Differential Regulation of G-protein-mediated Signaling by Chemokine Receptors*

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Monocyte chemoattractant protein-1 (MCP-1) is a member of a family of chemotactic cytokines that induce directed migration of leukocytes via activation of seven-transmembrane domain receptors. To identify G-proteins that couple to the two forms of the MCP-1 receptor, as well as to related chemokine receptors, we have performed cotransfection experiments in mammalian cells. In COS-7 cells, the type A and type B MCP-1 receptors coupled to G_{ai}, G_{aip}, and G_{ap}, whereas the macrophage inflammatory protein-1α/RANTES (regulated on activation, normal T cell-expressed and secreted) receptor (C-CR1) coupled only to G_{ai} and G_{aip} but failed to couple to G_{ap}. In HEK-293 cells, however, the MCP-1 receptors and C-CR1 coupled to G_{ap}, but failed to couple to G_{ai}. In contrast, the interleukin-8 and C5a receptors did not couple to G_{ai} in either COS-7 or HEK-293 cells but did couple to G_{ap}. Exchange of intracellular loops between the MCP-1 and interleukin-8 receptors to create chimeric receptors revealed that the third loop of the MCP-1 receptor accounted for virtually all of the coupling to G_{ap}. We conclude that the MCP-1 and related chemokine receptors couple to multiple G-proteins, that coupling is cell type-specific, and that the third intracellular loop of the C-C type receptors mediates G_{ap} coupling.

Chemokines (chemotactic cytokines) are low molecular weight proteins that are closely related in both primary amino acid sequence and tertiary structure (1–3). The chemokine family can be subdivided into two groups based on the presence or absence of an amino acid between the first two cysteines. In general, C-X-C family members are neutrophil agonists, and C-C members are mononuclear cell and basophil agonists. Interleukin-8 (IL-8) (1, 2) is the most thoroughly characterized member of the C-X-C or α-chemokine family; other members include growth regulatory gene, neutrophil-activating peptide-2, and platelet factor 4. IL-8 is a potent polymorphonuclear chemoattractant and agonist and has been implicated in neutrophil infiltration in the lung, liver, and spleen (4). Monocyte chemoattractant protein-1 (MCP-1) is the most extensively studied member of the C-C or β-chemokine family; other members include RANTES (regulated on activation, normal T cell-expressed and secreted), macrophage inflammatory proteins 1α and 1β (MIP-1α, MIP-1β), and eotaxin (5). MCP-1 is secreted by numerous cell types, including endothelial cells, epithelial cells, and hematopoietic cells, and is a potent chemoattractant for monocytes and CD45RO^+ lymphocytes (6). In vivo, MCP-1 has been implicated as an important factor in mediating monocyte infiltration in early atherosclerosis, as well as in a number of chronic inflammatory diseases (7, 8).

Because of the likely importance of the chemokines in a wide range of diseases, attention has recently been focused on the receptors that mediate chemokine responses. Several of these receptors have been cloned. In 1991, Gerard and Gerarddoned the receptor for the human complement fragment C5a and showed that it was a member of the G-protein-coupled, seven-transmembrane domain receptor family (9). Two highly homologous seven-transmembrane domain receptors for IL-8 have been cloned (IL-8RA and IL-8RB) and shown to be products of different genes (10, 11). Signaling by the IL-8 receptor in neutrophils was sensitive to inhibition by pertussis toxin (PTX), indicating that the receptor coupled at least in part to G_{i} (1, 12). A receptor for the C-C chemokine RANTES and MIP-1α, C-CR1, was cloned by Neote et al. (13) and Gao et al. (14), and we have recently cloned two alternatively spliced forms of the MCP-1 receptor (MCP-1RA and MCP-1RB) which differ only in their carboxyl-terminal tails (15). Signaling studies of the C-C chemokine receptors in transfected HEK-293 cells revealed potent, agonist-dependent inhibition of adenyl cyclase and mobilization of intracellular calcium, consistent with receptor coupling to G_{i} (16). In these studies, the calcium response was not, however, completely blocked by PTX, suggesting the involvement of additional G-proteins.

To identify PTX-resistant G-proteins that couple to the MCP-1 receptor, we have performed cotransfection experiments in COS-7 and HEK-293 cells. In this paper, we report that the C-C chemokine receptors, but not the IL-8 or C5a receptors, coupled to G_{ai} and that virtually all of this coupling could be attributed to the third intracellular loop of the receptor. The MCP-1 receptors, but not C-CR1, were found to couple to G_{ap} in COS-7 cells, but none of the C-C chemokine receptors coupled to G_{ap} in HEK-293 cells. These results suggest important differences in coupling between the C-X-C and C-C chemokine receptors.

EXPERIMENTAL PROCEDURES

Materials and Chemicals—The chemokines MCP-1, MIP-1α, RANTES, and IL-8 were obtained from R&D Systems, Inc. (Minneapolis). Lipofectamine, Opti-MEM, DMEM, and MEM with Earle's balanced salt were obtained from Life Technologies, Inc. PTX was purchased from List Biological Laboratories, Inc. (Eugene, OR). Fetal calf serum was obtained from Hyclone Laboratories (Logan, UT). myo-[2-^3H]inositol was obtained from DuPont NEN.
DNA Constructs—cDNAs of the MCP-1 receptor (types A and B) and C-CR1 were cloned as described (15). These constructs and the IL-8R construct consist of the prolactin signal sequence followed by a flag epitope and the receptor sequence (17). The expression vector for the human M1 muscarinic receptor was a generous gift from Dr. Wolf-gang Sadee, University of California, San Francisco, and all of the G-protein constructs were kindly provided by Dr. Bruce R. Conklin, Gladstone Institute of Cardiovascular Disease. The beta adrenergic receptor kinase 1 (βARK1) cDNA was a generous gift from Dr. Robert J. Lefkowitz, Duke University. The C5a receptor expression vector and the IL-8 receptor (type A) were generous gifts of Dr. Craig Gerard, Children’s Hospital, Boston, and Dr. William I. Woods, Genentech, South San Francisco, respectively. Chimeric receptors that exchanged intracellular loop 2 and 3 were created by overlapping polymerase chain reaction (18), as shown in Fig. 6.

Cell Culture and Transfection—COS-7 cells and human embryo kidney (HEK)-293 (CRL 1573) cells were obtained from the American Type Tissue Culture Collection (Bethesda, MD) and were grown in DMEM and MEM, respectively, with Earle’s balanced salt solution supplemented with 10% fetal calf serum, streptomycin (100 μg/ml), and penicillin (100 IU/ml) at 37°C in 5% CO2. cDNAs were transfected with Lipofectamine according to the manufacturer’s instructions. Briefly, cells were seeded in 24-well plates at a density of 4 × 105 cells/well and grown overnight. The cells were then washed with phosphate-buffered saline (PBS), and 0.3 μg of DNA mixed with 1.5 μl of Lipofectamine in 0.25 ml of Opti-MEM was added to each well. The total amount of DNA was maintained constant by adding DNA from an empty vector. After 5 h at 37°C, the medium was replaced with the medium containing 10% fetal calf serum.

Inositol Phosphate (IP) Formation Assay—Approximately 24 h after transfection, cells were labeled for 20–24 h with [3H]inositol (2 μCi/ml) in inositol-free medium containing 10% dialyzed fetal calf serum. Labeled cells were washed with inositol-free DMEM containing 10 mM LiCl and incubated at 37°C for 1 h with inositol-free DMEM containing 10 mM LiCl and the indicated agonist. IP formation was quantitated by counting [3H]inositol in the presence or absence of 100 ng/ml PTX and incubated in the presence of 10 mM LiCl for 1 h at 37°C with MCP-1 (100 nM), MIP-1α (100 nM), or carbacol (200 μM). Total [3H]inositol phosphate was measured as described under “Experimental Procedures.” Each data point was determined in triplicate, and the data shown are the means (± S.D.) of three independent experiments. The asterisks (*) indicate p < 0.05 versus control.

PTX. In contrast, little or no PI hydrolysis was detected in response to activation of C-CR1 by either MIP-1α or by RANTES. Cell surface expression of each of these epitope-tagged receptors was quantitated by ELISA, which revealed that C-CR1 was expressed essentially as well as MCP-1RB (see Fig. 4). The M1 muscarinic receptor couples exclusively via PTX-resistant Gαi, to hydrolyze PI, and as expected, signaling by M1 was not blocked by PTX pretreatment. These results indicate that both isoforms of the MCP-1 receptor couple to PTX-sensitive as well as PTX-resistant G-proteins to mediate IP release.

Hydrolysis of PI by Gαi-coupled receptors is thought to involve activation of phospholipase C by the βγ subunit of the G-protein complex (21). To determine if this mechanism was part of the signal transduction pathway, we cotransfected the cDNA for βARK1, which binds to and inactivates βγ subunits (22), with the cDNA for the MCP-1 receptor in COS-7 cells. In the presence of βARK1, MCP-1-induced IP release was blocked to approximately the same extent as by pretreatment of the cells with PTX (Fig. 2). These data are consistent with a model in which activation of Gαi releases the associated βγ subunit to activate phospholipase C and provides further evidence that both forms of the MCP-1 receptor couple, at least in part, to Gαi. As expected, overexpression of βARK1 did not affect carbacol-induced IP release in the cells transfected with the Gαq-coupled M1 muscarinic receptor. Similar results were obtained by cotransfection with transducin, which also binds and inactivates free βγ dimers (23, 24) (data not shown).

To identify G-proteins that couple to C-C chemokine receptors in a PTX-resistant manner, we cotransfected Gαq-subunits and receptors in COS-7 cells. Signaling by both forms of the MCP-1 receptor was enhanced significantly by coexpression of Gαq and Gα16 (Fig. 3). PI hydrolysis mediated by C-CR1 was potentiated by Gαq but not by Gα16. The chimeric G-protein Gαq5q16 has the carboxyl-terminal five amino acids of Gαi, which bind to the receptor, spliced onto Gαq (25). Cotransfection of Gαq5q16 also significantly potentiated signaling by each of the C-C chemokine receptors, consistent with coupling to Gαi. In contrast, IL-8RA did not induce PI turnover in COS-7 cells unless it was cotransfected with Gα16 or Gαq5q16. Furthermore, IL-8RA signaling was not enhanced by Gαq. Using an ELISA assay, we determined that cotransfection of the G-proteins did not alter the surface expression of any of the chemokine receptors (Fig. 4). We did note, however, that MCP-1RB was expressed at the cell surface at significantly lower levels than the other recep-
tors. These results suggest that both forms of the MCP-1 receptor couple efficiently to \( \text{G}_{q} \), \( \text{G}_{q16} \), and \( \text{G}_{i} \), whereas the IL-8 receptor has a preference for \( \text{G}_{q16} \) and \( \text{G}_{i} \).

To confirm these findings in a second cell type, we coexpressed the chemokine receptors and G-proteins in HEK-293 cells. In contrast to the results found in COS-7 cells, MCP-1-induced IP release was enhanced by cotransfection with \( \text{G}_{q} \) and \( \text{G}_{q16} \), but not by \( \text{G}_{q16} \) (Fig. 5). Similar results were obtained using C-CR1. Like the IL-8 receptor, the human C5a receptor signaled poorly in the absence of exogenous \( \text{G}_{q16} \), and signaling was not enhanced by coexpression of \( \text{G}_{q} \). Cotransfection of \( \text{G}_{q16} \) augmented signaling by the C5a receptor, as reported previously (26).

To identify the domain(s) of the MCP-1 receptor which bind to \( \text{G}_{q} \), we replaced the second and third intracellular loops of MCP-1RB with the corresponding regions of the IL-8RA (Fig. 6). As shown in Fig. 7, replacement of the 23-amino acid third intracellular loop of MCP-1RB with that of IL-8RA resulted in a chimera (MM8) which was phenotypically identical to IL-8RA in terms of signaling. Thus, MCP-1-dependent signaling was detected only in the presence of cotransfected \( \text{G}_{q16} \), and the receptor failed to couple to \( \text{G}_{q} \). Moreover, the complementary construct in which the third intracellular loop of MCP-1RB was substituted into IL-8RA (88M) resulted in a receptor in which signaling in response to IL-8 was indistinguishable from that of MCP-1RB. Note that exchange of this loop resulted in changes in only 14 amino acid residues since the carboxyl ends of the loops are virtually identical in the MCP-1 and IL-8 receptors (Fig. 6). In contrast, substitution of the second intracellular loop of IL-8RA into MCP-1RB (M8M) had no effect on MCP-1-dependent signaling. Thus, in the context of the receptor, the third intracellular loop of MCP-1RB was both necessary and
substituted in the third intracellular loop are has been interchanged. Amino acids that are identical or conservatively substituted in the second intracellular loop of the IL-8 receptor has been substituted. MM8 and 88M indicate chimeras in which the third intracellular loop has been substituted with MCP-1 receptor sequences, and 8 indicates IL-8 receptor sequences. The chimera M8M denotes the MCP-1 receptor into which the MCP-1 receptor couple identically to $G_{\alpha_16}$ and $G_{\alpha_i5}$, whereas the C5a receptor coupled to $G_{\alpha_16}$ but not $G_{\alpha_q}$. Receptor coupling to endogenous $G_{\alpha_16}$ probably accounts for the portion of PI turnover (approximately 50%) which is resistant to PTX.

Significant differences in G-protein coupling were found among the three C-C chemokine receptors and the IL-8 and C5a receptors. In COS-7 cells, both forms of the MCP-1 receptor, but not C-CR1, coupled to $G_{\alpha_16}$. Previous studies have demonstrated that a large number of receptors, including the $\beta_2$-adrenergic receptor, M2-muscarinic receptor, D1 dopamine receptor, $\mu$-opioid receptor, and thrombin receptor, couple to $G_{\alpha_16}$ in transiently transfected COS-7 cells (27). We next examined C-C chemokine receptor coupling in a second cell type, HEK-293. In contrast to COS-7 cells, the MCP-1 receptors failed to couple to $G_{\alpha_16}$ in transfected HEK-293 cells. The C5a receptor, previously shown to couple to $G_{\alpha_16}$ in this cell type (26), was used as a positive control. In HEK-293 cells, therefore, the C-C chemokine receptors coupled to $G_{\alpha_16}$, but not $G_{\alpha_q}$, whereas the C5a receptor coupled to $G_{\alpha_16}$ but failed to couple to $G_{\alpha_q}$.

In this paper we have used transient transfection of COS-7 and HEK-293 cells to investigate the signal transduction pathways of the MCP-1 and C-CR1 receptors. We have found that both forms of the MCP-1 receptor couple to at least three different G-proteins, $G_{\alpha_q}$, $G_{\alpha_16}$, and $G_\iota$, in COS-7 cells. The MIP-1α/RANTES receptor C-CR1 couples to $G_{\alpha_q}$ and $G_\iota$ but fails to couple to $G_{\alpha_16}$. The chimeric G-protein $G_{\alpha_16}$ is more effective than activation of phospholipase C via its $G_\iota$ portion (25). Since activation of phospholipase C by $G_{\alpha_q}$ but not $G_{\alpha_16}$ is generally more efficient than activation via $G_\iota$, this construct was used as a measure of $G_\iota$ coupling of each of the receptors. As expected, the MCP-1 receptors and C-CR1 coupled well to $G_{\alpha_16}$ to effect PI turnover. In contrast to the C-C chemokine receptors, the IL-8 and C5a receptors coupled to $G_{\alpha_q}$ and $G_{\alpha_16}$ but failed to couple to $G_{\alpha_q}$. Through the use of chimeric MCP-1/IL-8 receptors, we have shown that $G_{\alpha_16}$ coupling is determined completely by the third intracellular loop of the C-C chemokine receptors. We conclude that the chemokine receptors couple to multiple G-proteins, that receptor/G-protein pairings are highly cell type-specific, and that coupling to $G_{\alpha_q}$ may distinguish C-C from C-X-C chemokine receptors.

The type A and type B MCP-1 receptors are alternatively spliced variants of a single gene and differ only in their cytoplasmic tail (15). We had found previously that MCP-1RB coupled to $G_{\alpha_16}$ to inhibit adenylyl cyclase and mobilize intracellular calcium in transfected HEK-293 cells, but we were unable to study the signaling of MCP-1RA because cell surface expression was extremely poor in our stably transfected cell lines (16). In the current study we have shown that both forms of the receptor couple identically to $G_{\alpha_q}$, $G_\iota$, and $G_{\alpha_16}$. Receptor coupling to endogenous $G_{\alpha_q}$ probably accounts for the portion of PI turnover (approximately 50%) which is resistant to PTX.

FIG. 6. Exchange of intracellular loops between the MCP-1RB and IL-8RA. The sequences of the intracellular loops are shown. M designates MCP-1 receptor sequences, and 8 indicates IL-8 receptor sequences. The chimera M8M denotes the MCP-1 receptor into which the MCP-1 receptor has been substituted. MM8 and 88M indicate chimeras in which the third intracellular loop has been interchanged. Amino acids that are identical or conservatively substituted in the third intracellular loop are underlined.

G-protein Coupling in Chemokine Receptors

DISCUSSION

In this paper we have used transient transfection of COS-7 and HEK-293 cells to investigate the signal transduction pathways of the MCP-1 and C-CR1 receptors. We have found that both forms of the MCP-1 receptor couple to at least three different G-proteins, $G_{\alpha_q}$, $G_{\alpha_16}$, and $G_\iota$, in COS-7 cells. The MIP-1α/RANTES receptor C-CR1 couples to $G_{\alpha_q}$ and $G_\iota$ but fails to couple to $G_{\alpha_16}$. The chimeric G-protein $G_{\alpha_16}$ binds to $G_\iota$-coupled receptors via its carboxyl end and activates phospholipase C via its $G_{\alpha_q}$ portion (25). Since activation of phospholipase C by $G_{\alpha_q}$ is generally more efficient than activation via $G_\iota$, this construct was used as a measure of $G_\iota$ coupling of each of the receptors. As expected, the MCP-1 receptors and C-CR1 coupled well to $G_{\alpha_16}$ to effect PI turnover. In contrast to the C-C chemokine receptors, the IL-8 and C5a receptors coupled to $G_{\alpha_16}$ and $G_{\alpha_q}$ but failed to couple to $G_{\alpha_q}$. Through the use of chimeric MCP-1/IL-8 receptors, we have shown that $G_{\alpha_16}$ coupling is determined completely by the third intracellular loop of the C-C chemokine receptors. We conclude that the chemokine receptors couple to multiple G-proteins, that receptor/G-protein pairings are highly cell type-specific, and that coupling to $G_{\alpha_q}$ may distinguish C-C from C-X-C chemokine receptors.

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Significant differences in G-protein coupling were found among the three C-C chemokine receptors and the IL-8 and C5a receptors. In COS-7 cells, both forms of the MCP-1 receptor, but not C-CR1, coupled to $G_{\alpha_16}$. Previous work by several laboratories has demonstrated that a large number of receptors, including the $\beta_2$-adrenergic receptor, M2-muscarinic receptor, D1 dopamine receptor, $\mu$-opioid receptor, and thrombin receptor, couple to $G_{\alpha_16}$ in transiently transfected COS-7 cells (27). We next examined C-C chemokine receptor coupling in a second cell type, HEK-293. In contrast to COS-7 cells, the MCP-1 receptors failed to couple to $G_{\alpha_16}$ in transfected HEK-293 cells. The C5a receptor, previously shown to couple to $G_{\alpha_16}$ in this cell type (26), was used as a positive control. In HEK-293 cells, therefore, the C-C chemokine receptors coupled to $G_{\alpha_q}$, but not $G_{\alpha_16}$ whereas the C5a receptor coupled to $G_{\alpha_16}$ but failed to couple to $G_{\alpha_q}$.

It is likely that the promiscuous coupling of receptors to $G_{\alpha_16}$ in COS-7 cells was due, at least in part, to the high levels of G-protein expression achieved following transfection. In this regard, Chabre et al. (28) reported that the $\alpha_{2A}$-adrenergic receptor preferentially interacted with endogenous $G_{\alpha_q}$ but also coupled (with a higher EC$_{50}$) to transfected $G_{\alpha_q}$ and $G_{\alpha_16}$. It is also possible that selective expression of G-protein $\beta_2$ subunits may account for the differences in coupling observed between the COS-7 and HEK-293 cells. Kleuss et al. (37) (CK832) found
that in pituitary cells the y3 subtype was required for coupling to the somatostatin receptor, whereas the y4 subtype was required for coupling to the muscarinic receptor. It is not yet known which βγ subunits are present in COS-7 cells versus HEK-293 cells, nor is it known which βγ subunits are present in hematopoietic progenitor cells versus mature mononuclear cells. Taken together, these data indicate that chemokine receptor coupling is highly cell type-dependent and that in the case of recombinant receptors and G-proteins, signal transduction questions should be examined in multiple cell types.

The dramatic difference in coupling to Gαq between the C-C and C-X-C receptors provided an opportunity to identify the receptor site(s) interacting with Gαq. Extensive investigation of the adrenergic receptors has implicated the extended third intracellular loop, the second intracellular loop, and the carboxyl tail as contributors to the binding of G-proteins (for review, see Ref. 29). The high degree of amino acid sequence conservation in the third loop between the MCP-1 and C-CR1 receptors suggested that this domain might be critically involved in Gαq coupling. Consistent with this notion was the fact that the IL-8 and C5a receptors, which fail to couple to Gαq, bore little resemblance in the third loop to the C-C chemokine receptors. We therefore created a number of chimeric receptors that interchanged the second and third intracellular loops between the IL-8 receptor and MCP-1-IRB. Analysis of these constructs by agonist-dependent PI turnover revealed that virtually all of the Gαq coupling could be attributed to the third intracellular loop of the MCP-1 receptor. Comparison of the amino acid sequence in the third loop of the MCP-1 and IL-8 receptors further suggested that Gαq coupled to the amino-terminal portion of the loop, as the last 8 residues are either identical or conservatively substituted. We used a similar approach in an attempt to identify the receptor binding site for Gαq. Substitution of the third loop from C-CR1, which fails to couple to Gαq, into MCP-1-IRB (resulting in the chimera MIMC) did not, however, diminish coupling to Gαq (data not shown). We conclude, therefore, that although the third intracellular loop of the MCP-1 receptor is crucial for coupling to Gαq, it may not be for Gαq coupling. Experiments are currently in progress to determine if the second intracellular loop of the MCP-1 receptor interacts with Gαq.

Kuang et al. have recently reported that MCP-1IRB, but not MCP-1RA, coupled to Gαq (30), and thus concluded that the carboxyl-terminal tail of the receptor was critically involved in Gαq interactions. Our data do not support this conclusion, as we found that MCP-1RA and MCP-1-IRB coupled similarly to Gαq in COS-7 cells, and neither form of the receptor coupled to Gαq in HEK-293 cells. The failure of Kuang et al. to demonstrate MCP-1RA coupling to Gαq was probably due to the relatively lower levels of surface expression of MCP-1RA, compared with MCP-1-IRB, in transfected cells (Fig. 4). Kuang et al. also reported that neither of the MCP-1 receptors coupled to Gαq (30). Two lines of evidence from the present study indicate that both MCP-1RA and MCP-1-IRB are indeed Gαq-coupled. First, in the absence of exogenous G-proteins, both forms of the receptor mediate agonist-dependent PI turnover in COS-7 cells. Pretreatment of the cells with PTX blocked only 50% of the MCP-1-dependent PI turnover. This result raised the possibility that Gαq, which is PTX-resistant and is endogenously expressed in COS-7 cells, coupled to the MCP-1 receptor. Second, in cotransfection experiments, we have directly shown that both forms of the MCP-1 receptor, as well as C-CR1, couple to Gαq to enhance PI turnover. Similar results were obtained using transfected HEK-293 cells.

Cotransfection experiments are useful in that they reveal receptor/G-protein interactions that occur under conditions in which one or both are present at high concentrations. The coupling of G-proteins to receptors under physiological conditions may be much more stringent. Pretreatment of leukocytes with PTX abolishes the chemotactic response to MCP-1, IL-8, and formyl peptides almost completely, suggesting that coupling to Gαi is necessary and critically involved in mediating cell migration (31–33). Gα16 is present in hematopoietic progenitor cells, and its level in HL-60 cells falls dramatically after these cells are terminally differentiated (34). These data raise the intriguing possibility that PTX-resistant signaling pathways, and in particular coupling to Gα16, may be important in leukocyte maturation. MIP-1α and IL-8 have been recently shown to play roles in hematopoiesis (35, 36).

In summary, we have shown that the MCP-1 and MIP-1α/RANTES receptors couple to multiple G-proteins in transfected COS-7 and HEK-293 cells and that receptor/G-protein interactions are highly cell type-specific. The two forms of the MCP-1 receptor couple to the same G-proteins and in HEK-293 cells fail to couple to Gα16. Coupling to Gαq distinguishes the C-C chemokine receptors from the IL-8 and C5a receptors, as does coupling to Gα16 in HEK-293 cells. Virtually all of the Gαq coupling of the MCP-1 receptor, and most likely C-CR1 as well, can be attributed to the third intracellular loop. Since C-C chemokine receptors are found predominately in mononuclear cells, it is interesting to speculate that Gαq coupling may be particularly relevant in the setting of chronic inflammation. In contrast, since the IL-8 and C5a receptors are expressed well on polymorphonuclear leukocytes, Gαq coupling may be more important in acute inflammation. Pharmacological inactivation of leukocyte-specific G-proteins may thus provide an alternative and more specific means of treating inflammation.

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