SHORT COMMUNICATION

Cloning of STRL22, a New Human Gene Encoding a G-Protein-Coupled Receptor Related to Chemokine Receptors and Located on Chromosome 6q27

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Using PCR with pools of primers based on conserved sequences in chemokine receptors, we have cloned a human member of the G-protein-coupled receptor gene family. The new gene, which we have named STRL22, is predicted to encode a receptor related to chemokine receptors, including IL8RA (CXCR1), IL8RB (CXCR2), and CXCR4/fusin, and to the orphan receptors EBI1 and BLR1. Consistent with a role in leukocyte biology, STRL22 is expressed in lymphocytes and in lymphoid tissue. We have mapped STRL22 to chromosome 6q27. STRL22 cDNAs reflect alternative (or incomplete) mRNA processing in the 5′-nontranslated region, a phenomenon found in analysis of other chemoattractant receptor genes. In contrast to most chemokine receptor genes, the STRL22 coding sequence is not limited to a single exon. © 1997 Academic Press

The chemokines are a family of cytokines, many of whose members are increasingly recognized to have diverse effects on the physiology and pathophysiology of lymphocytes, including lymphocyte accumulation at sites of infection and inflammation (5), lymphocyte activation and proliferation (2), and lymphocyte infection by HIV-1 (4). The chemokines act through seven transmembrane domain G-protein-coupled receptors (GPCRs) displayed on their target cells (11). To date 10 human chemokine receptors have been described, some of which function as coreceptors for HIV-1 entry into cells (8).

To acquire new tools to study the roles of chemokines in lymphocyte biology, we designed experiments to identify novel chemokine receptors in human T cells. We prepared RNA from the F9 line of tumor infiltrating lymphocytes (TIL), which we had shown to respond to chemokines in assays for calcium flux and chemotaxis (10). This RNA was used in RT-PCR experiments with pools of degenerate primers, 5′GA(T/C)(C/A)G(G/A/T/C)TA(T/C)(T/C/G)TIGCIAT(T/C/A)GTICAIGC and 5′CCIA(T/C)(A/G)AAI(G/A)(C/T)(A/G)TAIA(T/A/G)IA(G/A/T/C)IGG(A/G)TT, based on conserved amino acid sequences (see below, Fig. 3) in the human chemokine receptors IL8RA[3], IL8RB, CCR1, and CCR2A and the mouse homologues of IL8RB and CCR1 (GenBank Accession Nos. M68932, M73969, L10918, U03882, L13239, and U28404, respectively).

PCR using an annealing temperature of 50°C yielded products of approximately 550 bp. The PCR products were analyzed by restriction digestion to identify and in some cases eliminate products from known receptor genes. Subfragments were isolated, inserted into the vector pBluescript, and sequenced. A novel sequence was discovered. A probe containing this sequence was used to screen a human genomic library in λ FIX II (Stratagene, La Jolla, CA) to isolate a gene that encoded a GPCR that we designated STRL22, for seven transmembrane domain receptor from lymphocytes, clone 22.

We sequenced the complete STRL22 open reading frame (ORF) from a 5.5-kb *Hin*dIII fragment obtained from one of the positive recombinant phages. We next screened 1.4×10^6 recombinant phages from a nonamplified Lambda ZAP (Stratagene) cDNA library that we prepared from poly(A)$^+$ RNA from the F9 TIL, and we identified 35 positive clones, indicating an abundance of 0.0025%. Fourteen cDNA clones were isolated and evaluated by a combination of restriction analysis and sequencing,

Sequence data from this article have been deposited with the GenBank/EMBL Data Libraries under Accession Nos. U68030 for the STRL22 3.0-kb cDNA, U68031 for the STRL22 genomic DNA, and U68032 for the 5′-nontranslated region of the STRL22 3.3-kb cDNA.

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[3] At the 1996 Gordon Conference on Chemotactic Cytokines, it was recommended that the receptors for IL-8 be referred to as CXCR1 and CXCR2 or as IL8RA and IL8RB, respectively, and that the other established chemokine receptors be designated CXCR3 (Mig/IP-10 receptor) and CCR1 through CCR5 (CC chemokine receptors). Subsequently SDF-1 was shown to be a ligand for HUMSTR/fusin, and HUMSTR was renamed CXCR4.

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A

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   1  CAAACGTTCCCAAATCTTCCCAGTCGGCTTGCAGAGACTCCTTGCTCCCAGGAGATAACCAGAAGCTGCATCTTATTGACAGATGGTCATCACATTGGTG  100
 101  AGCTGGAGTCATCAGATTGTGGGGCCCGGAGTGAGGCTGAAGGGAGTGGATCAGAGCACTGCCTGAGAGTCACCTCTACTTTCCTGCTACCGCTGCCTGT  200
 201  GAGCTGAAGGGGCTGAACCATACACTCCTTTTTCTACAACCAGCTTGCATTTTTTCTGCCCACAATGAGCGGGGAATCAATGAATTTCAGCGATGTTTTC  300
                ***                                                    MetSerGlyGluSerMetAsnPheSerAspValPhe
      .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .
 301  GACTCCAGTGAAGATTATTTTGTGTCAGTCAATACTTCATATTACTCAGTTGATTCTGAGATGTTACTGTGCTCCTTGCAGGAGGTCAGGCAGTTCTCCA  400
      AspSerSerGluAspTyrPheValSerValAsnThrSerTyrTyrSerValAspSerGluMetLeuLeuCysSerLeuGlnGluValArgGlnPheSerA
      .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .
 401  GGCTATTTGTACCGATTGCCTACTCCTTGATCTGTGTCTTTGGCCTCCTGGGGAATATTCTGGTGGTGATCACCTTTGCTTTTTATAAGAAGGCCAGGTC  500
      rgLeuPheValProIleAlaTyrSerLeuIleCysValPheGlyLeuLeuGlyAsnIleLeuValValIleThrPheAlaPheTyrLysLysAlaArgSe
      .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .
 501  TATGACAGACGTCTATCTCTTGAACATGGCCATTGCAGACATCCTCTTTGTTCTTACTCTCCCATTCTGGGCAGTGAGTCATGCCACTGGTGCGTGGGTT  600
      rMetThrAspValTyrLeuLeuAsnMetAlaIleAlaAspIleLeuPheValLeuThrLeuProPheTrpAlaValSerHisAlaThrGlyAlaTrpVal
      .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .
 601  TTCAGCAATGCCACGTGCAAGTTGCTAAAAGGCATCTATGCCATCAACTTTAACTGCGGGATGCTGCTCCTGACTTGCATTAGCATGGACCGGTACATCG  700
      PheSerAsnAlaThrCysLysLeuLeuLysGlyIleTyrAlaIleAsnPheAsnCysGlyMetLeuLeuLeuThrCysIleSerMetAspArgTyrIleA
      .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .
 701  CCATTGTACAGGCGACTAAGTCATTCCGGCTCCGATCCAGAACACTACCGCGCACGAAAATCATCTGCCTTGTTGTGTGGGGGCTGTCAGTCATCATCTC  800
      laIleValGlnAlaThrLysSerPheArgLeuArgSerArgThrLeuProArgThrLysIleIleCysLeuValValTrpGlyLeuSerValIleIleSe
      .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .
 801  CAGCTCAACTTTTGTCTTCAACCAAAAATACAACACCCAAGGCAGCGATGTCTGTGAACCCAAGTACCAGACTGTCTCGGAGCCCATCAGGTGGAAGCTG  900
      rSerSerThrPheValPheAsnGlnLysTyrAsnThrGlnGlySerAspValCysGluProLysTyrGlnThrValSerGluProIleArgTrpLysLeu
      .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .
 901  CTGATGTTGGGGCTTGAGCTACTCTTTGGTTTCTTTATCCCTTTGATGTTCATGATATTTTGTTACACGTTCATTGTCAAAACCTTGGTGCAAGCTCAGA 1000
      LeuMetLeuGlyLeuGluLeuLeuPheGlyPhePheIleProLeuMetPheMetIlePheCysTyrThrPheIleValLysThrLeuValGlnAlaGlnA
      .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .
1001  ATTCTAAAAGGCACAAAGCCATCCGTGTAATCATAGCTGTGGTGCTTGTGTTTCTGGCTTGTCAGATTCCTCATAACATGGTCCTGCTTGTGACGGCTGC 1100
      snSerLysArgHisLysAlaIleArgValIleIleAlaValValLeuValPheLeuAlaCysGlnIleProHisAsnMetValLeuLeuValThrAlaAl
      .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .
1101  AAATTTGGGTAAAATGAACCGATCCTGCCAGAGCGAAAAGCTAATTGGCTATACGAAAACTGTCACAGAAGTCCTGGCTTTCCTGCACTGCTGCCTGAAC 1200
      aAsnLeuGlyLysMetAsnArgSerCysGlnSerGluLysLeuIleGlyTyrThrLysThrValThrGluValLeuAlaPheLeuHisCysCysLeuAsn
      .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .
1201  CCTGTGCTCTACGCTTTTATTGGGCAGAAGTTCAGAAACTACTTTCTGAAGATCTTGAAGGACCTGTGGTGTGTGAGAAGGAAGTACAAGTCCTCAGGCT 1300
      ProValLeuTyrAlaPheIleGlyGlnLysPheArgAsnTyrPheLeuLysIleLeuLysAspLeuTrpCysValArgArgLysTyrLysSerSerGlyP
      .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .    .
1301  TCTCCTGTGCCGGGAGGTACTCAGAAAACATTTCTCGGCAGACCAGTGAGACCGCAGATAACGACAATGCGTCGTCCTTCACTATGTGATAGAAAGCTGA 1400
      heSerCysAlaGlyArgTyrSerGluAsnIleSerArgGlnThrSerGluThrAlaAspAsnAspAsnAlaSerSerPheThrMet***

1401  GTCTCCCTAAGGCATGTGTGAAACATACTCATAGATGTTATGCAAAAAAAAGTCTATGGCCAGGTATGCATGGAAAATGTGGGAATTAAGCAAAATCAAG 1500
1501  CAAGCCTCTCTCCTGCGGGACTTAACGTGCTCATGGGCTGTGTGATCTCTTCAGGGTGGGGTGGTCTCTGATAGGTAGCATTTTCCAGCACTTTGCAAGG 1600
1601  AATGTTTTGTAGCTCTAGGGTATATATCCGCCTGGCATTTCACAAAACAGCCTTTGGGAAATGCTGAATTAAAGTGAATTGTTGACAAATGTAAACATTT 1700
1701  TCAGAAATATTCATGAAGCGGTCACAGATCACAGTGTCTTTTGGTTACAGCACAAAATGATGGCAGTGGTTTGAAAAACTAAAACAGAAAAAAAAATGGA 1800
1801  AGCCAACACATCACTCATTTTAGGCAAATGTTTAAACATTTTTATCTATCAGAATGTTTATTGTTGCTGGTTATAAGCAGCAGGATTGGCCGGCTAGTGT 1900
1901  TTCCTCTCATTTCCCTTTGATACAGTCAACAAGCCTGACCCTGTAAAATGGAGGTGGAAAGACAAGCTCAAGTGTTCACAACCTGGAAGTGCTTCGGGAA 2000
2001  GAAGGGGACAATGGCAGAACAGGTGTTGGTGACAATTGTCACCAATTGGATAAAGCAGCTCAGGTTGTAGTGGGCCATTAGGAAACTGTCGGTTTGCTTT 2100
2101  GATTTCCCTGGGAGCTGTTCTCTGTCGTGAGTGTCTCTTGTCTAAACGTCCATTAAGCTGAGAGTGCTATGAAGACAGGATCTAGAATAATCTTGCTCAC 2200
2201  AGCTGTGCTCTGAGTGCCTAGCGGAGTTCCAGCAAACAAAATGGACTCAAGAGAGATTTGATTAATGAATCGTAATGAAGTTGGGGTTTATTGTACAGTT 2300
2301  TAAAATGTTAGATGTTTTTAATTTTTAAATAAATGGAATACTTTTTTTTTTTTAAAGAAAGCAACTTTACTGAGACAATGTAGAAAGAAGTTTTGTTC 2400
2401  CGTTTCTTTAATGTGGTTGAAGAGCAATGTGTGGCTGAAGACTTTTGTTATGAGGAGCTGCAGATTAGCTAGGGGACAGCTGGAATTATGCTGGCTTCTG 2500
2501  ATAATTATTTTAAAGGGGTCTGAAATTTGTGATGGAATCAGATTTTAACAGCTCTCTTCAATGACATAGAAAGTTCATGGAACTCATGTTTTTAAAGGGC 2600
2601  TATGTAAATATATGAACATTAGAAAAATAGCAACTTGTGTTACAAAAATACAAACACATGTTAGGAAGGTACTGTCATGGGCTAGGCATGGTGGCTCACA 2700
2701  CCTGTAATCCCAGCATTTTGGGAAGCTAAGATGGGTGGATCACTTGAGGTCAGGAGTTTGAGACCAGCCTGGCCAACATGGCGAAACCCCTCTCTACTAA 2800
2801  AAATACAAAAATTTGCCAGGCGTGGTGGCGGGTGCCTGTAATCCCAGCTACTTGGGAGGCTGAGGCAAGAGAATCGCTTGAACCCAGGAGGCAGAGGTTG 2900
2901  CAGTGAGCCGAGATCGTGCCATTGCACTCCAGCCTGGGTGACAGAGCGAGACTCCATCTCAAAAAAAAAAAAAAAAAA                    2978
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B

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                273                                                                                  274
.....ATGAGCGGG[GTAAGATTTTTATTTTTGGCAAGGGGTATAATTTGGGTTCACTGTGGCTACTTGAACACTACACTGCAGCTAACTCTATCTTTGTTTCCTTTCCAG]GAA.....
     MetSerGly                                                                                                  Glu.....
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C

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TGCAAACGTTCCCAAATCTTCCCAGTCGGCTTGCAGAGACTCCTTGCTCCCAGGAGATAACCAGGTAAAGGAGTATGAAAGTTTGGGTACAAACTCATTG  100
CTGCAAATTGAAAACCATGCAAAGGCTGTCTTCCTCTGGGGAGTTCAATGCCTCTCTTTTTCTTATCACTTTACCATTGGTTGGACTTTGATTGCAGGGA  200
TCCTACGATTACTCAATACCCTACAGGATATACATGGTTAACCATTTGCATTTGGGCAAATAGGCGTTACTTTTCAATAGGAAGTGGCAATCCAGAACTT  300
GCTTTTGGCAATTCTAGTAGCTCACCGCTTTTTTCTTAATGACTGCTAGAAGCTGCATCTTATTGACAGATGGTCATCACATTGGTGAGCTGGAGTCATC  400
AGATTGTGGGGCCCGGAGTGAGGCTGAAGGGAGTGGATCAGAGCACTGCCTGAGAGTCACCTCTACTTTCCTGCTACCGCTGCCTGTGAGCTGAAGGGGC  500
TGAACCATACACTCCTTTTTCTACAACCAGCTTGCATTTTTTCTGCCCACAATG
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FIG. 1. (**A**) Sequence of STRL22 cDNA and predicted amino acid sequence of the STRL22 receptor. The predicted initiator codon is in boldface. Asterisks indicate two terminator codons, one in-frame and upstream of the initiator codon and the second at the 3′ end of the ORF. The predicted *N*-glycosylation sites are underlined. (**B**) The sequence of the 96-bp intron between the third and the fourth codons. The intron sequence is bracketed, and the splice donor and splice acceptor dinucleotides are underlined. Numbers correspond to those in the cDNA sequence shown in (**A**). (**C**) The 5′-nontranslated region of the 3.3-kb cDNA clone of STRL22. The shaded sequence is the 285-bp insertion, which is absent from the cDNA shown in (**A**). Splice donor and splice acceptor dinucleotides are underlined. The initiator codon is in boldface.

and 2 cDNAs of 3.0 and 3.3 kb, which differed in the lengths of their 5′ ends, were analyzed in more detail. The sequence of the 3.0-kb cDNA is shown in Fig. 1A.

Comparison of cDNA and genomic sequences revealed the existence of two introns, a 96-bp intron between the third and fourth codons as shown in Fig. 1B and an intron of unknown length (not shown) between nucleotides 167 and 168 of the sequence shown in Fig. 1A. The cDNA sequence contains a 1122-bp ORF counting from a presumptive initiator methionine codon, a 264-bp 5′-nontranslated region, and a 1592-bp 3′-nontranslated region. The first methionine codon in the ORF conforms well to the empirical rules of Kozak (9)

for an initiator codon and begins a predicted protein of 374 amino acids.

It is of interest that the 3.0-kb cDNA contains an upstream ATG at positions 83–85. While upstream AUG triplets are generally uncommon in vertebrate mRNAs (9), they are not uncommon in the 5′-nontranslated regions of mRNAs for chemoattractant receptors (1, 12). The STRL22 AUG at 83–85 might affect translation efficiency from the AUG at 265–267, but initiation at the upstream AUG would be expected to be poor due to the C at position 80 (9). Moreover, the upstream ORF extends for only 27 codons before reaching a stop codon at positions 164–166.

The sequence of the STRL22 cDNA ORF and the corresponding sequence of the genomic DNA were found to be identical with the exception of the 96-bp intron as noted (Fig. 1B). In general, ORFs of the chemokine receptor genes are found on single exons (11). In this regard the STRL22 ORF is unusual, but similar to the ORFs for the related orphan receptors EBI1 (17) and BLR1 (6), which contain introns interrupting the amino-terminal coding regions. The 3′ end of the cDNA in Fig. 1A does not contain a poly(A) addition signal. Sequencing demonstrated that the poly(A) tract at the end of the cDNA is present in genomic DNA (not shown), indicating that first-strand priming occurred at an internal site and that the cDNA does not include the complete 3′-nontranslated region of the mRNA.

Restriction analysis and partial sequencing demonstrated that the 3.3-kb cDNA differed from the sequence shown in Fig. 1A due to a 285-bp insertion in the 5′-nontranslated region. This sequence, shown in Fig. 1C, is bounded by the canonical intronic splice donor and splice acceptor dinucleotides. Similar to our findings, IL8RB (CXCR2) demonstrates the use of alternative exons in the 5′-nontranslated region (1). We cannot rule out the possibility, however, that the 3.3-kb STRL22 cDNA represents an immature, incompletely processed mRNA, even though the intron separating the third and fourth codons and the intron between nucleotides 454 and 455 (the intron between nucleotides 167 and 168 of the sequence in Fig. 1A) had been removed. Since our analysis of the STRL22 genomic DNA did not extend beyond the intronic sequence immediately 5′ to nucleotide 455, we have no additional direct data on mRNA processing based on comparisons of the STRL22 gene and cDNAs.

The 5′-nontranslated region of the 3.3-kb clone contains six upstream AUGs, and the AUG at 117–119 is in a good context for initiation. Although these AUGs might be expected to affect translation efficiency of the STRL22 ORF, none alter the ORF, since stop codons in all three frames separate the predicted STRL22 initiator AUG from those upstream.

Southern analysis of human genomic DNA demonstrated a single STRL22 gene (Fig. 2). By PCR analysis of DNA prepared from a panel of human–hamster hybrid cell lines (BIOS Laboratories, New Haven, CT), STRL22 was localized to chromosome 6 (not shown). Using an 18-kb fragment obtained from a λ FIX recombinant, STRL22 was localized by FISH to 6q27 (Fig. 2). A search of the Genome Data Base failed to find other GPCRs at this location.

A comparison of the sequence of the predicted protein of STRL22 with the database sequences, updated as of August 20, 1996, using the BLAST program revealed identity with an unpublished sequence designated GPR-CY4, which had been submitted to GenBank while this work was in progress—except that the predicted sequence of GPR-CY4 differed from STRL22 at the amino terminus, apparently due to the assignment of the GPR-CY4 initiator methionine based on genomic sequences. STRL22 was most closely related to published sequences for the human GPCRs EBI1, IL8RB, IL8RA, BLR1, and CXCR4/fusin, based on the BLAST search of the sequence database and based on an analysis of multiple related receptor sequences using the PileUp program of the Wisconsin Sequence Analysis package of Genetics Computer Group (Madison, WI) (not shown). A comparison between STRL22 and the five closely related sequences is shown in Fig. 3. The percentages of identity between the complete amino acid sequences of STRL22 and the other receptors range from 39% for EBI1 to 28% for CXCR4/fusin. As is typical for the GPRC family, similarity between STRL22 and other receptor sequences is greatest in the transmembrane domains (TMDs).

In addition to the seven TMDs, a number of sequence motifs and residues have been identified as conserved in most GPCRs, and some have been identified to be of functional importance by mutagenesis (16). Most of these conserved motifs and residues are found in the STRL22 sequence, including amino-terminal domain sites for N-linked glycosylation, cysteines in extracellular loops one and two (C118 and C197), proline residues in TMDs V, VI, and VII (P226, P269, and P313), an asparagine in TMD I (N64), an aspartic acid in TMD II (D92) and adjacent aspartic acid and arginine residues following TMD III (D142, R143). Based on data for the β_2-adrenergic receptor, C336 in STRL22 may be palmitoylated (13). As for other GPCRs, the predicted STRL22 sequence contains numerous intracellular serine and threonine residues, particularly in the C-terminal region, that may serve as sites for phosphorylation (16).

While it is not possible by sequence alone to identify a GPCR unambiguously as a chemokine receptor, STRL22 does contain sequences typical of the chemokine receptor subclass, including an acidic N-terminal domain, a short and basic third intracellular loop, cysteines in the N-terminal domain (C36) and the third extracellular loop (C288), and a conserved region including the DRY sequence just following TMD III (11) (Fig. 3). In addition, STRL22, like all the chemokine receptors identified to date, contains an alanine (A150) in place of a proline residue found in the second intracellular loop in the vast majority of nonchemokine receptor GPCRs (15).

Northern blot analysis of poly(A)$^+$ RNA from human tissues showed prominent expression of STRL22 in

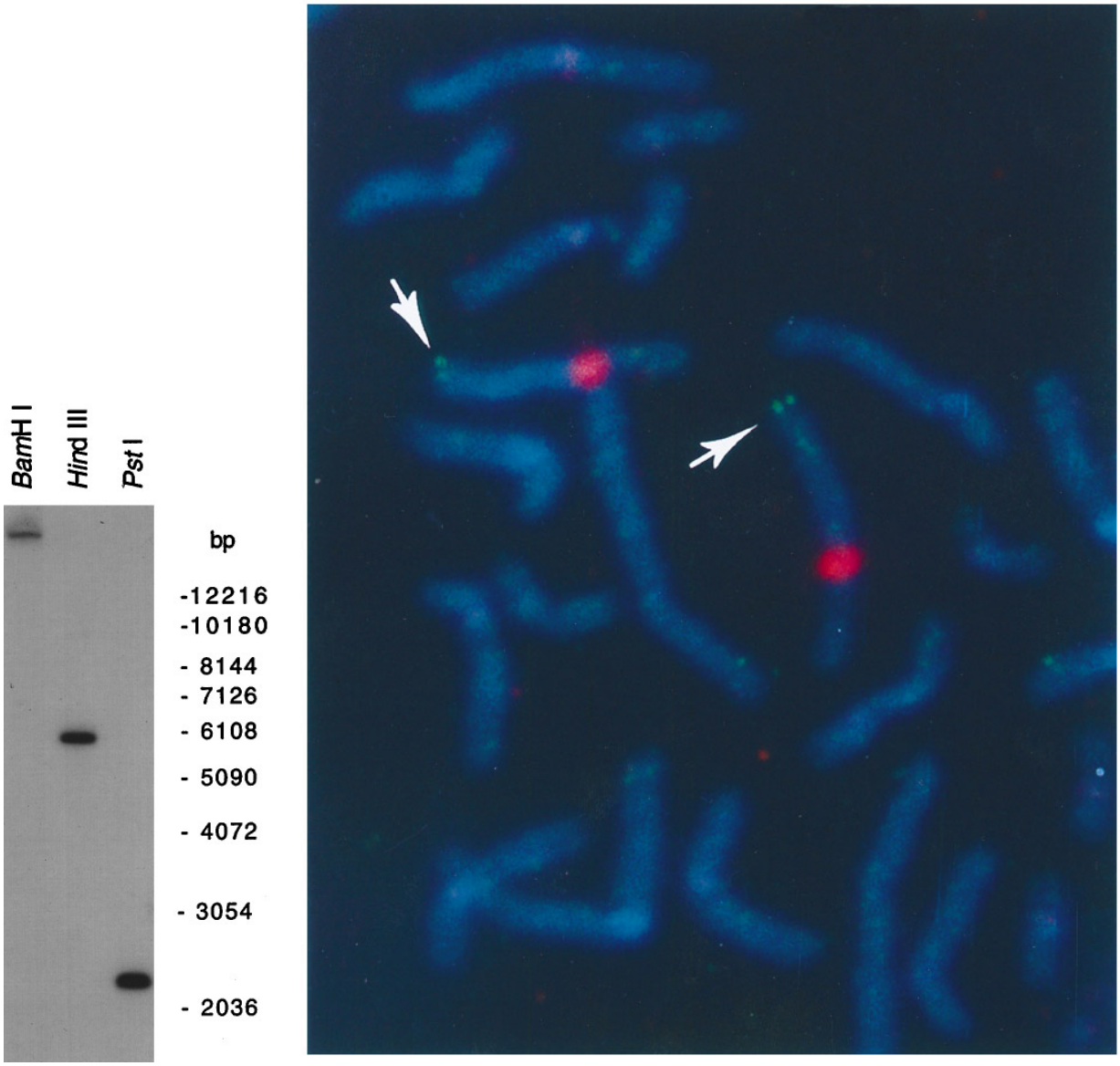
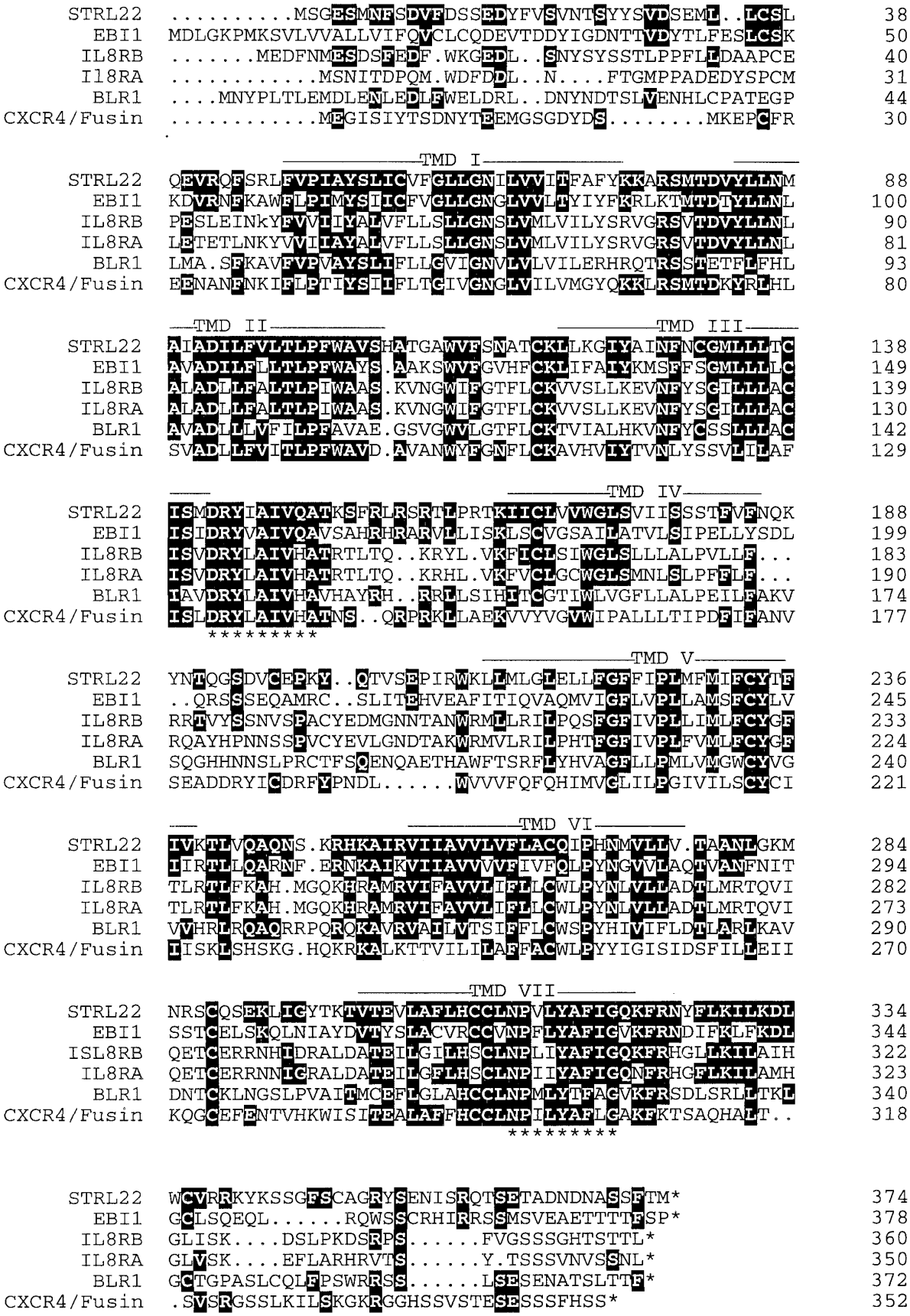


FIG. 2. (**Left**) Genomic Southern blot analysis of STRL22. Twenty micrograms of genomic DNA isolated from peripheral blood leukocytes was digested with restriction enzymes as indicated, separated by agarose gel electrophoresis, blotted onto Zeta Probe nylon membrane (Bio-Rad), and hybridized with ^{32}P-radiolabeled probes made from fragments of STRL22, including nucleotides 688–1208 (Fig. 1A). DNA molecular weight markers are indicated. (**Right**) Chromosomal localization of STRL22 by FISH. Hybridizations were performed in 50% formamide, 10% dextran sulfate, and 2× SSC on human metaphase chromosomes from PHA-stimulated peripheral blood lymphocytes using a digoxigenylated probe made from an 18-kb STRL22 genomic clone and a biotinylated alpha satellite/centromere probe (D6Z1) for chromosome 6. Hybridized probes were detected using fluoresceinated anti-digoxigenin antibodies and Texas red–avidin followed by counterstaining with DAPI. Specific labeling using the STRL22 probe was seen in 59 of a total of 80 metaphase cells analyzed. Twin-spot STRL22 signals at the termini of the long arms of chromosomes 6 are shown by the arrows. FISH was performed by Genome Systems, Inc. (St. Louis, MO).

lymphoid organs, particularly where the mature, responding cells are found, such as spleen, lymph node, and gut (Fig. 4). Among nonlymphoid organs, there was prominent expression in pancreas. In data not shown, STRL22 was expressed in lines of TIL and in an EBV-transformed B lymphoblastoid cell line, but not in a

FIG. 3. Alignment of the STRL22 predicted amino acid sequence with GPCRs EBI1, IL8RB, IL8RA, BLR1, and CXCR4/fusin. IL8RA is a receptor for IL8, IL8RB is a receptor for both IL8 and IL8-related chemokines (11), and CXCR4/fusin is a receptor for SDF-1 (3, 14) CXCR4 has been identified as a coreceptor for HIV-1 and is referred to in this context as "fusin" (8). The other sequences are orphan receptors presumed to function as chemokine receptors (6, 7, 17). GenBank/EMBL accession numbers for EBI1, BLR1, and CXCR4/fusin are L08176, X68149, and M99293, respectively, and accession numbers for IL8RB and IL8RA are noted in the text. Numbers at the right indicate the positions of the residues at the ends of the lines of sequence. Solid backgrounds highlight matches between STRL22 and the other receptors. The asterisks beneath the sequences indicate the positions of conserved residues used for designing pools of degenerate primers. Dots indicate gaps introduced for optimal alignment. Putative TMDs I–VII are indicated by bars. The alignment was generated using the PileUp program of the Wisconsin Sequence Analysis Package of Genetics Computer Group (Madison, WI).


STRL22 .....MSGESMNFSDVFDSSEDYFVSVNTSYYSVDSEML..LCSL 38
EBI1 MDLGKPMKSVLVVALLVIFQVCLCQDEVTDDYIGDNTTVDYTLFESLCSK 50
IL8RB .....MEDFNMESDSFEDF.WKGEDL..SNYSYSSTLPPFLLDAAPCE 40
Il8RA .....MSNITDPQM.WDFDDL..N....FTGMPPADEDYSPCM 31
BLR1 ....MNYPLTLEMDLENLEDLFWELDRL..DNYNDTSLVENHLCPATEGP 44
CXCR4/Fusin .....MEGISIYTSDNYTEEMGSGDYDS.....MKEPCFR 30
TMD I
STRL22 QEVRQFSRLFVPIAYSLICVFGLLGNILVVITFAFYKKARSMTDVYLLNM 88
EBI1 KDVRNFKAWFLPIMYSIICFVGLLGNGLVVLTYIYFKRLKTMTDTYLLNL 100
IL8RB PESLEINKYFVVIIYALVFLLSLLGNSLVMLVILYSRVGRSVTDVYLLNL 90
IL8RA LETETLNKYVVIIAYALVFLLSLLGNSLVMLVILYSRVGRSVTDVYLLNL 81
BLR1 LMA.SFKAVFVPVAYSLIFLLGVIGNVLVLVILERHRQTRSSTETFLFHL 93
CXCR4/Fusin EENANFNKIFLPTIYSIIFLTGIVGNGLVILVMGYQKKLRSMTDKYRLHL 80
TMD II
TMD III
STRL22 AIADILFVLTLPFWAVSHATGAWVFSNATCKLLKGIYAINFNCGMLLLTC 138
EBI1 AVADILFLLTLPFWAYSAAKSWVFGVHFCKLIFAIYKMSFFSGMLLLLC 149
IL8RB ALADLLFALTLPIWAASKVNGWIFGTFLCKVVSLLKEVNFYSGILLLAC 139
IL8RA ALADLLFALTLPIWAASKVNGWIFGTFLCKVVSLLKEVNFYSGILLLAC 130
BLR1 AVADLLLVFILPFAVAEGSVGWVLGTFLCKTVIALHKVNFYCSSLLLAC 142
CXCR4/Fusin SVADLLFVITLPFWAVDAVANWYFGNFLCKAVHVIYTVNLYSSVLILAF 129
TMD IV
STRL22 ISMDRYIAIVQATKSFRLRSRTLPRTKIICLVVWGLSVIISSSTFVFNQK 188
EBI1 ISIDRYVAIVQAVSAHRHRARVLLISKLSCVGSAILATVLSIPELLYSDL 199
IL8RB ISVDRYLAIVHATRTLTQ..KRYL.VKFICLSIWGLSLLLALPVLLF... 183
IL8RA ISVDRYLAIVHATRTLTQ..KRHL.VKFVCLGCWGLSMNLSLPFFLF... 190
BLR1 IAVDRYLAIVHAVHAYRH..RRLLSIHITCGTIWLVGFLLALPEILFAKV 174
CXCR4/Fusin ISLDRYLAIVHATNS..QRPRKLLAEKVVYVGVWIPALLLTIPDFIFANV 177
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TMD V
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EBI1 ..QRSSSEQAMRC..SLITEHVEAFITIQVAQMVIGFLVPLLAMSFCYLV 245
IL8RB RRTVYSSNVSPACYEDMGNNTANWRMLLRILPQSFGFIVPLLIMLFCYGF 233
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BLR1 SQGHHNNSLPRCTFSQENQAETHAWFTSRFLYHVAGFLLPMLVMGWCYVG 240
CXCR4/Fusin SEADDRYICDRFYPNDL.....WVVVFQFQHIMVGLILPGIVILSCYCI 221
TMD VI
STRL22 IVKTLVQAQNS.KRHKAIRVIIAVVLVFLACQIPHNMVLLV.TAANLGKM 284
EBI1 IIRTLLQARNF.ERNKAIKVIIAVVVVFIVFQLPYNGVVLAQTVANFNIT 294
IL8RB TLRTLFKAH.MGQKHRAMRVIFAVVLIFLLCWLPYNLVLLADTLMRTQVI 282
IL8RA TLRTLFKAH.MGQKHRAMRVIFAVVLIFLLCWLPYNLVLLADTLMRTQVI 273
BLR1 VVHRLRQAQRRPQRQKAVRVAILVTSIFFLCWSPYHIVIFLDTLARLKAV 290
CXCR4/Fusin IISKLSHSKG.HQKRKALKTTVILILAFFACWLPYYIGISIDSFILLEII 270
TMD VII
STRL22 NRSCQSEKLIGYTKTVTEVLAFLHCCLNPVLYAFIGQKFRNYFLKILKDL 334
EBI1 SSTCELSKQLNIAYDVTYSLACVRCCVNPFLYAFIGVKFRNDIFKLFKDL 344
ISL8RB QETCERRNHIDRALDATEILGILHSCLNPLIYAFIGQKFRHGLLKILAIH 322
IL8RA QETCERRNNIGRALDATEILGFLHSCLNPIIYAFIGQNFRHGFLKILAMH 323
BLR1 DNTCKLNGSLPVAITMCEFLGLAHCCLNPMLYTFAGVKFRSDLSRLLTKL 340
CXCR4/Fusin KQGCEFENTVHKWISITEALAFFHCCLNPILYAFLGAKFKTSAQHALT.. 318
****
STRL22 WCVRRKYKSSGFSCAGRYSENISRQTSETADNDNASSFTM* 374
EBI1 GCLSQEQL.....RQWSSCRHIRRSSMSVEAETTTTFSP* 378
IL8RB GLISK....DSLPKDSRPS.....FVGSSSGHTSTTL* 360
IL8RA GLVSK....EFLARHRVTS.....Y.TSSSVNVSSNL* 350
BLR1 GCTGPASLCQLFPSWRRSS.....LSESENATSLTTF* 372
CXCR4/Fusin .SVSRGSSLKILSKGKRGGHSSVSTESESSSFHSS* 352

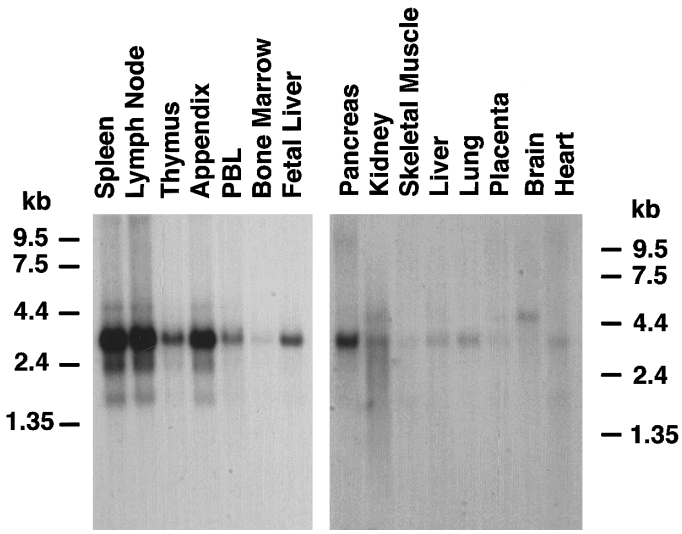

FIG. 4. Expression of STRL22 in human tissues. Blots were prepared by the supplier (Clontech, Palo Alto, CA) from 1.2% agarose–formaldehyde gels containing approximately 2 μg poly(A)$^+$ RNA per lane. Hybridizations were performed using a ^{32}P-labeled STRL22 ORF probe, and blots were washed according to the supplier's instructions, using a final wash of 0.1× SSC/0.1% SDS at 50°C. Membranes were exposed to film using an intensifying screen. The blot prepared from lymphoid tissue (**left**) was exposed overnight, and the blot from other selected tissues (**right**) was exposed for 7 days.

number of immortalized T cell lines, monocytes, or neutrophils. The major species of STRL22 mRNA had a mobility of approximately 3.6 kb, with a slower migrating minor species of approximately 4.4 kb that is preferentially expressed in brain (Fig. 4).

The predominant expression of STRL22 in immune tissue and lymphocytes supports the presumption that STRL22 is a chemokine receptor. The absence of detectable STRL22 expression in neutrophils and monocytes suggests that STRL22 may mediate a lymphocyte-specific chemokine activity. Studies to date testing STRL22-transfected human embryonic kidney 293 cell lines with a panel of chemokines including HuMig, IP-10, IL-8, MCP-1, MCP-2, MCP-3, MCP-4, platelet factor 4, MIP-1α, MIP-1β, RANTES, I-309, and lymphotactin have failed to identify an STRL22 agonist (F.L. and J.M.F., unpublished). Experiments to identify an agonist(s) will continue as new chemokines are discovered and become available.

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