Expression cloning of the murine and human interleukin 9 receptor cDNAs

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ABSTRACT   Interleukin 9 (IL-9) is a T-cell-derived lymphokine that induces the proliferation of various lymphoid and hemopoietic cells. A cDNA clone encoding the murine IL-9 receptor was isolated by expression cloning in COS cells and screening with ^{125}I-labeled IL-9. Transient expression of this cDNA produced high-affinity binding sites for IL-9. The predicted 52-kDa protein contains a putative signal peptide and a typical transmembrane domain. A cDNA for the human homologue was isolated by cross-hybridization. Transfection of this cDNA in a murine T-cell clone conferred responsiveness to human IL-9. Sequence analysis revealed that the IL-9 receptor belongs to the recently described hematopoietin receptor superfamily and is expressed in membrane-bound and soluble forms.

Interleukin 9 (IL-9) was originally identified as a growth factor for murine T helper cell clones (1, 2), murine mast cell lines (3, 4), and a human megakaryoblastic leukemia line (5). Subsequently, additional biological targets were discovered including murine fetal thymocytes (6), murine erythroid progenitors (7), human T-cell lines (8), and human erythroid and myeloid precursors (9, 10). Moreover, involvement of IL-9 in tumorigenesis has been recently suggested by the observations that a murine T helper clone becomes tumorigenic after transfection with the IL-9 cDNA (11) and that lymph nodes from patients with Hodgkin disease or large-cell anaplastic lymphoma express IL-9 constitutively (12).

Preliminary characterization of the IL-9 receptor on a murine T-cell clone has demonstrated the existence of saturable and specific binding sites with a K_d of ≈ 100 pM. Cross-linking analysis showed that the IL-9 receptor consists of a 64-kDa glycoprotein, the molecular mass of which is reduced to 54 kDa on treatment with N-glycosidase F (13).

We report here the expression cloning and sequence of a cDNA encoding the murine IL-9 receptor.[†] This cDNA was further used to identify a human homologue.[†]

MATERIALS AND METHODS

Construction of cDNA Libraries. Poly(A)$^+$ RNA extracted from the murine T-cell clone TS1 (1) was converted to double-stranded cDNA by the method of Gubler and Hoffman (14) with random hexanucleotide primers. EcoRI adaptors were attached and cDNA larger than 1.5 kilobases (kb) was isolated by fractionation on a 5–20% (wt/vol) potassium acetate gradient (15). The size-selected cDNA was inserted into the EcoRI site of the pCDSRα expression vector (16). Two additional cDNA libraries were generated in the BstXI site of the pCDM8 vector (15) with oligo(dT) or random primers. A human cDNA library was constructed by the same method in the pRC/RSV plasmid (Invitrogen, San Diego) with RNA from the megakaryoblastic leukemia cell line Mo7E (17).

Transient Expression and Binding Assays. Purified recombinant murine IL-9 was radiolabeled using the Bolton and Hunter reagent as described (18) to yield material with a specific activity of 2300 cpm/fmol. Screening of cDNA pools by direct expression in COS cells was performed essentially as described by Gearing et al. (19). The murine cDNA library was subfractionated into 100 pools (each containing ≈ 500 clones), and DNA from each pool was transfected by the DEAE-dextran/chloroquine method (15) into 1.5 × 10^5 COS cells seeded on glass microscope slides. After 48–72 h, the cells were incubated with 0.2 nM ^{125}I-labeled IL-9 for 3 h at 20°C, briefly washed, fixed, and dipped in liquid photographic emulsion (Kodak NTB2). After a 10-day exposure, the slides were developed and examined microscopically for the presence of autoradiographic grains. For subsequent screening and Scatchard analysis, binding assays were performed in culture dishes as described by Goodwin et al. (20).

Isolation of Full-Length Murine and Human cDNA Clones. Screening for additional murine IL-9 receptor cDNAs was performed by conventional methods (21) using the radiolabeled murine cDNA A1 as a probe. Human IL-9 receptor cDNA clones were obtained by hybridization with the same probe followed by washes under low-stringency conditions (2× standard saline citrate/0.1% SDS at 55°C).

Stable Expression and IL-9 Assay in TS1 Cells. TS1 T cells were transfected by the double-pulse technique using the Cellject electroporation system (Eurogentec, Belgium). TS1 cells (5 × 10^6) were resuspended at 37°C in 0.8 ml of Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol) fetal bovine serum, 50 mM 2-mercaptoethanol, 0.55 mM L-arginine, 0.24 mM L-asparagine, and 1.25 mM L-glutamine. Plasmid DNA (50 μg) was added to the cells in 0.4-cm cuvettes just before electroporation. After a double electric pulse (750 V/74 Ω/40 μF and 100 V/74 Ω/2100 μF), the cells were immediately diluted in fresh medium supplemented with mouse IL-9. After 24 h, the cells were washed and cultured in the presence of G418 (2.5 mg/ml, GIBCO) and mouse IL-9. Under these conditions, the frequency of transfection was ≈1/10,000. After G418 selection, transfected cells were maintained in human IL-9 and a TS1 proliferation assay was performed as described (1). Human recombinant IL-9 and mouse recombinant IL-9 were expressed in Spodoptera frugiperda cells using a baculovirus vector and purified as described (13). IL-9 units were defined on TS1 and on Mo7E cells for the murine and human proteins, respectively (1, 5).

RESULTS

Isolation of Murine IL-9 Receptor cDNAs. T-cell clone TS1 expressing ≈2000 high-affinity binding sites for IL-9 was

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Abbreviation: IL, interleukin.
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†The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M84746 for the murine IL-9 receptor and M84747 for the human IL-9 receptor).

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selected as a source of mRNA to construct a cDNA library in the expression vector pCDSRα. Plasmid DNA from pools of 500 clones was transfected into subconfluent monolayers of COS cells seeded on glass microscope slides. After 2 or 3 days, transfected cells were incubated with ^{125}I-labeled IL-9 and screened by autoradiography by the method of Gearing et al. (19). Two of 100 cDNA pools proved positive, respectively, for 1 and 33 cells out of the 1.5×10^5 transfected cells. The second pool was divided into 100 groups (each containing 15 clones) and finally into 100 single clones that were screened by conventional binding. This procedure led to the isolation of a cDNA clone, named p9RA1, containing a 1900-base-pair (bp) cDNA. To ensure that p9RA1 encoded the murine IL-9 receptor, binding studies were performed on transfected COS cells. Scatchard analysis demonstrated the presence of a single class of binding sites with a K_d value of 194 pM (Fig. 1), slightly higher than the dissociation constant measured on TS1 cells (67 pM).

RNA Blot Analysis and Isolation of Additional cDNAs. RNA blot analysis of TS1 cells with the 1.9-kb insert as a probe showed two major bands at about 4.5 and 2.5 kb and a minor band at 2 kb (Fig. 2). These transcripts were detected in another IL-9-responsive T-cell clone, TUC5.37, but not in other cell lines such as CTLL (an IL-2-dependent cytolytic T-cell line), MOPC104E (an IL-6-dependent plasmacytoma cell line), FDCP-1 (an IL-3-dependent myeloid cell line), and TUC13.1 (a T helper clone unresponsive to IL-9).

The p9RA1 cDNA was further used as a probe to obtain additional clones. Two oligo(dT)-primed cDNAs, p9RB1 and p9RB3 (1600 and 900 bp, respectively), and four random-primed clones, p9RC2, p9RC3, p9RC4, and p9RC9 (2000, 1000, 3000, and 2100 bp, respectively), were isolated and further analyzed. As was found for p9RA1, transient expression experiments performed with p9RC4, the largest cDNA, demonstrated high-affinity binding sites for IL-9 (K_d = 126 pM; data not shown) and RNA analysis showed the same three bands in RNA from IL-9-responsive cells.

Structure of the Murine IL-9 Receptor. The sequence of p9RC4, which turned out to be the most complete cDNA out of the seven clones sequenced, displayed a single open reading frame encoding a protein of 468 amino acids (Fig. 3). Two hydrophobic regions were predicted from the deduced amino acid sequence. The first at residues 15–40 probably corresponds to the signal peptide. Based on the probability weight matrix described by von Heijne (22), the potential cleavage site is assigned between positions 37 and 38. The
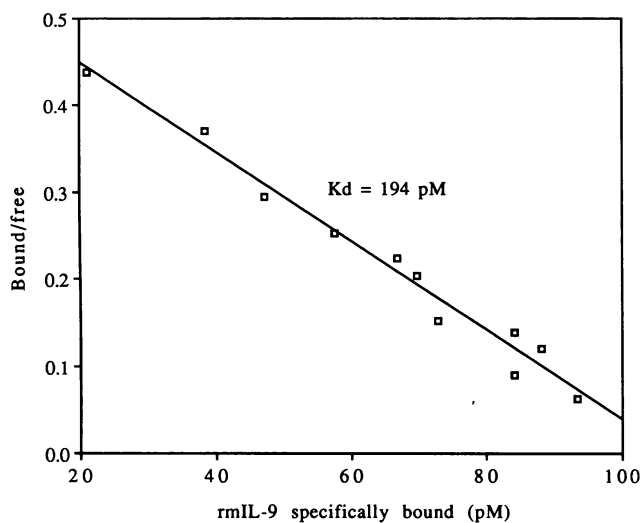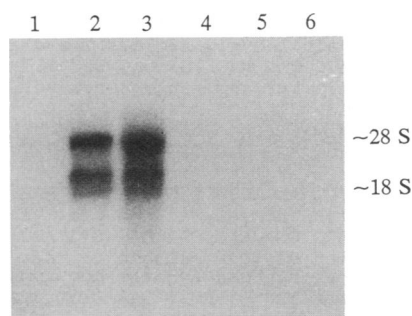

Fig. 2. RNA blot analysis of the murine IL-9 receptor transcripts. Poly(A)$^+$ RNA (1 μg per lane) isolated from TUC13.1 (lane 1), TUC5.37 (lane 2), TS1 (lane 3), CTLL (lane 4), MOPC104E (lane 5), and FDCP-1 (lane 6) was fractionated by agarose gel electrophoresis, transferred to a nitrocellulose filter, and hybridized with the 1.9-kb cDNA p9RA1. Size markers indicate the positions of 28S and 18S rRNA corresponding to 4.7 and 1.9 kb, respectively.

second hydrophobic region at amino acids 271–291 presumably constitutes the transmembrane domain.

The putative extracellular domain was composed of 233 amino acids and contained six cysteines and two potential N-linked glycosylation sites, in positions 116 and 155. A WSEWS motif, typical for the hematopoietin receptor superfamily (23), was found in positions 244–248, 26 residues upstream the transmembrane region. The cytoplasmic part of the protein was characterized by a high percentage of serine (13%) and proline (12.4%) residues and by the presence of three potential protein kinase C phosphorylation sites in positions 294, 416, and 465.

Comparison of the extracellular domains of the p9RC4 clone and other positive clones revealed the following features. In contrast to p9RC4 and p9RB1, the original clone p9RA1 and the p9RB3 cDNA contained an additional glutamine residue between Glu-192 and Ala-193 without any frameshift. Moreover, at the same site, a 22-nucleotide deletion was observed in p9RC2 clone. These findings, and the presence of a potential intronic sequence in p9RC9, suggest alternative splicing events.

In this respect, analysis of the p9RB3 clone implied the existence of a soluble form of the IL-9 receptor. p9RB3 cDNA indeed contained a large part of the extracellular domain but lacked nucleotides 648–1719 encoding the end of the extracellular domain, the transmembrane domain, and the cytoplasmic domain. The cytoplasmic domain of p9RA1 differed from that of all the other clones by a stop codon after Ala-378 followed by a 736-bp sequence unrelated to other cDNAs sequenced so far and possibly resulting from a cloning artefact.

A cDNA Encoding a Human IL-9 Receptor. A random-primed cDNA library prepared from polyadenylylated RNA of human megakaryoblastic leukemia Mo7E was screened by using the p9RA1 cDNA as a probe. Six cross-hybridizing clones, named ph9RA2, -3, -4, -5, -6, and -9, were isolated and sequenced. Although the coding sequence of the majority of these clones was interrupted by putative intronic sequences, the coding sequence of the ph9RA3 cDNA contained a 1566-bp open reading frame showing 66% identity with the murine p9RC4 sequence. Comparison of the murine and human deduced protein sequences is shown in Fig. 4. The predicted protein, containing 522 amino acids, showed 53% identity with its murine counterpart. The putative cleavage site for the signal peptide, predicted between positions 39 and 40, was conserved in the two species as well as the transmembrane domain, presumably spanning residues 271–291, the two potential N-glycosylation sites of the extracellular domain (positions 117 and 156), and the consensus sequence for the hematopoietic receptor superfamily. In contrast, only

Fig. 1. Binding characteristics of recombinant murine (rm) IL-9 receptor expressed in COS cells transfected with p9RA1 cDNA.

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1     CTCC ATG GCC CTG GGA AGA TGC ATT GCG GAA GGT TGG ACC TTG GAG AGA GTG GCG GTG AAA CAG GTC TCC TGG
           met ala leu gly arg cys ile ala glu gly trp thr leu glu arg val ala val lys gln val ser trp    23
74    TTC CTG ATC TAC AGC TGG GTC TGC TCT GGA GTC TGC CGG GGA GTC TCG GTC CCA GAG CAA GGA GGA GGA GGG CAG
           phe leu ile tyr ser trp val cys ser gly val cys arg gly val ser val pro glu gln gly gly gly gly gln  48
149   AAG GCT GGA GCA TTC ACC TGT CTC AGC AAT AGT ATT TAC AGG ATC GAC TGC CAC TGG TCG GCT CCA GAG CTG GGC
           lys ala gly ala phe thr cys leu ser asn ser ile tyr arg ile asp cys his trp ser ala pro glu leu gly  73
224   CAG GAA TCC AGG GCC TGG CTC CTC TTT ACC AGT AAC CAG GTG ACT GAA ATC AAA CAC AAA TGC ACC TTC TGG GAC
           gln glu ser arg ala trp leu leu phe thr ser asn gln val thr glu ile lys his lys cys thr phe trp asp  98
299   AGT ATG TGT ACC CTG GTG CTG CCT AAA GAG GAG GTG TTC TTA CCT TTT GAC AAC TTC ACC ATC ACA CTT CAC CGC
           ser met cys thr leu val leu pro lys glu glu val phe leu pro phe asp asn phe thr ile thr leu his arg  123
374   TGC ATC ATG GGA CAG GAA CAG GTC AGC CTG GTG GAC TCA CAG TAC CTG CCC AGG AGA CAC ATC AAG TTG GAC CCA
           cys ile met gly gln glu gln val ser leu val asp ser gln tyr leu pro arg arg his ile lys leu asp pro  148
449   CCC TCT GAT CTG CAG AGC AAT GTC AGC TCT GGG CGT TGT GTC CTG ACC TGG GGT ATC AAT CTT GCC CTG GAG CCA
           pro ser asp leu gln ser asn val ser ser gly arg cys val leu thr trp gly ile asn leu ala leu glu pro  173
524   TTG ATC ACA TCC CTC AGC TAC GAG CTG GCC TTC AAG AGG CAG GAA GAG GCC TGG GAG GCC CGG CAC AAG GAC CGT
           leu ile thr ser leu ser tyr glu leu ala phe lys arg gln glu glu ala trp glu ala arg his lys asp arg  198
599   ATC GTT GGA GTG ACC TGG CTC ATC CTT GAA GCC GTC GAA CTG AAT CCT TCC ATC TAC GAG GCC AGG CTG CGT
           ile val gly val thr trp leu ile leu glu ala val glu leu asn pro gly ser ile tyr glu ala arg leu arg  223
674   GTC CAG ATG ACT TTG GAG AGT TAT GAG GAC AAG ACA GAG GGG GAA TAT TAT AAG AGC CAT TGG AGT GAG TGG AGC
           val gln met thr leu glu ser tyr glu asp lys thr glu gly glu tyr tyr lys ser his trp ser glu trp ser  248
749   CAG CCC GTG TCC TTT CCT TCT CCC CAG AGG AGA CAG GGC CTC CTG GTC CCA CGC TGG CAA TGG TCA GCC AGC ATC
           gln pro val ser phe pro ser pro gln arg arg gln gly leu leu val pro arg trp gln trp ser ala ser ile  273
824   CTT GTA GTT GTG CCC ATC TTT CTT CTG CTG ACT GGC TTT GTC CAC CTT CTG TTC AAG CTG TCA CCC AGG CTG AAG
           leu val val val pro ile phe leu leu leu thr gly phe val his leu leu phe lys leu ser pro arg leu lys  298
899   AGA ATC TTT TAC CAG AAC ATT CCA TCT CCC GAG GCG TTC TTC CAT CCT CTC TAC AGT GTG TAC CAT GGG GAC TTC
           arg ile phe tyr gln asn ile pro ser pro glu ala phe phe his pro leu tyr ser val tyr his gly asp phe  323
974   CAG AGT TGG ACA GGG GCC CGC AGA GCC GGA CCA CAA GCA AGA CAG AAT GGT GTC AGT ACT TCA TCA GCA GGC TCA
           gln ser trp thr gly ala arg arg ala gly pro gln ala arg gln asn gly val ser thr ser ser ala gly ser  348
1049  GAG TCC AGC ATC TGG GAG GCC GTC GCC ACA CTC ACC TAT AGC CCG GCA TGC CCT GTG CAG TTT GCC TGC CTG AAG
           glu ser ser ile trp glu ala val ala thr leu thr tyr ser pro ala cys pro val gln phe ala cys leu lys  373
1124  TGG GAG GCC ACA GCC CCG GGC TTC CCA GGG CTC CCA GGC TCA GAG CAT GTG CTG CCG GCA GGG TGT CTG GAG TTG
           trp glu ala thr ala pro gly phe pro gly leu pro gly ser glu his val leu pro ala glu cys leu glu leu  398
1199  GAA GGA CAG CCA TCT GCC TAC CTG CCC CAG GAG GAC TGG GCC CCA CTG GGC TCT GCC AGG CCC CCT CCT CCA GAC
           glu gly gln pro ser ala tyr leu pro gln glu asp trp ala pro leu gly ser ala arg pro pro pro pro asp  423
1274  TCA GAC AGC GGC AGC AGC GAC TAT TGC ATG TTG GAC TGC TGT GAG GAA TGC CAC CTC TCA GCC TTC CCA GGA CAC
           ser asp ser gly ser ser asp tyr cys met leu asp cys cys glu glu cys his leu ser ala phe pro gly his  448
1349  ACC GAG AGT CCT GAG CTC ACG CTA GCT CAG CCT GTG GCC CTT CCT GTG TCC AGC AGG GCC TGA CACCTACCAAGGGATG
           thr glu ser pro glu leu thr leu ala gln pro val ala leu pro val ser ser arg ala ***               468
1428  TGGGCATTCTCTTCCCTCCTATCCTCGGATGGCACCAGACACAGTCTCTGCGTGTCTCTGCTAGGTGCACCATGTCTGTTTTGGGGAGATGAACGAAAGG
1528  CCCCAGGCTGACCCTGGGGTGCGTGTGGAACTCCGGAGAGGAGGCAGCTGTGCACGGATCAGAGGCAATGCGGATGGAAGCAGTAGACTGTGCCTTACCC
1628  CCCTGCTCTGCCTTTGTGGTGGGGATGCCTCCAGGGTCAGCATCTTAACATCGCCTTCGCTTCTCTTGTCTTTCTGGCTCTGTCCCAGGCCTGAAAAAAG
1728  AATGTGACAAGCAGCCTGGTCTGTTCTTCCACCCCTAAAGGGCTGGCCTGGGCCCAGGGACACTGATGAGACAACATTGGTGAAGTGTCCCTTTTCAGTG
1828  CCTTTCCCATTAAGACCAGAAGGGACGCTTTTGACTGCAGGCTGTGGGTGGCTGGGTACGGAGGGAATGATGGAGCTTTGAGCAGGTGGGGTTGTCCATC
1928  TTTGAGCTTTTGGGGTTCCAAGATCAGCTGGAAGGAGTCTCACCGACTGATTCAAAGAAGTCTTACCCATCTGTGATATTTTCTTTCCTGGTGCCGTGAT
2028  AAAACACCGTGACCAAAAATGACTTACAAAAGGAAGAGTTGGCTTGGTTTAAGGTTCCAGAGGTGTGGAGACATGGCAGCCAGCGGCACACATGGCAGTG
2128  AGGACAGGAAGCTGAGAGCTCACATCTCAACCAAAAGTTGAGTGAACTGAAAGTACTATCCCCTCCCCCACCCCAACTCCAGCAAGGCTCCACCCCCCTG
2228  AAGGTTCCATGCCTCCCTAAACAGCTCGGCCAAATAGAGACCAAGTGTTCAAATATCTGAGTCTGTGGGGGACATTTCTCATTCAAACCACTTCACTGCC
2328  CCCACTGTTCCTAGGAAAAAGCTGAAGCCAGGGCTACTGGACAGGGTTGGGAATGGCTATTTCTCAGCAGCCGGCCTGTGAAGAATGACGATGCCCCTAA
2428  CTGCCTTCTGAGGTAGCCTGGAGAGAGCTGTGGGTGGGCTAGAATGTGGCTGTTATTTTCCTAGGCTTGCCTAACAGAATACCAGAAACCGGGTGGCTAA
2528  TACCGTAGATTTGTTTTCTCTCTGCTCTGAGGTCCATGGGCTGGAATGGAGACACCAGGAAGGCCATGCTCCTGGAGGGAAGGGTTTGTTTCCAGCCCTG
2628  CTTCACATTCCCTCTGAGTGTCCTCTGCGTACTGAGACTAGATGCTACCCAATGACGGGACAGGTTGTGTCTCATACTCTTGCATGTGAGCAGAGATTGT
2728  GACCTAGAAGGCCAAAACAGGCTGAGCAGTTGGCCACGTTTGTGCAGATTTGGAGCCATAAAGCACTGGCCTAACAGAAGTCCTCTTGCTCCTTGAAGGG
2828  CTCTTAAAGGGCAAGCGTGGGAACAATGCTAGTGTGAGGACTATTTCCAAGCCTGTGACACCGTGCAAGAGACGGTCGAACTACTGCGCAGGGTCCATGG
2928  AGCGCAGAGTCCATGGAGCGCAGAGAGGAACTGCATGCAGCATTGTGAGCTCAGAGCTGGTGTCCTGGGAAGGCTCACAATGCTGACCCCAGG
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FIG. 3. Sequence of the murine IL-9 receptor cDNA. Position numbers on the left side for the nucleotide sequence and on the right side for the amino acid sequence are shown.

one of the three potential protein kinase C phosphorylation sites of the murine cytoplasmic domain was conserved in the human (position 294). The cytoplasmic part of the protein seemed less conserved and was significantly larger in the human sequence (231 versus 177 residues). This region also showed a high percentage of serine (11.2%), partially due to a stretch of nine successive serines at positions 431–439.

To ensure that ph9RA3 clone encodes a functional IL-9 receptor, murine TS1 cells were transfected with this cDNA and tested for their proliferative response to human IL-9. As shown in Fig. 5, the original murine cells, unresponsive to human IL-9 at 100 units/ml, became responsive after transfection with the human IL-9 receptor cDNA.

Analysis of clones ph9RA2, -4, -6, and -9 confirmed the protein sequence deduced from the ph9RA3 clone. However, a truncated protein was predicted from ph9RA5 cDNA, resulting from an 85-nucleotide deletion in the sequence encoding the intracellular domain. The putative protein would consist of 307 amino acids and would include the complete extracellular and transmembrane regions of the IL-9 receptor, 5 amino acids of the cytoplasmic domain, and 11 unrelated residues. Sequencing of additional clones has shown that this deletion is located at a splicing junction but the

relevance of this particular splicing pattern needs further investigation.

DISCUSSION

This report describes the molecular cloning of cDNAs encoding murine and human IL-9 receptors. Transfection of COS cells with the murine cDNA resulted in the expression of high-affinity binding sites for IL-9. The human IL-9 receptor cDNA described here was shown to encode a biologically active receptor since murine T cells became responsive to human IL-9 upon transfection with this cDNA.

The two proteins are 53% homologous, with 67% identity in the extracellular region. Homology searches in the GenBank and European Molecular Biology Laboratory DNA data bases and the National Biomedical Research Foundation protein data base (January 1992) revealed a significant similarity between the extracellular domain of the IL-9 receptors and several other recently cloned growth factor receptors. In particular, the presence of a WSEWS motif and of four cysteine residues with a fixed distance indicates that the IL-9 receptor is a member of the hematopoietin receptor superfamily (23).

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m MALGRCIAEGWTLERVAVKQV--SWFLIYSWVCSGVCRGVSVPEQGGGGQKAGAFTCLSNSIYRIDCHWSAPELGQESRAWLLFTSNQVTEIKHKCTF  96
  | ||||| |||||| |       | |     | || |||| | |          |||| | | ||||||||||||| |  ||||||||     |||
h MGLGRCIWEGWTLESEALRRDMGTWLLACICICTCVCLGVSVTGEGQGPR-SRTFTCLTNNILRIDCHWSAPELGQGSSPWLLFTSNQAPGGTHKCIL  97

m WDSMCTLVLPKEEVFLPFDNFTITLHRCIMGQEQVSLVDSQYLPRRHIKLDPPSDLQSNVSSGRCVLTWGINLALEPLITSLSYELAFKRQEEAWE-A  193
  | || ||| | |  |       | |||||| | | | |||||||| | |  |||||||    |   ||||||||||| ||| | ||| | ||||||| || || |
h RGSECTVVLPPEAVLVPSDNFTITFHHCMSGREQVSLVDPEYLPRRHVKLDPPSDLQSNISSGHCILTWSISPALEPMTTLLSYELAFKKQEEAWEQA  195

                                          ▓▓▓▓▓
m RHKDRIVGVTWLILEAVELNPGSIYEARLRVQM-TLESYEDKTEGEYYKSHWSEWSQPVSFPSPQRRQGLLVPRWQWSASILVVVPIFLLLTGFVHLL  290
  | | |||||||||||| || || | |||||||| |||      | | |        | || ||| | | | |    || | |||||||| ||
h QHRDHIVGVTWLILEAFELDPGFIHEARLRVQMATLEDDVV--EEERYTGQWSEWSQPVCFQAPQ-RQGPLIPPWGWPGNTLVAVSIFLLLTGPTYLL  290
                                              ▓▓▓▓▓

m FKLSPRLKRIFYQNIPSPEAFFHPLYSVYHGDFQSWTGARRAGPQARQNGVSTSSAGSESSIWEAVATLTYSPACPVQFACLKWEATAPGFPGLPG--  386
  |||||| |||||||| ||| || ||||| | || | || | ||  | |  | |  |  | || || ||| | |  |     | |   ||
h FKLSPRVKRIFYQNVPSPAMFFQPLYSVHNGNFQTWMGAHRAGVLLSQDCAGTPQGALEPCVQEATALLTCGPARPWKSVALEEEQEGPGTRLPGNLS  388

m SEHVLPAGCLELEGQPSAYLPQEDWAPLGSARPPPPDS---DSGSS--------DYCMLDCCEECHLSAFPGHTESPELTLAQPVALPVSSRA       468
  || ||||||| |   | |||||||||| || |||| | || | ||  |||| ||| || | |   | |
h SEDVLPAGCTEWRVQTLAYLPQEDWAPTSLTRPAPPDSEGSRSSSSSSSSSNNNNYCALGCYGGWHLSALPGNTQSSGPIPALACGLSCDHQGLETQQ  486

h GVAWVLAGHCQRPGLHEDLQGMLLPSVLSKARSWTF                                                                522
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FIG. 4. Alignment of cDNA-derived amino acid sequences for murine (m) and human (h) IL-9 receptors. Vertical lines indicate identical amino acids. The putative signal peptide and the transmembrane domain are indicated by horizontal lines and the consensus sequence for the hematopoietin receptor superfamily is indicated by a hatched box.

RNA analysis showed that the murine IL-9 receptor mRNA consists of at least three transcripts of different sizes probably due to alternative polyadenylylation signals and

FIG. 5. Response to human IL-9 of murine TS1 cells transfected with a human IL-9 receptor cDNA. Original murine TS1 cells (*Upper*) and TS1 cells transfected with human IL-9 receptor ph9RA3 cDNA (*Lower*) were cultured in the presence of increasing doses of purified murine (solid circles) or human IL-9 (open circles). After 3 days, the number of living cells was determined by a classical hexosaminidase assay.

alternative splicing, as suggested by sequencing of a variety of independent clones. In addition to membrane-bound receptor forms, we identified a murine cDNA encoding a putative soluble form of the protein resulting from the deletion of the sequences encoding the transmembrane and cytoplasmic domains. The possibility of a cloning artefact was excluded by the detection of both membrane-bound and soluble forms of the IL-9 receptor by a PCR performed on TS1 RNA (data not shown). Soluble receptor cDNAs have been described for murine IL-4 (24), human IL-5 (25), and human IL-7 receptors (20), and soluble IL-6 and interferon γ receptors have been found in human urine, presumably resulting from proteolytic cleavage (26). Besides their putative physiological role in the regulation of the immune responses, these molecules could provide us with highly specific tools for modulating the activity of their corresponding ligands, either by blocking the active site or by mediating interactions with another cell surface glycoprotein as was described for the IL-6 receptor (27).

The mechanisms of signal transduction by cytokine receptors are still poorly understood. A high percentage of serine and proline residues have been found in the cytoplasmic domain of some IL receptors (20, 24). Interestingly, both the murine and the human IL-9 receptors contain high numbers of serines and prolines. A particularly intriguing stretch of nine successive serines was found in the human protein. The functional significance of these residues and that of the potential protein kinase C phosphorylation site conserved in both proteins will need further investigation.

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