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Assignment of genes for interleukin-8 receptors (IL8R) A and B to human chromosome band 2q35

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Abstract. The human genes encoding the interleukin 8 receptors type A and B were assigned to chromosome 2 by polymerase chain reaction amplification and by Southern analysis of a panel of human × rodent somatic cell hybrid DNAs. The IL8R genes were further localized by in situ hybridization to band 2q35.

The chemoattractant molecules, interleukin-8 (IL8), the anaphylatoxin C5a, and the bacterially derived *N*-formyl peptides are important mediators of inflammation. Interaction of these ligands with specific receptors on phagocytic cells stimulates directed migration (chemotaxis) and the release of lysosomal granule contents. The chemoattractant receptors constitute a newly recognized subgroup of the rhodopsin superfamily of G-protein-coupled receptors (Boulay et al., 1990). The rhodopsin family is further characterized by a seven-transmembrane domain structure and includes receptors for the visual pigment opsin, the adrenergic hormones, several neurotransmitter molecules (including acetylcholine, substance K, substance P, and serotonin), and the olfactory receptor (Dohlman et al., 1991).

Receptors for several chemoattractant molecules have been molecularly cloned (Boulay et al., 1990; Gerard and Gerard, 1991; Holmes et al., 1991; Murphy and Tiffany, 1991), including two closely homologous cDNAs encoding receptors for IL8, designated here as type IL8RA and IL8RB, isolated from HL60 myelomonocytic cells and neutrophils, respectively. The IL8R cDNAs share 77% amino acid identity, including continuous identical regions of 105 and 64 amino acids. In this study we used Southern hybridization techniques with DNA isolated from a panel of human × rodent somatic cell hybrids and fluorescent in situ hybridization to determine the chromosomal localization of the IL8RA gene. In addition, the IL8RB gene was localized by polymerase chain reaction (PCR) amplification of the human gene from the hybrid DNA panel, as well as by fluorescent in situ hybridization.

Materials and methods

Degenerate oligonucleotide primers were prepared from homologous regions in the sixth and seventh transmembrane domains of the published sequences of the human *N*-formyl peptide (Boulay et al., 1990) and C5a receptors (Gerard and Gerard, 1991) and the rabbit IL8 receptor (Thomas et al., 1990). These primers were used in the PCR to amplify known and novel cDNAs from various RNA templates. A 155-bp cDNA encoding an apparently novel sequence with 55% homology to the published receptors was isolated. This cDNA was used to screen a custom-made plasmid cDNA library in the pCDM8 vector which had been prepared from U937 myelomonocytic cellular poly-A RNA (Invitrogen, San Diego, CA). A 1,552-bp clone was isolated, sequenced, and found to be identical to the recently published IL8RA clone. A 552-bp *Hha*I-*Hin*dIII fragment of this clone (nucleotides 698–1,250, bearing only 58% homology with the related region within the published sequence of IL8RB cDNA) was used for Southern analysis of human × rodent somatic cell hybrid DNA. These were SST-digested genomic DNAs from a panel of 16 mouse × human and 29 hamster × human hybrid DNAs, as well as human, mouse, and hamster control DNA. Hybridization was performed at 42 °C in a mixture containing 50% formamide (Hybrisol I, Oncor, Gaithersberg, MD). The blots were washed at 42, 48, and 55 °C in 2 × SSC, 0.1% SDS.

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The IL8RB gene was localized by PCR amplification from the somatic hybrid panel, followed by agarose gel electrophoresis. A 1,050-bp sequence was amplified with two synthetic oligonucleotides (5'-TTGCTGAAACTGAAGAA-3' and 5'-GACATTGACAGACGAAGA-3') from either cDNA or human genomic templates, suggesting that the open reading frame of the IL8RB gene is uninterrupted by an intronic sequence. The PCR products were further verified by Southern blotting and hybridization with a 245-bp *Acc*I fragment (nucleotides 1–245) of the IL8RB cDNA, a region having 60% nucleotide homology with the comparable region of the IL8RA cDNA. Hamster and mouse genomic DNA templates provided no significant bands on gel electrophoresis or Southern hybridization. Positive samples in the hybrid panel were confirmed in a second round of PCR.

The type A and B IL8R cDNAs were used to screen a human genomic library in λ-DASH (Stratagene, San Diego, CA), from which genomic clones were isolated. A 9-kb *Eco*RI fragment of the IL8RA clone containing the complete open reading frame in a single exon was used as a probe for in situ hybridization, utilizing a standard fluorescent technique (Tory et al., 1992). Two overlapping fragments (15 kb) resulting from *Sst*I and *Hin*dIII digests of the IL8RB genomic clone were used similarly for in situ hybridization. In brief, metaphase chromosomes were prepared from PHA-stimulated peripheral blood leukocytes. Probe DNA was labeled with biotin-11-dUTP using nick translation. Hybridization was performed at 37 °C for 16 h, followed by washes at 40 °C in a solution containing 50% formamide in 2 × SSC.

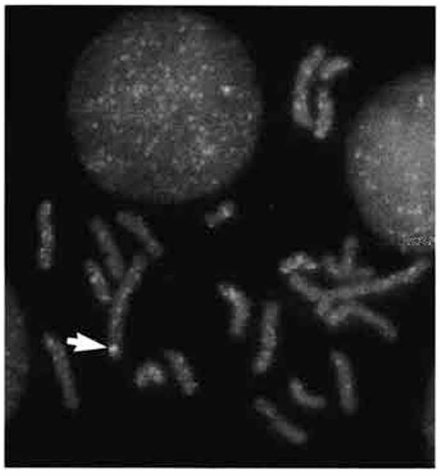

Fig. 1. Partial human metaphase following fluorescence in situ hybridization with an IL8RA genomic fragment as the probe; arrow indicates position of hybridization signal at chromosome band 2q35. The IL8RB gene co-localized to the same band in a similar experiment (data not shown).

Results and discussion

In the IL8RA Southern blot experiment, the labeled probe recognized only human genes with two detectable SST fragments (2.3 and 2.0 kb). The IL8RA bands exhibited the least discordance (8%) with chromosome 2 (χ^2 test, $P = 0.0001$). All other chromosomes were at least 17% discordant (Table I). PCR amplification for IL8RB produced hybridization-positive bands of the predicted size in 7/45 hybrids. The analysis also mapped IL8RB to chromosome 2 with a discordancy of 7% ($P < 0.001$).

Forty-two metaphase cells were examined following in situ hybridization with the IL8RA probe. Specific hybridization was noted at band 2q35 in 22 cells (Fig. 1), and no significant background was observed at any other chromosomal sites. The IL8RB probe also hybidized specifically to 2q35 in 44/55 metaphase cells.

Our results confirm and refine the recent localization of the IL8RA and IL8RB genes to 2q34→q35 (Ahuja et al., 1992). The high degree of amino acid homology and co-localization of the genes for the IL8 receptors suggest that these genes may have arisen by duplication. Further studies examining the genomic structure of the IL8R genes may clarify this relationship. The IL8 receptors are co-expressed on polymorphonuclear neutrophils and, because of their genomic proximity, may share common regulatory elements.

Table I. Segregation of human IL-8 receptor genes in human × rodent somatic cell hybrids

Human gene/ chromosome	Human chromosome																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
Type II IL8R[a]																							
+/+	3	4	4	5	2	6	4	5	4	3	6	5	4	4	3	4	1	7	4	0	4	4	9
+/–	6	3	5	4	7	3	5	4	4	6	3	4	5	3	4	4	7	2	5	7	4	5	0
–/+	1	0	8	11	2	7	5	6	5	4	13	3	7	14	7	5	1	9	3	9	11	7	29
–/–	31	33	25	21	30	25	25	27	28	26	20	29	22	17	26	27	31	23	28	23	19	25	3
Percent discordancy	17	8	31	37	22	24	26	24	22	26	38	17	32	45	28	23	20	27	20	41	40	29	71
Type I IL8R[b]																							
+/+	3	3	4	4	2	3	3	3	3	3	6	4	4	4	1	2	1	4	3	0	4	2	7
+/–	4	2	3	3	5	4	4	4	4	4	1	3	3	3	4	4	5	3	4	6	3	5	0
–/+	1	1	8	13	2	11	6	8	7	4	13	5	8	15	10	6	1	12	4	11	12	10	34
–/–	36	37	30	24	35	26	29	29	31	31	25	31	25	21	28	30	36	25	32	26	23	27	3
Percent discordancy	11	7	24	36	16	34	24	27	24	19	31	19	28	42	33	24	14	34	19	40	36	34	77

[a] Chromosome assignment by Southern hybridization.
[b] Chromosome assignment by PCR amplification.

The genes for several other members of the rhodopsin superfamily of receptors have been mapped to chromosome 2, including the receptor-like 1 alpha-2-adrenergic locus (ADRA2L1) on 2q and the luteinizing hormone/choriogonadotropin receptor gene (LHCGR) at band 2p21 (Spurr and White, 1991). Of particular interest is the recent localization of the gene for the human receptor for vasoactive intestinal peptide (VIPR1) to band 2q37 (Libert et al., 1991). This gene shares 30% amino acid identity with the IL8 receptors, is expressed on T and B cells (Finch et al., 1989), and mediates immunological, as well as neuroendocrine, functions (Goetzl et al., 1990). The VIPR1 gene may therefore be an intermediate in the evolution of the chemoattractant cytokine receptors from the rhodopsin superfamily. In contrast, the *N*-formyl peptide receptor gene (FPR1) and two closely related genes (FPRL1 and FPRL2; Murphy et al., 1992) and the C5a receptor gene (C5AR; Bao et al., 1992) have all recently been localized to chromosome 19. The *N*-formyl peptide and C5a receptors share 35% amino acid identity with each other and only approximately 23% homology with the IL8 receptors. These receptor clusters (IL8RA and IL8RB, on the one hand, and FPR1, FPRL1, FPRL2, and C5AR, on the other) may represent distinct evolutionary subfamilies within the chemoattractant receptor members of the rhodopsin superfamily.

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