Molecular Cloning and Functional Expression of Murine JE (Monocyte Chemoattractant Protein 1) and Murine Macrophage Inflammatory Protein 1α Receptors

EVIDENCE FOR TWO CLOSELY LINKED C-C CHEMOKINE RECEPTORS ON CHROMOSOME 9*

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We have isolated cDNA clones that encode two closely related, murine C-C chemokine receptors. Both receptors are members of the G-protein-coupled, seven-transmembrane domain family of receptors and are most closely related to the human monocyte chemotactrant protein 1 receptor. Expression of each of the receptors was detected in murine monocyte/macrophage cell lines, but not in nonhematopoietic lines. Expression of these receptors in Xenopus oocytes revealed that one receptor signaled in response to low nanomolar concentrations of murine JE, whereas the second receptor was activated by murine macrophage inflammatory protein (MIP) 1α and the human chemokines MIP-1β and RANTES. Binding studies revealed high affinity binding of radiolabeled mJE to the mJE receptor and murine MIP-1α to the second receptor. Chromosomal localization indicated that the two receptor genes were clustered within 80 kilobases of each other on mouse chromosome 9. Creation of receptor chimeras suggested that the amino terminus was critically involved in mediating signal transduction and ligand specificity of the mJE receptor, but not the mMIP-1α receptor. The identification and cloning of two functional murine chemokine receptors provides important new tools for investigating the roles of these potent cytokines in vivo.

Leukocyte trafficking plays an important role in immune system surveillance and chronic inflammation. Locally produced chemoattractant cytokines, known as chemokines, are thought to play a critical role in this directed migration (see Refs. 1–4 for recent reviews). Human monocyte chemoattractant protein 1 (hMCP-1)1 and its murine homolog, JE (mJE), are members of the C-C family of chemokines, in which the first two of four conserved cysteines are adjacent to each other. Other C-C chemokines include macrophage inflammatory protein 1α and 1β (MIP-1α, MIP-1β) and RANTES (regulated on activation, normal T cell-expressed). In general, C-C chemokines are potent monocyte and lymphocyte chemoattractants. A C-C chemokine that is a chemoattractant for eosinophils, eotaxin (5), has recently been described, as well as a novel lymphocyte chemokine containing two, rather than four cysteines, known as lymphotaxin (6).

The murine JE gene was originally identified by virtue of its dramatic induction in murine fibroblasts by platelet-derived growth factor and other growth factors (7). Characterization of the gene by Rollins et al. (8) revealed important similarities to known cytokines such as macrophage colony-stimulating factor, interferon-α, and interleukin (IL) 6. Murine JE and hMCP-1 are 62% identical over their amino-terminal domains, but mJE extends an additional 49 amino acids beyond the carboxyl end of hMCP-1. This carboxyl-terminal extension, which is extensively glycosylated, is not required for the chemoattractant activity of mJE (9). Further, mJE and hMCP-1 have similar chemoattractant activity for human monocytes (9). Murine JE is thus a structural and functional analog of hMCP-1.

MCP-1 has been implicated in the pathogenesis of diseases characterized by monocytic infiltrates, including psoriasis (10), pulmonary fibrosis (11), rheumatoid arthritis (12), and atherosclerosis (13, 14). In mice, mJE has been shown to be up-regulated by infusion of minimally oxidized low density lipoproteins (15) and thus may play a role in the accumulation of monocytic/macrophages in early atherosclerotic lesions. A possible role for mJE in tumor suppression in vivo was suggested by Rollins et al. (16) who found that expression of hMCP-1 or mJE in Chinese hamster ovary cells suppressed the ability of the cells to form tumors in nude mice.

Human receptors for IL-8 (Type A and Type B (17, 18)) and a single receptor that binds both RANTES and MIP-1α (19, 20) have been cloned and shown to be members of the seven-transmembrane domain superfamily of receptors. We have recently reported the cloning and expression of two alternatively spliced forms of the human MCP-1 receptor, which differ only in their terminal carboxyl tails (21) and which couple to Gi in vivo.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) U47035 (JE-R) and U47036 (mMIP1α-R).

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† The abbreviations used are: MCP-1, monocyte chemoattractant protein 1; IL, interleukin; RANTES, regulated on activation, normal T cell-expressed and secreted; MIP, macrophage inflammatory protein; PIPES, 1,4-piperazinediethanesulfonic acid.

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suggest that IL-8, and perhaps other chemokines, are involved in isis, as well as a decreased neutrophil response after injection of thioglycollate, was noted in mice in which the IL-8 receptor was deleted by homologous recombination (23). These studies suggest that IL-8, and perhaps other chemokines, are involved in the regulation of mollusks. Gao and Murphy (24) have very recently reported the cloning of a murine MIP-1α receptor, as well as two orphan receptors. In this paper, we report the cloning and functional expression of a murine JE/MCP-1 receptor, as well as a second, closely related receptor that signals in response to mMIP-1α, hMIP-1β, and hRANTES.

MATERIALS AND METHODS

Reagents—Recombinant chemokines were obtained from R & D Systems, Inc. (Minneapolis, MN). Initial experiments used full-length mouse (9) and rat JE purified from supernatants of stably transfected Chinese hamster ovary cells (kindly provided by T. Yoshimura, National Cancer Institute, Boston, MA). Murine JE expressed in E. coli was obtained from Escherichia coli (R & D Systems, Inc.) and used in subsequent experiments. No differences in activity were observed between the JE expressed in E. coli versus mammalian cells. LipofectAMINE and G418 sulfate were from Life Technologies, Inc. Restriction enzymes were from Boehringer Mannheim. 32Ca was obtained from Amersham. All other reagents and media were obtained from Sigma.

Tissue Culture, Calcium Fluorimetry, and Stable Transfections—WEHI 3, WEHI 274.1, and WEHI 265.1 cells were obtained from the American Type Culture Collection (Bethesda, MD) and were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum (HyClone Laboratories, Logan, UT) and 1% penicillin/streptomycin. Murine JE cDNA, which had been isolated from the murine JE receptor, was grown in RPMI 1640 containing 10% fetal calf serum and antibiotics. All other cell lines were grown in Dulbecco’s modified Eagle’s medium with 10% fetal calf serum plus antibiotics. For calcium fluorimetry, cells were grown to log phase, loaded with the calcium-specific dye Indo-1 AM (Molecular Probes, Eugene, OR), and assayed by spectrophotometry for changes in the concentration of intracellular calcium (Ca2+) in response to chemokines as described (22). To generate stable cell lines that expressed murine chemokine receptors, human embryonic kidney (HEK)-293 cells, obtained at passage 36 from the American Type Culture Collection, were grown in modified Eagle’s medium/Earle’s balanced salt solution with 10% fetal bovine serum (BioWhittaker, Walkersville, MD) and 1% penicillin/streptomycin. Murine JE cDNA, which had been isolated from the murine JE receptor, was grown in RPMI 1640 containing 10% fetal calf serum and antibiotics. All other cell lines were grown in Dulbecco’s modified Eagle’s medium with 10% fetal calf serum plus antibiotics. For calcium fluorimetry, cells were grown to log phase, loaded with the calcium-specific dye Indo-1 AM (Molecular Probes, Eugene, OR), and assayed by spectrophotometry for changes in the concentration of intracellular calcium (Ca2+) in response to chemokines as described (22). 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in 0.3 M sodium acetate, pH 5.2, to visualize ribosomal RNAs. The membrane was stripped by boiling for 10 min in water containing 0.5% SDS.

P1 Clones—Murine genomic bacteriophage P1 clones containing each of the two receptors were obtained using PCR primer pairs. A 129 ES mouse genomic library (average insert size of 85 kb) was screened by Genome Systems (St. Louis, MO) using primer pairs specific for each receptor. The mMIP-1a receptor (MIP-1a-R) primers were located at nucleotides 1015–1033 (sense) and 1144–1164 (antisense) of the cDNA of the mJE receptor primers at nucleotides 1360–1378 (sense) and 1593–1612 (antisense) of the cDNA. Of five positive clones for the mMIP-1a receptor, four also produced a specific product using the JE receptor primer pair. We further characterized two of the double positive clones by PCR and confirmed that both primer pairs amplified a specific product from each P1 clone. The PCR reactions included 0.19 nM of P1 DNA template, 100 μM concentration of each primer, and 2.5 units of Pfu DNA polymerase (Stratagene) in a volume of 100 μl. The PCR conditions were 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 50°C for 60 s, and 72°C for 2 min. PCR products were separated on a 1.8% agarose gel and stained with ethidium bromide.

Chromosomal Localization—The genes for mJE-R and mMIP-1a-R were mapped by linkage analysis of an interspecific backcross of (C57BL/6J × Mus spretus) F1 × C57BL/6J mice, constructed as described previously (28). The cross was previously typed for several hundred restriction fragment length variants (RFLVs) and simple sequence repeat polymorphisms (28). To identify informative RFLVs, DNAs from the parental strains were digested with various restriction enzymes revealed an RFLV for the mJE receptor gene using the enzyme HindIII; thus, following digestion, DNA from strain C57BL/6J exhibited bands of 16 kb and 7.2 kb, DNA from M. spretus exhibited a band of 6.4 kb, and DNA from F1 mice exhibited all three bands. The RFLVs were then examined in a set of backcross mice, and the segregation patterns were compared with those of previously typed markers.

Chimeric Receptors—The amino termini of the two murine receptors were interchanged by taking advantage of a conserved EcoRV site located within the putative first intracellular loop of each receptor (residue 92 of the mJE-R and residue 69 of the mMIP-1a-R). These chimeric receptors, designated mJE/mMIP-1a-R and mMIP-1a/mJE-R, were expressed and assayed for signaling in Xenopus oocytes as described above.

RESULTS

Cloning and Expression of Murine Chemokine Receptors—To identify cell lines expressing the mJE receptor, we screened murine monocyte/macrophage cell lines for responsiveness to mJE and related C-C chemokines. As shown in Fig. 1, mJE and, to a lesser extent, hMCP-1 induced a transient intracellular calcium flux in WEHI 274.1 cells. Calcium fluxes were not observed in WEHI 265.1, WEHI 3, or P388D1 cells (data not shown). To clone the mJE receptor, we constructed a cDNA library from the WEHI 274.1 cells and screened this library with a probe complementary to a highly conserved region of the human MCP-1R. A 2.9-kb cDNA clone was obtained that conferred mJE- and hMCP-1-dependent signaling, when expressed in Xenopus oocytes, as assayed by 45Ca2+ efflux (Fig. 2). This receptor was specific for J/EC/C/JP/MCP-1, as no calcium efflux was observed in response to the closely related C-C chemokines mMIP-1a, mMIP-1b, hRANTES, hMIP-1a, or hMIP-1b (Fig. 2A). In addition, HEK-293 cells stably expressing this cDNA also underwent a robust intracellular calcium flux in response to mJE (1–30 nM) (Fig. 3A), thus confirming that this receptor signals in mammalian cells. Human MCP-1 also elicited a calcium flux, although at higher concentrations than mJE (Fig. 3B). A small but reproducible signal was seen in response to mMIP-1a (100 nM), but not to hMIP-1α, hMIP-1β, or hRANTES (Fig. 3C). We therefore refer to this cDNA clone as the mJE receptor.

Screening of a mouse spleen library yielded a second cDNA clone that also hybridized strongly to the hMCP-1 receptor probe. In contrast to the mJE receptor, however, the receptor encoded by this cDNA signaled in response to mMIP-1a, hRANTES, and hMIP-1β, but did not respond to mJE, hMCP-1, mMIP-1b, or hMIP-1α (Fig. 4). We therefore refer to this receptor as the murine MIP-1α (mMIP-1α) receptor. The response of this receptor to murine, but not human MIP-1α, is intriguing, as the human MIP-1α/RANTES (C-C CKR-1) receptor

![Fig. 1. Calcium mobilization in WEHI 274.1 cells by mJE and hMCP-1. WEHI 274.1 cells were loaded with Indo-1 AM and challenged with mJE (30 nM) or hMCP-1 (100 nM) at the time indicated by the arrow.](http://www.jbc.org/)

![Fig. 2. Expression of the mJE receptor in Xenopus oocytes. A, specificity of the mJE receptor. All chemokines were used at a final concentration of 100 nM. B, dose-response curves for mJE and hMCP-1. All data points were determined in triplicate. The data shown are representative of three experiments.](http://www.jbc.org/)
responds equally well to both human and murine MIP-1α (19).

Binding of Radiolabeled Chemokines—Radiolabeled JE bound with high affinity to membranes prepared from HEK-293 cells expressing the putative JE receptor (Fig. 5A). Analysis of these binding data by the method of Scatchard revealed a dissociation constant (Kd) of 46 pM. In competition binding assays using 150 pM 125I-labeled JE, we observed very similar IC50 values for unlabeled JE (195 pM) and human MCP-1 (210 pM) (Fig. 5B). HEK-293 cells expressing the mMIP-1α receptor bound 125I-mMIP1α with high affinity (Kd = 640 pM) (Fig. 5C). In competition assays, we found that unlabeled hMIP-1β, as well as mMIP-1α, competed efficiently with 125I-mMIP-1α for binding to the receptor (data not shown).

Sequence Similarity of the Murine and Human C-C Chemo
kine Receptors—The murine JE receptor cDNA encoded a protein of 373 amino acids (Fig. 6). Hydropathy analysis of the predicted amino acid sequence revealed seven putative transmembrane domains and an extracellular amino terminus of 50 residues. The mJE receptor was closely related to the MCP-1 receptor, being 75% identical overall. However, the transmembrane (TM) domains and intracellular loops, particularly in the region of the second and third TMs, were almost identical. Conversely, the amino termini, the second and third extracellular loops, and the carboxyl-terminal tails were less well conserved between the two murine receptors (Fig. 6), suggesting that these regions may be involved in determining chemokine specificity.

Chromosomal Localization of mJE and mMIP-1α Receptors—Hybridization of murine genomic DNA with probes specific for the mJE and mMIP-1α receptors revealed single bands in each lane of the Southern blot, suggesting that each receptor is encoded by a single copy gene (Fig. 7). Rehybridization of this same blot with a probe derived from a highly conserved portion of the coding region of these two receptors (TM1 to TM3) failed to reveal additional bands, suggesting the absence of other closely related receptors (data not shown).

The chromosomal locations of the two genes were determined by linkage analysis of an interspecific backcross involving the parental mouse strains C57BL/6j and M. spreitus as described previously (28). Receptor-specific probes were used to identify informative RFLVs of the genes upon Southern hybridization. The segregation of the RFLV was examined in 65 (C57BL/6j × M. spreitus) F1 × C57BL/6j backcrossed mice. DNA from these mice has been typed previously for over 200 genetic markers spanning all chromosomes except the Y chromosome (28). The mJE receptor RFLV exhibited linkage with a number of mark-

Fig. 3. Calcium mobilization in 293 cells stably transfected with the mJE receptor cDNA. Dose-response curves to mJE (A), hMCP-1 (B), and other C-C chemokines (100 nM) (C).

Fig. 4. Expression of the murine MIP-1α receptor in Xenopus oocytes. A, specificity of the mMIP-1α receptor. The indicated chemokines were used at final concentrations of 100 nM. B, dose-response curves for mMIP-1α and hMIP-1β. All data points were determined in triplicate. The data shown are representative of three similar experiments.
ers on the distal portion of mouse chromosome 9, the nearest proximal marker being the microsatellite marker D9Mit19 (1 recombinant out of 65 animals) and the nearest distal marker being the random cDNA RFLV D9Ucla3 (1 recombinant out of 65 animals) (Fig. 8). The linkage was highly significant, as both markers exhibited logarithm of the odds scores exceeding 17.3. Analysis of the segregation of an RFLV for the mMIP-1α receptor gene revealed complete co-segregation with the mJE receptor RFLV (no recombination out of 65 animals). These results indicate that the genes for the mJE receptor and the mMIP-1α receptor are tightly linked on mouse chromosome 9 (Fig. 8). The results indicate the following order of markers typed on distal chromosome 9, with distances given in centimorgans:

\[ \text{centromere-} (D9Ucla2, D9Mit36) (5.9 ± 3.3) - (Jer, Mip1ar) (1.5 ± 1.5) - (D9Ucla3, D9Ucla5). \]

We designate the symbols Jer and Mip1ar for the JE receptor and MIP-1α receptor, respectively. This region of murine chromosome 9 is syntenic to human chromosome 3p21 (29).

Further evidence that the two receptors are closely linked was obtained by screening a murine 129ES genomic library for the presence of the cloned receptors. A, binding of chemokines to the cloned receptors. Radiolabeled mJE was added at the indicated concentrations to membranes prepared from HEK-293 cells stably expressing the mJE receptor. Nonspecific binding was determined by subtraction of the nonspecific binding from the total binding. The dissociation constant \( K_d \), determined by Scatchard analysis, was 46 ± 18 pM. Shown is one of three similar experiments. B, competition of mJE and hMCP-1 for the mJE receptor. Radiolabeled mJE (120 pM) was added to membranes prepared from HEK-293 cells stably expressing the mJE receptor. Unlabeled mJE and hMCP-1 were added at the indicated concentrations. The IC \( 50 \) values were 195 pM for mJE and 210 pM for hMCP-1. C, radiolabeled mMIP-1α was added at the indicated concentrations to HEK-293 cells stably expressing the mMIP-1α receptor. Nonspecific binding was determined by the addition of a 100-fold excess of unlabeled mMIP-1α. The apparent \( K_d \) was 640 pM.
Expression of the JE Receptor and the MIP-1α Receptor in Murine Monocytic Cell Lines—We examined a number of hematopoietic and nonhematopoietic murine cell lines for expression of the mJE receptor and the mMIP-1α receptor. As shown in Fig. 10, the mJE receptor mRNA was expressed by monocytic WEHI 274.1 cells and at lower levels by WEHI 265.1 and WEHI 3 cells. P388D1 cells, a macrophage-like line, and a variety of non-myeloid cell lines did not express detectable levels of the mJE receptor mRNA. Conversely, the mMIP-1α receptor message was expressed at high levels by P388D1 cells, at much lower levels by WEHI 265.1 cells, but was not detectable in the other cell lines tested (Fig. 10). These results are consistent with the observations that WEHI 274.1 cells respond to mJE (Fig. 1), and that P388D1 cells respond to hRANTES and mMIP-1α (Table II).

Receptor Chimeras—To investigate the role of the amino terminus in ligand recognition and signaling, we constructed chimeric receptors in which the amino termini, along with TM1, were interchanged between the mJE receptor and the mMIP-1α receptor. These chimeric receptors were expressed in Xenopus oocytes and assayed for signaling in response to various chemokines. As shown in Fig. 11, the chimeric mJE/MIP-1α receptor (amino terminus and TM1 from the mJE receptor spliced onto the mMIP-1α receptor) signaled well in response to mMIP-1α, and hRANTES, but did not signal in response to mJE or hMCP-1. This chimeric receptor, therefore, retained the same ligand specificity as the wild-type mMIP-1α receptor. In contrast, the complementary mMIP-1α/mJE receptor chimera failed to signal to any of the C-C chemokines (Fig. 11). Enzyme-linked immunosorbent assays confirmed that this chimera was expressed on the surface of microinjected oocytes at levels comparable to, or higher than, the wild-type receptors (data not shown). These data indicate, therefore, that the amino...
Murine JE has been implicated in models of disease characterized by prominent monocyte/macrophage infiltrates, but the mechanisms of monocyte activation and directed migration induced by mJE are not well understood. To gain insight into this phenomenon, and as a first step in genetic modification of the mJE receptor gene, we have cloned its cDNA from a murine monocytic cell line. Several lines of evidence support the conclusion that this cDNA encodes a murine JE receptor. First, injection into Xenopus oocytes of cRNA obtained from this clone conferred mJE/hMCP-1-dependent activation at low nanomolar concentrations. We have confirmed these results in transfected mammalian cells. In both Xenopus oocytes and HEK-293 cells expressing the JE receptor, calcium is mobilized much more efficiently by mJE as compared to hMCP-1. Similar results were obtained using wild-type WEHI 274.1 murine monocytes. Second, these responses are specific for mJE/hMCP-1, as other closely related chemokinines failed to induce signals. Third, radiolabeled JE bound with high affinity to HEK-293 cells transfected with this cDNA. Fourth, Northern blot analysis revealed high levels of expression of the receptor mRNA in monocytic cell lines that responded to mJE and little or no mRNA in lines that failed to respond to mJE. Finally, sequencing of the cDNA revealed a putative seven-transmembrane domain receptor with a predicted amino acid sequence that was 72% identical with the hMCP-1 receptor. We conclude, therefore, that this cDNA encodes a murine JE receptor.

The second receptor cloned in this study signaled well in response to low nanomolar concentrations of murine MIP-1α and also bound this chemokine with high affinity. It is likely, therefore, that mMIP-1α is the natural ligand for this receptor. The mMIP-1α receptor also signaled in response to human MIP-1β and thus represents the first example of a cloned receptor activated by MIP-1β. In addition, hMIP-1β competed well with radiolabeled mMIP-1α in receptor binding assays. Whether or not MIP-1β is a natural ligand for this receptor remains unclear, however, because the murine form of MIP-1β was not efficient at receptor activation. Similarly, this receptor was activated by human RANTES, and it will be interesting to determine if murine RANTES is a functional ligand.

The mJE and mMIP-1α receptors are almost completely identical in the putative transmembrane domains, as well as in the first extracellular loop. In addition, the second and third intracellular loops are nearly identical, suggesting that both receptors may couple to the same or very similar G-proteins. Interestingly, the murine MIP-1α receptor is more closely related to the MCP-1 receptor than to C-C CKR-1, the human receptor that binds and signals in response to MIP-1α and RANTES (19). These data suggest that the MIP-1α receptor is a novel receptor and not simply a murine homolog of the human MIP-1α/RANTES receptor. Based on primary sequence identity, it may in fact represent the murine form of a human MCP-1 receptor homolog. This receptor does not, however, signal in response to human or murine MCP-1. The mJE receptor signals primarily in response to mJE and hMCP-1 and, in this regard, is very reminiscent of the ligand specificity of the hMCP-1 receptor. We have recently found that hMCP-3, but not hMCP-2, is a functional ligand for the human MCP-1 receptor (30). It remains to be determined if the MARC/fic protein (31), which appears to be the murine homolog of hMCP-3, activates the mJE receptor.

The cloned mJE receptor bound mJE and hMCP-1 in a comparable manner, yet signaled much more efficiently in response to mJE as compared to hMCP-1. High affinity binding of the ligand to the receptor thus appears to be necessary, but not sufficient, to initiate signaling. These data are consistent with a model in which one portion of the receptor binds the ligand with high affinity, while a second receptor domain interacts with the chemokine to initiate signaling. Recent work in our laboratory on the binding of hMCP-1 to its receptor supports such a model,2 as does published work on the C5α receptor (32). It should be noted that hMCP-1 and mJE have been found to be equivalent in inducing chemotaxis of human monocytes (9). Thus, unlike the murine receptor, the human MCP-1 receptor may not distinguish between human and murine MCP-1. Studies are currently in progress in our laboratory comparing the binding and signaling properties of the human and murine MCP-1 receptors.

The mJE and mMIP-1α receptors appear to have arisen by gene duplication and may represent the first two members of a family of receptor genes clustered on chromosome 9. The evidence for this hypothesis includes the high degree of identity between these two receptors at the DNA sequence level, their co-segregation in a genetic cross, and their co-localization on a P1 bacteriophage clone. This area of mouse chromosome 9 is syntenic to human chromosome 3p21, where the hMIP-1α/ RANTES (20) and hMCP-1 receptor genes are found in close proximity. This region does not correspond to any mutations with obvious relevance to these receptors. In addition, Gao and Murphy (24) have very recently identified a murine MIP-1α receptor distinct from the receptors described in this paper, as well as two additional closely related murine receptors without identified ligands, all of which map to mouse chromosome 9. Other chemokine receptors have been localized to human chromosome 2q34-q35 in the case of the human type A and B IL-8 receptors (33) and to chromosome 19q13.3 for the formyl peptide and C5α receptors (34).

The amino-terminal domains represent the areas of greatest sequence divergence between the mJE and mMIP-1α receptors. The amino termini of the receptors for thrombin (35), thryrotrpin (36), C5a (37), and IL-8 (38) participate in the binding of their respective ligands. Taken together, these observations suggest that divergence of this domain in the mJE and mMIP-1α receptors may contribute to their different agonist specificities. To test this hypothesis, we constructed two chimeric receptors in which the amino-terminal domains were
exchanged between the mJE receptor and the mMIP-1 receptor. Analysis of the signaling properties of these two chimera in Xenopus oocytes indicated that the amino terminus of the mJE receptor, but not the mMIP-1 receptor, was critical for signaling. This result is in agreement with recent results obtained using the human MCP-1 receptor, and suggests that distinct mechanisms of ligand binding have evolved within the C-C chemokine receptor family. Since the first extracellular loop of the mJE receptor and mJE receptor are identical, it is likely that the second and third extracellular loops of the mMIP-1-R will be found to mediate ligand binding and specificity.

In summary, we have cloned two novel murine receptors that appear to define a family of C-C chemokine receptors clustered on chromosome 9. Through the construction of receptor chimeras, we have demonstrated that signaling of the mJE receptor, but not the mMIP-1 receptor, is critically dependent upon ligand interaction with the receptor amino terminus. The identification of the murine JE receptor represents an important step in the creation of genetically modified mice to probe the role of JE/MCP-1 in models of human disease.

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