

# HUMAN INTERLEUKIN 4 RECEPTOR CONFERS BIOLOGICAL RESPONSIVENESS AND DEFINES A NOVEL RECEPTOR SUPERFAMILY

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IL-4 is a T cell product originally described as B cell growth factor by virtue of its ability to co-stimulate B lymphocyte proliferation (1). It has broad effects on B cells, including the promotion of Ig class switching to IgE and IgG1 isotypes in activated B cells (2-7). In addition to regulating B lymphocyte growth and differentiation, IL-4 modulates the survival, proliferation, and differentiation of T lymphocytes, as well as a wide range of hematopoietic cell lineages, thereby contributing to allergic and other immune processes (8-10). It has recently been reported that murine IL-4 also exhibits antitumor effects in vivo (11). IL-4 mediates its effects through cell surface receptors; a single class of high affinity receptors for IL-4 has been detected on nearly all hematopoietic cells examined (12-17), and on many non-hematopoietic cell types as well. These include human fibroblasts, epithelial cells, and hepatic, bladder, and pancreatic tumor lines (13), as well as murine fibroblast, muscle, neuroblast, epithelial, and a variety of stromal cell lines (12, 16). The biological effect of IL-4 on most of these cells is unknown, but it has been reported that IL-4 can stimulate fibroblast proliferation, and as such may play a role in inflammation (18).

We recently reported the isolation and characterization of a murine IL-4 receptor (IL-4-R) cDNA (19), but the mechanism(s) by which IL-4-R mediate the pleiotropic effects of IL-4 remains largely unknown. In this report we describe the cloning of a human IL-4-R cDNA. The predicted amino acid sequence shows homology with four other recently cloned receptors, and thus defines a novel receptor superfamily. When transfected into a murine T cell line, the human IL-4-R cDNA confers responsiveness to human IL-4, demonstrating that the encoded protein contains sufficient information to transmit the IL-4 signal across the cell membrane. This expression system may prove useful for identifying components of the IL-4 signal transduction machinery.

## Materials and Methods

*Cells and Culture Conditions.* Human peripheral blood T cells (PBT)<sup>1</sup> were purified from

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<sup>1</sup> Abbreviation used in this paper: PBT, peripheral blood T cells.

three donors as described (20), and cultured in 1  $\mu\text{g/ml}$  PHA-P (Sigma Chemical Co., St. Louis, MO) for 44 h before RNA isolation. COS-7 cells were maintained in DMEM with 10% FCS and antibiotics. CTLL-2 cells were grown as described (21). CTLL-T22-8 and CTLL-neo lines were grown in the same medium with the addition of 2.0 mg/ml G418.

**RNA Isolation and cDNA Library Construction and Screening.** Pooled human PBLs were obtained by standard Ficoll purification, and were cultured in IL-2 for 6 d followed by stimulation with PMA and Con A for 8 h. Polyadenylated RNA was isolated and used to construct an oligo(dT)-primed cDNA library in  $\lambda\text{gt}10$  using a cDNA synthesis kit (Amersham Corp., Arlington Heights, IL). A hybridization probe was produced by synthesizing an unlabeled RNA transcript of the entire murine IL-4 receptor cDNA insert from clone 7B9-4 (19), followed by synthesis of a  $^{32}\text{P}$ -labeled single-stranded cDNA with reverse transcriptase using random primers (Boehringer Mannheim Biochemicals, Indianapolis, IN). Hybridization to plaque filters was carried out in 50% formamide, 0.4 M NaCl, 10 mM Pipes, pH 6.4, 1 mM EDTA, 0.2% SDS, 0.1 mg/ml salmon sperm DNA at 42°C, followed by washing in 2  $\times$  SSC, 0.2% SDS at 55°C. The T22-8 cDNA clone was isolated from a library similarly prepared from T22 cell polyadenylated RNA (22, 23) and screened with a  $^{32}\text{P}$ -labeled random-primed probe made from clone PBL-1.

**Subcloning, DNA Sequencing, and RNA Blot Hybridization.** These methods were carried out as previously described (19). RNA blot hybridization used antisense riboprobes derived from human IL-4-R clone PBL-1 and murine IL-4-R clone 7B9-2 (19) in 50% formamide, 5  $\times$  SSC, 0.5% SDS, 5  $\times$  Denhardt's, 50 mM Na(H)PO<sub>4</sub>, pH 7.0, 0.1 mg/ml salmon sperm DNA at 63°C followed by washing at 68°C in 0.1  $\times$  SSC, 0.2% SDS.

**Transfection, Metabolic Labeling, and Affinity Purification.** COS-7 cells were transfected (24) with 5  $\mu\text{g}$  of the mammalian expression vector pDC302 (19) or pDC302 containing the human IL-4-R cDNA clone T22-8. 3 d after transfection, cells were metabolically labeled with 100  $\mu\text{Ci}$  of [ $^{35}\text{S}$ ]methionine and cysteine (Trans  $^{35}\text{S}$ -label; ICN Biomedicals, Inc., Irvine, CA) for 1 h at 37°C, and then lysed (19). Lysates were incubated with a slurry of IL-4 Affigel as previously described for murine IL-4 receptor (19), except that recombinant human IL-4 synthesized in yeast (13) was coupled to the beads instead of recombinant murine IL-4. Incubation with the beads was with or without 10  $\mu\text{g}$  of uncoupled human IL-4. IL-4 Affigel beads were then washed (19), and eluted proteins were analyzed by SDS-PAGE under reducing conditions. CTLL-2 cells were electroporated (25) with 1  $\mu\text{g}$  pSV2neo (26) and 10  $\mu\text{g}$  of either pDC302 control plasmid or pDC302 containing the human IL-4-R cDNA T22-8. Cells were cultured as previously described (21), with the addition of 2.0 mg/ml of G418.

**Binding Assays.** Equilibrium binding studies were performed on CTLL-neo and CTLL-T22-8. Murine and human  $^{125}\text{I}$ -IL-4 were prepared as described (13, 16). Specific activities of 1  $\times 10^{16}$  cpm/mmol were routinely achieved for both preparations. Briefly, equilibrium binding analysis was done by incubating 2  $\times 10^6$  CTLL-neo or CTLL-T22-8 cells with various concentrations of murine  $^{125}\text{I}$ -IL-4 or human  $^{125}\text{I}$ -IL-4 for 1 h at 37°C in binding medium (RPMI medium containing 2.5% bovine serum albumin, 0.2% sodium azide, and 0.2 M Hepes, pH 7.4). Free and cell-bound  $^{125}\text{I}$ -IL-4 were separated by the phthalate oil method (16). Nonspecific binding of  $^{125}\text{I}$ -IL-4 was measured in the presence of 200-fold molar excess unlabeled IL-4. Binding data were analyzed with RS/1 software (Bolt, Beranek and Newman, Boston, MA).

**Proliferation Assays.** [ $^3\text{H}$ ]TdR incorporation studies were done by incubating 2  $\times 10^3$  CTLL-neo or CTLL-T22-8 cells in microtiter plates at 37°C in DMEM, 10% FCS, with various concentrations of recombinant murine IL-4 (16) or recombinant human IL-4 (13). In some experiments, cells were also cultured in the presence of various concentrations of anti-murine IL-4-R antibody (Beckmann, M. P., K. A. Schooley, B. Gallis, T. Vanden Bos, D. Friend, A. R. Alpert, R. Raunio, K. S. Prickett, P. E. Baker, and L. S. Park, manuscript submitted for publication.). After 20 h of culture, cells were pulsed with 1  $\mu\text{Ci}$  [ $^3\text{H}$ ]TdR (Amersham Corp., Arlington Heights, IL) for 4 h. Cells were harvested onto glass strips and [ $^3\text{H}$ ]TdR incorporation was determined on a Packard Tricarb 460 liquid scintillation counter. Results shown are duplicate, independent experiments.

## Results

*Characterization of Human IL-4 Receptor cDNA Clones.* A probe from the murine IL-4 receptor cDNA clone 7B9-4 (19) was used to isolate human IL-4-R cDNA clones from two libraries, one derived from human PBL, and the second from a CD4<sup>+</sup>/CD8<sup>-</sup> human T cell line, T22 (22, 23). A restriction map and two representative clones are diagrammed in Fig. 1 A. A full-length 3.6-kb cDNA clone, T22-8, contains an open reading frame encoding 825 amino acids flanked by 175 bp of 5' and 947 bp of 3' noncoding sequence (shown in Fig. 1 B). The predicted protein sequence exhibits 53% identity with the murine IL-4 receptor, and contains a predicted 25 amino acid signal peptide, 207 amino acid external domain, 24 amino acid transmembrane region, and 569 amino acid cytoplasmic domain. Three of six potential N-linked glycosylation sites are conserved between the human and murine sequences. Five of the seven cysteines present in the human IL-4-R extracellular domain are also found in the murine IL-4-R. These presumably contribute to protein structure, and take on added significance in view of their conservation in other related receptor sequences (see below).

The PBL-1 cDNA showed identical sequence to T22-8 except for a 68-bp insertion that terminates the open reading frame 45 amino acids downstream of the predicted NH<sub>2</sub> terminus of the mature protein. This insertion was found in other cDNA clones and therefore is not a cloning artifact but could represent unspliced intronic sequences.

*IL-4 Receptor Is A Member of a New Superfamily.* A search against the EMBL and Genbank DNA and National Biomedical Research Foundation protein databases (28) revealed that the human IL-4-R sequence is unique, as was found with the murine IL-4-R (19). However, in comparing the predicted amino acid sequence of the IL-4-R with the published sequences of several recently cloned receptors, statistically significant homology was found among the extracellular domains. These sequences are the human IL-6-R (29), the human IL-2-R  $\beta$  subunit (p75; reference 30), the murine erythropoietin receptor (31), and the rat prolactin receptor (32). Like the IL-4-R, these receptor sequences are unique in the databases, and do not contain tyrosine kinase domains or features that would classify them as members of other gene superfamilies (except IL-6-R, see below). Pairwise alignments of the predicted extracellular domains of the receptors were performed with the ALIGN program (33, 34), generating scores ranging from 3.9 to 8.8 (given in Table I). Scores >3.0 are statistically significant and indicate common ancestry.

Shown in Fig. 2 is an alignment of the extracellular domains of these receptor superfamily members. Invariant amino acids are designated in bold print, although many more amino acids are conserved among the sequences. Distinguishing features of the extracellular domains include four conserved cysteines and a COOH-terminal double tryptophan-serine motif (WSXWS; see asterisks).

*Expression of Human IL-4-R cDNA.* Proof that cDNA clone T22-8 encodes a biologically active human IL-4-R was provided by biochemical and functional analyses of the encoded protein in mammalian expression systems. COS-7 cells were transiently transfected with the mammalian expression vector pDC302 (19), or pDC302 containing cDNA clone T22-8, and were metabolically labeled with [<sup>35</sup>S]methio-



TABLE I  
Alignment Scores of the Predicted Extracellular Domains

	hIL-4-R	mIL-4-R	hIL-6-R	hIL-2-R $\beta$	mEPO-R
mIL-4-R	30.4				
hIL-6-R	6.5	6.3			
hIL-2-R $\beta$	6.1	4.7	4.8		
mEPO-R	6.7	4.8	8.1	7.7	
rPRL-R	3.9	4.5	8.3	4.6	8.8

Each receptor was compared using the ALIGN program, which computes the optimal alignment of two sequences and generates an alignment score (33, 34). A score > 3.0 SD is statistically significant. Amino acids 1-207 of the human IL-4-R (hIL-4-R; Fig. 1 B), 1-208 of murine IL-4-R (mIL-4-R; reference 19), 106-329 of human IL-6-R (hIL-6-R; reference 29), 1-214 of human IL-2-R $\beta$  subunit (hIL-2-R $\beta$ ; reference 30), 37-249 of murine erythropoietin receptor (mEPO-R; reference 31), and 20-229 of rat prolactin receptor (rPRL-R; reference 32) were compared.

nine and [<sup>35</sup>S]cysteine. Radiolabeled receptors were purified from cell lysates by affinity chromatography on human IL-4 coupled to Affigel, and subjected to SDS polyacrylamide gel electrophoresis. As shown in Fig. 3 A, a major IL-4 binding protein of 140 kD was produced in cells transfected with the T22-8 expression construct (lane 3) but not in cells transfected with vector DNA (lane 1). Addition of excess human IL-4 to the affinity purification (lanes 2 and 4) blocked the reactivity of the IL-4 Affigel with the expressed IL-4-R protein (lane 4). This size correlates well with the observed *M<sub>r</sub>* (139,000) of the native human IL-4 receptor on both gingival fibroblasts and the Raji B cell line (13). Similarly, the recently isolated full length murine IL-4 receptor cDNA encodes a 139 kD protein in transfected COS-7 cells (19).

*Human IL-4-R cDNA Confers Human IL-4 Responsiveness to Murine CTLL-2 Cells.* We next sought to determine whether the cloned human IL-4-R could mediate a biological response to IL-4. The cloned murine T cell line, CTLL-2 (35), typically exhibits 1,000-4,000 IL-4-Rs per cell (16) and shows short-term proliferation in response to murine IL-4 (10). Because murine and human IL-4 are species-specific with respect to receptor binding (13-16), CTLL-2 cells do not bind or respond to human IL-4. Thus, this cell line provides an ideal host for examining biological activity of the cloned human IL-4-R cDNA. A stable cell population (CTLL-T22-8) expressing the human IL-4-R was derived by cotransfection of CTLL-2 cells with the pDC302/T22-8 expression construct and the plasmid pSV2neo (which encodes resistance to neomycin; reference 26), followed by selection for growth in the presence of the antibiotic G418. A control line (CTLL-neo) was established by cotransfection of CTLL-2 cells with pDC302 vector and pSV2neo plasmid DNAs, followed by selection in G418.

FIGURE 1. Human IL-4-R cDNAs. (A) cDNA clones T22-8 and PBL-1 are diagrammed along with a restriction map. Bar denotes coding region. Location of 68-bp insertion in clone PBL-1 is shown ( $\nabla$ ). (B) Nucleotide and predicted amino acid sequence of clone T22-8. The predicted signal peptide and transmembrane region are marked by light and heavy underlining, respectively. Potential N-linked glycosylation sites and extracellular cysteines have asterisks and boxes, respectively. The dotted line marks the putative polyadenylation signal.

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huIL-4R MKVL-----QEP---TCVSDY-MSISTCEWKMNGPTNCSTELRLLYQLVFL-LSEAH
muIL-4R IKVL-----GEP---TCFSDY-IRTSTCEWFLDSAVIDCSSQLCLHYRLMFFEFSENL
huIL-2R $\beta$  AVN-----GTSQF-TCFYNS-RANISCVWSQDQALQ-DTSCQVHAWPDR--RRWNQ
huIL-6R VHLLVDVPPEEPQL-SCFRKSPLSNVCEWGPRSTPSLTTKAVLLVRKFQ--NSPAE
muEPOR SKAALLASRGSEELL-CFTQR-LEDLVCFWEEAAASSGMDFNYSFSYQLEGE--SRK-
raPRLR QSPPGKPEIHKCRSPD-KETFTCWWNPGLTGGGLPTNYSILTYSKEGE-KTTYE

-----TCIPENNGGAGCV-----CHLLMDDVVSADNYTLD---L----WAGQ--QLLWKGFSFKPSEHVKP
-----TCIPRNSASTVCV-----CHMEMNRPVQSDRYQME---L----WAEH--RQLWQGSFSPSGNVKP
-----TCELLPVSQASWA-----CNLILGAPDSQKLTVDIVTLRLVLCREGVVRVMAIQDFKPFENLRL
DFQEPK-QYSQESQKFS-----CQLAVPEGDSS-FYIVS---MCVASSVGS--KFSTQTFQCGGILQP
-----SCSLHQAPTVRGVSFRFCSL--PTADTSSFPVLE---LQVTEASGS---PRYHRIIHINEVLL
-----C-PDYKTSQPNPNS-----CFFSKQYTSIWKIYIIT---VNATNQMGSS---SSSDPLYVDVTYIVEP

RAPGNL--TVHTNVSD-TLL-LTWSNPYPPD-NYLYNHLTYAVNIWSENDFADFRINYVNTYLEPSLRI
LAPDNL--TLHTNVSD-EWL-LTWNNLYPSN-NLLYKDLISMVNI SREDNPAEFIVYNVTYKEPRLSF
MAPISL----QVVHVETHRCNISW-EISQAS-HYFERHLEFEARTLSPGHTWEEAPL-LTLKQKQEWI
DPPANIT--VTAVARNRRLSVWTDPPHSWN--SSFYRLRFELRYRA-ERSKTFTT--WMVKDLQHHC
DAPAGL---LARRAEEGSHVLRWLPPPGAPMTT---HIRYEVVDSAGNRAGGTQR--VEVLEGRTEC
EPPRNLTLVQKQKDKKTYLWVKWSPPTITDVKTGWFTMEYEIRLKP-EEAEWEI---HFTGHQTFQ

*****
AATLKSIGISYRARVRAW-A-QCYNTWSEWS-P---STKWHNSYREPFQEH
PINILMSGVYYTARVRVR-S-QILTWSEWS-P---SITWYNHFQLPLIQR
CLETLTPTDQYEFQVRVK-PLQGEFTWSEWSQP-----LAFRTKPAALGKDT
VIHDAWSGLRHVQLRAQ--EEFGQSEWSNS-PEAMGTPWTESRSPPAENEVST
VLSNLRGGTRYTFAVARMAEPSFSGFWSAWSEP---ASLLTASDLDP
KVFDLYPGQKYLVQTRCK-PD---HGYWSRWSQES-----SVEMPNDFTLKD

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FIGURE 2. Sequence alignment of extracellular domains. Amino acids conserved among all five receptors are shown in bold print. Alignment of the sequences specified in Table I was performed by computer using the algorithm of Feng and Doolittle (45), and then modified to more closely reflect the pairwise alignments produced by the ALIGN program. The alignment shown is not necessarily optimal for each individual pair of receptors.

Expression of murine and human IL-4-Rs in these lines was first examined by radiolabeled-IL-4 binding and Scatchard analysis. Representative binding studies (Fig. 3 B) indicate that both CTLL-neo cells (*top panel*) as well as CTLL-T22-8 cells (*middle panel*) have  $\sim 1,400$  murine IL-4-Rs on the cell surface with an affinity of  $\sim 4 \times 10^9/M$ . As expected, no apparent human  $^{125}I$ -IL-4 binding was detected on the CTLL-neo cells (not shown). However,  $>800$  human IL-4-Rs could be detected on the transfected CTLL-T22-8 cells (Fig. 3 B, *bottom panel*) with an affinity for human IL-4 of  $1.1 \times 10^{10}/M$ . These binding affinities are consistent with the values previously reported for the native murine (12, 14-16) and human (13, 17) IL-4-Rs.

Expression of murine and human IL-4-R mRNA was examined in the transfected lines by RNA gel blot analysis. A blot containing RNA from CTLL-T22-8 and CTLL-neo cells, and for comparison human peripheral blood T lymphocytes, was hybridized under stringent conditions with a human IL-4-R probe (Fig. 4 A). Shown in Fig. 4 B is a blot containing CTLL-T22-8 and CTLL-neo RNAs that was hybridized with a probe specific for murine IL-4-R mRNA. The murine probe detected only the endogenous 3.9-kb murine IL-4-R mRNA, which was expressed at similar levels in the two CTLL sublines. The human IL-4-R probe hybridized to a 4.0-kb endogenous IL-4-R mRNA in the human T cells, but did not detect the murine form in the CTLL-neo control line. In the CTLL-T22-8 transfectants the human

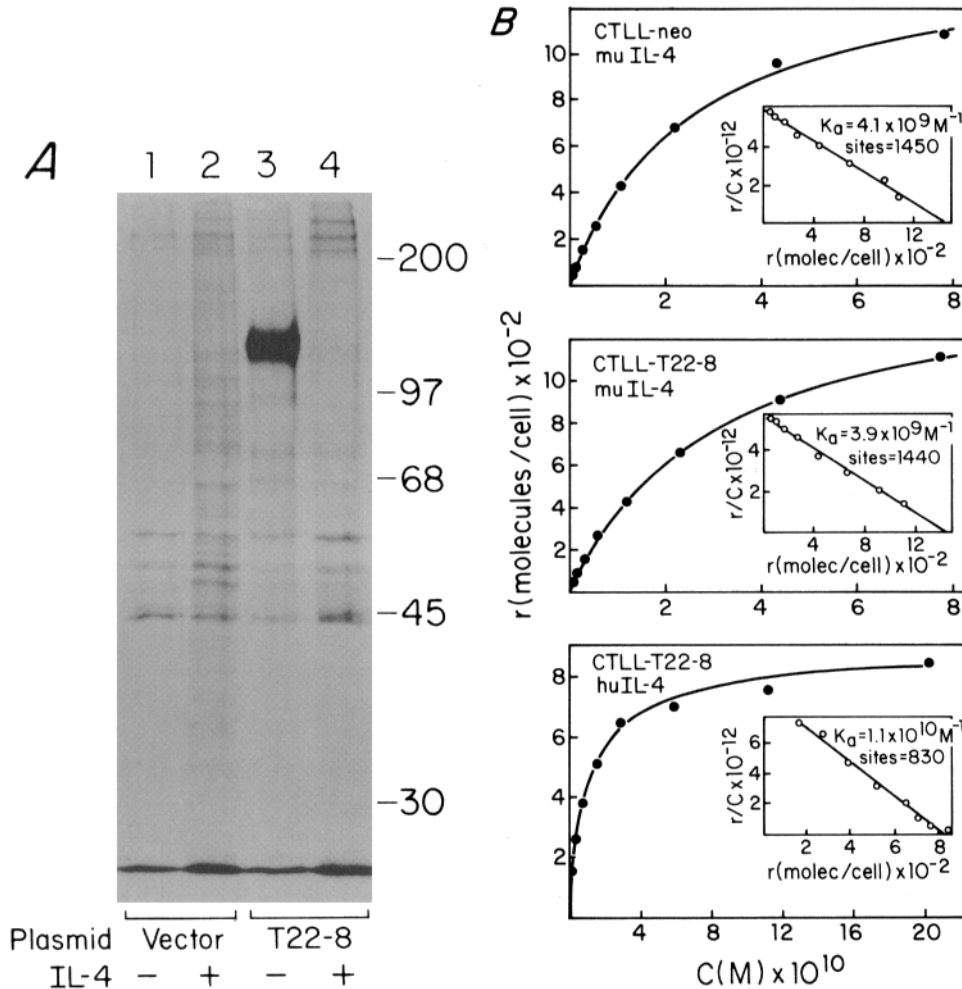


FIGURE 3. (A) Detection of the human IL-4-R expression product in transfected COS-7 cells. Radiolabeled lysates from COS-7 cells transfected with pDC302 vector DNA (vector lanes) or the pDC302-T22-8 expression construct (T22-8 lanes) were incubated with human IL-4 Affigel in the presence (+) or absence (-) of an excess of human IL-4, and the bound proteins were subjected to electrophoresis and autoradiography. The mobilities of molecular weight standards are indicated. (B) IL-4 binding characteristics of transfected CTLL-2 cells. For equilibrium binding studies CTLL-neo (*top*) or CTLL-T22-8 (*middle and bottom*) cells were incubated with various concentrations of murine  $^{125}\text{I}$ -IL-4 (*top and middle*) or human  $^{125}\text{I}$ -IL-4 (*bottom*) for 1 h as previously described (13). Nonspecific binding of  $^{125}\text{I}$ -IL-4 was measured in the presence of 200-fold molar excess of unlabeled IL-4. Binding data were analyzed with RS/1 software (Bolt, Beranek and Newman, Boston, MA).

probe hybridized to a 4.3-kb mRNA, consistent with the predicted size of the exogenous human IL-4-R mRNA, which should contain an additional 0.25 kb of 5' flanking sequences transcribed from the expression vector. Surprisingly, a 3.0-kb RNA species is also evident in the CTLL-T22-8 lane. This could result from any of several events such as aberrant splicing, gene rearrangement during integration into the

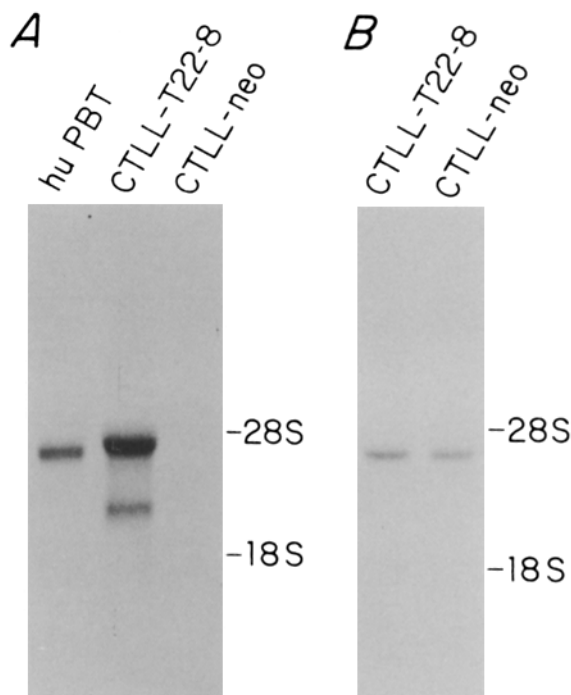


FIGURE 4. Expression of human IL-4-R mRNA in transfected CTLL-2 cells. RNA gel blots containing 8  $\mu$ g per lane total RNA from human peripheral blood T cells that had been cultured for 44 h in 1  $\mu$ g/ml of PHA (PBT, *A*), CTLL-T22-8 transformants (CTLL-T22-8, *A* and *B*), and CTLL-neo transformants (CTLL-neo, *A* and *B*) were probed for (*A*) human and (*B*) murine IL-4-R mRNA. The positions of 28S and 18S ribosomal RNAs are marked.

genome, or utilization of a cryptic polyadenylation signal within the cDNA. The sequence AGTAAA, which occurs at position 2540 (Fig. 1 *B*) might function as such a signal, although there is no evidence for its use in normal human cells.

The ability of the expressed human IL-4-Rs in CTLL-T22-8 cells to convey a biological signal across the membrane was assessed by quantifying the proliferative response of these cells to human IL-4. The results, comparing [ $^3$ H]thymidine incorporation in response to various concentrations of human and murine IL-4 in the two CTLL sublines, are shown in Fig. 5 *A*. While the CTLL-neo control line proliferated only in the presence of murine IL-4, the CTLL-T22-8 cells responded to both murine and human IL-4. Moreover, addition of anti-murine IL-4-R mAb (M1), which blocks murine IL-4 binding (Beckmann, M. P., et al., manuscript submitted for publication), abrogated the response to murine IL-4 in the CTLL-T22-8 cell line, while having no effect on the biological response to human IL-4 (Fig. 5 *B*). Similarly, the anti-murine IL-4-R antibody blocked the proliferative activity of murine IL-4 on the CTLL-neo cells (data not shown). These data demonstrate that the proliferative response of CTLL-T22-8 cells to human IL-4 was mediated through the human IL-4-R encoded by cDNA clone T22-8.

#### Discussion

We have isolated cDNA clones that hybridize with a murine IL-4 receptor probe. The protein sequence encoded by these clones matches closely with that determined for the murine IL-4-R (19), and its identity as the human IL-4-R is confirmed by functional expression of the cDNA in COS-7 and CTLL-2 cells. In murine cells



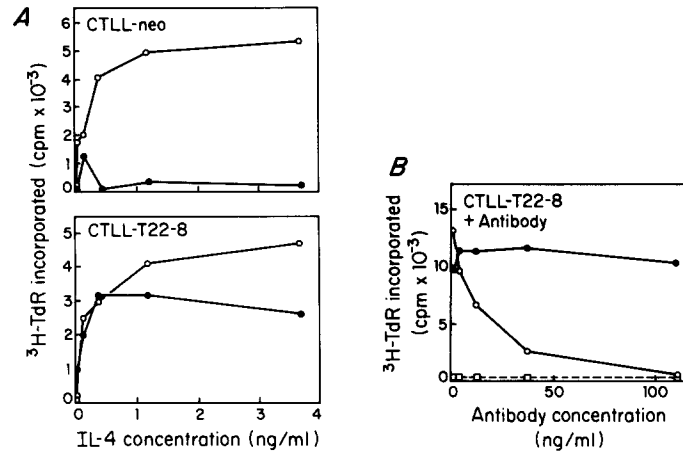


FIGURE 5. (A) Proliferative responsiveness of transfected CTLL-neo and CTLL-T22-8 cells (A and B, respectively) to recombinant murine IL-4 (O) or recombinant human IL-4 (●). (B) Mitogenic responsiveness of CTLL-T22-8 in the presence of anti-human IL-4-R antibody. CTLL-T22-8 cells were stimulated with 0.5 ng/ml of murine (O) or human (●) IL-4 in the presence of various concentrations of anti-murine IL-4-R antibody (1-140 ng/ml; reference 27). Anti-murine IL-4-R antibody alone (□) was used as a control.

we found an alternatively spliced mRNA that encoded a secreted form of the IL-4-R (19), but an analogous cDNA has not yet been isolated in the human system. However, if a secreted form of the human IL-4 receptor is expressed at the same low frequency as the murine secreted form ( $\sim 1$  in 15 cDNAs), then a large number of human IL-4-R cDNAs would have to be examined to rule out its occurrence. Alternatively, expression of secreted IL-4-Rs may be restricted to the murine species.

The sequence comparisons shown in Table I and Fig. 2 define the IL-4-R as a member of a novel superfamily of cytokine receptors. The ALIGN scores are in a range similar to that found among the members of the Ig gene superfamily. Because all of these receptors can be found on, and mediate effects on, cells of the hematopoietic lineage, we suggest the name, hematopoietin receptor superfamily. As additional members of this family are discovered and as receptor structures are examined, the significance of the conserved residues highlighted in Fig. 2 may become more evident.

It is interesting to note that these apparently structurally related receptors bind molecules that also share similar structures. The x-ray crystallographic data of IL-2 shows that it has a four-helix bundle core structure (36). IL-6 has also been modeled as a helix bundle protein (37). Although the structure of prolactin is not known, x-ray crystallographic data on the closely related protein, growth hormone, reveal that it has a four-helix bundle structure (38). Therefore, conservation of the binding region in this family of receptors may be due to the structural requirements necessary to bind a specific class of hormones, and would predict that erythropoietin and IL-4 may also have four-helix bundle structures.

Interestingly, the first 86 amino acids of the mature IL-6-R extracellular domain are unrelated in sequence to the hematopoietin receptor superfamily as determined with the UWGCG GAP program (28), which uses the same algorithm as ALIGN,

but has a gap length penalty and no end gap penalty. However, the NH<sub>2</sub>-terminal 81 amino acids of the mature IL-6-R align with the Ig superfamily C2 domain (29, 39), indicating that the extracellular portion of the IL-6-R has an NH<sub>2</sub>-terminal Ig-like domain, followed by a domain characteristic of the hematopoietin receptor superfamily. Whether IL-6 binding and biological responsiveness require both domains is not yet known.

The demonstration of signal transduction by the human IL-4-R in murine CTLL-2 cells in response to human IL-4 indicates that homology between the murine and human IL-4 receptor must be sufficient to allow interaction of the human IL-4-R with the murine proteins involved in transmitting the IL-4 signal to the nucleus. Presumably, the cytoplasmic domain functions in this capacity. The amino acid sequence identity between the predicted cytoplasmic domains of the human and murine IL-4-Rs is 54%, with the greatest conservation focused in two regions: amino acids 232-259 and 685-727 (Fig. 1 B). Additional evidence suggesting the importance of the cytoplasmic domain comes from previous experiments with CTLL-2 cells. CTLL-2 cell line derivatives that express a high number of endogenous murine IL-4-Rs with a truncated cytoplasmic domain no longer respond to murine IL-4 (19). This is in contrast to the IL-6 receptor, whose 86 amino acid cytoplasmic domain is dispensable for signal transduction (40).

Examination of the predicted cytoplasmic domain sequence of the human IL-4-R revealed no clues about the signaling mechanism. No homologies with protein kinases or sequences found at phosphorylation acceptor sites for protein tyrosine kinases or protein kinase C (41, 42) were observed.

No significant homologies between the predicted cytoplasmic domains of the IL-4-Rs and the other members of the hematopoietin receptor superfamily, which vary greatly in length, were observed using the ALIGN program. D'Andrea et al. (43) recently reported homology between the entire length of the  $\beta$  subunit of the IL-2-R and the erythropoietin receptor, which are roughly equivalent in size. In view of the similarities between IL-4-R and these other receptors, it will be interesting to determine whether components of the signaling machinery are shared among them. In addition, analysis of the genomic structure of these receptor genes might shed further light not only on their evolutionary relatedness, but on domain structural and functional similarities as well.

During the preparation of this paper, we became aware that Bazan (44) had also noticed the sequence similarities between the receptors described here (with the exception of IL-4-R).

### Summary

IL-4, a pleiotropic cytokine produced by T lymphocytes, plays an important role in immune responsiveness by regulating proliferation and differentiation of a variety of lymphoid and myeloid cells via binding to high affinity receptors. In this report we describe the isolation and functional expression of a human IL-4-R cDNA. When transfected into COS-7 cells, the cDNA encodes a 140-kD cell-surface protein. After transfection into a murine T cell line, the cDNA encodes a protein that binds human IL-4 with high affinity and can confer responsiveness to human IL-4. The predicted extracellular domain of the IL-4-R exhibits significant amino acid

sequence homology with the  $\beta$  subunit of the IL-2-R (p75), and the receptors for IL-6, erythropoietin, and prolactin. These receptors comprise a novel superfamily with extracellular domains characterized by four conserved cysteine residues and a double tryptophan-serine (WSXWS) motif located proximal to the transmembrane region.

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