Importance of the Amino Terminus of the Interleukin-8 Receptor in Ligand Interactions*

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Interleukin-8 (IL-8) and growth regulatory gene/melanoma growth stimulatory activity (GRO/MGSA) are small polypeptide molecules involved in the chemotactic response of certain cell types. Two receptors have been described which interact with IL-8, designated type 1 and type 2. IL-8 binds with high affinity to both receptors, whereas GRO/MGSA and neutrophil-activating peptide-2 demonstrate a high degree of binding only to the type 2 receptor. The two forms of IL-8 receptor are members of the rhodopsin seven-helix membrane-spanning superfamily, and share a high degree of overall homology, although the amino termini are very divergent. By using conserved restriction enzyme sites, a series of chimeric IL-8 receptor molecules were constructed between the type 1 and type 2 receptors and transfected into human 293 kidney epithelial cells. These chimeric molecules altered regions of the receptor presented to the ligand. The ability of the chimeric receptors to bind IL-8 was determined, as well as the ability of IL-8 and GRO/MGSA to inhibit radiolabeled IL-8 binding. The amino terminus of the IL-8 receptors was found to be important for differential binding of GRO/MGSA and IL-8. In addition, a series of peptides was also constructed to further investigate which residues of IL-8 receptor interact with IL-8. These peptides also identified the amino-terminal sequence of the IL-8 receptors as being important in interacting with IL-8.

Interleukin-8 (IL-8)1 and growth regulatory gene/melanoma growth stimulatory activity (GRO/MGSA) are members of a superfamily of cytokine polypeptides with molecular masses of about 10 kDa (Oppenheim et al., 1991). This superfamily is made up of proteins with substantial sequence and structural homology, in particular, the presence of four conserved cysteine residues. IL-8 and GRO/MGSA have been shown to have a variety of effects on a wide range of cell types. They act as chemotactic molecules on neutrophils (Yoshimura et al., 1987; Moser et al., 1991) and also produce a rise in cytosolic free calcium (Moser et al., 1991). GRO/MGSA can act as an autocrine mediator of melanoma cell growth (Richmond et al., 1988). The three-dimensional structure of IL-8 has been determined by both x-ray crystallography and NMR spectroscopy (Clore et al., 1990; Baldwin et al., 1991). It exists as a dimer of two identical subunits and may undergo a conformational change upon binding to the receptor (Clore and Gronenborn, 1991).

IL-8 and GRO/MGSA appear to bind to the same receptor since they have been shown to compete with one another for binding to neutrophils and the human myelomonocytic cell line, HL60 (Moser et al., 1991; Murphy and Tiffany, 1991; Oppenheim et al., 1991; Sager et al., 1992). The isolation of rabbit and human cDNA clones coding for proteins which bind IL-8 has been reported (Beckmann et al., 1991; Cerretti et al., 1992; Holmes et al., 1991; Murphy and Tiffany, 1991; Thomas et al., 1991). These receptors fall into two classes, designated type 1 and type 2. Both types of receptors are members of a family of proteins containing seven membrane-spanning helices that couple to guanine nucleotide binding proteins (G proteins) (Dohlman et al., 1987). The two types of receptors are similar, sharing about 74% amino acid identity; however, the amino termini of the different receptors are very different. Although initial reports indicated that the type 2 receptor was a low affinity receptor for IL-8 and GRO/MGSA (Murphy and Tiffany, 1991), recent experiments with both receptor forms produced in COS cells showed little difference in their affinity for IL-8 (Cerretti et al., 1992; Lee et al., 1992). There was, however, a very large difference in their interactions with GRO/MGSA and NAP-2. Although the type 1 receptor had little affinity for GRO/MGSA, the type 2 receptor bound GRO/MGSA and NAP-2 with high affinity.

Because of the similarity in their sequences, chimeric receptor molecules were constructed in order to determine the basis for the differential binding of IL-8 and GRO/MGSA. These chimeric molecules presented different combinations of amino acid residues to the ligands, allowing regions responsible for GRO/MGSA binding to be identified. Based on this information, peptides were synthesized which show the capacity to specifically inhibit IL-8 binding to the receptor. The amino-terminal residues of the IL-8 receptors were shown to be important in ligand interactions.

EXPERIMENTAL PROCEDURES

Plasmid Vectors and Restriction Enzymes—Expression vectors allowing high level production of rabbit type 1 IL-8 receptor (Beckmann et al., 1991) and human type 2 IL-8 receptor (Cerretti et al., 1992) have been described elsewhere. pDC303 (Maliszewski et al., 1990) was used for empty vector controls. Preparation of plasmid DNA, restriction digestions, and plasmid construction followed standard protocols (Ausubel et al., 1988). The DNA sequences of the recombinant vectors

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1 The abbreviations used are: IL-8, interleukin-8; G proteins, guanine nucleotide binding proteins; GM-CSF, granulocyte-macrophage colony-stimulating factor; GRO, growth regulatory gene; MGSA, melanoma growth stimulatory activity; NAP-2, neutrophil-activating peptide-2; PBS, phosphate-buffered saline.
were verified by deoxy sequencing (Sanger et al., 1977).

Construction of Chimeric IL-8 Receptor Molecules—Using restriction enzyme sites conserved between rabbit type 1 IL-8 receptor and human type 2 IL-8 receptor, chimeric tos of the two receptors were made, as summarized in Fig. 2. Chimera 1 contained the two genes fused in the Bpu1102I site, with the rabbit type 1 sequence upstream of the site and the rabbit type 1 sequence downstream. ARM was also used the HindII site and chimaera used the BamHI site, with rabbit type 1 sequences upstream and human type 2 downstream in each case. Chimera 4, 5, and 6 are the reciprocal constructions of chimeras 1, 2, and 3, respectively, with the human type 2 sequence upstream of the site and the rabbit type 1 sequence downstream. ARM was constructed using an oligonucleotide linker to remove the first 30 amino acids of rabbit type 1 IL-8 receptor. The location of the deletion is shown in Fig. 1. All the constructions used in this study, including rabbit type 1 IL-8 receptor and human type 2 IL-8 receptor, contain an octapeptide termed Flag (DYKDDDDK) at the amino terminus, which provides an antibody epitope following the recombinant proteins to be easily identified (Prickett et al., 1989).

Production and Labeling of Cytokines—Recombinant human IL-8 and human GRO/MGSA were synthesized in yeast and purified as described by Beckmann et al. (1989). Amino acid analysis was performed to determine protein concentration. IL-8 was tagged with 125I using Enzymobeads as described by Beckmann et al. (1991). The specific activities of the radioiodelabeled ligand was approximately 1 x 106 cpm/mmol. To verify that the radioiodelabeled IL-8 retained activity, it was shown to bind specifically to H660 cells after radioiodelabling. Following radioiodeliation, proteins were stored in 0.01 m acetic acid with 0.5 mg/ml bovine serum albumin, and 0.5 M NaCl was present in all buffers.

Transfections and Binding Assays—Human embryonic kidney 293 cells (ATCC CRL1573) were transfected with the recombinant plasmas using the DEAE-dextran method (Mossley et al., 1989). Two to three days after transfection, adherent monolayers of cells were assayed for direct IL-8 binding. For equilbrium binding studies, different concentrations of radioiodelabeled IL-8 in binding medium (RPMI 1640 medium containing 2.5% bovine serum albumin, 0.2% sodium bicarbonate, 0.05% pen-strep, and HEPES, pH 7.4) were added to the adherent cells. After 2 to 3 h at 4°C, cells were washed three times with binding medium, then twice with PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4, 7.4 mM KH2PO4, pH 7.4). The cells were harvested by trypsination. Non-specific binding was determined in the presence of 100-fold excess unlabeled IL-8. Cell-bound and free radioiodelabeled cytokines were quantified on a gamma counter. Binding studies performed on H660 cells were essentially as described by Cerretti et al. (1992). Inhibition of IL-8 binding was performed on 293 cells in a similar fashion except that 125I-IL-8 (8 x 105 cpm) was incubated with cells, and increasing amounts of unlabeled IL-8 or unlabeled GRO/MGSA were added. The amount of cell-bound and free radiolabeled IL-8 was then quantified as previously described (Cerretti et al., 1992), and binding constants were determined by Scatchard (1949) analysis. All binding data were analyzed using RS/1 (Bolt, Beranek and Newman, Boston, MA) run on a Microvax II, as previously described (Beckmann et al., 1991).

For IL-8 receptor peptide binding inhibition studies, dimethyl sulfoxide-induced H660 cells (5 x 105 cells) were incubated with 125I-IL-8 (4 x 105 cpm) and various concentrations of the peptides for 30 min at 37°C. Free and cell-bound IL-8 were separated by the phthalate acid method (Dower et al., 1984) and quantitated on a Packard Autoradiography counter.

Peptide Synthesis—Peptides derived from IL-8 receptor sequence were synthesized via Merrifield methodology using Fmoc (N-(9-fluorenyle) methoxycarbonyl) chemistry on an Applied Biosystems 430A peptide synthesizer.

RESULTS

Construction of Chimeric Molecules of Rabbit Type I and Human Type II IL-8 Receptors—The rabbit type 1 IL-8 receptor was used in this investigation because it was expressed on the surface of 293 cells at far greater levels than the human type 1 IL-8 receptor. There is little difference in the binding characteristics of the two forms of the type 1 receptor (Cerretti et al., 1992). An alignment of the protein sequence for the IL-8 receptors is shown in Fig. 1, as are the locations of the putative membrane spanning helices. The coding region for each receptor contains several conserved restriction enzyme sites. By using the restriction enzymes Bpu1102I, HindIII, and BamHI, chimeras of each receptor could be made which presented different extracellular regions. The relative locations of these restriction enzyme sites are shown in Fig. 1, and a schematic representation of the different chimeric receptors is shown in Fig. 2. The effect each region of the receptors had on the differential binding of GRO/MGSA and IL-8 could then be examined. Interestingly, the amino termini of the type 1 and type 2 receptors are the least conserved of any of the regions in the molecules. A recombinant receptor, ARM, was also constructed to investigate what effect deleting the amino terminus of the rabbit type 1 receptor had on ligand binding.

Production of Recombinant Receptors—Since the chimeric IL-8 receptors contained the antigenic Flag sequence at the amino terminus, immunoprecipitation with anti-Flag antibodies, followed by polyacrylamide gel electrophoresis, was undertaken to examine expression of the receptors in 293 cells. As can be seen in Fig. 3, all the constructions (the six chimeras and ARM) produced protein which was precipitated with anti-Flag antibody coupled to Protein G-Sepharose. Competition experiments revealed that only the major proteins seen at approximately 40 kDa are specific for the anti-Flag antibody (data not shown). The other higher molecular weight proteins were not specifically competed.

The rabbit type 1 IL-8 receptor and the ARM receptor both migrate close to their expected molecular masses, 41.6 and 38.2 kDa, respectively. Human type 2 IL-8 receptor migrates somewhat slower than rabbit type 1, even though its calculated molecular mass is 41.7 kDa. Chimeras 1, 2, and 3 migrate similarly to rabbit type 1 IL-8 receptor, while chimeras 4, 5, and 6 behave like human type 2 IL-8 receptor. This localizes the region responsible for the different mobilities to the amino termini of the receptors. Although several potential N-linked
glycosylation sites exist in the extracellular regions of the molecule (Fig. 1), the sizes of these proteins suggest that in transfected 293 cells the IL-8 receptors are not significantly glycosylated. In contrast to the IL-8 receptors expressed in human 293 cells, neutrophils produce IL-8 receptors with a molecular mass of 58 kDa, suggesting a greater degree of glycosylation (Samanta et al., 1989; Grob et al., 1990) in this cell type.

**Binding of IL-8 to the Chimeric Receptors**—Following production of rabbit type 1 IL-8 receptor, human type 2 IL-8 receptor, or the chimeric receptors in 293 cells, direct binding of radiolabeled IL-8 was performed in order to determine the affinity of the receptors for IL-8. The results are shown in Table I. Flag rabbit type 1 IL-8 receptor and Flag human type 2 IL-8 receptor have very similar affinities in these cells, with an apparent \( K_d \) of approximately \( 6.3 \times 10^{-10} \) M and \( 2.9 \times 10^{-9} \) M, respectively. These equilibrium constants are similar to those found for IL-8 receptors on neutrophils and to recombinant IL-8 receptors that lack the NH\(_2\)-terminal Flag sequence transfected into COS-7 cells (Cerretti et al., 1992), indicating that the Flag sequence does not interfere with ligand binding.

Only chimeras 1, 2, 3, and 6 appear to bind IL-8. These four molecules have a \( K_d \) within 3–4-fold of the wild-type molecules (Table I). This would indicate that the fusion of these regions from rabbit type 1 IL-8 receptor and human type 2 IL-8 receptor has not had a significant effect on the structure or activity of the molecules. ARM and chimeras 4 and 5 do not display any IL-8 binding. These three constructions produce protein, as evidenced by immunoprecipitations of metabolically labeled recombinant chimeric receptors (Fig. 3). Attempts to detect rabbit type 1 IL-8 receptor, human type 2 IL-8 receptor, or any IL-8 receptor chimera on the cell surface were unsuccessful. Cell surface labeling with \( ^{125}I \), followed by immunoprecipitation or cell surface staining with anti-Flag antibodies, was attempted with little success (data not shown). In contrast, a membrane-bound form of human GM-CSF receptor bearing an NH\(_2\)-terminal Flag sequence could be detected in these experiments. The inability to surface label the IL-8 receptors suggests that no accessible tyrosines are present. The Flag epitope, while accessible to solvent when fused to GM-CSF receptor, does not appear to be recognized by antibodies when expressed on the cell surface.
Fig. 2. Diagrams of the six chimeric IL-8 receptors. Chimeras 1–6 are shown, and the locations of the rabbit type 1 receptor and the human type 2 receptor sequences are shown. The membrane-spanning helices are shown as rectangles. The loops connecting the helices which correspond to rabbit type 1 receptor sequences are shown as cross-hatched lines and those which correspond to human type 2 receptor sequences are shown as black lines.

Fig. 3. Immunoprecipitation of wild-type and chimeric IL-8 receptors with anti-Flag antibodies. Following transfection with recombinant receptors and labeling with [35S]methionine and [35S]cysteine, the 293 cells were lysed. The lysates were incubated with anti-Flag antibodies, precipitated with Protein G-Sepharose, and electrophoresed on polyacrylamide gels. Lane 1, pDC800 (empty vector); lane 2, rabbit type 1 IL-8 receptor; lane 3, human type 2 IL-8 receptor; lane 4, chimera 1; lane 5, chimera 2; lane 6, chimera 3; lane 7, chimera 4; lane 8, chimera 5; lane 9, chimera 6; and lane 10, ARM.

fused to the IL-8 receptors. It is therefore difficult to conclude if ARM and chimeras 4 and 5 are present on the cell surface.

There may also be some modulation in the relative affinities of the chimeric receptors for IL-8. Chimera 6, which has the first 212 amino acids of the human type 2 IL-8 receptor fused to the last 146 amino acids of the rabbit type 1 IL-8 receptor, has an higher affinity for IL-8 than the reciprocal juxtaposition, chimera 3. It also displays a higher affinity than wild-type human type 2 IL-8 receptor, even though there are very few amino acid differences between chimera 6 and human type 2 IL-8 receptor. The intracellular loops of proteins in this superfamily have been implicated in the specificity of G protein coupling, which may affect the affinity of the receptor (Dohlman et al., 1987). The presence of a particular sequence in this region may affect the level of interaction with G proteins that are important for ligand-receptor associations.

There is usually a 5–7-fold difference between the number of rabbit type 1 IL-8 receptors and human type 2 IL-8 receptors found on the cell surface in transfected 293 cells (Table I). Combining different portions of rabbit type 1 IL-8 receptor and human type 2 IL-8 receptor had an effect on the production levels of the recombinant proteins. Chimera 1, which substituted the amino terminus of human type 2 IL-8 receptor with the amino-terminal region of rabbit type 1 IL-8 receptor, had over 10-fold more receptors on the cell surface than wild-type human type 2 IL-8 receptor (Table I). Chimeras 2 and 3 also have receptor numbers comparable to rabbit type 1 IL-8 receptor. The amino-terminal sequences of human type 2 IL-8 receptor appear to decrease the number of receptors.
TABLE 1

Binding of IL-8 and GRO/MGSA to chimeric IL-8 receptors

IL-8R expression constructs were transfected into 293 cells and 2 days after transfection cells were assessed for IL-8 binding as described under "Experimental Procedures." For saturation binding studies, data was analyzed according to the method of Scatchard (1949). For inhibition of IL-8 binding, cells were incubated with 8 x 10^{-10} M of IL-8 and various concentrations of unlabelled IL-8 or GRO/MGSA as described under "Experimental Procedures." Affinity constants (Kd) and inhibition constants (Ki) were calculated on RS/1 run on a Microvax II under the VMS operating system.

| IL-8 receptor | Kd (M) | No. of receptors | Ki (M) |
|---------------|--------|----------------|--------|
| Rabbit type 1 receptor | 6.3 x 10^{-10} | 260,000 | 1.4 x 10^{-9} |
| Human type 2 receptor | 2.9 x 10^{-9} | 57,000 | 9.1 x 10^{-2} |
| Chimera 1 | 7.0 x 10^{-10} | 600,000 | 4.8 x 10^{-4} |
| Chimera 2 | 3.7 x 10^{-10} | 260,000 | 2.2 x 10^{-10} |
| Chimera 3 | 8.3 x 10^{-9} | 310,000 | 2.7 x 10^{-8} |
| Chimera 4 | ND | ND | ND |
| Chimera 5 | ND | ND | ND |
| Chimera 6 | 6.7 x 10^{-9} | 57,000 | 1.2 x 10^{-2} |
| ΔRM | ND | ND | ND |
| Vector control | ND | ND | ND |

* Not detected.

** Based on extrapolation of binding curve.

Expression vector pDCS03 (Maliszewski et al., 1990) without a recombinant gene inserted.

found on the cell surface, while the corresponding sequences from rabbit type 1 IL-8 receptor result in protein which is more readily expressed.

Inhibition of IL-8 Binding to Chimeric Receptors—The ability of IL-8 to inhibit the binding of radiolabeled IL-8 to the different receptor molecules was also examined (Fig. 4). IL-8 binding can be completely inhibited for the two wild-type IL-8 receptors and the four chimeric IL-8 receptors (chimeras 1, 2, 3, and 6). The Kd of each receptor for IL-8 are similar (Table 1). Chimera 6 again displayed a higher affinity for IL-8 than the reciprocal construction (chimera 3) or even human type 2 IL-8 receptor.

Of interest was the ability of the different receptors to bind GRO/MGSA (Fig. 4). The affinity of the different IL-8 receptors for GRO/MGSA could be measured by observing the ability of GRO/MGSA to inhibit the binding of radiolabeled IL-8. Rabbit type 1 IL-8 receptor displayed little ability to bind GRO/MGSA, while human type 2 IL-8 receptor produced in 293 cells appears to effectively bind GRO/MGSA. The observed Kd was lower than the high affinity value seen on neutrophils (Cerretti et al., 1992). The interactions of GRO/MGSA with neutrophils can sometimes be fit to a monophasic curve, with a Kd of 1 x 10^{-9} M. For inhibition of IL-8 binding to neutrophils (Cerretti et al., 1992), the interactions of GRO/MGSA with neutrophils can sometimes be fit to a monophasic curve, with a Kd of 1 x 10^{-9} M. The human type 2 IL-8 receptor amino-terminal peptide (i8r-13) showed a small amount of inhibitory activity. The amino acid sequence of i8r-5 was scrambled to generate i8r-7. This peptide was used as a specificity control in examining the ability of the i8r-5 sequence to interact with the IL-8, rather than nonspecific aspects of the peptide such as charge. The i8r-7 peptide also showed little ability to inhibit IL-8 binding.

In order to identify which residues were important for the inhibitory activity, a series of peptides was synthesized which truncated the i8r-5 sequence from either end. Removing the last 11 amino acids from i8r-5 generated i8r-12, which resulted in greatly decreased inhibition of IL-8 binding (Table II). The minimal amount of inhibition observed is based on the extrapolation of the inhibition curve, which did not reach full saturation. Further truncations from either end resulted in the complete inability of the peptide to inhibit IL-8 binding.

DISCUSSION

Although the two forms of the IL-8 receptor share a large degree of sequence identity, they display differential binding properties for IL-8 and GRO/MGSA. The similarity of the two types of IL-8 receptor, and the ability to differentiate between them as a result of ligand binding, allowed construction of chimeric molecules of the type 1 and the type 2 receptors. Of the seven constructions made, three (ΔRM and chimeras 4 and 5) did not produce protein capable of ligand binding at the cell surface. Attempts to visualize the presence of these receptors on the cell surface by radiolabeling or by using anti-Flag antibodies have not been successful. Whether these three proteins are present on the cell surface and are incapable of binding any ligand, or whether there is a defect in their transport to the cell surface, cannot be determined. Although the four remaining constructions (chimeras 1, 2, 3, and 6) contain different combinations of rabbit type 1 IL-8 receptor and human type 2 IL-8 receptor sequences, they all bind IL-8. The amino-terminal regions of these three IL-8 receptors are dissimilar, but the determinants for high affinity binding are based on extrapola...
FIG. 4. Inhibition of 125I-IL-8 binding to wild-type and chimeric IL-8 receptors. Inhibition of radiolabeled 125I-IL-8 binding to transfected cells was determined as detailed under “Experimental Procedures.” ■, inhibition by IL-8; ●, inhibition by GRO/MGSA. A, rabbit type 1 IL-8 receptor; B, human type 2 IL-8 receptor; C, chimera 1; D, chimera 2; E, chimera 3; and F, chimera 6.

TABLE II
IL-8 receptor N-terminal peptide inhibition of 125I-IL-8 binding to HL60 cells

Different concentrations of each peptide were incubated with HL-60 cells as detailed under “Experimental Procedures.” Inhibition constants (K_i) were calculated on RS/1 run on a Microvax II under the VMS operating system.

| Peptide sequence | K_i (M) |
|------------------|---------|
| A. Amino terminal peptides | |
| Rabbit Type 1 | |
| i18r - 1 MEYVRNMTDLWTWFEDDEFANATGMPPVEKDSPLVVTQTLNK | 2.2 x 10^-6 |
| Human Type 1 | |
| i18r - 5 MSNITDPQWDFDDLNFTGMPPADEYSPCMLETETLNK | 1.7 x 10^-5 |
| Human Type 2 | |
| i18r - 13 MEYVSDFEDFWKGEDLSNSYSSLTPPFLLDAAPEPE | 1.5 x 10^-5 |
| Scrambled human Type 1 | |
| i18r - 7 MPADYSSWMENKNMTMGLNDEPCLMTDELDDPDFPTIDQ | 1.2 x 10^-5 |
| B. Truncated human type 1 IL-8 receptor peptides | |
| i18r - 12 MSNITDPQWDFDDLNFTGMPPADEYS | 1.1 x 10^-4 |
| i18r - 11 TDPQWDFDDLNFTGMPPADEYS | 1.5 x 10^-5 |
| i18r - 14 MSNITDPQWDFDDLNFTGMPPA | 1.0 x 10^-5 |
| i18r - 16 MSNITDPQWDFDD | ND |
| i18r - 17 TDPQWDFDDLNFTGMPPA | ND |
| i18r - 10 DFDDLNFTGMPPADEYS | ND |
| i18r - 9 DDLNFTGMPPADEYS | ND |

* Based on extrapolation of binding curve.

ND, not detected.

IL-8 binding are still present. The use of peptides corresponding to regions of the rabbit type 1 IL-8 receptor, the human type 1 IL-8 receptor and the human type 2 IL-8 receptor has shown that the the amino-terminal residues are important for interactions between IL-8 and its receptor. There is only 32% identity in this region between the rabbit type 1 IL-8 receptor and the type 2 IL-8 receptor, although both receptors bind IL-8. The other regions of the two molecules show 83% identity. The low degree of similarity between the rabbit type 1 IL-8 receptor, the human type 1 IL-8 receptor, and human type 2 IL-8 receptor at the amino terminus suggests that either the few residues which are conserved are responsible for ligand binding, or that the amino termini interact with ligand differently. All three wild-type IL-8 receptors have a large number of acidic amino acid residues which may play a role in interacting with the ligand.

The amino terminus of the human type 2 IL-8 receptor also seems to be important for determining the level of binding of
molecules may interact with G proteins involved in signal transduction. In other members of the G protein-coupled receptors, the intracellular loop following helix 5 appears to be important for interactions between G proteins and the receptor (Dohman et al., 1987). These G proteins may also affect the affinity of receptor-ligand interactions (Schumacher et al., 1992). As can be seen in Fig. 1, this loop is very short and the three wild-type IL-8 receptors share a great deal of amino acid conservation in this region. In fact, rabbit type 1 and human type 2 IL-8 receptors have only a single amino acid difference. Further investigations using mutagenesis should help determine whether the interaction of this loop with G proteins affects the affinity of the receptor for ligand, or whether another region at the carboxyl terminus is involved in ligand binding.

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