented eosinophil activation induced by

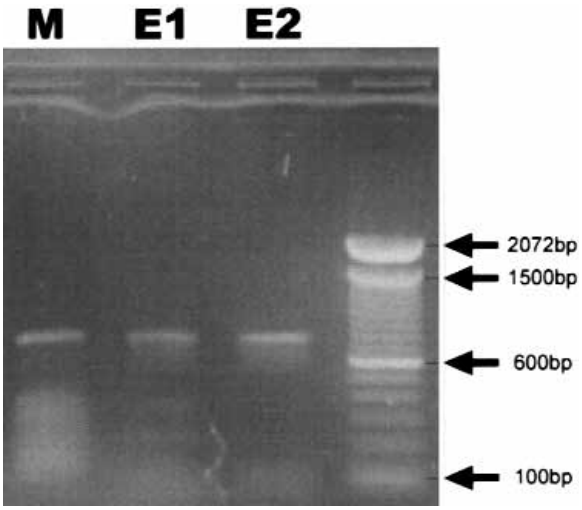

FIG 2. RT-PCR analysis. Reverse transcriptase reaction was performed on 1 μg of RNA derived from 2 eosinophil preparations from healthy donors *(E1, E2)* and from monocytes *(M)*. As expected, size determination after gel electrophoresis revealed a single band of approximately 700 bp.

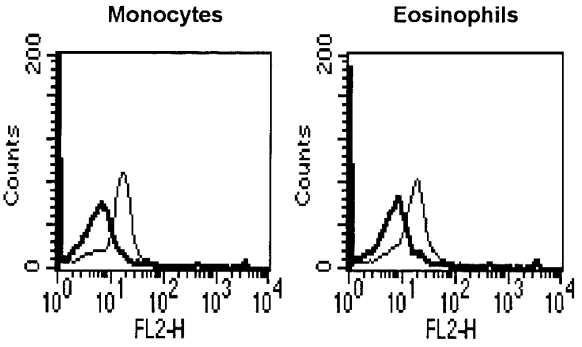

FIG 3. Cytofluorometry. CCR2 protein cell surface expression on eosinophils. Monocytes served as control. *Solid lines* indicate specific CCR2 mAb; *bold lines* indicate isotype-matched control. Data are representative of at least 3 independent experiments.

concentrations below 10 nmol/L of MCP-1 but failed to affect the second peak of the dose-response curve. In contrast to the first peak of the curve, the second peak was sensitive to blocking of CCR1 and CCR3, with higher potency of the anti-CCR3 blocking mAb (Fig 4). The control antibody did not produce such an effect (not shown).

Elevation of $[Ca^{2+}]_i$ in eosinophils by human MCP-1

In another experimental approach, changes in $[Ca^{2+}]_i$ flux were determined in single-cell measurements of eosinophils and monocytes. Incubation of cells with 1 nmol/L of MCP-1 significantly increased $[Ca^{2+}]_i$ in both cell populations. Maximal effects were seen after 20 seconds of stimulation with MCP-1. Further increases in the concentration of MCP-1 (10 nmol/L) elevated $[Ca^{2+}]_i$ in eosinophils only weakly and did not produce an additive effect in monocytes (Table I).

TABLE I. Intracellular Ca^{2+} concentrations in eosinophils and monocytes after MCP-1 stimulation

	Intracellular Ca^{2+} (nmol/L)			
	Monocytes		Eosinophils	
	Mean ± SEM	*P* value	Mean ± SEM	*P* value
Control	110.1 ± 6.78	—	134.8 ± 8.67	—
MCP-1 (1 nmol/L)	284.1 ± 47.9	0.0014	306.7 ± 45.1	0
MCP-1 (10 nmol/L)	254.2 ± 30.8	0	180.0 ± 23.0	0.0734

Statistical analysis: Mann-Whitney *U* test after Kruskal-Wallis ANOVA ($P < .001$; n = 9). *MCP-1,* Monocyte chemoattractant protein 1.

Signal transduction in eosinophils chemotaxis

Cells were incubated with optimal doses of the intracellular enzyme inhibitors staurosporine (10 ng/mL), tyrphostin-23 (10 ng/mL), IBMX (1 μmol/L), WTN (10 nmol/L), and GFX (500 nmol/L); thereafter, cells migrated toward several eosinophil chemoattractants. With the exception of MIP-1α, the chemotactic response of eosinophils to β-chemokines, PAF, or C5a was diminished by staurosporine. The effects of GFX, a highly selective protein kinase C (PKC) inhibitor, differed from those of staurosporine; GFX blocked the MIP-1α effect and failed to affect chemotaxis toward MCP-3, eotaxin, and C5a. Tyrphostin-23 blocked MCP-1-, MCP-4-, and Gro-α–induced migration, whereas WTN reduced chemotaxis toward MCP-1, MCP-2, MCP-4, and the eotaxins. Of all the inhibitors, IBMX, a phosphodiesterase IV inhibitor, was the only one that enhanced eosinophil migration—ie, toward MCP-3 and MIP-1α—and decreased chemotaxis only toward PAF (Table II).

DISCUSSION

MCP-1 triggers chemotaxis in eosinophils via the CCR2 receptor. Several chemoattractants were compared for their ability to induce eosinophil migration. As expected, eotaxin exhibited the highest potency in inducing eosinophil migration. With the exception of Gro-α, which showed a linear increase in dose response, all β-chemokines, C5a, and PAF were chemotactic for human eosinophils with bell-shaped dose-response curves, confirming interaction with their receptors and association of adequate signaling complexes. Surprisingly, we found a double-peaked dose-response curve for MCP-1-induced chemotaxis of human eosinophils. In contrast to eosinophils from a hypereosinophilic but nonatopic patient, receptor mRNA for CCR2 was recently identified in eosinophils from an atopic patient[17]; however, functional expression of the receptor on the surface of eosinophils was not investigated. We therefore performed RT-PCR experiments and detected CCR2 mRNA also in normal human eosinophils. FACS analyses confirmed expression of the receptor protein on the cell surface. The blocking antihuman CCR2 antibody (MCP-1R04),[18] which specifi-

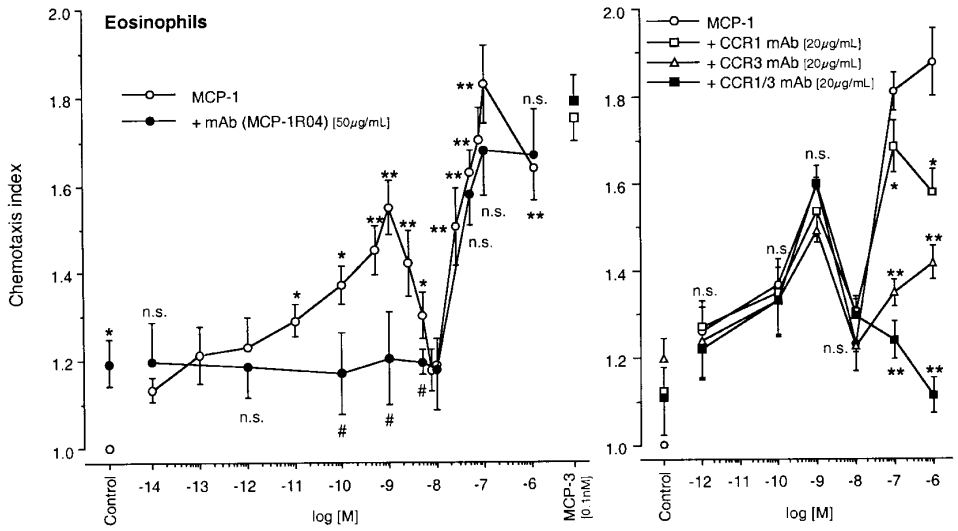

FIG 4. Effect of MCP-1 on eosinophil chemotaxis. Human eosinophils alone or preincubated with a specific anti-CCR1, anti-CCR2, or anti-CCR3 blocking mAb migrated for 60 minutes toward various concentrations of human MCP-1 in the lower well of a Boyden microchemotaxis chamber. MCP-3 (0.1 nmol/L) served as positive control. After fixing and staining, migration depth was quantified microscopically in the leading front assay. Data are expressed as means ± SEMs of the chemotaxis index; n = 5. Statistics: Mann-Whitney *U* test after Kruskal Wallis ANOVA ($P < .001$). *n.s.,* Not significant vs migration without mAb. #$P < .05$ vs migration with mAb. *$P < .05$; **$P < .01$ vs medium control.

TABLE II. Signaling characteristics: effect of intracellular enzyme blockers on eosinophil chemotaxis

		Cell preincubation				
Chemoattractant	**Medium**	**Staurosporine (10 ng/mL)**	**Tyrphostin-23 (10 ng/mL)**	**IBMX (1 μmol/L)**	**WTN (10 nmol/L)**	**GFX (500 nmol/L)**
Medium	1	**1.172**	0.980	1.017	1.026	**1.153**
MCP-1 (1 nmol/L)	1.572	**1.290**	**1.196**	1.586	**1.365**	**1.322**
MCP-2 (10 nmol/L)	1.365	**1.132**	1.307	1.315	**1.095**	**1.220**
MCP-3 (0.1 nmol/L)	1.71	**1.421**	1.650	**1.817**	1.671	1.675
MCP-4 (10 nmol/L)	1.647	**1.001**	**1.412**	1.554	**1.454**	**1.380**
Eotaxin (10 nmol/L)	2.153	**1.616**	2.095	2.185	**1.677**	2.091
Eotaxin-2 (10 nmol/L)	1.876	**1.551**	1.900	1.875	**1.605**	1.894
MIP-1α	1.554	1.647	1.593	**1.724**	1.517	**1.352**
PAF (10 nmol/L)	1.656	**1.166**	1.661	**1.417**	1.688	**1.344**
C5a (0.1 nmol/L)	1.593	**1.078**	1.662	1.684	1.578	1.655
Gro-α (1 μmol/L)	1.723	1.685	**1.422**	1.667	1.694	**1.52**

Statistical analysis: Mann-Whitney *U* test after Kruskal-Wallis ANOVA ($P < .001$; n = 5). Values were compared statistically with respect to unaffected chemotaxis toward the respective chemoattractant. Fields with *P* values less than .05 are shown in boldface.
C5a, Complement fragment 5a; *GFX,* bisindolylmaleimide I; *IBMX,* 3-isobutyl-1-methylxanthine; *MCP,* monocyte chemoattractant protein; *MIP-1α,* monocyte inflammatory protein 1α; *PAF,* platelet-activating factor; *WTN,* wortmannin.

cally recognizes the third extracellular loop (amino acids 273-292) of CCR2 protein, was also used in the chemotaxis assays. This antibody abolished the first peak, thus confirming functionally relevant CCR2 ligation by MCP-1, but it did not affect the second peak, which was induced by very high concentrations of MCP-1. The insensitivity of effects induced by high doses of MCP-1 to a specific CCR2 blocking mAb can be explained by the well-known phenomenon of receptor-sharing within the chemokine family.[8-10] In our study, further experiments on eosinophils revealed unspecific ligation of CCR1 and CCR3 by high concentrations of MCP-1. It is unlikely that cell contamination is responsible for the migratory response to MCP-1, inasmuch as monocyte contamination was <1% and neutrophils in the eosinophil preparations (<1%) would not respond to MCP-1.[22-24] The receptor specificity of the used anti-CCR2 mAb was shown in previous work[18]; it was confirmed here.

MCP-1 activates a CCR2 signaling pathway in human eosinophils. Activation of individual pathways used by a single receptor after chemokine ligation can be studied for the eotaxins, which bind only to CCR3.[25] Eotaxin- and eotaxin 2–induced eosinophil chemotaxis was blocked by staurosporine and WTN but not by GFX, sug-

gesting that PKC is not solely involved in CCR3 signaling and that additional activation of phosphatidylinositol-3-kinase (a WTN-sensitive enzyme) is needed. This was previously shown also for eotaxin-triggered oxidative burst in eosinophils.[26] In our study, MCP-1–induced signaling involved PKC (the staurosporine- and GFX-sensitive pathway), phosphatidylinositol 3,4,5-phosphate (the WTN-sensitive pathway), and tyrosine kinases. PKC-dependent activation of mitogen-activated protein kinase and WTN and tyrphostin sensitivity are implicated in MCP-1–induced and CCR2-mediated monocyte chemotaxis.[27] Given that this signal transduction pathway was found in our study to be also responsible for MCP-1-induced chemotaxis of eosinophils, this might additionally confirm expression of CCR2 in eosinophils.

Both MCP-2 and MCP-3 are ligands for CCR1, CCR2, and CCR3, and MCP-4 binds to CCR2 and CCR3[1]; thus the signal transduction patterns induced in eosinophils are difficult to interpret, because simultaneous receptor binding, and thus postreceptor desensitization, cannot be excluded. Attention must therefore be directed to the chemokine and not to a single receptor that induces signaling events. This also applies to MIP-1α, which is a ligand for CCR1, CCR4, and CCR5.[8-10] Intracellular enzymes that affect PAF-, C5a-, and Groα–stimulated chemotaxis have recently been described; the descriptions concur with our results.[28-30]

MCP-1 increases intracellular Ca^{2+} in eosinophils. To further confirm functional CCR2 expression, MCP-1 was tested in another assay. Before becoming motile, the cell assumes a polarized morphology, which might be reinforced during chemotaxis by redistribution of intracellular Ca^{2+} stores, cytoskeletal constituents, and chemoattractant receptors. In 9 independent experiments, stimulation of eosinophils with MCP-1 raised $[Ca^{2+}]_i$, maximum levels being observed 20 seconds after cell treatment. Because $[Ca^{2+}]_i$ elevation is a crucial step in receptor-mediated stimulation of migrating cells,[31] this sensitive assay demonstrates the response of human eosinophils to low MCP-1 concentrations and confirms results obtained in the chemotaxis assay.

Our observation of CCR2 expression by eosinophils coincides with recent studies in an in vivo mouse model in which MCP-1 neutralization before antigen challenge reduced eosinophil accumulation in the lung by 80% and bronchial hyperreactivity by approximately 70% without hindering RANTES and eotaxin expression.[6] It nonetheless contradicts studies showing lack of chemotactic response to MCP-1 by eosinophils[13-16]; the differences might be explained by the use of different assay systems or the narrower MCP-1 dose range analyzed in the other studies.[13,16]

In summary, we report CCR2 mRNA expression, MCP-1-induced and CCR2-mediated chemotaxis, MCP-1-triggered intracellular Ca^{2+} release, and signaling characteristics of CCR2 in normal human eosinophils, thus providing evidence of functional expression of the receptor in these cells. In addition to contributing to a better understanding of eosinophil biology, this study—particularly our analysis of the complex situation of eosinophil signal transduction—might be of use in identifying effective therapeutic targets in eosinophilia-associated inflammatory responses.

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