## A receptor for interleukin 10 is related to interferon receptors

(cytokine receptors/Ba/F3 cells)

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ABSTRACT We isolated cDNAs encoding a mouse interleukin 10 receptor (mIL-10R) from mouse mast cell and macrophage cell lines. The two cDNAs are substantially identical and express an ~110-kDa polypeptide in COS7 cells, which binds mIL-10 specifically. A mouse pro-B-cell line (Ba/F3) expressing transfected recombinant mIL-10R binds IL-10 with high affinity (~70 pM) and proliferates in response to mIL-10. mIL-10R is structurally related to interferon receptors (IFNRs). Since IL-10 inhibits macrophage activation by IFN- $\gamma$ , a possible implication of this relationship is interaction of IL-10R and IFN- $\gamma$ R or their signaling pathways.

Interleukin 10 (IL-10) is a cytokine produced by activated T cells, B cells, keratinocytes, and monocytes/macrophages (1). Mouse and human IL-10 (mIL-10, hIL-10) inhibit cytokine synthesis by activated T cells (2–4), natural killer cells (5), and monocytes/macrophages (6-8) and block the ability of macrophages to act as antigen-presenting (3, 4) or costimulatory (5) cells. Like other cytokines, IL-10 has multiple activities, including costimulation of proliferation and differentiation of human B cells (9), mouse thymocytes, T cells (10-12), and mast cells (13), up-regulation of class II major histocompatibility complex (MHC) expression on mouse B cells (14), and sustaining viability of mouse mast cell lines and mouse B cells in vitro (14, 15). IL-10 is homologous to an Epstein-Barr virus gene, BCRF1 (15, 16). BCRF1 (viral IL-10, vIL-10) exhibits some but not all of the activities of IL-10 on mouse and human cells (1). The activities of IL-10 are likely mediated via cell surface receptors (IL-10Rs); here we describe detection of mIL-10R and report the structure and expression of mIL-10R cDNA clones.§ Recombinant mIL-10R expressed in the IL-3-dependent mouse pro-B-cell line Ba/F3 binds IL-10 with high affinity and transduces a growth signal in response to mIL-10. We found unexpectedly that mIL-10R is a member of the class II [interferon receptor (IFNR)-like] subgroup of the cytokine receptor (CR) family.

## **MATERIALS AND METHODS**

Cell Lines and Antibodies. MC/9 and Ba/F3 cells have been described (13, 17). J774 cells were from the American Type Culture Collection. Cell lines used in RNA blot analysis were from A. Miyajima, L. Lanier, D. Rennick, and G. Zurawski (DNAX). Anti-mIL-10 (2A5) and isotype control (GL113) monoclonal antibodies (mAbs) were from J. Abrams and J. Silver. Anti-FLAG mAbs M1 and M2 were purchased from IBI-Kodak. Streptavidin-phycoerythrin (SA-PE) was from Becton Dickinson.

Structure and Expression of FLAG-mIL-10. A leader sequence and FLAG (18) peptide sequence were fused to the mIL-10 coding region (1, 16) by PCR: MALPVTALLLPLA-

LLLHAARP (signal sequence)—DYKDDDDK (FLAG)—QYSREDNNCTH . . . (mIL-10). The PCR product was cloned in the pcDSR $\alpha$ 296 vector (19). Expression of FLAG-mIL-10 in COS7 cells was analyzed by [<sup>35</sup>S]methionine labeling/SDS/PAGE and by ELISA with anti-FLAG mAb M2 and anti-mIL-10 (16).

**Detection of mIL-10R.** Fluorescence-activated cell sorting (FACS). Cells were washed twice with FACS buffer (Hanks' balanced salt solution/3% fetal calf serum/0.01% NaN<sub>3</sub>) and incubated with 3–30 nM FLAG-mIL-10 on ice for 1 hr. Cells were pelleted and subjected to cross-linking with 0.2 mM bis(sulfosuccinimidyl) suberate (BS<sup>3</sup>; Pierce) as described (20) and then washed once and resuspended in FACS buffer containing 20  $\mu$ g of biotinylated mAb M1 per ml. After 30 min on ice, cells were washed and incubated with SA-PE and then washed twice prior to FACS analysis or fluorescence microscopy.

Ligand binding. Transfected Ba/F3 cells expressing recombinant mIL-10R (see below) were incubated as triplicate samples with 4–530 pM <sup>125</sup>I-labeled hIL-10 ( $^{125}I$ -hIL-10) [ $\approx 100 \mu$ Ci/µg (1 Ci = 37 GBq); DuPont/NEN] in the presence or absence of 150 nM hIL-10 (T. Nagabhushan, Schering-Plough) for 4 hr at 4°C in RPMI 1640 medium/2% bovine serum albumin/0.02% sodium azide and then pelleted through a mixture of phthalate oils as described (21–23). 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 were subtracted to obtain specific binding cpm. Values for K<sub>d</sub> and receptor number were obtained by Scatchard analysis as described (21).

SDS/PAGE. Cells  $(5-15 \times 10^7 \text{ MC}/9)$ , after the fifth cycle of sorting, or  $1-4 \times 10^6$  transfected COS7) were incubated with <sup>35</sup>S-labeled FLAG-mIL-10 (<sup>35</sup>S-FLAG-mIL-10) (16)  $(3-5 \text{ nM}; \pm 100 \times \text{ molar excess mIL-10 as competitor})$  on ice for 1 hr and then pelleted and cross-linked as above, except with ethylene glycolbis(sulfosuccinimidylsuccinate) (Pierce) instead of BS<sup>3</sup>. Cells were washed, resuspended in FACS buffer, and lysed by addition of an equal volume of  $2 \times 1$  lysis buffer [2% Triton X-100/40 mM Tris HCl, pH 7.4/40 mM EDTA/20 mM EGTA/120 mM NaCl/10 µg (each) of leupeptin, pepstatin, and aprotinin per ml/~1 mg of soybean trypsin inhibitor per ml/2 mM phenylmethylsulfonyl fluoride/1 mg of iodoacetamide per ml/1 mg of phenanthroline per ml] for 0.5-1 hr on ice. Nuclei and debris were pelleted, and the cleared lysate was pretreated overnight at 4°C with Pansorbin (Calbiochem) to which had been bound rabbit

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Abbreviations: IL, interleukin; IFN, interferon; PE, phycoerythrin; FACS, fluorescence-activated cell sorting; CR, cytokine receptor; mAb, monoclonal antibody; SA-PE, streptavidin-phycoerythrin conjugate; h, human; m, mouse; v, viral; R, receptor; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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<sup>&</sup>lt;sup>§</sup>The sequence reported in this paper has been deposited in the GenBank data base (accession no. L12120).

anti-mouse IgG. The Pansorbin was removed by centrifugation and the supernatant was subjected to immunoprecipitation with 20  $\mu$ g of anti-FLAG mAb M2 per ml followed by fresh rabbit anti-mouse IgG/Pansorbin. After several washes, the Pansorbin was boiled for 3 min in reducing sample buffer, and the eluate was analyzed by 7.5% SDS/ PAGE and autoradiography.

**cDNA Libraries.** cDNA libraries were prepared using the SuperScript plasmid system (BRL), but using *BstXI* instead of *Sal* I adaptors (Invitrogen). cDNA was size-selected on Chromaspin-1000 (Clontech) and ligated into *Not* I–*BstXI*-digested pJFE14 (24). The MC/9-b2 and J774-a1 cDNA libraries contained  $5.5 \times 10^6$  and  $2 \times 10^6$  independent clones, respectively.

Isolation of mIL-10R cDNA Clones. mIL-10R cDNA clones were isolated by incubating COS7 cells transfected with cDNA library DNA with FLAG-mIL-10 followed by crosslinking and selection (panning) of COS7 cells expressing mIL-10R on plates coated with anti-FLAG mAb (25, 26). After three or four cycles of transfection and panning, the recovered plasmid DNA was highly enriched for mIL-10R cDNAs. Sequence data were obtained as described (15) and analyzed using software from IntelliGenetics.

Analysis of mIL-10R mRNA Expression. mIL-10R mRNA expression was detected by RNA blot (15). mIL-10R RNA was also detected in ethidium bromide-stained gels as an  $\approx$ 500-bp amplified fragment by PCR. PCR primers were 5'-AGGCAGAGGCAGCAGCAGGCCCAGCAGAATGCT-3' (sense) and 5'-TGGAGCCTGGCTAGCTGGTCACAGTAG-GTCT-3' (antisense).

**Expression of mIL-10R in Transfected Ba/F3 Cells.** The mIL-10R cDNA pMR29 was cotransfected into Ba/F3 cells (17) with a plasmid containing a gene for neomycin resistance. Transfectants were selected in 1 mg of G418 per ml as described (17) and mIL-10R-expressing cells (BaMR29a) were twice sorted and expanded in culture prior to use in experiments. Ba/F3 cells transfected with the neomycin-resistance plasmid alone (BaF-Neo) served as a negative control. Transfected cells (5000 per well) were tested for response to mIL-10 in 100- $\mu$ l cultures of RPMI 1640 medium/10% fetal calf serum/50  $\mu$ M 2-mercaptoethanol with mIL-10 and/or 10  $\mu$ g/ml mAb for 48 hr. Cell proliferation was measured by a colorimetric assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma) (17, 27).

## RESULTS

**Detection of mIL-10R-Expressing Cells.** We expressed a "FLAG" (18) epitope-tagged mIL-10 that was detected by anti-FLAG mAbs. The biological activities of FLAG-mIL-10—assessed by inhibition of cytokine synthesis by an activated T-cell clone (2, 3) and costimulation of proliferation of the MC/9 mast cell line (13, 15)—were indistinguishable from wild-type mIL-10. Furthermore, anti-FLAG mAbs M1 and M2 added to bioassays *in vitro* did not neutralize the biological activities of FLAG-mIL-10 (data not shown).

Cells and cell lines representative of mIL-10-responsive cells were tested for ability to bind FLAG-mIL-10. A mast cell line (MC/9) and a macrophage cell line (J774) were found to give reproducible but low levels of specific FLAG-mIL-10 binding that were reduced to background by excess mIL-10 as competitor (Fig. 1A). MC/9 cells were routinely grown in the presence of mIL-3, mIL-4, and mIL-10 to achieve optimal cell proliferation, but we found that culturing MC/9 cells in the absence of mIL-10 for ~48 hr prior to assay resulted in superior signals in FACS analysis. This observation suggested that only "unoccupied" mIL-10R could be readily detected by this method or, alternatively, that mIL-10R may undergo ligandinduced down-modulation on MC/9 cells. MC/9 and J774 were subjected to repeated cycles of sorting and selection of the



FIG. 1. Detection of mIL-10R on mouse mast cell (MC/9) and macrophage (J774) cell lines and transfected COS7 cells expressing mIL-10R. FACS histograms are shown for the original MC/9 and J774 cells (A) and for cells after two (J774) or three (MC/9) cycles of sorting and enrichment (B) and compared to results obtained in the presence of a 100-fold molar excess of mIL-10; each panel shows detection of FLAG-mIL-10 bound to IL-10R (right histogram) reduced to background by competition with a 100-fold excess of mIL-10 (left histogram). A portion of the former is shaded to indicate the degree of enhanced IL-10R expression achieved by sorting. Immunofluorescence micrographs of COS7 cells expressing mIL-10R (pMR29) in the absence (C) and presence (D) of mIL-10 competitor are also shown.

3-5% most brightly staining cells (28, 29). Sublines MC/9-b2 (three cycles) and J774-a1 (two cycles) expressing a significantly higher number of receptors were obtained (Fig. 1*B*) and cDNA libraries were prepared from these cells.

**Expression of mIL-10R cDNA Clones.** Two  $\approx$ 3.6-kb cDNA clones, pM3.14 and pJ18, were isolated from the MC/9-b2 and J774-a1 libraries, respectively. COS7 cells transfected with the mIL-10R cDNAs specifically bound FLAG-mIL-10 (Fig. 1 C and D). In contrast to MC/9 and J774 cells, use of the cross-linking reagent was not required for detection of mIL-10R expressed on COS7 cells, probably because of increased avidity for ligand due to higher IL-10R density. hIL-10 also competed effectively with FLAG-mIL-10 for binding to COS-mIL-10R (not shown), consistent with hIL-10's activity on mouse cells (1). Binding of FLAG-mIL-10 to mIL-10R-expressing COS7 cells was not inhibited by a 100-fold molar excess of mIL-2, mIL-3, mIL-4, mIL-5, mIL-6, mIL-7, mIFN- $\alpha$ , mIFN- $\gamma$ , or vIL-10 (not shown).

mIL-10R was visualized as a 120- to 140-kDa protein by cross-linking <sup>35</sup>S-FLAG-mIL-10 bound to mIL-10R expressed on MC/9 and transfected COS7 cells (Fig. 2). No



FIG. 2. Detection of mIL-10R expression by MC/9 cells (fifth cycle of sorting) and pMR29-transfected COS7 cells by cross-linking to <sup>35</sup>S-FLAG-mIL-10. The MC/9 samples are immunoprecipitates and COS7 samples are cleared lysates. The prominent bands near the 49-kDa standard are cross-linked FLAG-mIL-10 homodimers.

such species was detected in mock-transfected COS7 cells (not shown). Subtraction of the mass of a single FLAGmIL-10 polypeptide chain ( $\approx 20$  kDa) gave the estimated size of mIL-10R as  $\approx 110$  kDa. IL-10 is a noncovalent homodimer (1, 21); longer exposures revealed a much fainter  $\approx 160$ -kDa band that we attributed to mIL-10R cross-linked to two FLAG-mIL-10 polypeptides.

mIL-10R Is Related to IFNRs. A 700-bp EcoRI-Sma I fragment from the 5' end of pM3.14 was used as a hybridization probe to isolate another clone from the MC/9-b2 cDNA library (pMR29) that was then used for sequence analysis. pMR29 encoded an open reading frame of 576 amino acids (Fig. 3), including a putative signal peptide sequence of 16 amino acids (34), a 222-amino acid extracellular domain, a transmembrane segment of 24 amino acids (35), and a cytoplasmic domain of 314 amino acids. The calculated molecular mass of mIL-10R is only  $\approx 63$  kDa, in contrast to the observed size of  $\approx 110$  kDa, suggesting that, as observed with other CRs (36), mIL-10R may be glycosylated at one or more of the four potential sites identified (Fig. 3). The nucleotide and predicted protein sequences of mIL-10R were novel, having neither identity nor close primary sequence relationship to any sequences in the GenBank (Release 74), Protein Identification Resource (Release 34), and Swissprot Protein Sequence (Release 23) data bases. pM3.14 and pJ18 differed only at the 3' end, and their open reading frames were identical.

IL-10 is predicted to be in the four  $\alpha$ -helix bundle cytokine family (37), and most CRs for these cytokines (for example, IL-2-IL-7 and granulocyte and granulocyte/macrophage colonv-stimulating factors) are in the class I group of the CR superfamily (31). However, mIL-10R lacks the two distinctive sequence motifs of four conserved cysteines (paired sequentially), a tryptophan in the N-terminal portion (C-X9-20-C-X-W-X<sub>22-36</sub>-C-X<sub>8-25</sub>-C, where X is any amino acid), and a nearly invariant "WSXWS" box near the C terminus of the extracellular binding domains of class I CRs (31, 38). In contrast, the structure of mIL-10R is most similar to class II CRs, which include IFNRs, a viral IFNR homolog, and tissue factor (30, 31). Structurally, the extracellular portion of mIL-10R consists of two homologous segments of  $\approx$ 110 amino acids similar in size to the immunoglobulin-like ligand binding domains of the growth hormone receptor (31, 33). The first class II CR domain has two conserved tryptophans and the second cysteine pair of class I CRs, whereas a unique disulfide loop is formed in the second domain (31) as shown in Fig. 3.

mIL-10R mRNA Expression. RNA blot analysis revealed an  $\approx$ 3.6-kb mIL-10R mRNA species (Fig. 4) in a number of cells or cell lines, including cells known to respond to mIL-10 [mouse B cells and thymocytes, MC/9 cells, and the macrophage line IG.18LA (3)]. mIL-10R mRNA was detected in NFS60 by PCR. No mIL-10R expression was detected by RNA blot, FACS, or PCR in EL4.E1, P815, Ltk<sup>-</sup>, and Ba/F3 cells. Enhanced IL-10R expression by MC/9-b2 cells was not accompanied by markedly increased mRNA expression, suggesting that higher mIL-10R expression by these cells might be due to reduced receptor recycling or to posttranslational events. We also analyzed restriction enzyme digests of genomic DNA from three mouse strains (BALB/ CyJ, SJA/9, P/J) by hybridization to the entire pMR29 cDNA insert: identical band patterns were observed, suggesting that the mIL-10R gene is likely present in a single copy (not shown).

mIL-10R Transduces a Growth Signal in an IL-3-Dependent Cell Line. The mIL-10R cDNA pMR29 was transfected into

mIL-10R	MLSRLLPFLVTISSLS
IL-10R ≱ Myx IFNRh mIFNgR	้ง ที่มี เรา Edpepp สามาร์ เป็นการ์ข้อมา เรา เป็น เป็น เป็น เป็น เป็น เป็น เป็น เป็น
mIFNaR D1 mIFNaR D2	1 ENLIKPIENIIDIVIIJDDNYTILKAVSSHG.ESMIGSVIFSAEVITKDEAKAVILKVPEGOHTITIKGERPISLD.TNVYIKTORRVIRAEGGNSTISSANNEV.DPFIPFTTA 203 MPVDGNIGVNGADGKSVVILVAVILA SADVIGRAGUN POVSKSSGSSHSDKAVPEDITAANVADTIKUVEGOD TVVTGTELIVADASGS VI
mIL-10R	1 LEFIAYGTELPSPSYWWFEARFFOHLLHWKPIPNOSESTYYEVALKOY GNSTWNDIHICRKAQALSOUTTETLDLYHRSYGYFARYRAVDNSOYSWWTTETRFTVDEVIL
Class II Moti	" 19 GSVN PGLKTN SSKE KFTKE RSPE, TFSCHWTDEV, KN LGPIGLF TRRNTGE WTGEWK, EPPDYV, NSCYFNSSF, TSIWIPECIKLTSNGG, TVDEKCFSVDEIV
11.10R #	
Myx IFNRh	81 RESPREVOLVATGT SVEVLANDEV VIA. GOEVSVYGHSFCDYURGENT HELFSKNKR. AEYVYPGRY. CONVEC. ARSIDSO. ESVEATAVLTYG. DSYRSEAGVEVENPELAKREV
mIFNgR	104 KV OPPOLEIRRKKEEOLSVLVFHPEVVVNGESOGTMFGDGSTC. YTEDWTVVEHNRSGEILHTKHTVEKEECNETLCELNISVSTLDSAVOISS. FWQVATEKSKOVGIPPFHODRK
mIFNaR D1	101 HMSPPEVALEAEDK AILVAISPPGQDGM
mIFNaR D2	306 LPPPPVUTVTAMSD TULVTVMVCQDSTCD
mIL-10R	112 LTV DSVT LKAMDGI I YOT HPPRPTIT PA GDE YEQV FK. DLRV VAISI RKFSELK. NATKRVKQET FTUTVPI GVRKFCVKVLPRLE. SRINKA EWSEECULITTEQYF
Class II Moti	insert Y
hGHR	124 QPDEPEIANLWTLLN+DIQVRWEARARNADI
mIL-10R	220 VTN <mark>LSILVISMLLFCGILVCLVLQWYI</mark> RHPGKLPTVLVFKKPHDFFPANPLCPETPDAIHIVDLEVFPKVSLELRDSVLHGSTDSGFGSGKPSLQTEESQFLLPGSHPQIQGTLGKEESPGL
mIL-10R	342 QATCGDNTDSGICLQEPGLHSSMGPAWKQQLGYTHQDQDDSDVNLVQNSPGQPKYTQDASALGHVCLLEPKAP£EKDQVMVTFQGYQKQTRWKAEAAGPAECLDEEIPLTDAFDPELGVHLQ
mll - 10B	ASA DDI AWPPPALAA GYI KOESOGMA SA PPOT PS DOWNOI TEEWSI I GYVSCEDI SI ESWDEAUYI DDI DCGAADOGLI DSI COM VTI DI I OSI OVEC"

FIG. 3. Predicted amino acid sequence of mIL-10R. The putative signal peptide and hydrophobic transmembrane segment are in bold type and are boxed. The extracellular domains of mIL-10R are aligned with homologous sequences from the myxoma virus IFNR homolog (Myx IFNRh) (30) and the mIFN- $\gamma$ R and mIFN- $\alpha$ R (31, 32); the IFN- $\alpha$ R chain features a duplicated binding segment (D1 and D2). Two tiers of aligned chains marked binding domains 1 and 2 refer to the structurally similar domains of the extracellular segments of all CRs (31, 33). Class II CRs have a characteristic set of identifying sequence motifs, emphasized by the identical matches (reverse lettering); conservative substitutions in four of the five IFNR-like sequences are boxed. A prototypical class I CR, the human growth hormone receptor (33), is aligned below the class II sequences to highlight conserved and distinct sequence features. The cytoplasmic segment of mIL-10R follows the transmembrane helix; there are limited regions of similarity to the analogous domains of the IFN- $\gamma$ R and IFN- $\alpha$ Rs (not shown). Potential N-linked glycosylation sites (N X S/T) of mIL-10R were identified at amino acid residues 34, 50, 97, and 166. Numbers in the human growth hormone receptor sequence represent insertions of the indicated number of amino acids that are not depicted.



FIG. 4. mIL-10R expression by various cell lines (RNA blot). The 3.6-kb mIL-10R mRNA (upper) and actin mRNA (lower) are shown. J774-a3 cells were from the fourth cycle of sorting. IG.18LA is a thymic stromal macrophage line that responds to IL-10 (3). Poly(A)<sup>+</sup> RNA from these two cell lines was used; the remaining samples were total RNA, including a pre-mast cell (IC2), mast cells (PT18, MC/9), myeloid leukemia cells (FDC-P1), two T-cell lines (CTLL, HT2), fresh low-density splenic B cells, and small, dense, resting splenic B cells stimulated with mIL-4 and lipopolysaccharide (LPS). Mouse thymocytes were isolated from 3- to 4-week-old BALB/c mice. Superscript a, low-density splenic B cells stimulated by Percoll density gradient (14); superscript b, small, dense, resting splenic B cells stimulated by LPS (50  $\mu$ g/ml) and mIL-4 (100 units/ml) for 48 hr.

Ba/F3 cells, which expressed little or no mIL-10R (Fig. 4). The resulting mIL-10R<sup>+</sup> cell line BaMR29a (Fig. 5A) was tested for ability to bind <sup>125</sup>I-hIL-10 and respond to mIL-10. BaMR29a cells expressed about 4000 mIL-10Rs and bound <sup>125</sup>I-hIL-10 with a  $K_d$  of about 70 pM, a relatively high affinity among CRs (Fig. 5B). Neither BaF-Neo nor parental Ba/F3 cells gave a proliferative response to mIL-10, but BaMR29a cells responded to mIL-10 in a dose-dependent fashion in an MTT assay (Fig. 5C) and by [<sup>3</sup>H]thymidine incorporation (not shown). The response was inhibited by a neutralizing anti-mIL-10 mAb and thus was specific for mIL-10.

BaMR29a cells also responded to hIL-10 (not shown), as expected since hIL-10 is active on mouse cells (1).

## DISCUSSION

We have demonstrated the existence of receptors for IL-10. Using epitope-tagged mIL-10, we identified cells expressing mIL-10R and isolated cDNA clones encoding mIL-10R. COS7 cells transfected with mIL-10R cDNA clones express cell surface receptors that bind mIL-10 and hIL-10 specifically. The recombinant mIL-10R is indistinguishable in size from mIL-10R



FIG. 5. Expression of mIL-10R in transfected Ba/F3 cells and their response to mIL-10. (A) FACS analysis of mIL-10R expression by BaMR29a cells as detected by binding of FLAG-mIL-10 (shaded) or FLAG-mIL-10 in the presence of a 100-fold excess of mIL-10. BaF-Neo cells did not bind FLAG-mIL-10 (not shown). (B) Scatchard analysis of <sup>125</sup>I-hIL-10 binding to BaMR29a cells. The data were plotted and analyzed by a linear least-squares fit and gave a  $K_d$  of  $\approx$ 70 pM,  $\approx$ 4000 mIL-10Rs per cell, and a coefficient of variation r = 0.989. (C) Response of BaMR29a (mIL10) and BaF-Neo (BaF3-neo) cells to mIL-10 as measured by MTT assay. Responses in the presence of 10  $\mu$ g of anti-mIL-10 per ml (2A5) or isotype control (GL113) mAb are shown. Error bars indicate the range of duplicate samples. mIL-10 units are based on a cytokine synthesis inhibition assay for mIL-10 (3); a concentration of 1 unit/ml gives a half-maximal response.

expressed by cells from which the cDNA clone was isolated (MC/9) (Fig. 2). mIL-10R mRNA was expressed in all cells examined that were known to respond to mIL-10 (Fig. 4). Moreover, mIL-10 stimulated proliferation of Ba/F3 cells expressing recombinant mIL-10R (Fig. 5). We are not yet certain whether the recombinant mIL-10R is itself sufficient to transduce a signal to the cell in response to ligand binding or if additional required receptor or signaling components are provided in trans by Ba/F3 cells. However, we conclude that the mIL-10R that we identified is a functional receptor for IL-10, because it binds the ligand specifically and can mediate transduction of a biological response to IL-10.

It was not possible to obtain accurate ligand affinity measurements for mIL-10R using FLAG-mIL-10. However, Tan et al. (21) have detected mIL-10R and hIL-10R using <sup>125</sup>I-labeled hIL-10. Recombinant mIL-10R expressed by BaMR29a cells binds <sup>125</sup>I-hIL-10 with a  $K_d$  of  $\approx$ 70 pM (Fig. 5C). Tan et al. (21) observed that the  $K_d$  for mIL-10R on MC/9 cells is similarly in the 50-170 pM range and have obtained similar results for recombinant mIL-10R expressed by our cDNA clone on COS7 cells (J. Tan & C.-C. Chou, personal communication). This value is likely similar to the affinity of mIL-10R for mIL-10, since the specific activities of mIL-10 and hIL-10 on mouse cells are similar (1), and reflects a relatively high affinity compared to other CRs (38), which have  $K_d$  values ranging from 30 pM for the high-affinity erythropoietin receptor to 120 nM for the low-affinity hIL-3 receptor.

Since the  $K_d$  for recombinant mIL-10R and mIL-10R on MC/9 cells are similar, it is possible that mIL-10R encoded by the cDNA clones accounts for most or all mIL-10R on MC/9 cells (Fig. 2). That binding of FLAG-mIL-10 to recombinant mIL-10R was not inhibited by excess vIL-10 is also consistent with the lack of activity of vIL-10 on MC/9 cells (15); moreover, we have so far been unable to detect significant levels of binding of FLAG-vIL-10 to COS-mIL-10R (Y.L. and K.W.M., unpublished data). However, like the cellular cytokine, vIL-10 inhibits macrophage activation and stimulates human and mouse B cells (1), suggesting the possible existence of additional IL-10R components on B cells and activated macrophages.

Macrophage activation by a number of stimuli, including IFN- $\gamma$ , is inhibited by IL-10 (1). In this context, the observed relationship of IL-10R to IFNR suggests several possibilities. First, in view of the demonstration of shared subunits among receptors for different cytokines (39), 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 mIL-10 binding to recombinant mIL-10R, and up to a 100-fold excess of hIL-10 did not compete for binding to hIFN- $\gamma R$  on U937 cells (C.-C. Chou & C. Lunn, personal communication). However, the IL-3, IL-5, and granulocyte/macrophage colony-stimulating factor receptor system (39) shows that although a group of CRs shares a common secondary chain, each CR is only capable of binding its cognate cytokine. A second possibility is that IL-10R activation might directly antagonize the IFNR signal transduction pathway (40, 41), possibly by interacting with one or more intracellular components. Evaluation of these possibilities awaits further characterization of the structure and signal transduction mechanisms of IL-10R and IFNR.

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