Structure and Functional Expression of a Human Interleukin-8 Receptor

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Interleukin-8 (IL-8) is a member of a family of pro-inflammatory cytokines. Although the best characterized activities of IL-8 include the chemoattraction and activation of neutrophils, other members of this family have a wide range of specific actions including the chemotaxis and activation of monocytes, the selective chemotaxis of memory T cells, the inhibition of hematopoietic stem cell proliferation, and the induction of neutrophil infiltration in vivo. A complementary DNA encoding the IL-8 receptor from human neutrophils has now been isolated. The amino acid sequence shows that the receptor is a member of the superfamily of receptors that couple to guanine nucleotide binding proteins (G proteins). The sequence is 29% identical to that of receptors for the other neutrophil chemoattractants, fMet-Leu-Phe and C5a. Mammalian cells transfected with the IL-8 receptor cDNA clone bind IL-8 with high affinity and respond specifically to IL-8 by transiently mobilizing calcium. The IL-8 receptor may be part of a subfamily of related G protein–coupled receptors that transduce signals for the IL-8 family of pro-inflammatory cytokines.

INTERLEUKIN-8 (IL-8), WHICH WAS initially identified as a chemoattractant for neutrophils, also has a wide range of pro-inflammatory effects that include stimulation of neutrophil degranulation and increased expression of the cell adhesion molecule Mac-1 (CDIIb/CD18) and the complement receptor CR1 (*1*). Treatment of neutrophils with IL-8 also inhibits their adherence to activated endothelial cells (*2*). IL-8 is a member of a family comprised of ten or more pro-inflammatory cytokines that have a molecular size of about 10 kD and similar structural features. The family can be divided into a class of cytokines in which two of the conserved cysteine residues are separated by another amino acid (C-X-C) and into a class in which these cysteines are adjacent (C-C). The C-X-C class includes IL-8 and platelet factor 4, which are encoded by genes on human chromosome 4; the C-C class includes RANTES and macrophage chemotactic and activating factor (MACF), which are encoded by genes on chromosome 17 (*1*). Some members of the family [melanoma growth stimulating activity (MGSA) and macrophage inflammatory protein 2 (MIP-2)] compete with IL-8 for binding to neutrophils (*1*) suggesting that these molecules bind a common receptor. The small molecular size of IL-8 has allowed the rapid determination of its three-dimensional structure based on both high resolution crystallographic and NMR data (*3*). In order to better understand the range of activities exhibited by this family of cytokines, we have begun to characterize the family of receptors with which they interact, beginning with the IL-8 receptor. A cDNA clone encoding the IL-8 receptor from human neutrophils was isolated by expression cloning. The amino acid sequence encoded by this clone shows that the IL-8 receptor is a member of the superfamily of G protein–linked receptors that contain seven transmembrane domains (*4*), rather than being a member of the superfamilies of growth hormone-hematopoeitic receptors (*5*) or tyrosine kinase receptors (*6*), which have only one (or two) transmembrane domains but also bind small protein ligands.

An expression cloning strategy (*7, 8*) was used to isolate cDNA clones encoding the IL-8 receptor. A cDNA library constructed from human neutrophil mRNA in the mammalian expression vector pRK5B was transfected into COS-7 cells as pools of 2500 clones, and the cells were screened for binding of ^{125}I-labeled IL-8 (*9*). One pool that contained the desired DNA was partitioned into smaller pools and retransfected until a pure clone (pRK5B.il8r1.1) was obtained. COS cells transfected with the isolated cDNA clone specifically bound IL-8 (Fig. 1). The dissociation constant (K_d) for IL-8 binding was 3.6 nM, which is within the range of 0.8 to 4 nM reported for IL-8 binding to human neutrophils (*10, 11*). The chemotactic peptide fMet-Leu-Phe (fMLP) did not compete with IL-8 for binding to these transfected cells.

The DNA sequence (*12*) of the isolated cDNA clone (GenBank accession number M68932) contains a single long open reading frame beginning with a methionine codon and a sequence closely matching the consensus expected for a translation initiation site (*13*). This open reading frame encodes a protein of 350 amino acids. The amino acid sequence (Fig. 2) shares several features with the G protein–coupled receptors of the rhodopsin superfamily, including seven hydrophobic domains that are presumed to span the cell membrane and N-linked glycosylation sites near the NH_2-terminus (*4*). The translated molecular size of the receptor is about 40 kD, somewhat smaller than the reported molecular size of 58 to 67 kD for the receptor from human neutrophils on the basis of cross-linking data (*10, 11*). Glycosylation would be expected to account for some or all of the apparent difference in sizes. The IL-8 receptor from human neutrophils is glycosylated (*11*) and a total of five potential N-linked glycosylation sites are found on extracellular regions of the amino acid sequence (Fig. 2).

The encoded amino acid sequence is similar to that of the fMLP receptor from rabbit (*14*). This similarity (79% amino acid identity overall with multiple matches of more than 20 contiguous amino acids) indicates that these two sequences may encode homologs of the same receptor in these species. The human fMLP receptor has also been cloned (*15*); it has only 26% amino acid identity with the rabbit fMLP receptor (and 29% identity with the human IL-8 receptor presented here). The considerable divergence between the rabbit and human fMLP receptor sequences has led to the suggestion that

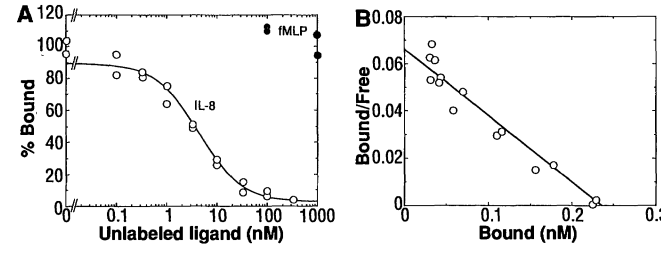

Fig. 1. High affinity binding of ^{125}I-labeled IL-8 to COS cells transfected with clone pRK5B.il8r1.1. (**A**) Competition with unlabeled IL-8 (○) or fMLP (●). (**B**) Scatchard analysis of the IL-8 binding data; apparent K_d = 3.6 nM with an average of 820,000 binding sites per cell (uncorrected for transfection efficiency). Similar analysis of binding to human neutrophils gave a K_d = 1.1 nM with 31,000 binding sites per cell. One day after electroporation of DNA from the clone pRK5B.il8r1.1 into COS-7 cells, binding competition assays were performed on cells in six-well dishes (about 175,000 cells per dish) in the medium described (*22*) for 2 hours at 4°C. The wells were then washed, and the cells were harvested with trypsin and counted. No specific binding was detected in wells containing cells transfected with DNA from the vector pRK5B. Neutrophil binding assays were performed as described (*22*) but for 2 hours at 4°C.

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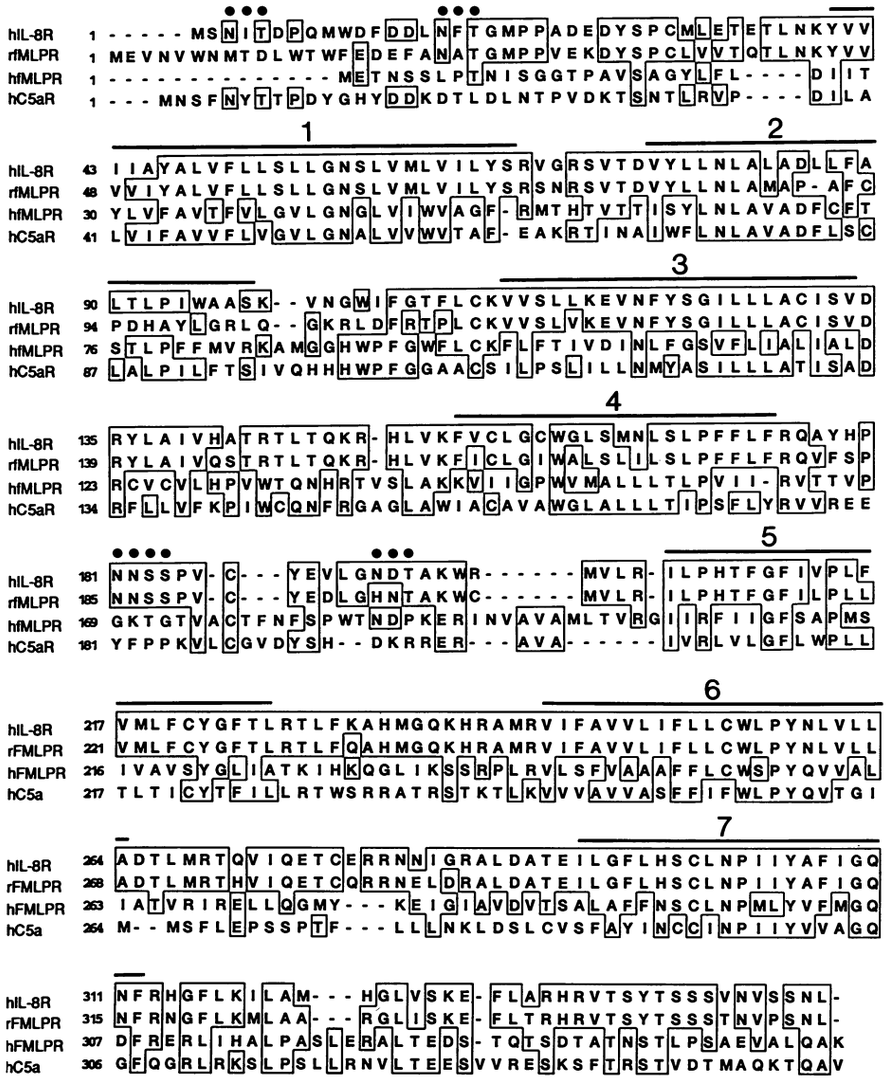

Fig. 2. Amino acid sequence of the human IL-8 receptor encoded by the cDNA insert of clone pRK5B.il8r1.1. Shown is an alignment with the rabbit fMLP receptor (*14*) and with the human neutrophil receptors for fMLP (*15*) and C5a (*19*). The seven putative transmembrane domains are overlined. The IL-8 receptor contains two potential N-linked glycosylation sites in the first extracellular region and three more in the third extracellular loop (shown by dots). Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

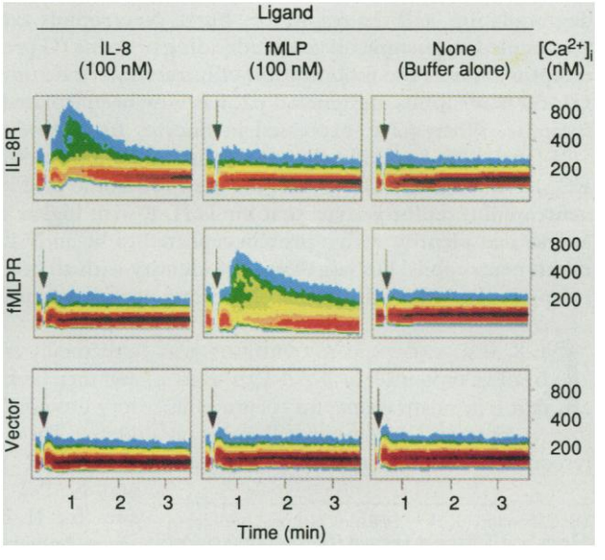

Fig. 3. Density plots of the intracellular Ca^{2+} response of transfected human IL-8 and fMLP receptors to their ligands by flow cytometry. Ligand was added at the arrow. Human 293 cells were transfected by electroporation (*23*) with a vector encoding the IL-8 receptor (clone pRK5B.il8r1.1) or fMLP receptor [human fMLP receptor cDNA (*15*) in the vector pRK5], or with vector (pRK5B) DNA. After 2 days, the cells were incubated with indo-1 acetoxymethyl ester (2 μM) in RPMI medium for 30 min at 37°C. Intracellular Ca^{2+} was measured with a Coulter 753 flow cytometer from the ratio of fluorescence at 405 and 525 nm (*24*). Colors represent the proportion of cells with the indicated $[Ca^{2+}]_i$ at each period of sampling (3 s); white, 0–2%; green, 4–6%; light green, 6–8%; yellow, 8–10%; orange, 10–14%, red, 14–22%; black, >22%.

these may be two distinct isoforms of the fMLP receptor (*14*).

Neutrophils respond to the chemoattractants IL-8 and fMLP with a rapid transient increase in the concentration of intracellular free Ca^{2+} (*16*). To verify that the clone isolated here encodes the IL-8 receptor, we measured the intracellular Ca^{2+} response of cells transfected with this clone to IL-8 and fMLP. Cells were also transfected with a vector encoding the human fMLP receptor or with the expression vector alone as controls. Cells transfected with the clone encoding the IL-8 receptor responded to IL-8 with a transient increase in intracellular Ca^{2+} (Fig. 3). No response to fMLP was detected. Conversely, cells transfected with the vector encoding the human fMLP receptor responded to fMLP but not to IL-8. No response to either chemoattractant was detected in cells transfected with the expression vector alone. Only a subset of the cells responded to the stimuli in these experiments because only a portion of the cells were transfected. These results demonstrate that the cloned receptors function to initiate signaling in response to ligand binding.

No binding was detected of [^{3}H]fMLP to cells expressing the IL-8 receptor or of ^{125}I-labeled IL-8 to cells expressing the human fMLP receptor (*17*). This result is consistent with the lack of competition between IL-8 and fMLP for binding to neutrophils (*1*). The binding and Ca^{2+} response data demonstrate the specificity of the two human receptors for their respective ligands and provide evidence that the cDNA we have cloned encodes the IL-8 receptor. The similarity of the sequence of the human IL-8 receptor with the sequence previously identified as a rabbit fMLP receptor (*14*) suggests that the rabbit sequence may actually encode an IL-8 receptor.

Hybridization of the cloned cDNA for the IL-8 receptor to human neutrophil mRNA, showed bands of 2.4 and 3.0 kb and a fainter band at 3.5 kb (Fig. 4). It is clear from the sequence of additional clones (*18*) that the mRNA for the receptor has a 3′ untranslated region of at least 1.1 kb; the multiple RNA bands could be due to multiple polyadenylation sites. No hybridization

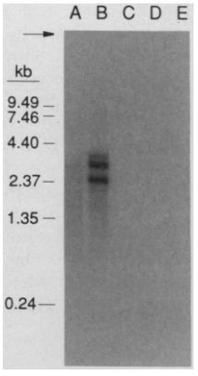

Fig. 4. RNA hybridization on blots with a cDNA probe from the human IL-8 receptor. Lane A, 5 μg of RNA not polyadenylated from human neutrophils; B, 2 μg of polyadenylated [poly(A)$^+$] RNA from human neutrophils; C, 2 μg of poly(A)$^+$ RNA from U937 cells; D, 2 μg of poly(A)$^+$ RNA from U266 cells; E, 2 μg of poly(A)$^+$ RNA from Jurkat cells. RNA was separated by size by electrophoresis on a formaldehyde (1%) gel (*25*), transferred to nitrocellulose, hybridized to the full-length cDNA insert from clone pRK5B.il8r1.1, and washed in 30 mM sodium chloride, 3 mM trisodium citrate at 55°C.

was detected to mRNA from U266 or Jurkat cell lines, which are of the B cell and T cell lineages (Fig. 4). IL-8 does not bind to cells from these lineages (*11*). No hybridization to mRNA from the monocyte cell line U937 was detected, although low levels of IL-8 binding to these cells has been reported (*11*).

Alignment of the sequences of the human receptors for the three neutrophil chemoattractants IL-8, fMLP (*15*), and C5a (*19*) shows the similarity (29 to 34% amino acid identity) of these G protein–coupled receptors (Fig. 2). The third intracellular loop of receptors in this subfamily is shorter than that in other G protein–coupled receptors such as the β-adrenergic (*4*) or muscarinic acetylcholine receptors (*20*). This loop contains determinants at least partially responsible for the binding of G proteins to the receptors (*4*). The intracellular COOH-terminal region of the IL-8 receptor has little similarity to those of the fMLP and C5a receptors but it does contain several serine and threonine residues that may function as phosphorylation sites. Like the C5a receptor (*19*), the NH_2-terminal extracellular region of the IL-8 receptor has several acidic residues. These amino acids may participate in the binding of IL-8, which is quite basic (pI ~9.5), to the receptor. The amino acid sequence presented here is 77% identical with a receptor sequence presented in the accompanying paper (*21*) including consecutive amino acids matches of 105 and 64 amino acids. These two sequences may be members of a family of related receptors for the IL-8 family of cytokines.

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26. The authors thank D. V. Goeddel for discussions and development of the expression cloning vector, pRK5B, C. Nelson for providing the human neutrophils, E. Luis for the IL-8, G. Bennett for the iodinations, and J. Borree for assistance with flow cytometry.

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Cloning of Complementary DNA Encoding a Functional Human Interleukin-8 Receptor

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Interleukin-8 (IL-8) is an inflammatory cytokine that activates neutrophil chemotaxis, degranulation, and the respiratory burst. Neutrophils express receptors for IL-8 that are coupled to guanine nucleotide-binding proteins (G proteins); binding of IL-8 to its receptor induces the mobilization of intracellular calcium stores. A cDNA clone from HL-60 neutrophils, designated p2, has now been isolated that encodes a human IL-8 receptor. When p2 is expressed in oocytes from *Xenopus laevis*, the oocytes bind ^{125}I-labeled IL-8 specifically and respond to IL-8 by mobilizing calcium stores with an EC_{50} of 20 nM. This IL-8 receptor has 77% amino acid identity with a second human neutrophil receptor isotype that binds IL-8 with higher affinity. It also exhibits 69% amino acid identity with a protein reported to be an N-formyl peptide receptor from rabbit neutrophils, but less than 30% identity with all other known G protein–coupled receptors, including the human N-formyl peptide receptor.

IL-8, ALSO KNOWN AS NEUTROPHIL ACTIVATING PROTEIN–1 or NAP-1, is a potent chemoattractant for neutrophils that is produced by many cell types in response to inflammatory stimuli (*1*). IL-8 is structurally and functionally related to several members of the macrophage inflammatory protein–2 (or MIP-2) family of cytokines. These include MIP-2, MGSA (melanoma growth-stimulating activity), and NAP-2 (*2–4*). High affinity binding sites for IL-8 have been found on transformed myeloid precursor cells such as HL-60 and THP-1 as well as on neutrophils (*5, 6*). NAP-2 and MGSA compete with IL-8

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