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Cloning and Characterization of a Novel Class I Cytokine Receptor

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The human gp130 cDNA sequence was used as a query to search an expressed sequence tag database (dbEST) to identify cDNA sequences with similarity to the cytokine class I receptor family. A novel class I cytokine receptor was identified in a human infant brain cDNA library and was named WSX-1. Full-length cDNA sequences for human and murine WSX-1 were isolated and characterized. The WSX-1 cDNA encodes a 636 amino acid transmembrane protein with an extracellular domain of 482 amino acids and a cytoplasmic domain of 96 amino acids. The structure of the WSX-1 protein most closely resembles that of gp130. Northern blot analysis indicates high levels of expression in thymus, spleen, lymph node, and peripheral blood leukocytes, suggesting a role for WSX-1 in modulation of the immune response.

Cytokines regulate the growth and differentiation of cells in the hematopoietic and immune systems. These growth factors are involved in a wide range of physiological processes, including hematopoiesis, cell differentiation, and the initiation, maintenance and modulation of host defense mechanisms (1). Identifying novel members of the cytokine family and their receptors is of interest because of the vital role cytokines play in regulating biological responses.

Although many cytokines of the hematopoietic and immune systems adopt a highly conserved four-helix bundle tertiary structure, they exhibit a very low level of primary amino acid sequence identity (2). Due to this low degree of primary sequence similarity, identification of novel cytokines by homology-based cloning methods has proven to be very difficult. An alternative approach to cloning novel cytokines is to first clone orphan cytokine receptors, which are much more easily identified by primary amino acid sequence, followed by use of the cloned receptor for identification of the corresponding ligand.

Cytokine signals of the immune and hematopoietic systems are mediated through specific receptor complexes, the majority of whose components are members of the class I cytokine receptor family. This class of receptors includes the receptors for Interleukin 2 (IL)-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-12, IL-13, IL-15, Erythropoietin (EPO), Granulocyte colony-stimulating factor (G-CSF), Granulocyte-macrophage colony-stimulating factor (GM-CSF), Leukemia inhibitory factor (LIF), Oncostatin M (OM), Cardiotrophin-I (CT-1), Ciliary neurotrophic factor (CNTF), Thrombopoietin (TPO), and Leptin. Members of this family of receptors are readily identified by a conserved cytokine binding domain of 200 amino acid residues in the extracellular portion of the receptor. This cytokine binding domain is comprised of two fibronectin type III domains of approximately 100 amino acids each, the N-terminal domain containing conserved cysteine residues while the highly conserved Trp-Ser-X-Trp-Ser (WSXWS) sequence motif is present in the C-terminal domain (3-5). This 200 amino acid cytokine binding domain is duplicated in some of the receptors, while other receptors have added additional structural domains to their extracellular region, such as classical fibronectin type III repeats or immunoglobulin-like (Ig) domains (4).

Many of the class I cytokine receptors are heterodimers composed of at least two distinct receptor chains, the α and β subunits. The α subunits are primary cytokine binding proteins, and the β subunits are required for formation of high affinity binding sites as well as for signal transduction. The signal transducing subunit is often common to multiple cytokines and the cytokine receptors can be divided into functional subfamilies based on the formation of heterodimers with common signal transducers. The IL-3β common subunit (IL-3βc) acts as the signaling chain for the IL-3, IL-5, and GM-CSF receptor subfamily (6), the IL-2γ receptor

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serves as the affinity converter for the IL-2, IL-4, IL-7, IL-9, IL-15 receptor subfamily (7), and gp130 is shared by the receptors for IL-6, IL-11, CT-1, CNTF, LIF, and OM (7,8).

The conserved structural elements of the class I cytokine receptor family make it possible to clone new members of this family on the basis of primary amino acid homology (9,10). This present study describes a new homology-based method for cloning receptors of the class I cytokine family. The gp130 sequence was used as a query to search for homologous sequences in the public database of EST cDNA sequences. This resulted in the identification, isolation, and subsequent characterization of a novel WSXWS type I cytokine receptor cDNA from human fetal brain. The present study describes the primary amino acid sequence, domain structure, tissue distribution, and chromosomal localization for this novel receptor which we have named WSX-1.

MATERIALS AND METHODS

Query. The publicly available partial cDNA sequence database dbEST was searched with a modified version of the tFASTA program (11) using ktup=1. Scores were sorted by optimized score. Human gp130 (12) was used as the query sequence and resulted in the identification of EST75457 as having significant similarity to the query sequence.

Isolation of the human WSX-1 cDNA. Marathon-ready human fetal brain cDNA (ClonTech Laboratories) was amplified in 5′ and 3′ rapid amplification of cDNA ends (RACE) (13) reactions using primers designed to EST73457 in conjunction with specific AP1 and AP2 primers (ClonTech Laboratories). The 5′ end of the cDNA was generated in a RACE PCR reaction with an antisense EST73457 specific primer 5′GGTTCCCTGATACCCACACAT3′ and the AP1 primer. The resulting 780 bp product was then reamplified with "nested" internal primers to this PCR product, 5′GGGTCAGGGGTATGGTCTTCA3′ and the AP2 primer. The 3′ end of the cDNA was generated in a RACE PCR reaction with a sense EST73457 primer 5′ACCCCTGACCCCTGTTGAGAT3′ and the AP1 primer. The resulting 1440 bp product was then reamplified with "nested" internal primers to this PCR sequence, 5′GCCTTCTGCTCCAAAAGATGT3′ and the AP2 primer. The 5′ and 3′ RACE products extended the cDNA sequence and together created a full-length PCR derived sequence. The 5′ RACE product was random-primed and labeled with ^{32}P, and used to screen by hybridization a cDNA library made from the K562 (ATCC CCL 243) cell line, described below. Full-length cDNA clones were isolated from this library by hybridization screening at high stringency and the DNA was sequenced. These clones were found to be identical to the sequence generated by PCR from human fetal brain with one exception; the K562 cDNA encoded an additional 58 amino acids in the cytoplasmic domain.

Construction of a K562 library. Total RNA was isolated from K562 cells using guanidine isothiocyanate followed by CsCl centrifugation (14). Poly (A)$^+$ RNA was selected using an OLIGOTEX-dT-mRNA isolation kit (Qiagen). First-strand cDNA was synthesized from 14 μg of poly (A)$^+$ RNA using a primer encoding a *Xho*I site, 5′GTCGGTGCTCAGCATTCACTACTCGAGGGTTTTTTTTTTTTTTTT3′. Second-strand cDNA was synthesized (15), *Eco*RI adapters were ligated to the cDNA, and the cDNA was digested with *Xho*I to generate *Eco*RI and *Xho*I cohesive ends. A lambda phage library was prepared by ligating the *Eco*RI-*Xho*I cDNA into Lambda ZAP II phage arms (Stratagene Cloning Systems).

Isolation of the murine WSX-1 cDNA. Oligos 5′CCATACCCCTGACCCCTGTTGAGAT3′ and 5′CAGAGGTTCCCTGATACCCACACAT3′ were designed to predicted conserved regions of the human WSX-1 cDNA sequence and used to screen by PCR a murine spleen cDNA library. To construct the library, cDNA from murine spleen (prepared essentially as for the K562 library) was cloned into the mammalian expression vector pHZ-1. The pHZ-1 vector contains the murine metallothionein-1 promoter and a cloning cassette flanked by the bacteriophage T7 promoter and both human growth hormone and bacteriophage T7 terminators. Additional elements of pHZ-1 include an *E. coli* origin of replication, a bacterial beta-lactamase gene, and a mammalian selection unit comprising the SV40 promoter and origin, a neomycin resistance gene, and the SV40 transcription terminator. The library was transformed into *E. coli* DH10b cells, divided into 29 pools of 2500 clones each, and each pool examined by PCR. Annealing conditions for PCR were optimized for cross-species hybridization between human primers and murine cDNA. PCR conditions were denaturation for 30 sec at 94°C, annealing for 30 sec at 55°C, and extension at 72°C for 1 minute. Two positive pools were identified and one pool was further divided and screened by PCR until a single colony containing murine WSX-1 cDNA was identified. The cDNA was sequenced and determined to encode murine WSX-1, but lacked codons corresponding to the N-terminal five amino acids of human WSX-1. A full-length murine cDNA was then isolated from a cDNA library made from Ba/F3 cells (16). Pools from the library were used to transform *E. Coli* DH10b cells and were screened by PCR using primers designed to the murine cDNA sequence 5′ATTCCCCGGGAACAGTTCACC3′ and 5′GACGGTCCCCGATACCCACAC3′. Screening 51 pools of 10,000 colonies each resulted in two positive pools. Plasmid DNA from the positive pools was used to transform *E.coli* and the resulting colonies screened by hybridization using a ^{32}P random-primed probe generated from the PCR product. Two positive clones were sequenced and both were found to encode full-length murine WSX-1 cDNA.

Northern analysis. Human Multiple Tissue Northern Blots (Human I, Human II and Human III from ClonTech Laboratories) were probed with a 160 bp PCR product generated from human placenta cDNA with oligos to human WSX-1, 5′ACCCCTGACCCCTGTTGAGAT3′ and 5′GGTTCCCTGATACCCACACAT3′. The human placenta cDNA was provided as a control in a Marathon cDNA Amplification Kit (ClonTech Laboratories). The blots were hybridized overnight in ExpressHyb (ClonTech Laboratories) with a ^{32}P random-primed probe and washed at 50°C in 0.1 × SSC, .1%SDS, followed by exposure to X-ray film.

Total RNA was isolated from cell sorted human peripheral blood cells, CD4+, CD8+ and CD19+ (CellPro Incorporated), and from a 7-day human mixed-leukocyte reaction (MLR) using guanidine isothiocynate followed by CsCl centrifugation. Poly (A)$^+$ RNA was selected from total RNA using oligo d(T) cellulose chromatography (17). Two μg of poly (A)$^+$ RNA was separated by electrophoresis in a 1.5% agarose formaldehyde gel and transferred to Hybond N+ (Amersham) nylon filters. The Northern blot was hybridized and washed in a similar manner to the Human Multiple Tissue Northern Blots above, using a ^{32}P random-primed 450 bp fragment generated from human WSX-1 cDNA by PCR with oligos 5′CGGAATTCGGCCATTCCTCGGGAACAGC3′ and 5′GGTTCCCTGATACCCACACAT3′.

Chromosomal localization. The Human/Rodent Somatic Cell Hybrid Panel 2 (National Institute of General Medical Sciences, Coriell Institute for Medical Research, Camden, NJ) was used to identify the somatic hybrid cell lines that contained the human WSX-1 gene. PCR amplification was performed using 100 ng of DNA isolated from each somatic hybrid with WSX-1 specific primers, 5′CCATACCCCTGACCCCTGTTGAGAT3′ and 5′CAGAGGTTCCCTGATACCCACACAT3′. PCR conditions were as follows; an initial 5 min denaturation at 95°C, followed by 35 cycles of 30 sec at 95°C, 2 min at 65°C and 30 sec at 72°C, followed by an extension of 7 min at 72°C. Subchromosomal mapping was performed with the commercially available version of the Stanford G3 Radiation Hybrid Mapping

Panel (Research Genetics, Inc.). PCR amplification was performed using 25 ng of DNA from each hybrid cell line and the same primers used above. PCR conditions were as follows; an initial 5 min denaturation at 95°C, followed by 35 cycles of 1 min at 95°C, 1 min at 65°C and 1.5 min at 72°C, followed by an extension of 7 min at 72°C. Linkage analysis and subsequent chromosomal localization were obtained through use of publicly available servers (Stanford Human Genome Center, Stanford University School of Medicine, Palo Alto, CA and LDB, The Genetic Location Database, University of Southhampton, UK).

RESULTS

Identification and Isolation of a Full-Length Clone for WSX-1

The human gp130 cDNA sequence was used as a query to search the expressed sequence tag database (dbEST) to identify ESTs with homology to the cytokine class I receptor family. EST73457 from human infant brain demonstrated significant similarity to gp130 and contained the five amino acid sequence Trp-Gly-Glu-Trp-Ser (WGEWS) which fits the WSXWS motif and is a hallmark of the hematopoietic class I cytokine receptor family. PCR primers were designed to EST73457 and rapid amplification of cDNA ends (RACE) was used to extend the cDNA sequence using human fetal brain cDNA as a template. The resulting PCR product was used as a hybridization probe to obtain a full-length clone from a cDNA library of the human erythroleukemia K562 cell line. The cDNA clones isolated from K562 cells were found to be identical to the sequence generated by PCR from human fetal brain with one exception; the cytoplasmic domain of the cDNA from K562 cells encoded an additional 58 amino acids (residues 567-624, Fig. 1). This difference in the cytoplasmic domain most likely represents an isoform of WSX-1 that is the result of an alternate splicing event.

The human WSX-1 cDNA contains an open reading frame of 1908 nucleotides which encodes a protein of 636 amino acids in length (Fig. 1). The predicted primary sequence includes a potential hydrophobic signal peptide, followed by a classical cytokine binding domain containing two conserved cysteine residues and a WSXWS motif, then a region consisting of three fibronectin type III domain repeats, a 26 amino acid transmembrane domain and a 96 amino acid cytoplasmic domain. In addition to the WSXWS motif and the two conserved cysteines of the cytokine binding domain, there are other conserved residues of this domain that are believed to serve a structural role in class I cytokine receptors; a proline at residue 131, a tryptophan at residue 151, and a tyrosine at residue 202 (4). Examination of the cytoplasmic domain of WSX-1 shows a membrane-proximal box 1 motif (Apolar-X-X-X-Aliphatic-Pro-X-Pro) (residues 554-561) which is believed to be important for association with Janus kinases (18).

Since Northern blots indicated spleen as an abundant source of WSX-1 mRNA, murine spleen cDNA was chosen as a source for isolation of the murine ortholog of WSX-1. PCR primers designed to conserved regions of the human cDNA sequence were used with PCR to identify clones from a murine spleen cDNA library. The PCR reactions used a low stringency annealing temperature of 55°C to allow for cross-species hybridization between the human primers and the murine cDNA. When the spleen cDNA clones were sequenced they were identified as the murine version of WSX-1, but lacked codons corresponding to the N-terminal five amino acids of human WSX-1. The resulting spleen cDNA sequence was then used to design specific primers to the murine cDNA, which were subsequently used with PCR to isolate full-length cDNA clones from a library made from the murine pro-B cell line Ba/F3.

The human WSX-1 receptor has 63% sequence identity at the amino acid level to murine WSX-1 (Fig. 2). There are seven potential N-linked glycosylation sites (Asn-X-Ser/Thr) for human WSX-1, five of these are conserved in the murine WSX-1 sequence.

Tissue Distribution of WSX-1

Northern blot analysis was used to determine the tissue distribution of WSX-1. A transcript of 3.5 kilobases (kb) was identified in all tissues examined, except placenta, where a much smaller transcript of 1.0 kb was observed (Fig. 3). The smaller transcript could represent a soluble form of the WSX-1 receptor, however it appears to be too small to encode the entire extracellular domain of WSX-1. Probing of a similar but different Northern blot showed no evidence of this smaller transcript in placenta (data not shown), indicating that expression of this 1.0 kb transcript is highly variable. Although WSX-1 mRNA appears to be present at low levels in all tissues probed, it is expressed at much higher levels in lymphoid tissues, specifically thymus, spleen, lymph node and peripheral blood leukocytes. In addition to lymphoid tissues, mRNA expression of WSX-1 was found in various immunocompetent primary cells and cell lines. To further examine the role of WSX-1 in the lymphoid system, we analyzed expression of WSX-1 in T cells versus B cells. RNA levels were determined by Northern blot for human primary peripheral blood cells sorted for CD4+ T cell subsets, CD8+ T cell subsets, or for CD19+ B cells (Fig. 4). CD4+ sorted-cells showed a much higher level of expression than CD8+ T cells, suggesting a pos-

FIG. 1. Nucleotide sequence and predicted amino acid sequence of the cDNA for human WSX-1. The signal peptide is underlined, potential N-linked glycosylation sites appear in bold and underscored with stars, the hallmark WSXWS is underscored with plus signs, the putative transmembrane region is doubly underlined, and the cytoplasmic Box 1 is underscored with asterisks.

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ACGAGGCGGAGGCGGCCTGCCGGGGTGGTTCGGCTTCCCGTTGCCGCCTCGGGCGCTGTACCCAGAGCTCGAAGAGGAGCAGCGCGGCCG

CGCGGACCCGGCAAGGCTGGGCCGGACTCGGGGCTCCCGAGGGACGCCATGCGGGGAGGCAGGGGCGCCCCTTTCTGGCTGTGGCCGCTG
                                                  M  R  G  G  R  G  A  P  F  W  L  W  P  L
                                                                                           14

CCCAAGCTGGCGCTGCTGCCTCTGTTGTGGGTGCTTTTCCAGCGGACGCGTCCCCAGGGCAGCGCCGGGCCACTGCAGTGCTACGGAGTT
P  K  L  A  L  L  P  L  L  W  V  L  F  Q  R  T  R  P    Q  G  S  A  G  P  L  Q  C  Y  G  V
                                                                                           44

GGACCCTTGGGCGACTTGAACTGCTCGTGGGAGCCTCTTGGGGACCTGGGAGCCCCCTCCGAGTTACACCTCCAGAGCCAAAAGTACCGT
G  P  L  G  D  L    N  C  S  W  E  P  L  G  D  L  G  A  P  S  E  L  H  L  Q  S  Q  K  Y  R
                     *                                                                     74

TCCAACAAAACCCAGACTGTGGCAGTGGCAGCCGGACGGAGCTGGGTGGCCATTCCTCGGGAACAGCTCACCATGTCTGACAAACTCCTT
S    N  K  T  Q  T  V  A  V  A  A  G  R  S  W  V  A  I  P  R  E  Q  L  T  M  S  D  K  L  L
     *                                                                                    104

GTCTGGGGCACTAAGGCAGGCCAGCCTCTCTGGCCCCCCGTCTTCGTGAACCTAGAAACCCAAATGAAGCCAAACGCCCCCCGGCTGGGC
V  W  G  T  K  A  G  Q  P  L  W  P  P  V  F  V  N  L  E  T  Q  M  K  P  N  A  P  R  L  G
                                                                                          134

CCTGACGTGGACTTTTCCGAGGATGACCCCCTGGAGGCCACTGTCCATTGGGCCCCACCTACATGGCCATCTCATAAAGTTCTGATCTGC
P  D  V  D  F  S  E  D  D  P  L  E  A  T  V  H  W  A  P  P  T  W  P  S  H  K  V  L  I  C
                                                                                          164

CAGTTCCACTACCGAAGATGTCAGGAGGCGGCCTGGACCCTGCTGGAACCGGAGCTGAAGACCATACCCCTGACCCCTGTTGAGATCCAA
Q  F  H  Y  R  R  C  Q  E  A  A  W  T  L  L  E  P  E  L  K  T  I  P  L  T  P  V  E  I  Q
                                                                                          194

GATTTGGAGCTAGCCACTGGCTACAAAGTGTATGGCCGCTGCCGGATGGAGAAAGAAGAGGATTTGTGGGGCGAGTGGAGCCCCATTTTG
D  L  E  L  A  T  G  Y  K  V  Y  G  R  C  R  M  E  K  E  E  D  L  W  G  E  W  S  P  I  L
                                                                  +++++++++++++++     224

TCCTTCCAGACACCGCCTTCTGCTCCAAAAGATGTGTGGGTATCAGGGAACCTCTGTGGGACGCCTGGAGGAGAGGAACCTTTGCTTCTA
S  F  Q  T  P  P  S  A  P  K  D  V  W  V  S  G  N  L  C  G  T  P  G  G  E  E  P  L  L  L
                                                                                          254

TGGAAGGCCCCAGGGCCCTGTGTGCAGGTGAGCTACAAAGTCTGGTTCTGGGTTGGAGGTCGTGAGCTGAGTCCAGAAGGAATTACCTGC
W  K  A  P  G  P  C  V  Q  V  S  Y  K  V  W  F  W  V  G  G  R  E  L  S  P  E  G  I  T  C
                                                                                          284

TGCTGCTCCCTAATTCCCAGTGGGGCGGAGTGGGCCAGGGTGTCCGCTGTCAACGCCACAAGCTGGGAGCCTCTCACCAACCTCTCTTTG
C  C  S  L  I  P  S  G  A  E  W  A  R  V  S  A  V    N  A  T  S  W  E  P  L  T    N  L  S  L
                                                     *                       *            314

GTCTGCTTGGATTCAGCCTCTGCCCCCCGTAGCGTGGCAGTCAGCAGCATCGCTGGGAGCACGGAGCTACTGGTGACCTGGCAACCGGGG
V  C  L  D  S  A  S  A  P  R  S  V  A  V  S  S  I  A  G  S  T  E  L  L  V  T  W  Q  P  G
                                                                                          344

CCTGGGGAACCACTGGAGCATGTAGTGGACTGGGCTCGAGATGGGGACCCCCTGGAGAAACTCAACTGGGTCCGGCTTCCCCCTGGGAAC
P  G  E  P  L  E  H  V  V  D  W  A  R  D  G  D  P  L  E  K  L  N  W  V  R  L  P  P  G    N
                                                                                          *
                                                                                          374

CTCAGTGCTCTGTTACCAGGGAATTTCACTGTCGGGGTCCCCTATCGAATCACTGTGACCGCAGTCTCTGCTTCAGGCTTGGCCTCTGCA
L  S  A  L  L  P  G    N  F  T  V  G  V  P  Y  R  I  T  V  T  A  V  S  A  S  G  L  A  S  A
                       *                                                                  404

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S  S  V  W  G  F  R  E  E  L  A  P  L  V  G  P  T  L  W  R  L  Q  D  A  P  P  G  T  P  A
                                                                                          434

ATAGCGTGGGGAGAGGTCCCAAGGCACCAGCTTCGAGGCCACCTCACCCACTACACCTTGTGTGCACAGAGTGGAACCAGCCCCTCCGTC
I  A  W  G  E  V  P  R  H  Q  L  R  G  H  L  T  H  Y  T  L  C  A  Q  S  G  T  S  P  S  V
                                                                                          464

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C  M    N  V  S  G  N  T  Q  S  V  T  L  P  D  L  P  W  G  P  C  E  L  W  V  T  A  S  T  I
          *                                                                               494

GCTGGACAGGGCCCTCCTGGTCCCATCCTCCGGCTTCATCTACCAGATAACACCCTGAGGTGGAAAGTTCTGCCAGGCATCCTATTCTTG
A  G  Q  G  P  P  G  P  I  L  R  L  H  L  P  D  N  T  L  R  W  K  V  L  P  G  I  L  F  L
                                                            ============================524

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W  G  L  F  L  L  G  C  G  L  S  L  A  T  S  G  R  C  Y  H  L  R  H  K  V  L  P  R  W  V
=================================================                                         *
                                                                                          554

TGGGAGAAAGTTCCTGATCCTGCCAACAGCAGTTCAGGCCAGCCCCACATGGAGCAAGTACCTGAGGCCCAGCCCCTTGGGGACTTGCCC
W  E  K  V  P  D  P  A  N  S  S  S  G  Q  P  H  M  E  Q  V  P  E  A  Q  P  L  G  D  L  P
********************                                                                  584

ATCCTGGAAGTGGAGGAGATGGAGCCCCCGCCGGTTATGGAGTCCTCCCAGCCCGCCCAGGCCACCGCCCCGCTTGACTCTGGGTATGAG
I  L  E  V  E  E  M  E  P  P  P  V  M  E  S  S  Q  P  A  Q  A  T  A  P  L  D  S  G    Y  E
                                                                                          614

AAGCACTTCCTGCCCACACCTGAGGAGCTGGGCCTTCTGGGGCCCCCCAGGCCACAGGTTCTGGCCTGAACCACACGTCTGGCTGGGGGC
K  H  F  L  P  T  P  E  E  L  G  L  L  G  P  P  R  P  Q  V  L  A  .
                                                                636

TGCCAGCCAGGCTAGAGGGATGCTCATGCAGGTTGCACCCCAGTCCTGGATTAGCCCTCTTGATGGATGAAGACACTGAGGACTCAGAGA
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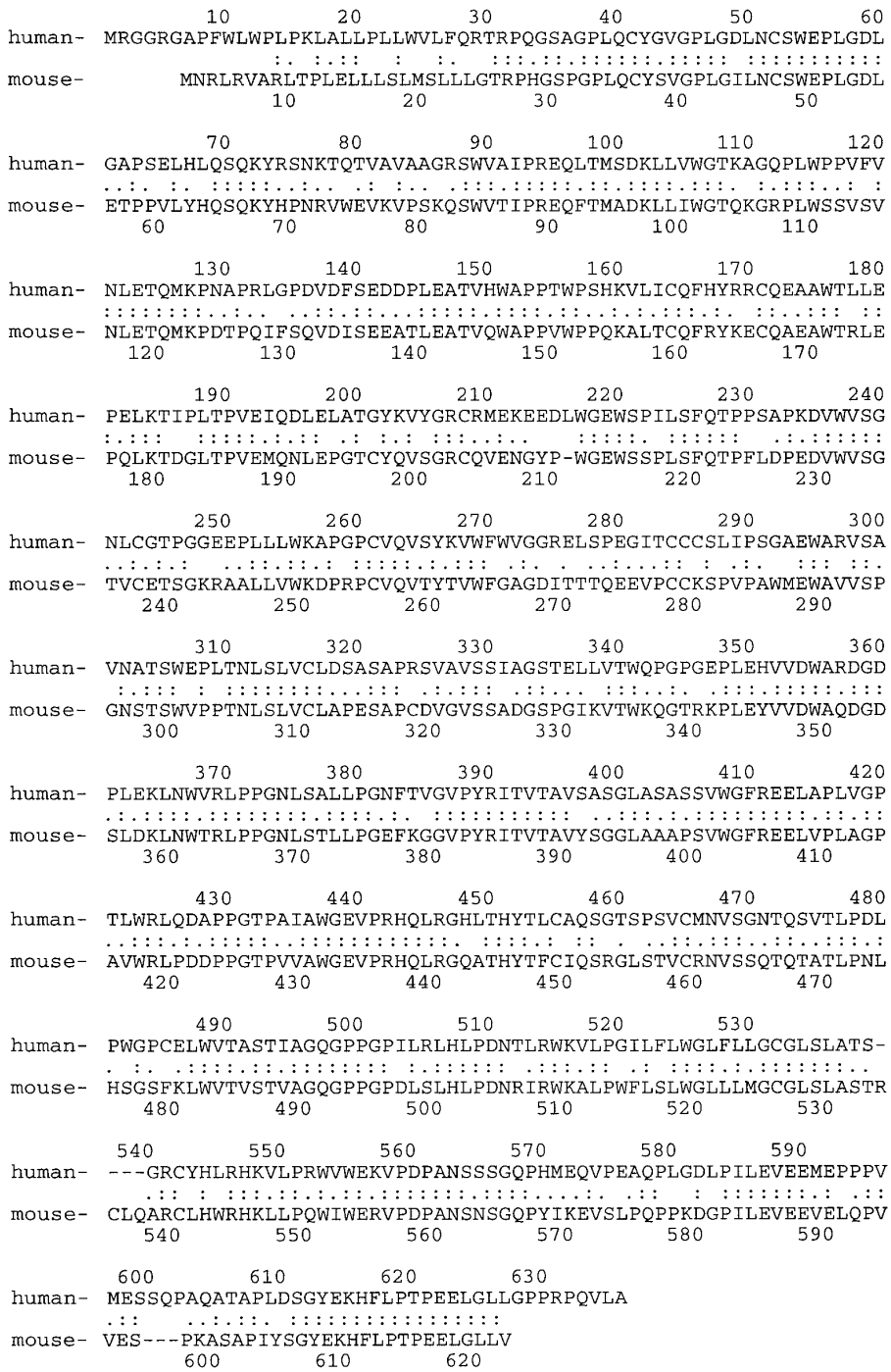

FIG. 2. Alignment of the amino acid sequence of human and murine WSX-1. The L-FASTA program was used to construct the alignment. Identical amino acids between the human and murine sequences are shown with double dots, conserved residues are marked with single dots. The sequence data have been deposited with GenBank data bases under accession number AF053004 for human WSX-1 and AF053005 for murine WSX-1.

sible role for the receptor in T cell helper function versus T cell suppressor function. WSX-1 also appeared to be expressed in CD19+ B cells, although at much lower levels than CD4+ T cells, indicating a possible role in B cell function. The CD19+ B cell population was less than 80% pure and may have been contaminated with T cells as well as other cell types, so low levels of expression seen here could be attributed to contaminating cell types and not B cells. An even higher level of expression was seen for a 7-day human allogeneic mixed-leukocyte

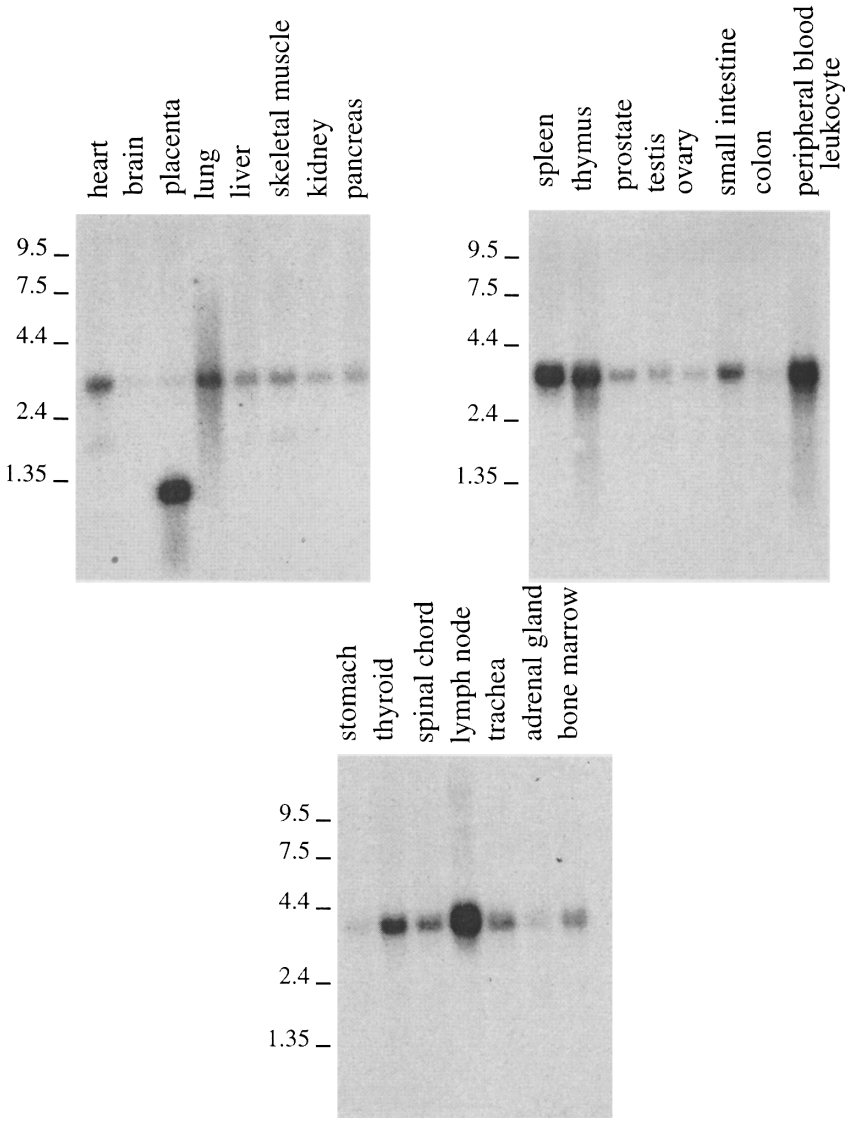

FIG. 3. Northern blot hybridization of a human WSX-1 probe with poly $(A)^+$ RNA from human tissues. Two ug of poly $(A)^+$ RNA is loaded in each lane. Mobilities for the DNA molecular weight markers are indicated in kilobases.

reaction (MLR), indicating that WSX-1 is expressed in activated peripheral blood mononuclear cells (PBMC). A high percentage of activated PBMC are T cells, further suggesting expression of WSX-1 on T cells (Fig. 4).

Chromosomal Localization

The WSX-1 gene was mapped to human chromosome 19 using PCR on the National Institute of General Medical Sciences Human/Rodent Somatic Cell Hybrid Panel 2. Subchromosomal mapping of the WSX-1 gene was achieved using linkage results obtained from the commercially available version of the Stanford G3 radiation hybrid panel and was positioned in the 19p13.11 region on the integrated LDB chromosome map 19 (data not shown).

DISCUSSION

Many cytokines have been identified by their activity on hematopoietic progenitor cells or their biological action on lymphoid cells. In turn, most of the cytokine receptors have been functionally identified by their ability to bind to previously identified cytokines (9). A homology-based cloning method would allow for cloning of receptors whose ligands have not yet been identified. In addition, identification of a cytokine receptor and its tissue distribution can help to elucidate a biological role for the ligand, since the receptor localization identifies the site of action of the ligand.

One method has been reported for cDNA cloning of novel class I cytokine receptors where oligonucleotides

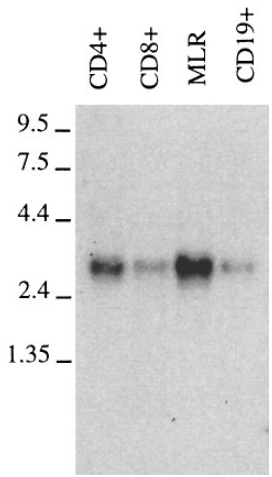

FIG. 4. Northern blot hybridization of a human WSX-1 probe with poly $(A)^+$ RNA from human PBMC CD4+, CD8+ or CD19+ sorted cells, or a mixed-leukocyte reaction (MLR). Two ug of poly $(A)^+$ RNA is loaded in each lane. Mobilities for the DNA molecular weight markers are indicated in kilobases.

designed to the WSXWS motif were used as hybridization probes. This resulted in identification of a novel cDNA for the IL-11 α receptor (9). Another recent report describes cloning of the Oncostatin M receptor β (OSMRβ) from genomic DNA by performing PCR with degenerate oligonucleotide primers designed to conserved regions of class I cytokine receptors (10). We have extended this technology to searching EST databases using conserved cytokine receptor motifs. In this manner, numerous cDNA sequences can be searched in a short period of time, providing a substitute for labor intensive hybridization techniques and extensive DNA sequencing. Database mining can be extended to libraries of EST sequences derived from rare cell types or subtracted cDNA sources, allowing for the cloning of rare cytokine receptor cDNAs and the subsequent identification of their ligands. In this study, database mining of dbEST has resulted in the cloning of a novel class I cytokine receptor, WSX-1, from a human infant brain library.

The full-length cDNA sequence for WSX-1 encodes a cytokine receptor with an extracellular domain consisting of the conserved 200 amino acid WSXWS-containing cytokine binding domain and an additional three fibronectin type III domains. When compared to other members of the class I cytokine receptor family, WSX-1 shows the strongest structural similarity to members of the gp130 subfamily, specifically gp130 (12), LIF receptor (19), G-CSF receptor (20), and the IL-12β1 (21) and IL-12β2 receptors (22) (Fig. 5). WSX-1 as well as all of these receptors of the gp130 subfamily are distinguished from other class I cytokine receptors by having an extracellular domain consisting of three fibronectin domains in addition to the cytokine binding domain. Of the previously identified class I cytokine receptors, WSX-1 demonstrates the strongest similarity to gp130 with a 19% amino acid identity over 551 residues.

WSX-1 as a Signaling Receptor Subunit

For many class I cytokine receptor systems, two membrane-bound receptor chains are required for high affinity binding of the ligand and for signal transduction; a ligand-specific α subunit and a common β signal transducing subunit (5, 23). From examination of the structure of WSX-1 it is not readily apparent whether this is a ligand-binding α receptor subunit or a signal-transducing β subunit. One structural requirement for a signal transducing chain would be a moderately long cytoplasmic domain, since the α receptor subunits of this family tend to have very short cytoplasmic domains and the signaling β receptors encode much longer cytoplasmic regions. For example, the α chains for the IL-11 receptor and the IL-6 receptor have cytoplasmic domains of 39 and 75 amino acids in length, respectively (9, 24), while the CNTF α receptor is attached to the cell membrane via a glycosylphosphoinositol linkage and has no cytoplasmic domain at all (25). For the β signaling receptor subunits, the cytoplasmic domain ranges from 277 amino acids for gp130 and 239 amino acids for the LIF receptor (12, 19), to only 120 amino acids for mpl (TPO receptor) (26) and 187 residues for the G-CSF receptor (20). The cytoplasmic domain of WSX-1 was found to be 96 amino acids in length, which could be of sufficient length to contain the necessary information to transduce a signal.

An additional requirement for a signal transducing β subunit is the presence of conserved tyrosine residues in the cytoplasmic domain. Although type I cytokine receptor subunits do not possess intrinsic tyrosine kinase activity, phosphorylation of tyrosine residues follows activation of the receptor (7). Examination of the WSX-1 cytoplasmic domain reveals that human WSX-1 has two tyrosine residues present in the cytoplasmic domain which could be phosphorylated; for murine WSX-1 there are three tyrosines residues with one of these conserved between the human and murine sequences.

One other criteria for assessing signal transduction would be the presence of Box 1 and Box 2 domains in the cytoplasmic region. For the class I cytokine receptors, signal transduction is a result of ligand binding which initiates multimerization of receptor subunits and activation of receptor-associated tyrosine kinases, in particular the Janus kinases. While the cytoplasmic domains of the class I cytokine receptors do not appear to contain any known signal transduction motifs, there exist two membrane-proximal elements in the cytoplasmic domain termed Box 1 and Box 2, that appear to be conserved among members of the cytokine receptor superfamily. The Box 1 motif (Apolar-X-X-X-Aliphatic-

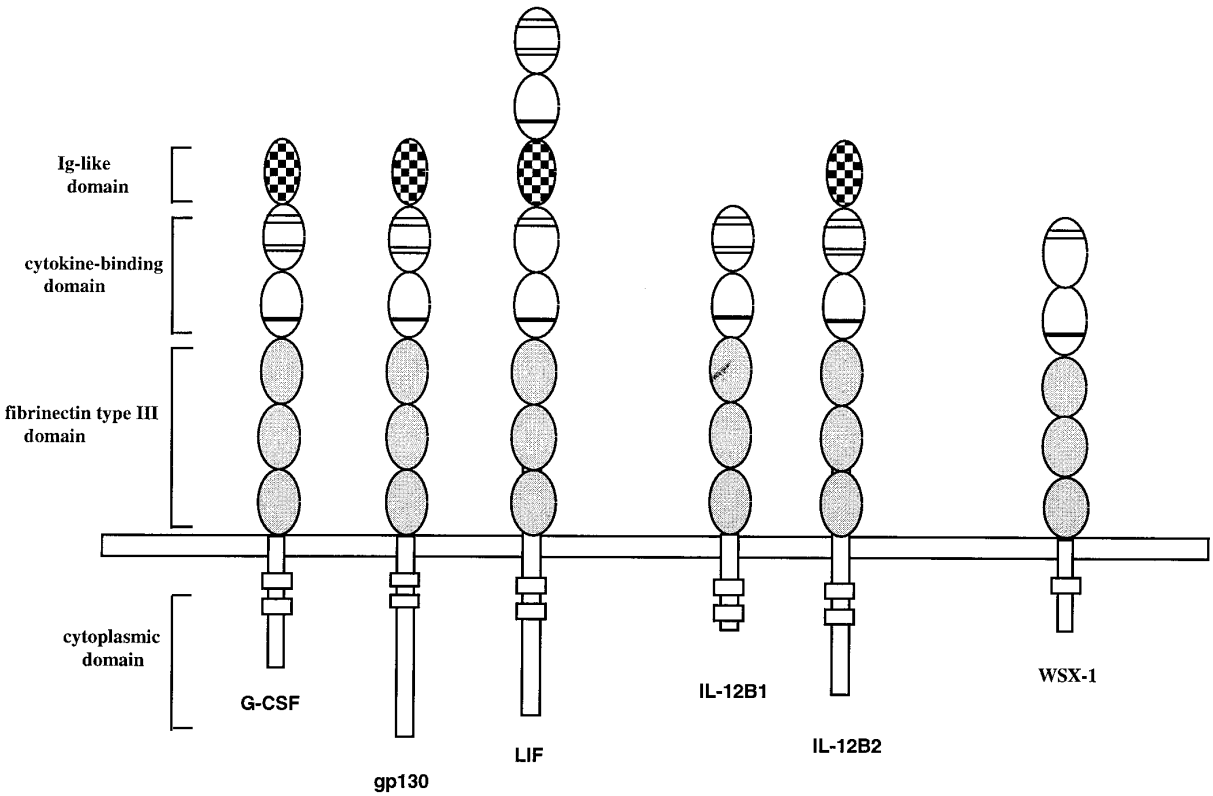

FIG. 5. Schematic representation of receptors of the gp130 subfamily. Ig-like domains are checkered, cytokine binding domains are open circles, and fibronectin type III repeats are shaded circles. Within the cytokine binding domain, paired cysteine residues are depicted with double lines while the WSXWS motif is shown by a heavy single line. The Box 1 and Box 2 regions in the cytoplasmic domain are designated by horizontal open rectangles.

Pro-X-Pro) is believed to be important for association with Janus kinases. The Box 2 motif lacks a true consensus sequence, although proline is frequently followed by acidic residues (18, 27). The cytoplasmic domain of WSX-1 contains a membrane-proximal Box 1 motif, but no obvious Box 2 motif appears to be present. The minimal segment of the cytoplasmic domain believed to be essential for supporting signaling and cell proliferation includes both the Box 1 and Box 2 regions, suggesting that WSX-1 may not be independently capable of signal transduction.

Functional Complex for WSX-1

From the structure of WSX-1, it is difficult to determine whether this is a ligand-binding α receptor subunit or a signal transducing β subunit. If a β subunit is necessary for WSX-1 to form a high affinity signaling receptor, then receptors of the gp130 subfamily would be a likely choice for a signaling partner since WSX-1 demonstrates a strong homology to this subfamily. For many members of the gp130 subfamily, gp130 acts as a common signal transducer (27, 28). As a result of ligand binding to the α receptor chain, signaling is triggered by the formation of homodimers of gp130 or heterodimers between gp130 and LIF receptor. In the case of IL-6 and IL-11, the ligand specific α receptors plus their ligands induce gp130 homodimers (29). For CT-1, CNTF, LIF and OM, heterodimers between gp130 and LIF receptor are involved in forming a high-affinity signaling receptor complex. This would suggest that either gp130 or gp130 plus the LIF receptor could form a high-affinity complex with WSX-1 and act as signal transducers.

Although the gp130 subfamily has the strongest structural homology to WSX-1, examination of the WSX-1 extracellular domain reveals that it is lacking an Ig-like domain that is present in the other receptors of this subfamily (Fig. 5). The only other cytokine receptor with this structure of three fibronectin type III domains, but no Ig domain, is the IL-12β1 receptor (21). High affinity binding of the IL-12 ligand to its receptor complex requires heterodimerization of the IL-12β1 receptor with the IL-12β2 receptor (22). Since a signal transducer chain can often be utilized by multiple cytokines, it is possible that the IL-12β2 receptor could serve as a signaling partner for WSX-1 as well as for IL-12 β1 receptor.

Possible Function of Receptor/Ligand

Northern blot analysis of WSX-1 showed a high level of expression in lymphoid tissues, specifically thymus, spleen, lymph node and peripheral blood leukocytes. High levels of expression of this receptor in the thymus could indicate a role for WSX-1 in early thymocyte development, while localization to other lymphoid tissues may indicate a role for WSX-1 in regulation of the immune response. Expression of WSX-1 on activated PBMC of a human allogeneic MLR, as well as increased levels of expression for T cells versus B cells, further suggests a role for WSX-1 in cell-mediated immune responses (Fig 4).

Since WSX-1 is a novel cytokine class I receptor, it would be of considerable interest to identify the ligand for WSX-1 and define its role in the hematopoietic and immune systems. We have utilized a functional activity assay to screen known cytokines for their ability to stimulate the WSX-1 receptor (data not shown). Since the β receptor subunit for WSX-1 is unknown, activity of the WSX-1 receptor was assessed in two different receptor configurations. In one case, WSX-1 was co-expressed with gp130 where gp130 could serve as the β receptor signaling subunit. Alternatively, a chimeric receptor was designed where the extracellular domain of WSX-1 was fused to the cytoplasmic domain of the mpl receptor, thus relying on signaling through the mpl receptor. When numerous cytokines were tested for ability to stimulate these receptor complexes, IL-4 was the only cytokine which demonstrated any activity in these functional assays. Although these results are not conclusive, one interpretation would be that WSX-1 is part of a novel receptor complex for IL-4. We are currently conducting experiments to identify the ligand for WSX-1 and at the same time investigating IL-4 as a possible ligand for WSX-1.

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