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Cloning and Characterization of a Novel Human Chemokine Receptor

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The present study reports the identification of a human gene, HCR, which encodes a novel human chemokine receptor. The partial sequence of the HCR gene was first found in a human neutrophil cDNA library. With the use of an expressed sequence tag (EST) probe from the neutrophil library, the full length HCR cDNA was isolated. The open reading frame of HCR cDNA predicts a protein of 345 amino acids with seven transmembrane domain topography. The HCR gene exhibits good homology to human MIP-1a receptor with 43.1% amino acid identity and 64.4% amino acid similarity and also shows considerable sequence homology to other human chemokine receptors such as the MCP-3 receptor, MCP-5 receptor, and MCP-1 receptor. Northern blot analysis suggests that HCR gene is expressed abundantly in immunal tissues such as spleen, fetal liver, lymph node, and bone marrow. Strong expression was also found in human lung and heart. A chromosome mapping study indicated that HCR gene is positioned within human chromosome band Xq13. Our result suggests that HCR gene is a novel putative chemokine receptor.

Chemokines are a family of secreted proteins that activate and mobilize cells of the immune system during inflammation. These inflammatory processes include those caused by viral, bacterial and parasitic infection, allergic and asthmatic reactions, atherosclerosis and arthritis (1,2). Based on the four distinctive, conserved cysteine residues in their amino acid sequences, chemokines are classified as CXC (or a) chemokines with the first two cysteines separated by one amino acid, or CC (or b) chemokines with the first two cysteines adjacent. Lymphotactin is a newly found chemokine which contains only two of the four conserved cysteine residues and is referred to as a C chemokine (1,2,3). CXC chemokines mainly activate and recruit neutrophils in acute inflammatory diseases while CC chemokines predominantly activate other leukocytes such as monocytes, T cells, eosinophils and basophils in chronic inflammatory disorders (1,2).

The activation and mobilization effects of chemokines on immunal cells are mediated by a group of chemokine receptors. These receptors are members of the G protein-coupled receptor family and have seven transmembrane elements (3,4,5). In addition to binding chemokines, chemokine receptors are also the binding sites and the cell entry factors of some pathogens such as human immunodeficiency virus (6,7,8) and plasmodium vivax which is the major cause of malaria (9). The chemokine receptors and chemokines are therefore considered as the molecular targets for the therapeutic drugs. In the present study, we report the cloning and characterization of a novel human putative chemokine receptor.

MATERIALS AND METHODS

Isolation and sequencing of HNFDS78 genomic clone. Express sequence tag (EST) analysis of a cDNA clone HNFDS78, which is Oligo (dT)-primed and constructed in Uni-ZAPXR vector (Stratagene), had been obtained from a human neutrophil cDNA library (10,11,12). This EST was not a full length gene and had about 800 bp sequence with significant homology to human chemokine receptors. The EST fragment was released from HNFDS78 cDNA plasmid digesting with Xho I and EcoR I enzymes, purified with GENECLEAN II Kit (BIO 101), labeled with [^{32}P] a-dCTP and used as the probe for the screening of full length HNFDS78 gene. A genomic clone corresponding to HNFDS78 was isolated by plaque hybridization of the human Lambda DASH II genomic library (Stratagene). The library (2×10^6 phage) was plated at a density of 5×10^4 plaque forming unit/150 mm plate for primary screening. The plaques were transferred onto nylon transfer membrane (Amersham). The membranes/filters were prehybridized at 42°C for a minimum of 2 hours in 50% formamide buffer with 50% formamide, 5×SSC, 2× Denhardts', 1% SDS, 20 mM NaH_2PO4, and 250 μg/ml denatured salmon sperm DNA. Hybridization was performed using the same 50% formamide buffer at 42°C for 18 hours using the [^{32}P] a-dCTP-labeled HNFDS78 probe (Prime-It II Random Primer Labeling Kit, Stratagene). Following hybridization, the filters were rinsed twice with 2×SSC containing 0.1% SDS at room temperature for 10 minutes, and then washed twice at 42°C for 10 minutes in 0.2×SSC containing 0.1% SDS at high stringency. The filters were exposed to Kodak

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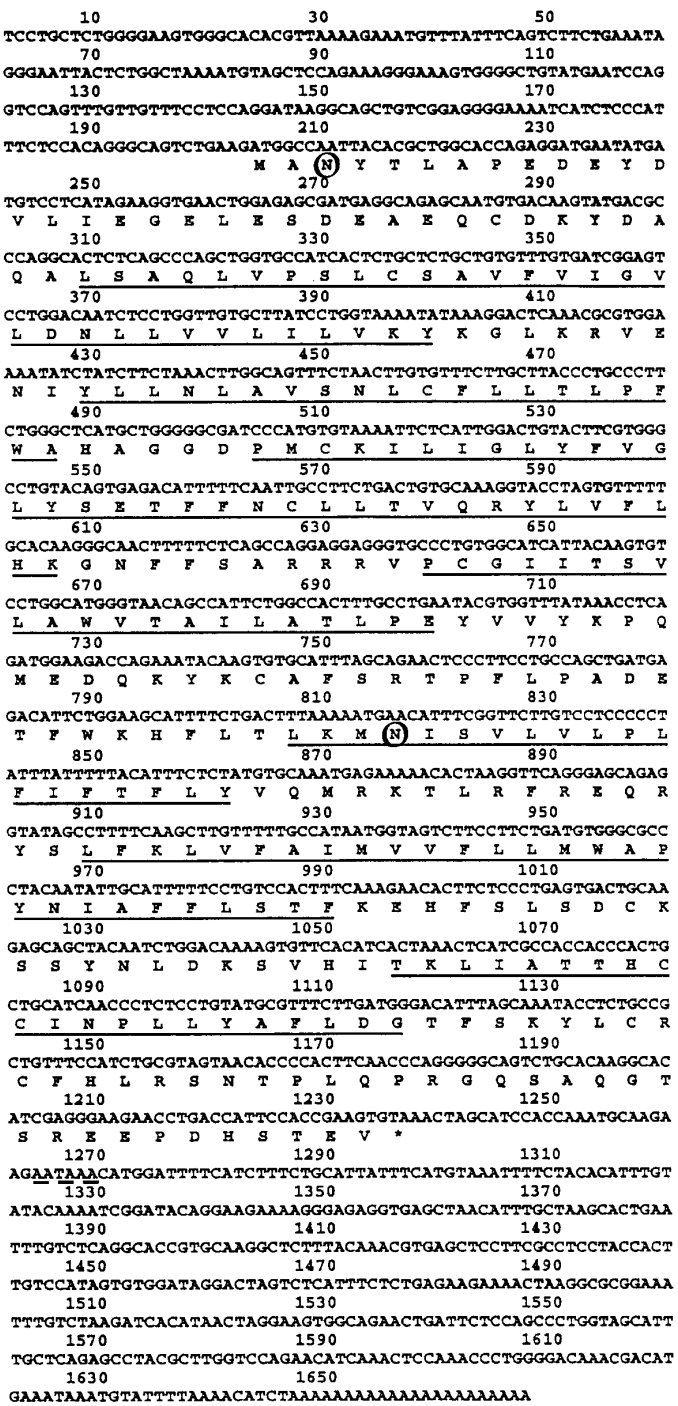

FIG. 1. cDNA sequence and deduced amino acid sequence of HCR gene. The potential transmembrane elements are underlined with a solid line. Potential sites for post-translational N-linked glycosylation are circled, and the polyadenylation signal is underlined with a dotted line. The nucleotide sequence of HCR has been submitted to the GenBank and has the accession number U97123.

autoradiographic film overnight at −80°C with an intensifying screen. To proceed to a secondary screening, putative positive clones were picked, and put into 500 μl of SM buffer with 5.8 gm NaCl/L, 2.0 gm $MgSO_4$/L, 50 ml 1M Tris (pH 7.5)/L, and 5 ml 2% gelatin/L and 10 μl of chloroform. Secondary screening was completed with the same method as primary screening. These conventional DNA recombinant techniques were repeated until positive clones had been obtained (13). Positive lambda phages in SM buffer were titered, and then plated in duplicate. Once bacteriophage suspensions were recovered and pooled in sterile conical tube, 5% of chloroform was added to each tube. Then, the tubes were shaken gently, incubated for 15 minutes at room temperature, and centrifuged for 15 minutes at 2000 × g. Supernatants were transferred to new sterile tubes to which 5% polyethylene glycol (M.W. 8,000) and 5% of NaCl were added. The tubes were gently shaken and incubated at 37°C for 30 minutes. Next, they were centrifuged for 20 minutes at 10,000 × g. Supernatants were discarded. The genomic DNA pellets were resuspended in TE buffer, extracted twice with phenol:chloroform [1:1 (v/v)], and precipitated by ethanol. Pellets were resuspended in sterile water. A few positive clones were obtained, the longest of which was sequenced to completion using an ABI sequencer (10).

Northern blot analysis. The fragment of HNFDS78 cDNA containing the appropriate open reading frame was released with EcoR I and Xho I, labeled with [^{32}P] a-dCTP (Amersham) and used as cDNA probe. Human Multiple Tissue Northern (MTN) Blots, containing 2 μg of poly $(A)^+$ RNA per lane from different tissues, were obtained from Clontech. These blots were hybridized to the [^{32}P] a-dCTP labeled cDNA probe at 65°C for 18 hours with Church buffer consisting of 1% BSA, 250 mM NaH_2PO4 (pH 7.2), and 7% SDS. The blots were rinsed at room temperature in 2×SSC containing 0.1% SDS for 10 minutes, and the final wash was performed at 65°C for 10 minutes in 0.2×SSC containing 0.1% SDS. These blots were then exposed at −80°C overnight to a Kodak autoradiography film with an intensifying screen (13).

Chromosome mapping. A genomic clone containing the HNFDS78 gene was nick-translated using Digoxigenin-dUTP (Boehringer Mannheim) and fluorescence *in situ* hybridization was done as detailed in Johnson et al., 1991b (15). Individual chromosomes were counterstained with DAPI and color digital images, containing both DAPI and gene signal, were recorded using a triple-band pass filter set (Chroma Technology, Inc., Brattleburo, VT) in combination with a charged coupled-device camera (Photometrics, Inc., Tucson, AZ) and variable excitation wave length filters (16). Images were analyzed using the ISEE software package (Inovision Corp., Durham, N.C.).

RESULTS AND DISCUSSION

We used expressed sequence tag (EST) analysis of cDNA clones from a human neutrophil cDNA library and identified an 800 bp clone (HNFDS78) which has significant homology to human chemokine receptors. Since the sequence homology of the clone suggested that it encoded a partial sequence of a chemokine receptor, it was used as a probe for the screening of a full length gene against a human genomic library.

Using a radio-labeled HNFDS78 probe, we identified a genomic clone encoding a full length gene corresponding to HNFDS78 in the human Lambda DASH II genomic library. This clone was named HCR (Human Chemokine Receptor) because it has good homology to human chemokine receptors. The entire sequence of HCR encodes 1.6 Kb nucleotide sequence with a single open reading frame of 1035 nucleotides, and a 408 nucleotide 3′ end untranslated sequence (obtained from cDNA sequence, Fig. 1). A typical polyadenylation signal (AATAAA) (17) is located downstream from the first in-frame

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  5 TLAPEDEYDVLIEGELESDEAEQCDKYDAQALSAQLVPSLCSAVFVIGVL 54  HCR
    | ...::||  ...|:: ::|..|:| :..|::|||:|.|:| |||||::
  3 TPNTTEDYD..TTTEFDYGDATPCQKVNERAFGAQLLPPLYSLVFVIGLV 50  MIP-1a
            .         .         .         .         .
 55 DNLLVVLILVKYKGLKRVENIYLLNLAVSNLCFLLTLPFWAH.......A 97
    :|:||||:||.|| ||.:..|||||||:|:| ||:||||| .       .
 51 GNILVVLVLVQYKRLKNMTSIYLLNLAISDLLFLFTLPFWIDYKLKDDWV 100
            .         .         .         .         .
 98 GGDPMCKILIGLYFVGLYSETFFNCLLTVQRYLVFLHKGNFFSARRRVPC 147
     ||:||||| |:|:.|||||.|| .|||::|||.::| .  :.| | |.
101 FGDAMCKILSGFYYTGLYSEIFFIILLTIDRYLAIVHAVFALRA.RTVTF 149
            .         .         .         .         .
148 GIITSVLAWVTAILATLPEYVVYKPQMEDQKYKCAFSRTPFLPADETFWK 197
    |:|||:: |. ||||.:|:. . |.| |  ...|.:   .| ...   ||
150 GVITSIIIWALAILASMPGLYFSKTQWEFTHHTCSL...HFPHESLREWK 196
            .         .         .         .         .
198 HFLTLKMNISVLVLPLFIFTFLYVQMRKTL..RFREQRYSLFKLVFAIMV 245
    | .||:|: .|||||:::.: |..: |.|  | .|.: . .:|:|.||:
197 LFQALKLNLFGLVLPLLVMIICYTGIIKILLRRPNEKKSKAVRLIFVIMI 246
            .         .         .         .         .
246 VFLLMWAPYNIAFFLSTFKEHFSLSDCKSSYNLDKSVHITKLIATTHCCI 295
    :|:|:|.|||:.:::|.|.: :   :|. | :|| .|::|.:|| ||||:
247 IFFLFWTPYNLTILISVFQDFLFTHECEQSRHLDLAVQVTEVIAYTHCCV 296
            .         .         .         .         .
296 NPLLYAFLDGTFSKYLCRCFHLRSNTPLQPRGQ.........SAQGTSR 335
    ||::|||::: |.||| . || | ...| .: .          |. :.|
297 NPVIYAFVGERFRKYLRQLFHRRVAVHLVKWLPFLSVDRLERVSSTSPST 346

336 EEPDHSTEV 344
    :|.: |.:.
347 GEHELSAGF 355
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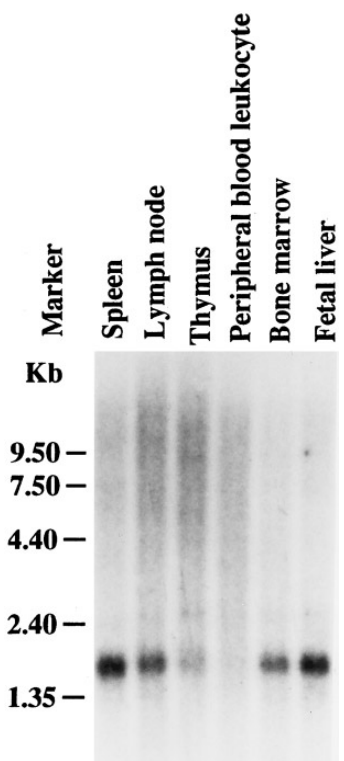
FIG. 2. Amino acid sequence alignment of HCR gene (top sequence) with human MIP-1a receptor (bottom sequence). Dots indicate the gaps that were inserted to optimize the alignment.



stop codon TAA. The open reading frame sequence of HCR predicts a protein of 345 amino acids with a molecular weight of 39.5 KD. Hydrophobicity analysis of the HCR amino acid sequence revealed a seven-transmembrane domain topography. Two potential N-glycosylation sites are at positions of amino acids 3 and 205. The deduced protein sequence of HCR, at the amino acid level, is most closely related to human MIP-1a receptor, CKR1 (18), with 43.1% amino acid identity and 64.4% amino acid similarity over the entire sequence (Fig. 2). The HCR amino acid sequence also shows similar homology to MCP-3 receptor with 42.7% amino acid identity (19), to MCP-5 receptor with 42.4% amino acid identity (6,7,20), and to MCP-1 receptor with 40.5% amino acid identity (21,22).

To determine the distribution of HCR in human tissues, Northern blot analysis of poly(A)$^+$ RNA from different human tissues was performed with the HNFDS78 cDNA probe at high stringency (Fig. 3). The major mRNA transcript for HCR mRNA was approxi-

FIG. 3. Distribution of HCR gene in human tissues. Two Clontech human multiple tissue Northern blots were probed HCR gene fragment (released by EcoR I and Xho I) according to the manufacturer's instructions, washed at high stringency and exposed to Kodak film at −80°C for 48 hours. Each lane contains 2 μg poly (A)$^+$ RNA.

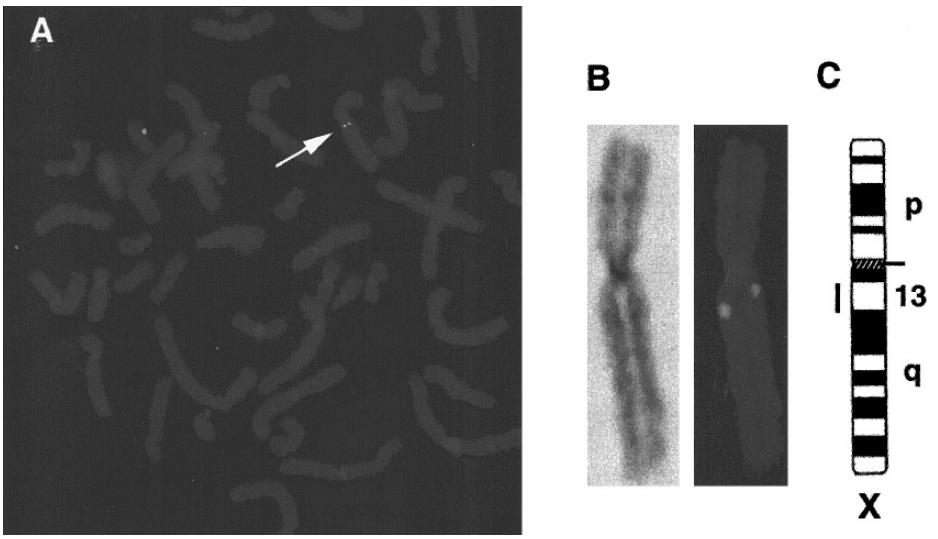


FIG. 4. Fluorescence in situ hybridization mapping of the HCR gene. A genomic clone containing the HCR gene was hybridized to normal human male chromosomes. (A) A chromosome spread from a single cell showing hybridization to the q arm of chromosome X (arrow). (B) Example of a single chromosome X with hybridization signal. (C) idiogram of chromosome X showing the position of the HCR gene.

mately 1.7 Kb and was expressed predominantly in spleen, fetal liver, lymph node, bone marrow, lung and heart. The HCR mRNA was also expressed to a lesser extent in thymus and placenta, and only marginal expression was found in the skeletal muscle. No expression of HCR mRNA transcript was shown in pancreas, kidney, adult liver, brain, and peripheral blood leukocyte.

To determine the precise chromosomal location of the HCR gene we did single-gene fluorescence *in situ* hybridization to human chromosome metaphase spreads (14) (Fig. 4). Approximately 15 spreads were analyzed by eye, most of which had a doublet signal characteristic of genuine hybridization on chromosome X. Doublet signal was not detected on any other chromosome. Detailed analysis of 12 individual chromosomes, using fluorescence banding combined with high-resolution image analysis, indicated that the HCR gene is positioned within band Xq13.

Chemokine receptors are involved in a wide range of physiological and pathological conditions, which include various stages of inflammation, virus and bacterial infections, tumor immunity, heamatopoeisis and angiogenesis (1–5). Defining the molecular basis of chemokine receptors has greatly improved our understanding of the functions of chemokine receptors. The rational design of chemokine receptor antagonists, based on the chemokine receptor structure and sequence, may ultimately lead to the development of therapeutic pharmaceuticals. In the present study, we describe the identification and characterization of a new member of chemokine receptor family, HCR. This gene is expressed abundantly in some immunal tissues such as spleen, fetal liver, lymph node and bone marrow, suggesting that it may be involved in immunal disease conditions. The novel finding in the present study also provides valuable information for understanding the chemokine receptor family and to search the potential targets for the development of therapeutic drugs.

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