

# Expression Cloning and Characterization of a Human IL-10 Receptor<sup>1</sup>

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cDNA clones encoding a human IL-10R (hIL-10R) from a Burkitt lymphoma cell line, BJAB, express a 90 to 110 kDa polypeptide in COS7 cells that binds hIL-10 specifically. The predicted amino acid sequence of hIL-10R is 60% identical and 73% similar to mouse IL-10R (mIL-10R). rIL-10R expressed in an IL-3-dependent mouse pro-B cell line (Ba/F3) binds hIL-10 with high affinity (200 to 250 pM), and the transfected cells exhibit a proliferative response to hIL-10. Mouse IL-10 does not bind to hIL-10R, and hIL-10R-expressing Ba/F3 cells do not respond to the mouse cytokine, observations consistent with the known species specificity of IL-10. Expression of hIL-10R mRNA seems to be restricted mainly to human hemopoietic cells and cell lines. In a number of human T cell clones, expression of hIL-10R mRNA is down-regulated after activation of the cells with anti-CD3 Ab and phorbol ester. The hIL-10R gene is on human chromosome 11. Like mIL-10R, hIL-10R is structurally related to IFNR. Because IL-10 inhibits macrophage activation by IFN- $\gamma$ , this relationship suggests possible shared receptor or signal transduction pathway components. *Journal of Immunology*, 1994, 152: 1821.

**I**L-10 is a cytokine produced by B cells, T helper cells, monocytes/macrophages, and keratinocytes (1–3). Human and mouse IL-10 (hIL-10, mIL-10)<sup>3</sup> inhibit cytokine synthesis by activated T cells (4–6), NK cells (7), and monocytes/macrophages (8–11). In addition to this inhibitory activity, IL-10 costimulates proliferation and differentiation of human B cells (12), mouse thymocytes, mouse T cells (13–15) and mast cells (16), up-regulates class II MHC expression on mouse B cells (17), and sustains viability of mouse mast cell lines and mouse B cells in vitro (17, 18). Unlike hIL-10, mIL-10 is species-specific and is only active on mouse cells (1, 19). At least two herpesviruses, EBV and equine herpesvirus type 2, encode viral homologues of IL-10 (18, 20, 21). The protein encoded by the EBV homologue, BCRF1, exhibits

some but not all of the activities of IL-10 on both mouse and human cells, and is now called viral IL-10 (vIL-10) (1). vIL-10 and hIL-10 have been demonstrated to play important roles in stimulation of growth and transformation of human B cells by EBV (22, 23).

The different activities of IL-10 are likely mediated by IL-10Rs expressed at the cell surface. We have reported recently the identification and cloning of mIL-10R (24). Here we describe isolation and functional expression of hIL-10R cDNA clones from a human Burkitt lymphoma cell line. The rhIL-10R cDNA has 70% DNA sequence homology (5'-untranslated region and predicted protein-coding region) to mIL-10R, with 60% identity between the predicted amino acid sequences. Although mIL-10R binds both the mouse and human cytokines (24), hIL-10R is species-specific, binding only hIL-10. Like mIL-10R, hIL-10R is structurally related to IFNR, and thus is a new member of the class II (IFNR-like) subgroup of the cytokine receptor family.

## Materials and Methods

### Cytokines

Purified rhIL-10 was from Dr. T. L. Nagabhushan, Schering-Plough Research Institute (Kenilworth, NJ). Purified mIL-10 was provided by Dr. Satish Menon (DNAX, Palo Alto, CA). Concentrated COS7 transfection supernatants served as a source of vIL-10 (7, 19). <sup>125</sup>I-labeled hIL-10 with sp. act. of ~100  $\mu$ Ci/ $\mu$ g was from Dupont-NEN (North Billerica,

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<sup>3</sup> Abbreviations used in this paper: hIL-10, human IL-10; mIL-10, mouse IL-10; vIL-10, viral IL-10; CR, cytokine receptor; CSIF, cytokine synthesis inhibitory factor; LGL, large granular lymphocyte; SA-PE, streptavidin-phycoerythrin conjugate; GM-CSF, granulocyte-macrophage colony-stimulating factor.

MA). Cytokines used in competition experiments were provided by various investigators at DNAX.

### Cell lines and Abs

BJAB cells were provided by Dr. J. Banchemareau (Schering-Plough; Dardilly, France) and maintained in RPMI 1640 supplemented with 10% FCS (R10 medium). Ba/F3 cells were provided by Dr. T. Kitamura (DNAX) and maintained in R10 + 50  $\mu$ M  $\beta$ -mercaptoethanol and 10 ng/ml mIL-3 (BaF medium). U937 cells were obtained from the American Type Culture Collection and were cultured in R10 with or without 1.5% DMSO. DMSO induces differentiation of U937 cells to cells with properties characteristic of mature macrophages (25, 26). RPMI 8866 cells and Daudi (Burkitt lymphoma) cells were as described (27) and were all maintained in R10 + 50  $\mu$ M  $\beta$ -mercaptoethanol. COS7 cells were maintained in DMEM supplemented with 5% FCS. Transfection of COS7 and Ba/F3 cells were performed as described (24, 28). Anti-hIL-10 (12G8) and its isotype control (GL117) mAb were provided by Dr. J. Abrams and Mr. J. Silver (DNAX). The 12G8 mAb also reacts with mIL-10 (K. W. Moore, unpublished observation). Anti-FLAG mAb M1 and M2 were purchased from IBI-Kodak (Rochester, NY). SA-PE and mAb used in magnetic-bead depletion experiments were purchased from Becton Dickinson (Mountain View, CA).

### Purification of human cells for RNA isolation

Human PBMC were isolated from buffy coats from healthy donors by centrifugation over Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) (7). PBMC were incubated in cell culture dishes for 40 to 60 min at 37°C. Non-adherent cells were washed away, and the adherent cells were directly lysed on the plates with guanidinium thiocyanate for RNA preparation as described previously (18, 20). Human monocytes/macrophages of 80 to 90% purity were isolated by adherence to gelatin-coated plates as described (7, 29).

T cells and LGL cells were isolated from PBMC after adherence, passage over a nylon wool column, density centrifugation on a percoll gradient, and negative selection by magnetic beads. Briefly, PBMC were incubated at  $10^6$  cells/100-mm tissue culture dish (Becton Dickinson, Lincoln Park, NJ) for 45 min at 37°C. Nonadherent cells were removed and passed over a nylon wool column (Robbins Scientific, Sunnyvale, CA). Cells were centrifuged, resuspended in 30% percoll, loaded on a 40% percoll gradient, and centrifuged for 25 min at  $1400 \times g$ . The pellet consisted of small resting T cells, which were >98% CD2<sup>+</sup>, CD3<sup>+</sup>. The interphase, containing LGL, was incubated with saturating concentrations of anti-CD14 (Leu-M3), anti-CD3 (Leu-4), anti-CD4 (Leu-3a), anti-CD19 (Leu-12), and anti-CD20 (Leu16) mAb for 30 min at 4°C, washed, and incubated with sheep anti-mouse IgG-coated magnetic beads (Dynabeads M450, Dynal A. S., Oslo, Norway) at a bead to cell ratio of 20:1. The mixture was incubated with gentle shaking for 30 min at 4°C, and rosetted cells were removed with the magnetic particle concentrator according to the manufacturer's recommendations. The resulting population was 80 to 90% CD16<sup>+</sup>, CD56<sup>+</sup>.

B cells were isolated from mononuclear cells obtained from spleens of healthy donors by centrifugation through Ficoll-Hypaque. Mononuclear cells were incubated with saturating concentrations of anti-CD2 (Leu-5b), anti-CD3 (Leu-4), anti-CD4 (Leu-3a), anti-CD8 (Leu-2a), anti-CD14 (Leu-M3), anti-CD16 (Leu-11a), and anti-CD56 (Leu-19) mAb for 30 min at 4°C and subjected to negative selection with magnetic beads as described above. The resulting population was >95% CD19<sup>+</sup>, CD20<sup>+</sup>.

### Structure and expression of FLAG-hIL-10

A 21 amino acid signal sequence and 8 amino acid FLAG peptide sequence (30) were fused to the hIL-10 coding region at the N-terminus by PCR as described previously (24, 31): MALPVTALLPLALLHAARP (signal peptide sequence)–DYKDDDDK (FLAG)–SPGQGTQSENS... (hIL-10). The PCR product was cloned in the pcDSR $\alpha$ 296 vector (32). Transient expression of FLAG-hIL-10 in COS7 cells was analyzed by <sup>35</sup>S-methionine-labeling/SDS-PAGE (20), and by ELISA with anti-FLAG mAb M1 as the coating mAb and anti-hIL-10 as the detecting mAb (10, 24). The expression level of FLAG-hIL-10 was the same as hIL-10, and its biological activities were indistinguishable from hIL-10 as assessed in the CSIF assay (4, 5) and by costimulation of proliferation of mouse MC/9 mast cells in the presence of IL-4 (16, 18). Furthermore,

anti-FLAG mAb did not block the biologic activities of FLAG-hIL-10 when added to CSIF or MC/9 bioassays in vitro (data not shown).

### Detection of hIL-10R

**FACS.** Human cells and cell lines were incubated with 30 to 60 nM FLAG-hIL-10 in FACS buffer (HBSS supplemented with 3% FCS, 0.02% NaN<sub>3</sub>) on ice for 1 h. After staining, cells were pelleted and resuspended directly in cross-linking solution (Dulbecco's phosphate-buffered saline/50 mM HEPES pH 8.3/0.2 mM BS<sup>3</sup> (Pierce Chemical Co., Rockford, IL)) for 30 min on ice. Excess BS<sup>3</sup> was quenched by adding Tris-HCl pH 8.0 to a final concentration of 50 mM. Cells were then washed twice in FACS buffer, incubated with 10  $\mu$ g/ml biotinylated M1 mAb for 30 min on ice, then washed and incubated with SA-PE prior to FACS analysis as described (24). To determine specificity of binding, COS7 or Ba/F3 cells expressing rhIL-10R were incubated with 5 nM FLAG-hIL-10 in the presence or absence of a 100-fold molar excess of the competing cytokine, then subjected to further treatment and FACS analysis as above.

**Ligand binding.** Transfected Ba/F3 cells expressing rhIL-10R (see below) were incubated as triplicate samples ( $10^6$  cells/sample) with 4-530 pM <sup>125</sup>I-labeled hIL-10 in the presence or absence of 150 nM hIL-10 for 4 h at 4°C in RPMI 1640, 2% BSA, 0.02% sodium azide, and then pelleted through a mixture of phthalate oils as described (24, 33, 34). The cell pellet and supernatant were assessed for bound and free <sup>125</sup>I cpm, respectively. Nonspecific binding cpm in samples containing unlabeled hIL-10 competitor were subtracted to obtain specific binding cpm. Values for  $K_d$  and receptor number were obtained by Scatchard analysis (24, 33, 34).

**SDS-PAGE.** The detailed procedure was described previously (24). Briefly, hIL-10R expressed by BJAB cells and hIL-10R cDNA-transfected COS7 cells was bound to <sup>35</sup>S-methionine-labeled FLAG-hIL-10 and cross-linked with sulfo-EGS (Pierce Chemical Co., Rockford, IL), followed by lysis in a buffer containing 1% Triton X-100 and a mixture of protease inhibitors. Cleared lysates or anti-FLAG mAb M2 immunoprecipitates were analyzed by 7.5% SDS-PAGE and autoradiography.

### cDNA library construction

A BJAB cDNA library of approximately  $10^7$  independent clones was prepared using the Super Script Plasmid System (BRL, Gaithersburg, MD), except that *Bst*XI adaptors instead of *Sal*I adaptors were used. Double-stranded cDNA was size-selected on Chromaspin-1000 columns (Clontech, Palo Alto, CA), ligated into the expression vector pJFE14 (35), and transformed into *Escherichia coli* DH10B cells.

### Expression cloning of hIL-10R cDNA

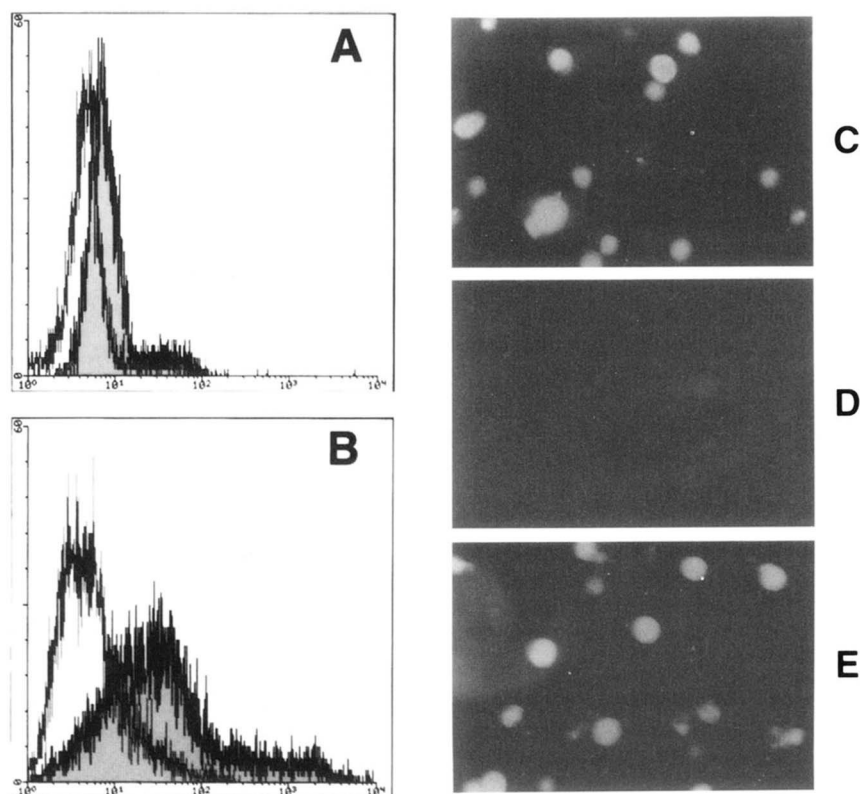
Screening of the BJAB cDNA library was performed by several rounds of transfection and selection by panning (24, 36–38). In the first round,  $4.5 \times 10^7$  COS7 cells were transfected with the BJAB cDNA library by electroporation. Three days after transfection, COS7 cells were harvested from the plates with 0.5 mM EDTA/0.02% NaN<sub>3</sub> and incubated with 30 to 60 nM FLAG-hIL-10 for 1 h on ice, followed by cross-linking with BS<sup>3</sup>. COS7 cells expressing hIL-10R were then selected on plates coated with anti-FLAG mAbs M1 and M2. Plasmid DNA recovered from the selected cells was transformed into *E. coli* DH10B cells by electroporation and reintroduced into COS7 cells by spheroplast fusion for subsequent rounds of panning. After three cycles of panning, DNAs were prepared from randomly picked *E. coli* and digested with *Xho*I and *Nor*I to release cDNA inserts. Approximately one-third of the clones analyzed had the same 3.6 kb insert, and two of them (pYLB5, pYLB6) were chosen for analysis by expression in COS7 cells.

An *Xba*I/*Pvu*II DNA fragment from the 5'-end of pYLB5 was used as a probe to isolate another clone (pSW8.1) from the original BJAB cDNA library. The DNA sequence of hIL-10R was determined by using pSW8.1 subclones as templates as described (18) and analyzed using software from Intelligenetics, Inc. (Mountain View, CA).

### Analysis of hIL-10R mRNA expression

hIL-10R mRNA expression in various human tissues was tested by probing human multiple-tissue Northern blots (Clontech, Palo Alto, CA) with

**FIGURE 1.** Detection of hIL-10R on human Burkitt lymphoma cell line (BJAB), and transfected COS7 cells expressing hIL-10R. FACS histograms are shown for BJAB (A) and for COS7 cells transfected with pYLB5 (B), and compared with results obtained in the presence of a 100-fold molar excess hIL-10; each panel shows detection of FLAG-hIL-10 bound to hIL-10R (right histogram) reduced to background by competition with 100-fold molar excess of hIL-10 (left histogram). Immunofluorescence micrographs of COS7 cells expressing hIL-10R (the same cells as in B) in the absence (C) and presence (D) of hIL-10 competitor and in the presence of mIL-10 competitor (E) are also shown.



the complete hIL-10R cDNA (*XhoI-NotI* fragment) according to the manufacturer's instructions. Expression of hIL-10R mRNA in human cells/cell lines was detected by RNA blot hybridization as described (18). Filters were probed first with hIL-10R and subsequently with human  $\beta$ -actin (Clontech, Palo Alto, CA). hIL-10R RNA blots required 24 to 72 h exposure times;  $\beta$ -actin required 2 to 3 h. Quantitative measurements of hIL-10R hybridization signals were made on a phosphorimager (Molecular Dynamics, Sunnyvale, CA) with reference to the  $\beta$ -actin hybridization signal as a standard. After subtraction of background from the hIL-10R and  $\beta$ -actin signals, the ratio of their intensities was calculated for each sample for purposes of comparison.

#### Expression of hIL-10R in transfected Ba/F3 cells

The hIL-10R cDNA pSW8.1 was transfected into Ba/F3 cells along with a plasmid encoding neomycin resistance as described (24, 28). G418-resistant hIL-10R-expressing cells (Ba8.1) were twice sorted for hIL-10R expression and expanded in culture (24). Ba/F3 and Ba8.1 cells were tested for binding to various concentrations of FLAG-hIL-10 and analyzed by FACS (24). Their responsiveness to hIL-10 and mIL-10 was tested as follows (24): 2000 to 5000 cells were cultured with various amounts of IL-10 in a total volume of 100  $\mu$ l in 96-well culture plates for 48 h at 37°C. A 10  $\mu$ l aliquot of Alamar Blue dye solution (Alamar Biosciences, Sacramento, CA) was added to each well. After 10 to 14 h incubation, the response was determined by measuring  $A_{570}-A_{600}$ .

#### Chromosomal localization of the hIL-10R gene

The chromosomal location of the hIL-10R gene was determined by hybridization to *MspI* digests of genomic DNA from a panel of human-hamster somatic cell hybrids on a filter purchased from BIOS Corporation (New Haven, CT). The full-length hIL-10R cDNA was used as a probe.

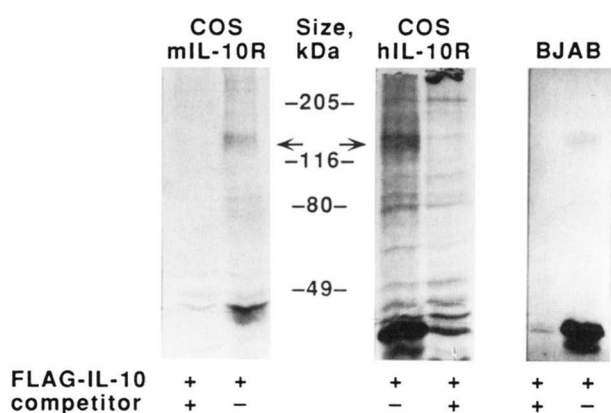
## Results

### Detection of hIL-10R on BJAB cells

hIL-10-responsive cells, which included human adherent cells from PBMC from healthy human donors (7) and human cell lines, were tested for their ability to bind FLAG-hIL-10. A Burkitt lymphoma cell line, BJAB, was found to give reproducible but low levels of specific FLAG-hIL-10 binding that were reduced to background by excess hIL-10 as competitor (Fig. 1A). The level of this specific staining increased when BJAB cells were transferred to fresh culture medium and maintained at low cell density 14 to 16 h before analysis, probably because production of low but detectable amounts of hIL-10 by BJAB cells themselves leads to substantial occupancy of hIL-10R by endogenous hIL-10. This observation suggested that only unoccupied hIL-10R could be readily detected by FLAG-hIL-10, or alternatively that hIL-10R may undergo ligand-induced down-modulation on BJAB cells.

### Expression of hIL-10R clones

COS7 cells transfected with candidate hIL-10R cDNA clones (*Materials and Methods*) were assessed for ability to bind FLAG-hIL-10. Figure 1B shows specific staining of COS7 cells expressing the pYLB5 clone, which was reduced to background in the presence of excess hIL-10 (Fig. 1B) but not mIL-10 (data not shown). Fluorescence



**FIGURE 2.** Detection of hIL-10R expression by BJAB cells and pSW8.1-transfected COS7 cells by cross-linking to  $^{35}\text{S}$ -labeled FLAG-hIL-10. The BJAB samples are immunoprecipitates and COS7 samples are cleared lysates. The hIL-10R and mIL-10R cross-linked to FLAG-hIL-10 or FLAG-mIL-10 (24), respectively, are indicated by arrows. The prominent bands near the 49 kDa standard are cross-linked FLAG-hIL-10 and FLAG-mIL-10 homodimers, as noted earlier (24).

microscopy of pYLB5-transfected COS7 cells also showed specific staining with FLAG-hIL-10 that was not inhibited by excess mIL-10 (Fig. 1C, D, E). In contrast to BJAB cells, use of the cross-linking reagent was not required for detection of hIL-10R expressed on COS7 cells, probably because of increased avidity for ligand due to higher hIL-10R density.

hIL-10R was visualized as a 120 to 140 kDa protein by cross-linking  $^{35}\text{S}$ -labeled FLAG-hIL-10 bound to hIL-10R expressed on both BJAB and hIL-10R cDNA-transfected COS7 cells (Fig. 2). This protein is the same size as mIL-10R detected on mIL-10R-transfected COS7 cells (24). No such protein species was detected in mock-transfected COS7 cells (not shown). Subtracting the mass of one ( $\sim 20$  kDa) or two ( $\sim 40$  kDa) FLAG-hIL-10 peptide chains gave the estimated size of hIL-10R as 90 to 110 kDa.

#### Sequence analysis of hIL-10R cDNA

To avoid possible mutations of cDNAs due to serial passages in COS7 cells during cDNA library screening, a fragment from the 5'-end of pYLB5 was used as a hybridization probe to isolate another clone from the original BJAB cDNA library (pSW8.1), which was then used for DNA sequence analysis. The DNA sequence<sup>3</sup> contained an open reading frame encoding a protein of 578 amino acids, including a putative signal peptide sequence of 21 amino acids (39), a 215 amino acid extracellular domain, a transmembrane segment of 25 amino acids, and a cytoplasmic domain of 317 amino acids. The calculated molecular mass of hIL-10R from the deduced amino acid se-

quence is  $\sim 61$  kDa, in contrast to the observed size of 90 to 110 kDa (Fig. 2). This suggested that, like mIL-10R (24), and as observed with other CRs (40), hIL-10R may be glycosylated at one or more of the six potential sites identified in the extracellular domain (Fig. 3). The nucleotide sequence of pSW8.1 is 70% homologous to the mIL-10R cDNA in the 5'-untranslated and predicted protein-coding regions. The predicted amino acid sequences of hIL-10R and mIL-10R are 60% identical; if chemically similar amino acids are included, the homology (similarity) is  $\sim 73\%$  (Fig. 3).

IL-10 was predicted to be a member of the four  $\alpha$ -helix bundle cytokine family (41), and most receptors for these cytokines (for example, IL-2, -3, -4, -5, -6, and IL-7; G-CSF; GM-CSF) are in the class I group of the CR superfamily (42). However, like mIL-10R, the structure of hIL-10R is more similar to the smaller group of class II CRs that includes IFNR, a viral IFNR homolog, and tissue factor (42–44). The extracellular portion of hIL-10R may be considered as two homologous segments of  $\sim 110$  amino acids that are similar to the size of the immunoglobulin-like ligand-binding domains of the growth hormone receptor (42, 45). The first class II CR domain features two conserved tryptophans and the second cysteine pair of class I CRs; however hIL-10R, unlike mIL-10R, lacks this cysteine pair (Fig. 3).

#### Functional expression of hIL-10R in Ba/F3 cells

The hIL-10R cDNA pSW8.1 was transfected along with a plasmid encoding a neomycin resistance gene into a mouse pro-B cell line, Ba/F3, which expresses little or no mIL-10R (24). G418-resistant cells expressing rhIL-10R (Ba8.1) were isolated by FACS (Fig. 4A, B), expanded in culture, and tested for ability to bind  $^{125}\text{I}$ -labeled hIL-10 (Scatchard analysis, Fig. 4C) and respond to either hIL-10 or mIL-10 (proliferation assay; Fig. 4D). The negative control Neo<sup>r</sup> plasmid-transfected Ba/F3 cell line (BaF-Neo) neither binds hIL-10 (Fig. 4B), nor responds to hIL-10 in the proliferation assay (Fig. 4D).

Ba8.1 cells expressed about 7000 receptors per cell and bound  $^{125}\text{I}$ -labeled hIL-10 with a  $K_d$  of 200 to 250 pM (Fig. 4C). This is similar to the  $K_d$  of 50 to 200 pM of hIL-10R reported by Tan et al., for the human JY cell line (46), and somewhat higher than  $\sim 70$  pM reported for mIL-10R (24). This value reflects a relatively high affinity compared with other CRs (47), which have  $K_d$  values ranging from 30 pM for the high-affinity erythropoietin receptor to 120 nM for the low-affinity hIL-3R.

Neither BaF-Neo nor parental Ba/F3 cells gave a proliferative response to hIL-10 or mIL-10, but Ba8.1 cells, like Ba/F3 transfectants expressing mIL-10R (24), responded to hIL-10 in an assay measuring proliferation and viability (Fig. 4D). The response was inhibited by a neutralizing anti-hIL-10 mAb (Fig. 4D) and thus was specific

<sup>3</sup>The sequence reported in this paper has been deposited in the Genbank database, Accession No. U00672.

**FIGURE 3.** Comparison of the predicted amino acid sequences of hIL-10R (upper) and mIL-10R (lower). The predicted signal sequence cleavage sites are indicated by arrows. The hydrophobic membrane-spanning regions are indicated by dashed boxes and six potential sites (N-X-S/T) of N-glycosylation in hIL-10R are boxed. Amino acid residues that define IL-10R as a member of the IFNR family (42) are underlined and in boldface. Chemically similar amino acids that are conserved among the IFNR family are underlined. Alignment of the mIL-10R sequence with other IFNR family members was presented elsewhere (24).

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      ↓
MLPCLVLLAALLSLRLGSDAHGTLPSPPS-VWFEAEFFHHILHPTIPNQSESTCYE
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
MLSRLLPFLVTISSLSLEFIAYGTLPSP-SYVWFPEARFFQHILHWPINQSESTYYE
      ↑
VALLRYGIES-HNSISNC--SQTLSYDLTAVTLDLYH-SNGYRARVRAVDGSRHSNWTV
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
VALKQYCN-STWNDIHCCKAQAALSCDLTTFTLDLYHRSYGYRARVRAVDNSQYSNWTI

TNTRFSVDEVTLTVGSGVNLEIHNGFILGKIQLPRPKMAPANDTYESIFSHFREYEIAI
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
TETRTFTVDEVILTVDSTLKAMDGIYGTIHPPRPTITPAGDEYEQVFKDLRVYKISI

RKVPG--NFTFTHKKVKHENFSLT-SGEVGEFCVQVKPSVASRSNKGMSKEECI-SLTR
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
RKFSSELNAT--KRVKQETFTL-TVPIGVRKFCVKVLPRLSRINKAEWSEECILLITTE

QYFTVTNVIIFFAFVLLSGALAYCLALQLYVRRRKKLPVLLFKKPSPIFISQRP-S
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
QYFTVTNLSILVISMLLFCGIL-VCLVLQWYIRHPGKLPVLVFKKPHDF-FPAN-PLC

PETQDTIHPLDEEAFKVSPELKNLD--LHGSTDSGFGSTKPSLQTEEPQFLPDHPQA
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
PETPDAIHIVDLEVFPKVSLELR--DSVLHGSTDSGFGSGKPSLQTEESQFLPGSHPQI

DRTLGNGEPPVLGDSCSSGSS--NSTDSGICLQEPSTL-SPSTGPTWEQVGSNSRGQDDSG
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
QGTGLKEESPGLQATC--G--DN-TDSGICLQEPGLHS-SMGPAWKQQLGYTHQDQDDSD

IDLQVNSEGRAGDTQGGSSALGHHSPEPEVPGE--DPAAVAFQGYLRQTRC-AEEKATKT
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
VNLVQNSPGQPKYTQDASALGHVCLLEPKAP-EEKDQVMVTFQGYQKQTRWKA-EAAGPA

GCLEESPLTDGLGPKFGRCLVDEAGLH--PPALAKGYLKQDPLEMTLASSGAPTQWQWQ
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
ECLDEEIPLTDAFDPELGVHLQDD--LAWPPPALAAGYLKQESQGMASAPPGTSPSRQWQ

PTEEWSSLLALSSCSDLGI-SDWSFAHDLAPLGCVAAPGGGLGSFNSDLVTLPLISSLQS
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
LTEEWSSLLGVVSCEDLSIES-WRFAHKLDPLDCGAAPGGLLDSLGSNLVTLPLISSLQV

SE    578
|
EE    575

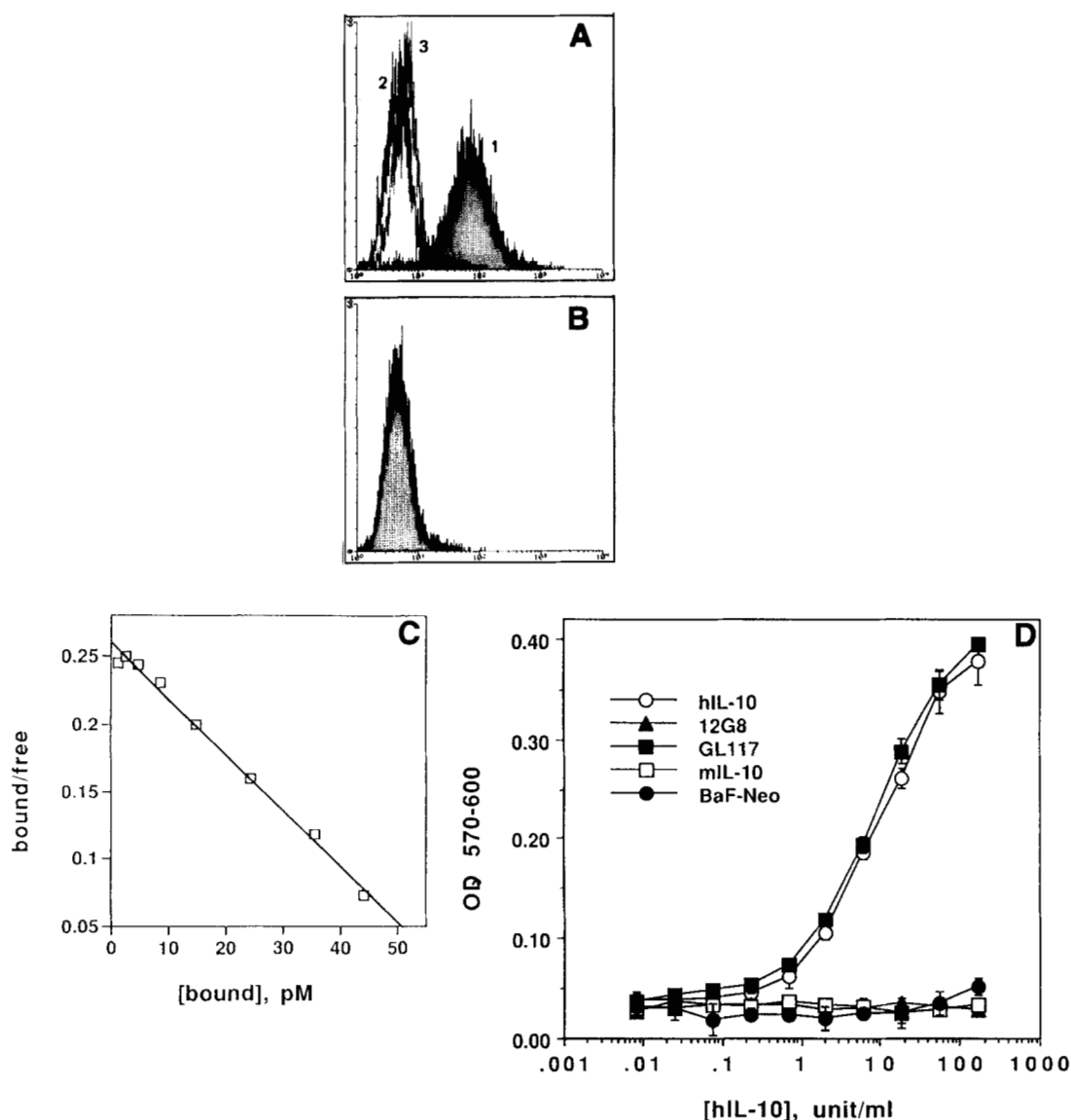
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for hIL-10. Ba8.1 cells did not respond to mIL-10, consistent with observations that mIL-10 is species-specific (1) and does not bind to hIL-10R (Fig. 1). Moreover, binding of FLAG-hIL-10 to hIL-10R expressed on Ba8.1 cells was not inhibited by 100-fold molar excess of hIL-1, hIL-2, hIL-3, hIL-4, and hIL-5; binding was also not inhibited by hIL-7, hIL-13, hGM-CSF, hIFN- $\alpha$ , hIFN- $\gamma$ , hTNF- $\alpha$ , mIL-10, or vIL-10 (data not shown).

#### *hIL-10R mRNA expression*

RNA blot analysis revealed a 3.6-kb hIL-10R mRNA species in a number of human tissues including spleen, thymus, and PBMC (Fig. 5A). Non-hemopoietic tissues revealed only faint hybridization to the hIL-10R probe, including pancreas, skeletal muscle, brain, heart, and kidney (not shown). Placenta, lung, and liver showed intermediate levels of hIL-10R mRNA. Possibly, much of the signal detected in these samples could be due to contaminating blood cells present during isolation of the tissues.

Human cells that express high levels of hIL-10R mRNA include monocytes, B cells, LGL, and T cells isolated from fresh PBMC or spleen (Fig. 5B). Cell lines in which the 3.6-kb mRNA was detected were BJAB, DMSO-differentiated U937 cells (Fig. 5B), NK cell lines (data not shown), and various cloned human T cell lines (48) (Fig. 5C). All cell populations and cell lines known to respond to IL-10, including B cells (12), monocytes/macrophages and DMSO-differentiated U937 cells (6, 10), and T cells and T cell clones (48), expressed readily detectable levels of hIL-10R mRNA, as also observed for mIL-10R (24). Activation of human T cell clones of several phenotypes, including Th0-like CD8<sup>+</sup> clones (GM16, GM59) and Th1-like (CR329), Th2-like (AA111), and Th0-like (HG120) CD4<sup>+</sup> clones, by anti-CD3 and PMA was associated consistently with down-regulation of the level of hIL-10R mRNA detected in the cells (Fig. 5C and data not shown). With reference to  $\beta$ -actin mRNA as standard, the decrease in hIL-10R mRNA ranged from 2- to 10-fold in the samples examined, with the CD8<sup>+</sup> Th0 clones tending to exhibit the more marked decreases in hIL-10R mRNA level.



**FIGURE 4.** Expression of hIL-10R in transfected Ba/F3 cells and their response to mIL-10, hIL-10. (A) FACS analysis of hIL-10R expression by Ba8.1 cells as detected by binding of FLAG-hIL-10 (histogram 1, *shaded*), FLAG-mIL-10 (histogram 2), or FLAG-hIL-10 in the presence of 100-fold excess hIL-10 (histogram 3). (B) FACS analysis of hIL-10R expression by BaF-Neo cells as detected by the FLAG-hIL-10. (C) Scatchard analysis of  $^{125}\text{I}$ -labeled hIL-10 binding to Ba8.1 cells. Data were plotted and analyzed by a linear least-squares fit, and in the particular experiment shown gave a  $K_d$  value of  $\sim 250$  pM,  $\sim 6000$  receptors/cell, and a coefficient of variation  $r = 0.997$ . Data from repeat experiments suggested  $K_d = 200$  to  $250$  pM, with 6000 to 8000 hIL-10R/cell. (D) Response of Ba8.1 and BaF-Neo cells to mIL-10 and hIL-10. The responses of Ba8.1 cells in the presence of  $10 \mu\text{g/ml}$  anti-hIL-10 (12G8) or isotype control (GL117) mAb are also shown. hIL-10 units were based on a CSIF assay using human PBMC (7, 10, 18).

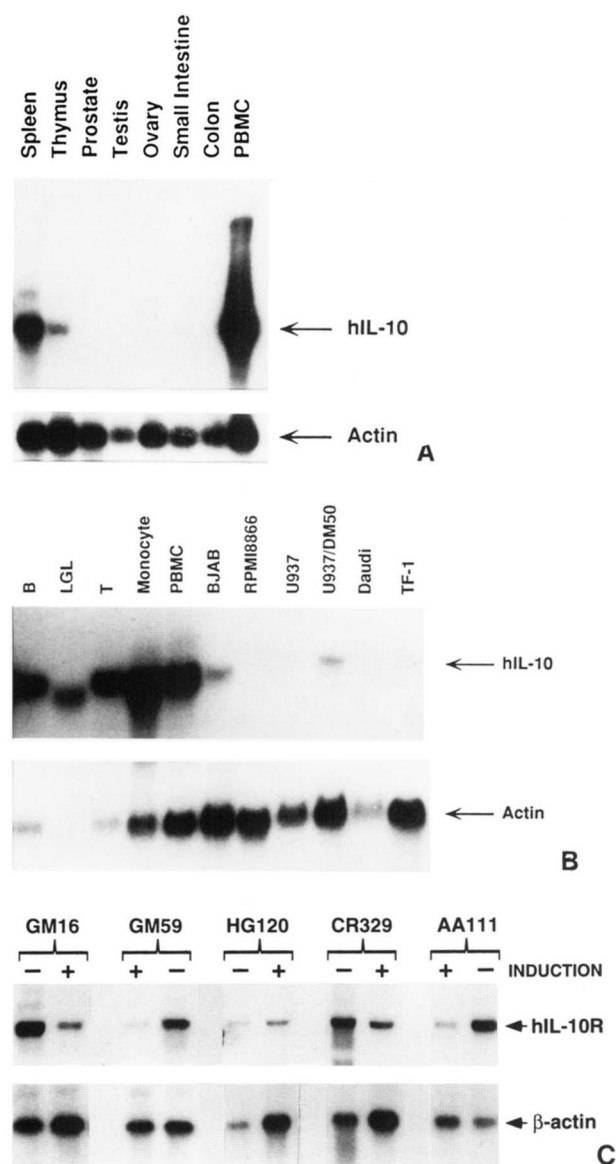
Little or no hIL-10R mRNA was detected in RPMI 8866, Daudi, undifferentiated U937, and TF1 cells on an RNA blot by autoradiogram. Furthermore, TF1 cells, which express receptors for and respond to a variety of human cytokines, including IL-1, IL-3, IL-4, IL-5, IL-6, IL-9, IL-11, IL-13, GM-CSF, and erythropoietin (49), did not exhibit a detectable response to hIL-10 (Y. Liu and K. W. Moore, unpublished observation). Analysis of the hIL-

10R and  $\beta$ -actin RNA blots using the phosphorimager suggested that expression of hIL-10R mRNA by these cell lines was at least several 100-fold below the level observed in the hIL-10R $^{+}$  populations.

#### *Chromosomal localization of the hIL-10R gene*

We determined the chromosomal location of the hIL-10R gene by hybridizing the hIL-10R cDNA probe to *Msp*I





**FIGURE 5.** hIL-10R mRNA expression in various human tissues and cells. The 3.6 kb hIL-10R mRNA (upper band) and actin mRNA (lower band) are shown. (A) Human multiple tissue RNA blot (Clontech, Palo Alto, CA): each lane contains 2 µg poly(A)<sup>+</sup> RNA. (B) 10 µg total RNA from cells shown was used in each lane, except for TF-1 cells (2 µg poly(A)<sup>+</sup> RNA). U937/DMSO RNA was from U937 cells that were cultured with 1.5% DMSO for three days before RNA extraction (25, 26). The apparent different size of hIL-10R mRNA in LGL is a gel artifact; longer exposures showed that the mobility of β-actin mRNA was similarly affected (not shown). (C) 10 µg total RNA from T cell clones shown was used in each lane. T cell clones were either resting (–) or induced (+) with anti-CD3 and PMA as described (18, 48) prior to RNA isolation.

digests of DNAs from a human-hamster hybrid cell line panel. The hIL-10R probe hybridizes prominently to a *MspI* fragment of ~3.2 kb, along with several smaller, less intense bands in human genomic DNA. Only cell line

1049, containing human chromosomes 5 and 11, was positive when hybridized with hIL-10R cDNA (not shown). However, all other lines containing chromosome 5 did not hybridize to the hIL-10R cDNA probe (not shown). Therefore, we concluded that the hIL-10R gene is located on chromosome 11.

## Discussion

With the use of epitope-tagged hIL-10, we identified a cell line expressing hIL-10R and isolated cDNA clones encoding hIL-10R. hIL-10R has 70% and 60% sequence identity to mIL-10R at the nucleic acid and protein levels, respectively (24). COS7 and Ba/F3 cells transfected with hIL-10R cDNA clones express cell-surface receptors that bind hIL-10 (but not mIL-10) specifically with high affinity, and Ba/F3 cells expressing rhIL-10R are stimulated by hIL-10 but not mIL-10 (Fig. 4). rhIL-10R is indistinguishable in size from hIL-10R expressed by the cells from which the cDNA was isolated, and moreover, is similar in size to mIL-10R (Fig. 2). hIL-10R mRNA was present in all cells examined that are known to respond to hIL-10 (Fig. 5). We thus conclude that the hIL-10R identified is a functional receptor for hIL-10, because it binds ligand specifically, mediates transduction of a biologic response to hIL-10, and exhibits the known species-specificity of the human receptor (1, 46).

We have observed significant levels of hIL-10R mRNA expression by T cells and NK/LGL cells, cells about which relatively little is known concerning their direct responses to IL-10. IL-10 inhibits cytokine synthesis by both T cells and NK cells, but these inhibitory effects are indirect, mediated via the monocyte/macrophage costimulatory cell (1). However, hIL-10 does enhance generation of lymphokine-activated killer activity from resting NK cells in response to IL-2 (7), and also has demonstrable inhibitory effects on IL-2 production by subsets of human T cells and T cell clones (48, 50). In addition, mIL-10 costimulates growth of mouse thymocytes and T cells (14), and enhances generation of cytotoxic T cell activity (15). NK/LGL cells express a significant level of hIL-10R mRNA, especially when compared with the β-actin reference standard (Fig. 5B). T cells and T cell clones also express readily detectable hIL-10R mRNA, which is down-regulated in the latter in response to activation by anti-CD3 and PMA (Fig. 5A, C). We do not presently know the significance of the observed down-regulation of hIL-10R mRNA expression, especially because it is unclear whether even an 8- to 10-fold lower hIL-10R number would significantly impair the ability of activated T cells to respond to IL-10. Loss of IL-10R expression by activated T cells might render these cells insensitive to inhibition by IL-10 except for indirect effects mediated by macrophages/monocytes. In any case, the expression of significant levels of hIL-10R mRNA by T and NK cells and its regulation by activation stimuli suggest possible

additional, as yet uncharacterized effects of IL-10 on T cells and NK cells.

The  $K_d$  value of hIL-10R is larger than that obtained for  $^{125}$ I-labeled hIL-10 binding to mIL-10R (~70 pM) expressed on transfected Ba/F3 (BaMR29) cells (24); this is consistent with the observation that hIL-10 has an order of magnitude greater sp. act. in stimulation of BaMR29 cells compared with Ba8.1 cells (A. Ho and K. W. Moore, unpublished observation). This finding may suggest an intrinsically higher affinity of mIL-10R for ligand. Alternatively, it is possible that additional receptor or signalling components provided in *trans* by (mouse) Ba/F3 cells interact much more efficiently with mIL-10R than with hIL-10R due to the species difference.

Like the cellular cytokine, vIL-10 inhibits macrophage activation and stimulates human and mouse B cells (1). The finding that vIL-10 at 100-fold molar excess did not detectably compete with FLAG-hIL-10 for binding to hIL-10R, although at 500-fold molar excess vIL-10 competed slightly with FLAG-hIL-10 (data not shown), thus provides further evidence for the possible existence of additional IL-10R components on human B cells and activated monocytes. Moreover, we have so far been unable to detect significant levels of binding of FLAG-vIL-10 to either Ba8.1 or COS7 cells expressing hIL-10R using FACS analysis.

hIL-10R, along with mIL-10R (24), are new members of the class II subgroup of CR, the IFNR family. Many of the activating effects of IFN- $\gamma$  on macrophages are inhibited by IL-10 (1). The structures of the ligand-binding chains of IFN- $\alpha\beta$ R and IFN- $\gamma$ R are known (43, 51, 52), but biologic and genetic evidence has been accumulated for the existence of an additional IFNR polypeptide(s) involved in transduction of a biologic signal (see for example (53)). In this light, and in view of the demonstration of shared subunits among receptors for different cytokines (54, 55), it is possible that IL-10R could likewise share a second receptor chain with an IFNR. If so, IL-10 and IFN- $\gamma$  might compete for binding to each other's receptors, but as noted above, neither IFN- $\alpha$  nor IFN- $\gamma$  competed for hIL-10 binding to rhIL-10R. However, in the IL3, IL5, and GM-CSF receptor system (45) a group of CRs shares a common secondary chain, but each CR is only capable of binding its respective cytokine. Further possibilities are that the IL-10 and IFN signal transduction pathways may share common features, or that the IL-10/IL-10R interaction might directly antagonize the IFNR signal transduction pathway (56, 57), perhaps by interacting with or sequestering one or more of its components. These suggestions are plausible in view of recent evidence that IL-10 and IFN- $\gamma$  both activate a transcription factor that interacts with IFN-response elements in the Fc $\gamma$ RI gene (58) and induce Fc $\gamma$ R expression (59). Further characterization of the structure and signal transduction mechanisms of IL-10R and IFNR may illuminate these possibilities.

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