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Reverse Transcription-PCR Cloning of Macrophage Inflammatory Protein 1α Receptor and C-X-C Chemokine Receptor

Kwi-Ok Oh, Yak-Hoon Kwak, Seung-Ryong Jeong, Dong-Whan Lee, Seo-Young So, Yo-Han Song[+], Kack-Kyun Kim*

*Department of Pharmacology and [+]Department of Oral Microbiology, College of Dentistry, Chonbuk National University, and *Department of Oral Microbiology, College of Dentistry, Seoul National University*

The chemokines are a superfamily of 8-10 KDa soluble proteins that have been implicated in chemotaxis and a wide range of inflammatory and immune reactions. They are classified into two branches C-C and C-X-C superfamily according to their primary structure. MIP-1α is a member of C-C chemokine superfamily. For the cloning of murine(m) MIP-1α receptor(R), we used synthetic oligonucleotides corresponding to transmembrane(TM) conserved sequences of already cloned human(h)IL-8-R as primers, and performed RT-PCR amplification using murine macrophage cell mRNA. Among 5' RT-PCR products, we isolated a homologous cDNA to hIL-8-R that presumably is a putative mIL-8-R cDNA. While this study was being undertaken, cloning of hMIP-1α-R has been done in other laboratory. We, therefore, synthesized oligonucleotides corresponding to hMIP-1α-R TM sequences and performed RT-PCR cloning of mMIP-1α-R from murine cytolytic T lymphocyte mRNA, using the oligonucleotides as primers. One putative mMIP-1α-R cDNA was isolated. cDNA library of CTLL-R8 cell was screened with this cDNA as a probe, and one positive clone was identified.

Key words : *chemokine, receptor, macrophage inflammatory protein Iα receptor, RT-PCR cloning*

Introduction

The chemokines (for chemoattractant and cytokine activity) are a superfamily of 8-10 KDa soluble proteins that have been implicated in chemotaxis and a wide range of inflammatory and immune reactions (Oppenheim *et al.*, 1991). The chemokines can be divided into two subfamilies (α or C-X-C and β or C-C) based on overall sequence homology, the disposition of the first two of four conserved cysteine residues, and the chromosomal location of the corresponding genes. Most C-X-C chemokines, such as interleukin 8 (IL-8), melanocyte growth-stimulatory activity (MGSA), neutrophil-activating peptide 2, neutrophil-activating protein derived from epithelial cells (ENA-78), attract neutrophils but not monocytes (Baggiolini *et al.*, 1989; Matsushima and Oppenheim, 1989; Walz *et al.*, 1992; Yoshimura *et al.*, 1987), whereas C-C chemokines, including such molecules as monocyte chemotactic protein 1 (MCP-1), RANTES, and macrophage inflammatory protein 1α and 1β (MIP-1α and MIP-1β), attract monocyte but not neutrophils (Leonard and Yoshimura, 1990; Matsushima *et al.*, 1989; Schall *et al.*, 1990). Certain chemokines have chemoattractant properties for T lymphocytes : MIP-1α is a chemoattractant for $CD8^+$ T cells (Taub *et al.*, 1993), and RANTES for memory T cells *in vitro* (Schall *et al.*, 1990), and IL-8 has been reported to attract T cells *in vivo* and *in vitro* (Larsen *et al.*, 1989). C-C chemokines, particularly MIP-1α and RANTES, also have effects on other blood leukocyte. MIP-1α activates macrophage (Fahey *et al.*, 1992), RANTES and MIP-1α are direct mediators of the release of histamine from basophils and mast cells (Kuna *et al.*, 1992a, 1992b; Bischoff *et al.*, 1992; Alam *et al.*, 1992), RANTES and MIP-1α are chemoattractants and activators of eosinophils (Kameyoshi *et al.*, 1992; Rot *et al.*, 1992). Also MIP-1α has been reported to suppress the proliferation of bone marrow stem cell (Graham *et al.*, 1990; Broxmeyer *et al.*, 1990), and cytolytic T lymphocyte (Oh *et al.*, 1991).

Two distinct IL-8 receptors(Rs) exhibiting a 77% amino acid identity have been molecularly cloned from human neutrophils (Holmes *et al.*, 1991; Murphy and Tiffany, 1991). This structural feature is characteristic to the superfamily of guanine nucleotide-binding protein (G-protein)-coupled receptors that have seven transmembrane(TM)

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domains. The receptor sequence of IL-8 is sufficiently similar to what had been identified as an isoform of the bacterial formylated tripeptide fMet-Leu-Phe(fMLP)-R and C5a-R (Holmes *et al.*, 1991; Murphy and Tiffany, 1991). Based on competition binding assay using ^{125}I-IL-8, the receptors for IL-8 are clearly different from the receptors for other cytokines, and only three of seven tested chemokines compete in ^{125}I-IL-8 binding, suggesting that the receptors for the various chemokines are not all the same but individually specific (Samanta *et al.*, 1989). Human MCP-1 has been reported to bind to monocytes with affinity of 2 nM (Valente *et al.*, 1991; Yoshimura and Leonard, 1990). The detection of the receptors for ACT-2 (Napolitano *et al.*, 1990) and LD-78 (Nakao *et al.*, 1990) on blood leukocytes has also been reported. MIP-1α-R was identified on a mouse T cell and a macrophage cell line with an affinity of 1.5 and 0.9 nM, respectively (Oh *et al.*, 1991).

While chemokines have probably evolved to alert immune system to microbial invasions of host, there has been, very recently, new information based on the study of chemokine receptors which have indicated that microbes may use the chemokine system to the disadvantage of the host through molecular mimicry (Ahuja *et al.*, 1994). However, since the biological activities of these cytokines are not yet established, the functional consequences of ligand binding to these receptors remains to be defined. Recently, the authors have detected MIP-1α-R on a various immune and inflammatory cell lines (Oh *et al.*, 1993). In the present study, for the cloning of murine(m)MIP-1α-R, we used synthetic oligonucleotides corresponding to conserved TM sequences of already cloned human(h) IL-8-R as primers, and performed RT-PCR amplification using murine macrophage cell mRNA. While this study was being undertaken, cloning of hMIP-1α-R has been done by Neote and colleagues (Neote *et al.*, 1993). We, therefore, took advantage of this and synthesized oligonucleotides corresponding to hMIP-1α-R TM sequences and performed an independent study to clone mMIP-1α-R. We now report here a partial characterization of cDNA clones encoding the mMIP-1α-R and a novel C-X-C-R that might be a putative mIL-8-R.

Materials and Methods

Cells

The murine cytolytic T lymphocyte (CTL) line CTLL-R8 was grown in RPMI 1640 (GIBCO Laboratories) containing 10% fetal bovine serum (FBS), 100 units/ml penicillin plus 100 μg/ml streptomycin. The murine CTL line CTLL-2 was grown in same condition with additional supplement of 100 units/ml recombinant IL-2. The murine macrophage cell line RAW 264.7 and human promyelocytic cell line HL-60 were grown in DMEM containing 10% FBS and antibiotics as described above. CTLL-R8 cells were stimulated with 5 μg/ml Con A (Sigma), for 16 h. RAW 264.7 cells were stimulated with 1 μg/ml LPS from *Escherichia coli* 0127:B8 (Sigma), for 18 h.

Reverse Transcription(RT)-PCR, subcloning, and sequencing

Total RNA from CTLL-2, CTLL-R8, HL-60, and RAW 264.7 was prepared as described previously (Chomczynski and Sacchi, 1987) and used for poly $(A)^+$ RNA selection which were used as substrates in reverse transcription. Degenerate 24-mer oligonucleotides corresponding to conserved TM region 6 (TM6) and TM7 of hIL-8-R were used as primers in the RT-PCR of RAW 264.7 mRNA. Base sequences of synthetic oligonucleotides were as follows.

Oligomer-IL-8-R-A :
5'-CATCTTCGCCGTCGTACTCATCTT-3'
Oligomer-IL-8-R-B :
5'-AAGTTTTGGCCAATGAAGGCGTAG-3'

Degenerate 20-mer and 36-mer oligonucleotides corresponding to TM3-adjacent region (388-407) and TM7-adjacent region (877-912) of hMIP-1α-R were used as primers in the RT-PCR of CTLL-2 mRNA. The same experiment was duplicated with human promyelocytic cell line HL-60 as an hMIP-1α-R-positive control. Base sequences of synthetic oligonucleotides were as follows

Oligomer-MIP-A :
5'-GACAGGTACCTGGCCATCGT-3
Oligomer-MIP-B :
5'-AACGAAGGCGTAGATCACTGGGTTGACACAGCAGTG-3'

Oligonucleotides and poly$(A)^+$ RNA were reacted with Superscript as recommended by the manufacturer (BRL) and incubated with RNase H (Epicenter Technologies) at 37℃ for 20 min. PCR conditions were as follows.

3 cycles : 94℃-1 min 37℃-1 min 72℃- 1 min
15 cycles : 94℃-1 min 50℃-1 min 72℃- 1 min
15 cycles : 94℃-1 min 50℃-1 min 72℃- 2 min
1 cycle : 72℃-10 min

PCR products were gel purified and subcloned into pGEM3 vector. Plasmid DNA was isolated using the Qiagen kit (Qiagen Inc.) as recommended by the manufacturer. Sequencing was performed with the Sequenase kit (US Biochemicals) by dideoxy chain termination method (Sanger *et al.*, 1977).

Screening of cDNA library

First and second strand cDNA were synthesized from Con A stimulated CTLL-R8 poly(A)$^+$ RNA by using reverse transcriptase and DNA polymerase sequentially as recommended by manufacturer (Pharmacia). cDNAs were ligated into λgt11/*Eco*RI site and packaged by addition of packaging extract recommended by manufacturer (Stratagene). λGt11 cDNA library was plating on 150 mm agar plates (3×10^4 plaques/plate) and *in situ* hybridization was performed with cloned RT-PCR product as a probe. Hybridized plaque and adjacent 10 plaques were separated and amplified. Then, phage DNA was isolated and analyzed by Southern blot with cloned RT-PCR product as a probe.

Results

Isolation of C-X-C chemokine receptor (putative mIL-8-R) cDNA from RAW 264.7

Originally we have tried to do RT-PCR cloning of novel chemokine receptor, especially MIP-1α-R, using synthetic oligonucleotides of conserved sequences of already cloned hIL-8-R as primers. We used RAW 264.7 and CTLL-R8 cells which had been shown to have a single class of high affinity MIP-1α-R on their surfaces with 380 and 1200 binding sites per cell, respectively (Oh *et al.*, 1991). However we got the RT-PCR products only from RAW 264.7 cell. As shown in Fig. 1, electrophoresis of RT-PCR products from RAW 264.7 mRNA demonstrated five bands migrated to the position of approximately 500, 400, 330, 200, and 150 base pairs(bp). Next, we purified each bands from acrylamide gel, subcloned, and analyzed their base sequences. Among five products, only

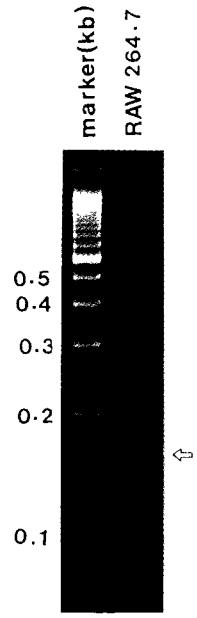

Fig. 1. RT-PCR product of putative C-X-C chemokine receptor mRNA. RNA was extracted from murine macrophage cell line RAW264.7. Oligonucleotides corresponding to conserved sequences of hIL-8-R were used as primers. Arrow indicates partial length cDNA of putative IL-8-R.

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hIL-8R : 5'-FLLCWLPYNLVLLADTLMRTQVIQET
mIL-8R : 5'-FLLCWLPYNLVLFTDTLMRTKLIKET

         -CERRNNIGRALDATEILGFLHSCLNP-3'
         -CERRDDIDKALNATEILGFLHSCLNP-3'
```

Fig. 2. Comparison of deduced amino acid sequences of human and putative mIL-8-R.

150 bp cDNA showed 85% sequence identity with optimally aligned base sequence of hIL-8-R and, presumably, is a partial cDNA of putative mIL-8-R (Fig. 2).

Isolation of C-C chemokine receptor (putative mMIP-1α-R) cDNA from CTLL-2

Results of our previous receptor binding study of CTLL-2 with ^{125}I-MIP-1α indicated the presence of approximately 3000-4000 MIP-1α binding sites per cell (Oh *et al.*, 1993). So we decided to use CTLL-2 for mMIP-1α-R RT-PCR cloning instead of RAW264.7. Furthermore, CTL line CTLL-2 was thought to be preferable to RAW264.7 for MIP-1α-R cloning, because activated macrophage cells are known to express many different kinds of chemokine receptors which would hamper the finding of scarce message of MIP-1α-R. As a hMIP-1α-R-positive con-

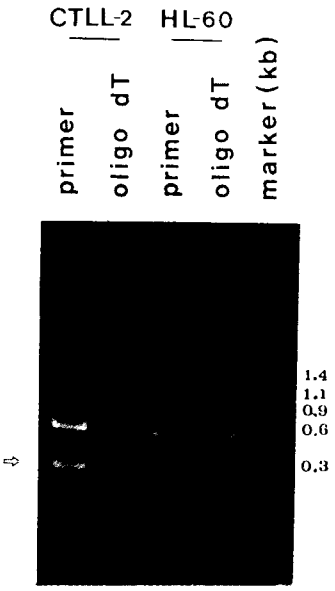

Fig. 3. RT-PCR product of putative C-C chemokine receptor mRNA. RNA was extracted from murine cytolytic T cell CTLL-2. Oligonucleotides corresponding to 388-407 and 877-912 bps of hMIP-1α-R sequence were used as primers. Arrow indicates partial length cDNA of putative mMIP-1α-R.
*Oligo dT: commercial oligo(dT) was used as primer for reverse transcription

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Human MIP-1α/RANTES receptor mRNA
Identities 37/39(94%)

Query :  39-CAGCCACTGCTGTGTCAACCCA
             ||  ||||||||||||||||||
             ||  ||||||||||||||||||
Subject : 873-CACGCACTGCTGTGTCAACCCA

          -GTGATCTACGCCTTCGT - 1
           |||||||||||||||||
           |||||||||||||||||
          -GTGATCTACGCCTTCGT-911
```

Fig. 4. Comparison of base sequences of hMIP-1α-R and putative mMIP-1α-R cDNA.

trol, we have done same RT-PCR experiment with human HL-60 cell, on which the existence of MIP-1α-R had been demonstrated (Neote *et al.*, 1993).

Electrophoresis pictures of RT-PCR products from CTLL-2 and HL-60 mRNA showed similarity in the presence of two cDNA bands at the position of approximately 200 and 500 bp (Fig. 3). To test the specificity of synthetic primer, we did parallel PCR experiments with cDNAs which were prepared with commercial oligo(dT) oligonucleotide as a primer as well as with those prepared with oligomer-MIP-B. As shown in Fig. 3, both the oligomer-MIP-B and oligo(dT) resulted in the identical RT-PCR products with same band pattern. This shows that oligomer-MIP-A and B were specific. Two cDNA bands were gel purified and subcloned. After their sequences were analyzed, it was revealed that 37 of 39 sequences in 200 bp cDNA was identical to optimally aligned hMIP-1α-R cDNA sequence (873-911) (Fig. 4).

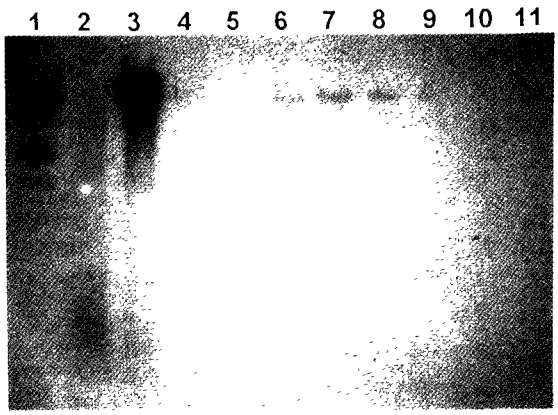

Fig. 5. Southern hybridization of phage DNA from one positive plaque (lane 3) and the adjacent plaques.
Lane 1 λ/HindIII marker, Lane 2-11 phage DNA

Screening of cDNA library

λGt11/CTLL-R8 cDNA library was screened by *in situ* hybridization with 200 bp RT-PCR product (putative mMIP-1α-R cDNA) as a probe. One plaque was hybridized with 200 bp cDNA as a result of screening total 210,000 plaques. A positive plaque and the adjacent 10 plaques were separated and analyzed in Southern blot. Only one phage DNA (Fig. 5, lane 3) was hybridized with a probe and this could be a mMIP-1α-R cDNA.

Discussion

Various receptors have been cloned by cDNA expression or RT-PCR amplification method. Among the cytokine receptors IL-1-R and IL-8-R (Sims *et al.*, 1988; Yamasaki *et al.*, 1988) were the first whose cDNAs were cloned by expression cloning technique. However, cDNA expression cloning is not a proper method for cloning the receptor that has low expression message (Taga and Kishimoto, 1992). Recently, much attention has been focused on cloning the various cytokine receptor cDNAs by using the RT-PCR amplification method (Kawasaki, 1990). Two distinct IL-8-Rs exhibiting a 77% amino acid identity have been molecularly cloned from neutrophils (Holmes *et al.*, 1991; Murphy and Tiffany, 1991). The primary sequences show

that IL-8Rs contain a seven-pass motif consisting of seven hydrophobic membrane-spanning domains. This structural feature is characteristic of the superfamily of guanine nucleotide-binding protein (G protein)-coupled receptors, indicating that IL-8Rs may constitute a subfamily of this large supergene family. The cDNA of only one C-C chemokine receptor (hMIP-1α/RANTES-R) has been isolated (Neote *et al.*, 1993) and this also belongs to the G protein-coupled receptor superfamily, a feature consistent with the fact that many of the functions of C-C chemokines can be blocked by pertussis toxin (Kuna *et al.*, 1992b). The known identity of amino acid sequence of hMIP-1α-R and IL-8-R is ~30% (Holmes *et al.*, 1991; Murphy and Tiffany, 1991, Neote *et al.*, 1993). The sequence similarity among various chemokines and chemoattractant molecule receptors is higher in the TM regions, especially TM2, TM6, and TM7, and the greatest divergence is seen in the second intracellular loop (Neote *et al.*, 1993). The highly conserved motif is at the end of TM3, DRYLAIVHA, and could represent a functionally important domain necessary for receptor function. We utilized this domain to design mMIP-1α-R cloning strategy, that is, oligomer-MIP-A synthesis. The chemoattractant receptors for anaphylatoxin C5a (Gerard and Gerard, 1991) and fMLP (Boulay *et al.*, 1990) do not have the conserved motif DRYLAIVHA, although both of them are G protein-coupled seven transmembrane spanning receptor. In the present study, for the prevention of amplification of various chemokines and other chemoattractant receptor mRNAs, we used relatively less conserved domain (TM7 adjacent region) to design another oligonucleotide primer oligomer-MIP-B that enabled us to isolate only two cDNA products from CTL mRNA.

The distribution of MIP-1α-R RNA is consistent with a functional role for the cloned MIP-1α-R in neutrophil, monocyte, and B and T lymphocytes. Northern blot data from cell lines suggest that the gene may also be expressed in myeloid precursor cells (Neote *et al.*, 1993). If so, the receptor could mediate the reported regulatory effects of MIP-1α on myelopoiesis (Oppenheim *et al.*, 1991). Human and murine MIP-1α have been shown to have a number of activities. Besides proinflammatory effects, MIP-1α has also been described as an endogenous pyrogen that acts in a prostaglandin-independent manner (Davatelis *et al.*, 1989); more recently, it has been shown to activate basophils and mast cells (Alam *et al.*, 1992). MIP-1α and homologous protein MIP-1β have very complicated relationship in their biological activities. Broxmeyer *et al.* (1990) has shown that MIP-1β can block the inhibitory effects of MIP-1α on stem cell proliferation. And MIP-1β, when present in excess compared with MIP-1α, block the activating effects of MIP-1α on macrophages (Fahey *et al.*, 1992). Furthermore, our previous study indicated two molecules compete the same binding sites (Oh *et al.*, 1993). Thus, we might envisage MIP-1α-R playing a role since it binds to MIP-1β but transmits a poor Ca^{2+} signal. In the case of inhibitory effect on stem cell proliferation, it is also possible that MIP-1α-R is coupled to both adenylyl cyclase and phospholipase C, as it is known that cAMP can act as a growth-inhibitory signal in some hematopoietic cells (Neote *et al.*, 1993).

Accumulating evidence suggest that monocyte express a shared MIP-1α/MIP-1β receptor and a shared RANTES/MIP-1α/MCP-1 receptor, and a restricted MCP-1 receptor (Ahuja *et al.*, 1994). And MIP-1α-R transduces a strong Ca^{2+} signal in response to low concentration of RANTES as well as MIP-1α. Several observations suggest that RANTES interact differently with MIP-1α-R than does MIP-1α, although precise interaction between ligand and receptor is not known.

A striking homology between the open reading frame in cytomegalovirus (CMV) and hMIP-1α-R was noted (Neote *et al.*, 1993). There is nearly 50% amino acid identity and this observation suggests that CMV-infected cells could bind to C-C chemokines as part of strategy to evade immune antiviral responses. If the value of chemokines to CMV can be defined, perhaps unsuspected physiological functions may be discovered for them. Moreover, the copied immunomodulatory genes of viruses may serve as useful starting point for developing new treatment for inflammation and viral infections. In addition to this, information of multiple ligand specificity of C-C receptors and agonist/antagonist profiles of each of the ligands enable us to get deeper understanding of interaction of chemokines and immune cells.

References

Ahuja, S.K., Gao, J.L. and Murphy, P.M.: Chemokine receptors and molecular mimicry. [Review]. *Immunol.*

Today **15**: 281-287, 1994.

Alam, R., Forsythe, P.A., Stafford, S., Lett-Brown, M.A. and Grant, J.A.: Macrophage inflammatory protein-1α activates basophils and mast cells. *J. Exp. Med.* **176**: 781-786, 1992.

Baggiolini, M., Walz, A. and Kunkel, S.L.: Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. [Review]. *J. Clin. Invest.* **84**: 1045-1049, 1989.

Bischoff, S.C., Krieger, M., Brunner, T. and Dahinden, C.A.: Monocyte chemotactic protein 1 is a potent activator of human basophils. *J. Exp. Med.* **175**: 1271-1275, 1992.

Boulay, F., Tardif, M., Brouchon, L. and Vignais, P.: The human N-formylpeptide receptor. Characterization of two cDNA isolates and evidence for a new subfamily of G-protein-coupled receptors. *Biochemistry* **29**: 11123-11133, 1990.

Broxmeyer, H.E., Sherry, B., Lu, L., Cooper, S., Oh, K. O., Tekamp-Olson, P., Kwon, B.S. and Cerami, A.: Enhancing and suppressing effects of recombinant murine macrophage inflammatory proteins on colony formation *in vitro* by bone marrow myeloid progenitor cells. *Blood* **76**: 1110-1116, 1990.

Chomczynski, P. and Sacchi, N.: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**: 156-159, 1987.

Davatelis, G., Wolpe, S.D., Sherry, B., Dayer, J.M., Chicheportiche, R. and Cerami, A.: Macrophage inflammatory protein-1: a prostaglandin-independent endogenous pyrogen. *Science* **243**: 1066-1068, 1989.

Fahey, T.J., Tracey, K.J., Tekamp-Olson, P., Cousens, L.S., Jones, W.G., Shires, G.T., Cerami, A. and Sherry, B.: Macrophage inflammatory protein 1 modulates macrophage function. *J. Immunol.* **148**: 2764-2769, 1992.

Gerard, N.P. and Gerard, C.: The chemotactic receptor for human C5a anaphylatoxin. *Nature* **349**: 614-617, 1991.

Graham, G.J., Wright, E.G., Hewick, R., Wolpe, S.D., Wilkie, N.M., Donaldson, D., Lorimore, S. and Pragnell, I.B.: Identification and characterization of an inhibitor of haemopoietic stem cell proliferation. *Nature* **344**: 442-444, 1990.

Holmes, W.E., Lee, J., Kuang, W.J., Rice, G.C. and Wood, W.I.: Structure and functional expression of a human interleukin-8 receptor. *Science* **253**: 1278-1280, 1991.

Kameyoshi, Y., Dorschner, A., Mallet, A.I., Christophers, E. and Schroder, J.M.: Cytokine RANTES released by thrombin-stimulated platelets is a potent attractant for human eosinophils. *J. Exp. Med.* **176**: 587-592, 1992.

Kuna, P., Reddigari, S.R., Rucinski, D., Oppenheim, J. J. and Kaplan, A.P.: Monocyte chemotactic and activating factor is a potent histamine- releasing factor for human basophils. *J. Exp. Med.* **175**: 489-493, 1992a.

Kuna, P., Reddigari, S.R., Schall, T.J., Rucinski, D., Viksman, M.Y. and Kaplan, A.P.: RANTES, a monocyte and T lymphocyte chemotactic cytokine releases histamine from human basophils. *J. Immunol.* **149**: 636-642, 1992b.

Larsen, C.G., Anderson, A.O., Appella, E., Oppenheim, J.J. and Matsushima, K.: The neutrophil-activating protein (NAP-1) is also chemotactic for T lymphocytes. *Science* **243**: 1464-1466, 1989.

Leonard, E.J. and Yoshimura, T.: Human monocyte chemoattractant protein-1 (MCP-1). [Review]. *Immunol. Today* **11**: 97-101, 1990.

Matsushima, K., Larsen, C.G., DuBois, G.C. and Oppenheim, J.J.: Purification and characterization of a novel monocyte chemotactic and activating factor produced by a human myelomonocytic cell line. *J. Exp. Med.* **169**: 1485-1490, 1989.

Matsushima, K. and Oppenheim, J.J.: Interleukin 8 and MCAF: novel inflammatory cytokines inducible by IL 1 and TNF. [Review]. *Cytokine* **1**: 2-13, 1989.

Murphy, P.M. and Tiffany, H.L.: Cloning of complementary DNA encoding a functional human interleukin-8 receptor. *Science* **253**: 1280-1283, 1991.

Nakao, M., Nomiyama, H. and Shimada, K.: Structures of human genes coding for cytokine LD78 and their expression. *Mol. Cell. Biol.* **10**: 3646-3658, 1990.

Napolitano, M., Seamon, K.B. and Leonard, W.J.: Identification of cell surface receptors for the Act-2 cytokine. *J. Exp. Med.* **172**: 285-289, 1990.

Neote, K., DiGregorio, D., Mak, J.Y., Horuk, R. and Schall, T.J.: Molecular cloning, functional expression, and signaling characteristics of a C-C chemokine receptor. *Cell* **72**: 415-425, 1993.

Oh, K.O., Zhou, Z., Kim, K.K., Samanta, H., Fraser, M., Kim, Y.J., Broxmeyer, H.E. and Kwon, B.S.: Identification of cell surface receptors for murine macrophage inflammatory protein-1α. *J. Immunol.* **147**: 2978-2983, 1991.

Oh, K.O., Jeong, S.R., Yu, K.R., Oh, H.J., Lee, D.W., Lee, S.H., Kwak, Y.H., Kim, H.S., and Kwon, B.S.: Receptor binding characteristics of macrophage inflammatory protein 1α and 1β. *J. Oral Biol.* **17**(2): 93-100, 1993.

Oppenheim, J.J., Zachariae, C.O., Mukaida, N. and Matsushima, K.: Properties of the novel proinflammatory supergene "intercrine" cytokine family. [Review]. *Annu. Rev. Immunol.* **9**: 617-648, 1991.

Rot, A., Krieger, M., Brunner, T., Bischoff, S.C., Schall, T.J. and Dahinden, C.A.: RANTES and macrophage inflammatory protein 1α induce the migration and

activation of normal human eosinophil granulocytes. *J. Exp. Med.* **176**: 1489-1495, 1992.

Samanta, A.K., Oppenheim, J.J. and Matsushima, K.: Identification and characterization of specific receptors for monocyte-derived neutrophil chemotactic factor (MDNCF) on human neutrophils. *J. Exp. Med.* **169**: 1185-1189, 1989.

Sanger, F., Nicklen, S. and Coulson, A.R.: DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* **74**: 5463-5467, 1977.

Schall, T.J., Bacon, K., Toy, K.J. and Goeddel, D.V.: Selective attraction of monocytes and T lymphocytes of the memory phenotype by cytokine RANTES. *Nature* **347**: 669-671, 1990.

Sims, J.E., March, C.J., Cosman, D., Widmer, M.B., MacDonald, H.R., McMahan, C.J., Grubin, C.E., Wignall, J.M., Jackson, J.L., Call, S.M. and et al., : cDNA expression cloning of the IL-1 receptor, a member of the immunoglobulin superfamily. *Science* **241**: 585-589, 1988.

Taga, T. and Kishimoto, T.: Cytokine receptors and signal transduction. [Review]. *FASEB J.* **6**: 3387-3396, 1992.

Taub, D.D., Conlon, K., Lloyd, A.R., Oppenheim, J.J. and Kelvin, D.J.: Preferential migration of activated $CD4^+$ and $CD8^+$ T cells in response to MIP-1α and MIP-1β. *Science* **260**: 355-358, 1993.

Valente, A.J., Rozek, M.M., Schwartz, C.J. and Graves, D.T.: Characterization of monocyte chemotactic protein-1 binding to human monocytes. *Biochem. Biophys. Res. Commun.* **176**: 309-314, 1991.

Walz, A., Burgener, R., Car, B., Baggiolini, M., Kunkel, S.L. and Strieter, R.M.: Structure and neutrophil-activating properties of a novel inflammatory peptide (ENA-78) with homology to interleukin 8. *J. Exp. Med.* **174**: 1355-1362, 1991.

Yamasaki, K., Taga, T., Hirata, Y., Yawata, H., Kawanishi, Y., Seed, B., Taniguchi, T., Hirano, T. and Kishimoto, T.: Cloning and expression of the human interleukin-6 (BSF-2/IFN-2) receptor. *Science* **241**: 825-828, 1988.

Yoshimura, T. and Leonard, E.J.: Identification of high affinity receptors for human monocyte chemoattractant protein-1 on human monocytes. *J. Immunol.* **145**: 292-297, 1990.

Yoshimura, T., Matsushima, K., Tanaka, S., Robinson, E.A., Appella, E., Oppenheim, J.J. and Leonard, E. J.: Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines. *Proc. Natl. Acad. Sci. U.S.A.* **84**: 9233-9237, 1987.