Molecular Cloning and Functional Expression of a New Human CC-Chemokine Receptor Gene[†]

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ABSTRACT: The cloning of several receptors activated by either CC or CXC chemokines and belonging to the G protein-coupled family of receptors has been reported recently. In the present work, we describe the cloning of a human gene, named ChemR13, encoding a new CC-chemokine receptor. The gene encodes a protein of 352 amino acids with a calculated molecular mass of 40 600 Da and displaying a single potential site for N-linked glycosylation. Using a set of overlapping lambda clones, the genomic organisation of the locus was investigated, demonstrating that the ChemR13 gene is physically linked, and in the same orientation, as the CC-CKR2 gene that encodes a receptor for the monocyte chemoattractant protein-1 (MCP-1). A distance of 17.5 kb separates the two coding regions, which share 75% identity in nucleic acid and amino acid sequences. Human ChemR13 was functionally expressed in a stably transfected CHO-K1 cell line. Physiological responses to chemokines were monitored using a microphysiometer. Macrophage inflammatory protein-1α (MIP-1α) was the most potent agonist. MIP-1β and RANTES were also active at physiological concentrations. The other CC-chemokines, MCP-1, MCP-2 and MCP-3, as well as CXC-chemokines (IL-8, GROα) had no effect. ChemR13 receptor transcripts were detected by Northern blotting in the promyeloblastic cell line KG-1A, suggesting a potential role in the control of granulocytic lineage proliferation or differentiation. ChemR13 is thus a new member of the growing family of chemokine receptors that mediate the recruitment of cells involved in immune and inflammatory processes. Being the fifth functionally identified receptor in his class, this new CC-chemokine receptor (CC-CKR) is tentatively designated CC-CKR5.

Chemotactic cytokines, or chemokines, are small signaling proteins that can be divided in two subfamilies (CC- and CXC-chemokines) depending on the relative position of the first two conserved cysteines. Interleukin 8 (IL-8)[1] is the most studied of these proteins, but a large number of chemokines (RANTES, MCP-1, MCP-2, MCP-3, GROα, GROβ, GROγ, MIP-1α and -β, etc.) has now been described (Baggiolini et al., 1994). Chemokines play fundamental roles in the physiology of acute and chronic inflammatory processes as well as in the pathological dysregulations of these processes, by attracting and simulating specific subsets of leukocytes (Oppenheim et al., 1991). RANTES, for example, is a chemoattractant for monocytes, memory T-cells and eosinophils and induces the release of histamine by basophils. MCP-1, released by smooth muscle cells in arteriosclerotic lesions, is considered as the factor (or one of the factors) responsible for macrophage attraction and, therefore, for the progressive aggravation of the lesions (Baggiolini et al., 1994). Recent studies have demonstrated that the actions of CC- and CXC-chemokines are mediated by subfamilies of G protein-coupled receptors. To date, despite the numerous functions attributed to chemokines and the increasing number of biologically active ligands, only six functional receptors have been identified in human. Two receptors for interleukin-8 (IL-8) have been described (Holmes et al., 1991; Murphy & Tiffany, 1991). One (IL-8RA) binds IL-8 specifically, while the other (IL-8RB) binds IL-8 and other CXC-chemokines, like GRO. Among receptors binding CC-chemokines, a receptor, designated CC-chemokine receptor 1 (CC-CKR1), binds both RANTES and MIP-1α (Neote et al., 1993), and the CC-CKR2 receptor binds MCP-1 and MCP-3 (Charo et al., 1994; Yamagami et al., 1994; Franci et al., 1995). Two additional CC-chemokine receptors were cloned recently. The CC-CKR3 receptor was initially reported to be activated by RANTES, MIP-1α, and MIP-1β, but these data have been retracted (Combadiere et al., 1995, and correction). The CC-CKR4 receptor responds

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[1] Abbreviations: BSA, bovine serum albumin; CHO, Chinese hamster ovary; CKR, chemokine receptor; EC_{50}, concentration producing 50% of the maximal response; GRO, growth-related gene product; IL-8, interleukin 8; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; PCR, polymerase chain reaction; PKC, protein kinase C; RANTES, regulated on activation normal T-cell expressed and secreted.

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to MIP-1α, RANTES, and MCP-1 (Power et al., 1995). In addition to these six functional receptors, a number of orphan receptors have been cloned from human and other species that are structurally related to either CC- or CXC-chemokine receptors. These include the human BLR1 (Dobner et al., 1992), EBI1 (Birkenbach et al., 1993), LCR1 (Jazin et al., 1993), the mouse MIP-1αRL1 and MIP-1αRL2 (Gao & Muphy, 1995), and the bovine PPR1 (Matsuoka et al., 1993). Their respective ligand(s) and function(s) are unknown.

In the present study, we report the molecular cloning and functional expression of a new human receptor, ChemR13, belonging to the CC-chemokine receptor family. The gene encoding ChemR13 is physically linked with the CC-CKR2 receptor gene in the human genome. The ChemR13 receptor, stably transfected in CHO-K1 cells, was found to be stimulated by MIP-1α, MIP-1β, and RANTES, while MCP-1, MCP-2, MCP-3, IL-8, and GROα had no effect.

EXPERIMENTAL PROCEDURES

Materials. Recombinant human chemokines, including MCP-1, MIP-1α, MIP-1β, RANTES, IL-8, and GROα were obtained from R&D Systems (London, U.K.). MCP-2 and MCP-3 were a gift of J. Van Damme, University of Leuven, Belgium. [^{125}I]MIP-1α (specific activity, 2200 Ci/mmol) was obtained from Dupont NEN (Brussels, Belgium). Chemokines obtained from R&D Systems were reported by the supplier as >97% pure on SDS−PAGE (sodium dodecyl sulfate−polyacrylamide gel electrophoresis) and biologically active on a bioassay specific for each ligand. The lyophilized chemokines were dissolved as 100 μg/mL solutions in sterile phosphate-buffered saline (PBS) and stored at −20 °C in aliquots. Chemokines were diluted to the working concentration immediately before use. All cell lines used in the present study were obtained from the ATCC (Rockville, MD).

Cloning and Sequencing. The mouse MOP020 clone was obtained by low stringency polymerase chain reaction, as described previously (Libert et al., 1989; Parmentier et al., 1989), using genomic DNA as template. A human genomic DNA library (Stratagene, La Jolla, CA) constructed in the lambda DASH vector was screened at low stringency (Sambrook et al., 1989) with the MOP020 (511 bp) probe. The positive clones were purified to homogeneity and analyzed by Southern blotting. The restriction map of the locus was determined, and a relevant *Xba*I fragment of 4400 bp was subcloned in pBluescript SK+ (Stratagene). Sequencing was performed on both strands after subcloning in M13mp derivatives, using fluorescent primers and an automated DNA sequencer (Applied Biosystem 370A). Sequence handling and data analysis were carried out using the DNASIS/PROSIS software (Hitachi) and the GCG software package (Genetics Computer Group, Madison, WI).

Expression in Cell Lines. The entire coding region was amplified by PCR as a 1056 bp fragment, using primers including respectively the *Bam*HI and *Xba*I recognition sequences, and cloned in the corresponding sites of the eukaryotic expression vector pcDNA3 (Invitrogen, San Diego, CA). The resulting construct was verified by sequencing and transfected in CHO-K1 cells as described (Perret et al., 1990). Two days after transfection, selection for stably transfected cell lines was initiated by the addition of 400 μg/mL G418 (Gibco), and resistant clones were isolated at day 10. CHO-K1 cells were cultured using Ham's F12 medium, as previously described (Perret et al., 1990; Desarnaud et al., 1994). The expression level of the ChemR13 receptor in the various cell clones was estimated by detecting the corresponding transcripts by Northern blotting, on total RNA prepared from the cells (see below).

Binding Assays. Stably transfected CHO-K1 cells expressing the ChemR13 receptor were grown to confluence and released from culture dishes by incubation in phosphate-buffered saline (PBS) supplemented with 1 mM EDTA. Cells were collected by low speed centrifugation and counted in a Neubaeur cell. Binding assays were performed in polyethylene minisorp tubes (Nunc) in a final volume of 200 μL of PBS containing 0.2% bovine serum albumin and 10^6 cells, in the presence of [^{125}I]MIP-1α. Nonspecific binding was determinated by the addition of 10 nM unlabeled MIP-1α. The concentration of labeled ligand was 0.4 nM (around 100 000 cpm per tube). The incubation was carried out for 2 h at 4 °C and was stopped by the rapid addition of 4 mL of ice-cold buffer and immediate collection of cells by vacuum filtration through GF/B glass fiber filters (Whatmann) presoaked in 0.5% polyethyleneinimine (Sigma). Filters were washed three times with 4 mL of ice-cold buffer and counted in a gamma counter.

Biological Activity. The CHO-K1 cell lines stably transfected with the pcDNA3/ChemR13 construct or wild-type CHO-K1 cells (used as controls) were plated onto the membrane of Transwell cell capsules (Molecular Devices), at a density of 2.5×10^5 cells/well in Ham's F12 medium. The next day, the capsules were transferred in a microphysiometer (Cytosensor, Molecular Devices), and the cells were allowed to equilibrate for approximately 2 h by perifusion of 1 mM phosphate-buffered RPMI-1640 medium (pH 7.4) containing 0.2% BSA. Cells were then exposed to various chemokines diluted in the same medium, for a 2 min duration. Acidification rates were measured at 1 min intervals.

Northern Blotting. Total RNA was isolated from transfected CHO-K1 cell lines, from a panel of human cell lines of hematopoietic origin and from a panel of dog tissues, using the RNeasy kit (Qiagen). RNA samples (10 μg per lane) were fractionated on a 1% agarose gel and transferred to nylon membranes (Pall Biodyne A, Glen Cove, NY) as described (Sambrook et al., 1989). Probes were α-^{32}P-labeled by random priming (Feinberg & Vogelstein, 1983) and used for blot hybridization.

RESULTS AND DISCUSSION

Cloning and Structural Analysis. The sequence homology characterizing genes encoding G protein-coupled receptors has allowed the cloning by low stringency polymerase chain reaction (PCR) of new members of this gene family (Libert et al., 1989; Parmentier et al., 1989). One of the clones, named MOP020, was amplified from mouse genomic DNA by using degenerate oligonucleotides corresponding to conserved regions in the second (IYIFNLA) and seventh (NPVLYAF) transmembrane segments of opioid and somatostatin receptors (Mollereau et al., 1994). MOP020 presented strong similarities with characterized chemokine receptors, sharing 80% identity with the CC-CKR2 receptor (Charo et al., 1994), 65% identity with the CC-CKR1 receptor (Neote et al., 1993), and 51% identity with IL-8

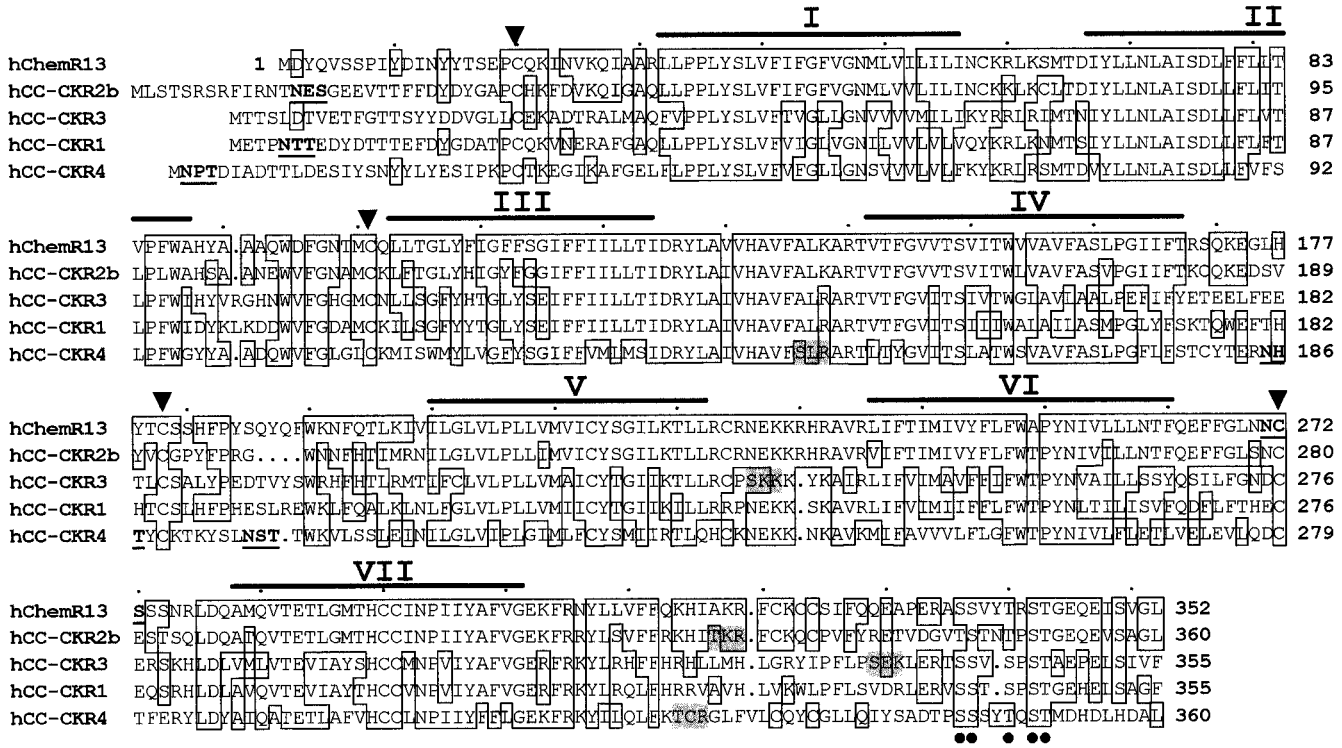

FIGURE 1: Primary structure of the new human chemokine receptor ChemR13. The amino acid sequence is aligned with that of the human CC-CKR1, CC-CKR2B, CC-CKR3, and CC-CKR4 receptors. Amino acids identical with the ChemR13 sequence are boxed. Numbering is relative to the initiation codons, and a dot tags every tenth residue of ChemR13. Putative transmembrane segments are overlined and numbered with the roman numbers I−VII. Potential N-linked glycosylation sites in the N-terminal extracellular segment and in the third extracellular loop are represented in bold and underlined. Potential sites of phosphorylation by protein kinase C (S/T X R/K motifs) in the second and third intracellular loop and C terminus are enhanced by a gray background. The conserved serine and threonine residues located at the C terminus and that are potential sites of phosphorylation by the family of G protein-coupled receptor kinases are indicated by filled circles. The conserved cysteine residues are indicated by inverted triangles. The nucleotide sequence encoding the novel receptor has been deposited with the Genbank/EMBL data libraries under accession number X91492.

receptors (Holmes et al., 1991; Murphy & Tiffany, 1991). The clone was used as a probe to screen a human genomic library. A total of 16 lambda phage clones were isolated. A restriction pattern was established for each clone and sequence data were obtained from a selected subset. It was found that the 16 clones constituted a single contig (see below) in which two related genes were included. One of the coding sequences (data not shown) was identical to the reported cDNA encoding the CC-CKR2 receptor (Charo et al., 1994; Yamagani et al., 1994). A 4.4 kb *Xba*I fragment of a representative clone containing the second region of hybridization was subcloned in pBluescript SK+. Sequencing revealed a novel gene, named ChemR13, sharing 84% identity with the MOP020 probe, suggesting that MOP020 is the mouse ortholog of ChemR13. MOP020 does not correspond to any of the three mouse β chemokine receptor genes cloned recently (Gao & Murphy, 1995), demonstrating the existence of a fourth murine β chemokine receptor.

The sequence of ChemR13 revealed a single open reading frame of 352 codons (Figure 1) encoding a protein of 40 600 Da. The sequence surrounding the proposed initiation codon is in agreement with the consensus as described by Kozak (1989), since the nucleotide in −3 is a purine. The hydropathy profile (not shown) of the deduced amino acid sequence is consistent with the existence of seven transmembrane segments. Alignment of the ChemR13 amino acid sequence with that of other functionally characterized human CC-chemokine receptors is represented in Figure 1. The highest similarity is found with the CC-CKR2 receptor (Charo et al., 1994) that shares 76% identical residues. There is also 56% identity with the CC-CKR1 receptor (Neote et al., 1993), 58% with the CC-CKR3 (Combadiere et al., 1993), and 49% with the CC-CKR4 (Power et al., 1995). ChemR13 represents therefore a new member of the CC-chemokine receptor group (Murphy, 1994). Like the related CC-CKR1 and IL-8 receptors (Holmes et al., 1991; Murphy & Tiffany, 1991; Neote et al., 1993; Gao et al., 1993), the coding region of ChemR13 appears as intronless.

Sequence similarities within the chemokine receptor family are higher in the transmembrane-spanning domains and in intracellular loops. As an example, the identity score between ChemR13 and CC-CKR2 goes up to 92% when considering the transmembrane segments only. Lower similarities are found in the N-terminal extracellular domain and in the extracellular loops. The N-terminal domain of the IL-8 and CC-CKR2 receptors has been shown to be essential for interaction with the ligand (Hébert et al., 1993; Gong & Clark-Lewis, 1995). The variability of this region among CC-chemokine receptors presumably contributes to the specificity toward the various ligands of the family.

A single potential site for N-linked glycosylation was identified in the third extracellular loop of ChemR13 (Figure 1). No glycosylation site was found in the N-terminal domain of the receptor, where most G protein-coupled receptors do contain such N-linked glycosylation consensus sequences. The CC-CKR1 and CC-CKR2 receptors do present such an N-linked glycosylation site in their N-terminal domain (Neote et al., 1993; Charo et al., 1994). By contrast, the CC-CKR3 receptor (Combadiere et al., 1995) does not display glycosylation sites either in the N-terminus

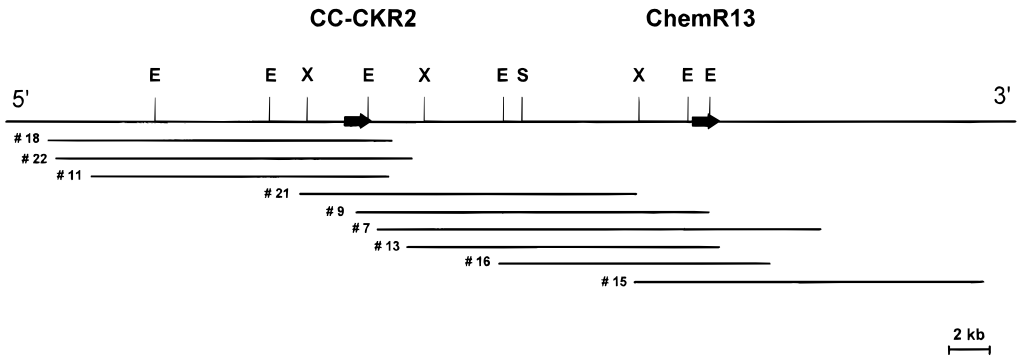

FIGURE 2: Chromosomal organization of the human CC-CKR2 and ChemR13 chemokine receptor genes. The upper line represents genomic DNA. The arrows indicate the position of the two intronless coding regions. *Eco*RI (E), *Xba*I (X), and *Sal*I (S) restriction sites are represented. Additional restriction sites could be present in the 3′ region of the locus. The lower lines represent the respective position and size of the 9 overlapping lambda clones covering this genomic region.

or in extracellular loops. ChemR13 contains four cysteines in its extracellular segments, and all four are conserved in the other CC- and CXC-chemokine receptors (Figure 1). The cysteines located in the first and second extracellular loops are present in most G protein-coupled receptors and are believed to form a disulfide bridge stabilizing the receptor structure (Strader et al., 1994). The two other cysteines, in the N-terminal segment and in the third extracellular loop could similarly form a stabilizing bridge specific to the chemokine receptor family (Hébert et al., 1993). The intracellular domains of ChemR13 do not include potential sites for phosphorylation by protein kinase C (PKC) or protein kinase A. PKC sites, involved in heterologous desensitization, are frequent in the third intracellular loop and C-terminus of G protein-coupled receptors. Such sites, present in CC-CKR3 and CC-CKR2, are represented in Figure 1. CC-CKR1 is also devoid of PKC sites. In contrast, all CC-chemokine receptors are rich in serine and threonine residues in the C-terminal domain. These residues represent potential phosphorylation sites by the family of G protein-coupled receptor kinases and are probably involved in homologous desensitization (Strader et al., 1994). Five of these S/T residues are perfectly aligned in all five receptors (Figure 1).

Physical Linkage of the ChemR13 and CC-CKR2 Genes. As stated above, the 16 clones isolated with the MOP020 probe corresponded to a single contig containing the ChemR13 and CC-CKR2 genes. The organization of this contig was investigated in order to characterize the physical linkage between the two receptor genes in the human genome. A combination of restriction mapping, Southern blotting, fragment subcloning, and partial sequencing allowed to determine the respective borders and overlaps of all clones. Out of the 16 clones, nineturned out to be characterized by a specific restriction map, and their organization is depicted in Figure 2. Four of these clones (nos. 11, 18, 21, 22) contain the CC-CKR2 gene alone, four clones (nos. 7, 13, 15, 16) contain the ChemR13 gene alone, and one clone (no. 9) contains part of both coding sequences. The CC-CKR2 and ChemR13 genes are organized in tandem, ChemR13 being located downstream of CC-CKR2. The distance separating CC-CKR2 and ChemR13 open reading frames is 17.5 kb. The chromosomal localization of the tandem is presently unknown. Other chemokine receptors have been located in the human genome: the CC-CKR1 gene was localized by fluorescence in situ hybridization to the p21 region of human chromosome 3 (Gao et al., 1993). The two IL-8 receptor genes and their pseudogene have been shown to be clustered on the human 2q34-q35 region (Ahuja et al., 1992). Future studies will demonstrate if CC-chemokine receptor genes do form large clusters in the genome, as do the genes encoding their ligands (Baggiolini et al., 1994).

Functional Expression and Pharmacology of the ChemR13 Receptor. Stable CHO-K1 cell lines expressing the ChemR13 receptor were established and were screened on the basis of the level of ChemR13 transcripts as determined by Northern blotting. Three clones were selected and tested for biological responses in a microphysiometer, using various CC- and CXC-chemokines as potential agonists. Wild-type CHO-K1 cells were used as control to ensure that the observed responses were specific for the transfected receptor and did not result from the activation of endogenous receptors. The microphysiometer allows the real time detection of receptor activation, by measuring the modifications of cell metabolism resulting from the stimulation of intracellular cascades (Owicki & Parce, 1992). Several studies have already demonstrated the usefulness of microphysiometry in the field of chemokine receptors. Modifications of the metabolic activity in response to CC-chemokines were monitored in human monocytes using this system (Vaddi & Newton, 1994). Similarly, the functional response of the human monocytic cell line THP-1 to the MCP-1 and MCP-3 chemokines was measured by using the microphysiometer (Pleass et al., 1995). The estimation of the EC_{50} for both proteins, using this procedure, was in agreement with the values obtained by monitoring the intracellular calcium in other studies (Charo et al., 1994; Franci et al., 1995).

Ligands belonging to the CC- and CXC-chemokine families were tested on ChemR13 transfected CHO-K1 cells. MIP-1α, MIP-1β, and RANTES, used at a 30 nM concentration, were found to be potent activators of the new receptor (Figure 3). The CC-chemokines MCP-1, MCP-2, and MCP-3 and the CXC-chemokines GROα and IL-8 had no effect on the metabolic activity at the same concentration. Recombinant IL-8 (inactive on ChemR13) was able to produce a 60% increase in metabolic activity in a CHO-K1 cell line transfected with the IL-8A interleukin receptor (Mollereau et al., 1993), demonstrating the biological activity of the ligand (data not shown). The biological activity of the MCP-2 and MCP-3 preparations as provided by J. Van Damme have been widely documented (Alam et al., 1994; Sozzani et al., 1994). MIP-1α, MIP-1β, and RANTES were inactive on the wild-type CHO-K1 cells. On the ChemR13 transfected cell line, the three active ligands (MIP-1α, MIP-

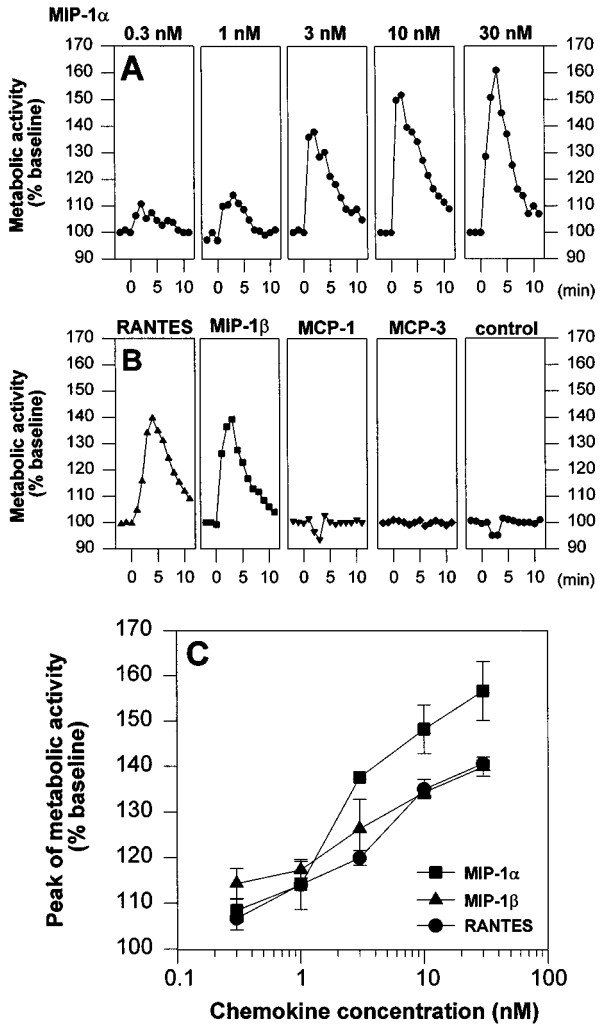

FIGURE 3: Functional expression of the human ChemR13 receptor in a CHO-K1 cell line. Metabolic activity of the recombinant cell line (and of CHO-K1 cells as control) was monitored using a microphysiometer, in response to various chemokines. (A) Functional response to various concentrations of MIP-1α. The abscissa indicates time after addition of the agonist that is left for 2 min in the presence of the cells before being washed out. (B) Exemplative recordings with high concentrations (30 nM) of the other chemokines that act as agonists on the ChemR13 receptor (MIP-1α and RANTES) or do not induce responses (MCP-1 and MCP-3). The control panel illustrates the absence of effect of MIP-1α (30 nM) on the wild-type CHO-K1 cell line. (C) Concentration-effect curve obtained with the three agonists of the ChemR13 receptor: MIP-1α, MIP-1β, and RANTES. The ordinate represents the peak value of metabolic activity following a 2-min perfusion of the ligands at concentrations ranging from 0.3 to 30 nM. All data points represent the mean and standard deviation from triplicate experiments performed on independent wells.

1β, and RANTES) caused a rapid increase in acidification rate, reaching a maximum by the second or third minute after perfusion of the ligand. The acidification rate returned to basal level within 10 min. The timing of the cellular response is similar to that reported for chemokines on their natural receptors in human monocytes (Vaddi & Newton, 1994). When agonists were applied repeatedly, the response strongly decreased as compared to the first stimulation, suggesting the desensitization of the receptor (not shown). All measurements were therefore obtained from the first stimulation of each capsule. The concentration-effect relationship was evaluated for the three active ligands in the 0.3−30 nM range (Figure 3B,C). The rank order of potency

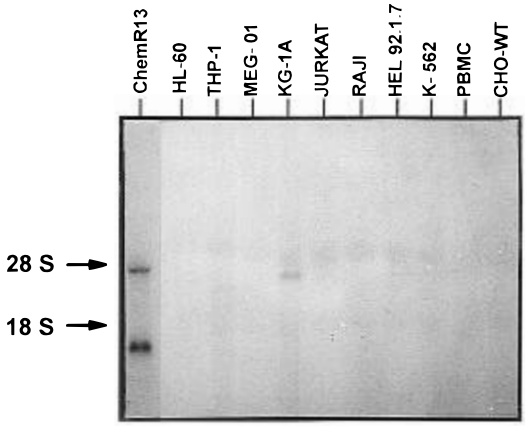

FIGURE 4: Distribution of mRNA encoding the ChemR13 receptor in a panel of human cell lines of hematopoietic origin. Each lane contains 10 μg of total RNA. The lineages to which the cell lines belong are described in the text. Peripheral blood mononuclear cells (PBMC) are also included. The blot was hybridized with a ChemR13 DNA probe, and the autoradiograph was carried for 8 days. The position of 18S and 28S ribosomal RNA bands is indicated by arrows. Identical results were obtained in two independent experiments. A band of 4.4 kb was detected in the KG-1a promyeloblastic cell line. The CHO-K1 cell line expressing the ChemR13 receptor was used as positive control (ChemR13) and wild-type CHO cells as negative control (CHO-WT). Two bands of 5.6 and 1.8 kb were found in the ChemR13 expressing CHO cell line. The quality of RNA preparation was assessed by hybridization with a β-actin probe (not shown).

was MIP-1α > MIP-1β = RANTES. At 30 nM concentrations, the effect of MIP-1α appeared to saturate (at 156% of baseline level), while the response curves for MIP-1β and RANTES were still in the ascending phase. Higher concentrations of chemokines could, however, not be used. The EC_{50} was estimated around 3 nM for MIP-1α. The concentrations necessary for obtaining a biological response as determined by using the microphysiometer are in the same range as those promoting intracellular calcium mobilization through the CC-CKR1 (EC_{50} for MIP-1α: ~1 nM) and the CC-CKR2A and B (EC_{50} for MCP-1: 10−15 nM) receptors (Neote et al., 1993; Charo et al., 1994). Among the presently reported receptors, ChemR13 is the only one responding to physiological concentrations of MIP-1β, as 10 nM elicits a significant increase in cell metabolism. These data suggest that ChemR13 could be (one of) the physiological receptor(s) for MIP-1β *in vivo*.

Binding experiments using [^{125}I]-human MIP-1α as ligand did not allow us to demonstrate specific binding to ChemR13 expressing CHO-K1 cells, using as much as 0.4 nM radioligand and 1 million transfected cells per tube. Failure to obtain binding data could be attributed to a relatively low affinity of the receptor for MIP-1α and possibly to an inadequate receptor expression level in our CHO cell line.

Northern Blotting Analysis. Northern blotting performed on a panel of dog tissues did not allow to detect transcripts for ChemR13 (not shown). Given the role of the chemokine receptor family in mediating chemoattraction and activation of various classes of cells involved in inflammatory and immune responses, the probe was also used to detect specific transcripts in a panel of human cell lines of hematopoietic origin (Figure 4). The panel included lymphoblastic (Raji) and T lymphoblastic (Jurkat) cell lines, promyeloblastic (KG-1A) and promyelocytic (HL-60) cell lines, a monocytic (THP-1) cell line, an erythroleukemia (HEL 92.1.7) cell line,

a megakaryoblastic (MEG-01) cell line, and a myelogenous leukemia (K-562) cell line. Human peripheral blood mononuclear cells (PBMC), including mature monocytes and lymphocytes, were also tested. ChemR13 transcripts (4.4 kb) could be detected only in the KG-1A promyeloblastic cell line but were not found in the promyelocytic cell line HL-60, in PBMC, or in any of the other cell lines tested. These results suggest that the ChemR13 receptor could be expressed in precursors of the granulocytic lineage. CC-chemokines are known to stimulate mature granulocytes (McColl et al., 1993; Rot et al., 1992; Kuna et al., 1992; Alam et al., 1992). However, recent data have also demonstrated that CC- and CXC-chemokines regulate the proliferation of mouse and human myeloid progenitor cells (Broxmeyer et al., 1990, 1993). Nevertheless, in order to confirm a potential role of ChemR13 in this process, the expression of the receptor in normal precursor cells of the granulocyte lineage will have to be confirmed.

As a conclusion, we have cloned a human gene, named ChemR13, encoding a novel CC-chemokine receptor, that responds to low nanomolar concentrations of MIP-1α, MIP-1β, and RANTES. This ChemR13 receptor gene is intronless in its coding sequence, and is located at a 17.5 kb distance downstream of the CC-CKR2 gene. This novel gene was found to be expressed in the human KG-1A promyeloblastic cell line. According to the nomenclature prevailing at the time of submission, this new CC-chemokine receptor is tentatively designated CC-CKR5.

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