

## Characterization of Interleukin-10 Receptors on Human and Mouse Cells\*

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Jimmy C. Tan, Stephen R. Indelicato, Satwant K. Narula, Paul J. Zavodny, and Chuan-Chu Chou†

From Schering-Plough Research Institute, Kenilworth, New Jersey 07033-0530

**Human interleukin (IL)-10 is a pleiotropic cytokine acting on a variety of immune cells. Here we show that the protein can be enzymatically iodinated to high specific radioactivity with retention of biological activity. The radiolabeled ligand binds specifically to its receptor in several mouse and human cell lines, notably human B-lymphoma line JY and mouse mast cell line MC/9. Human IL-10 apparently binds as a dimer to a single class of receptor in both the JY and MC/9 cell lines with a  $K_d$  in the 50–200 pM range. Interestingly, mouse IL-10 was capable of blocking binding of human IL-10 to mouse but not human cells. There appears to be at most only a few hundred IL-10 receptors/cell for both mouse and human cell lines examined. Chemical cross-linking of the radioiodinated hIL-10 to JY and MC/9 cells revealed a common protein complex with an apparent molecular mass of about 97 kDa. Additional high molecular weight complexes were detected with JY but not MC/9 cells.**

Interleukin (IL)-10 is a cytokine produced by B cells, T helper cells, and cells of the monocyte/macrophage lineage that exhibits diverse activities on different cell types (for reviews, see Refs. 1–3). IL-10 was initially reported as an activity produced by mouse TH2 cells that inhibited the production of other cytokines, notably interferon- $\gamma$ , in TH1 cells (4). This cytokine synthesis inhibitory factor activity was the basis for the isolation of the mouse IL (mIL)-10 cDNA by expression cloning; the mouse cDNA was subsequently used to isolate the human IL (hIL)-10 clone (5, 6). On lipopolysaccharide-stimulated monocytes, IL-10 inhibits the synthesis of other cytokines, down-regulates the expression of major histocompatibility complex class II antigens, and suppresses the release of reactive oxygen intermediates (7–9). These effects reflect deactivation of macrophage activity. In addition, IL-10 can act as a growth co-factor for mature and immature mouse thymocytes as well as a differentiation factor for cytotoxic T cells (10–12). It also enhances the viability and increases major histocompatibility complex class II expression of small dense splenic mouse B cells (13).

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† To whom correspondence and reprint requests should be addressed: Dept. of Biotechnology/Molecular Biology, Schering-Plough Research Inst., Kenilworth, NJ 07033-0530. Tel.: 908-298-3064; Fax: 908-298-3083.

<sup>1</sup> The abbreviations used are: IL, interleukin; hIL, human interleukin; mIL, mouse interleukin; CHO, Chinese hamster ovary; FBS, fetal bovine serum; GM-CSF, granulocyte macrophage colony-stimulating factor; PBS, phosphate-buffered saline; MTT, 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide.

Finally, in combination with IL-3 or IL-4, IL-10 is able to enhance the growth of mouse mast cells (14). Thus, IL-10 can exert both inhibitory and stimulatory activities on different cell types.

While all of these studies have helped elucidate the biological functions of IL-10, the IL-10 receptor remains largely uncharacterized. Here we report the initial biochemical characterization of the IL-10 receptor. We show that human IL-10, expressed in CHO cells, can be radioactively labeled to high specific activity without loss of biological activity. This labeled ligand was used to characterize specific binding to both human and mouse cell lines.

### MATERIALS AND METHODS

**Cell Lines and Tissue Culture**—MC/9 cells (ATCC CRL1649) were routinely grown in Dulbecco's modified Eagle's medium with 10% fetal bovine serum (FBS) containing 3–5% mitogen-stimulated spleen-conditioned media, 100 units/ml mIL-4, 10 units/ml Pen/Strep, 2 mM glutamine, 1 mM sodium pyruvate, 1  $\times$  minimum Eagle's medium essential and nonessential amino acids, 1  $\times$  minimum Eagle's medium vitamins, 50  $\mu$ M  $\beta$ -mercaptoethanol, 6 mg/liter folic acid, 116 mg/liter L-arginine, and 36 mg/liter L-asparagine. TF-1 cells (15) were grown in RPMI 1640 medium with 10% FBS and 1  $\mu$ g/l recombinant human GM-CSF. JY cells (provided by J. de Vries, DNAX, Palo Alto, CA) were grown in Dulbecco's modified Eagle's medium with 10% FBS, 6 mM glutamine, and antibiotics. The other cell lines (Ramos (ATCC CRL 1596), WEHI 265.1 (TIB 204), U937 (CRL 1593), HL-60 (CCL 240), JD (CRL 8163), Jiyoye (CCL 87), THP-1 (TIB202), B-JAB (provided by J. Blanchereau, Schering-Plough France), BH-5, and C10 (provided by W. Tadmori, SPRI)) were grown in RPMI with 10% FBS, 6 mM glutamine, and antibiotics. In addition, culture media for BH-5 and THP-1 cells were supplemented with 50  $\mu$ M  $\beta$ -mercaptoethanol. All tissue culture reagents were from Life Technologies, Inc.

**Cytokines and Antibodies**—Recombinant CHO-derived human IL-10 and IL-5, as well as *Escherichia coli*-derived human GM-CSF, interferon- $\gamma$ , and mouse IL-10 were supplied by SPRI. The specific biological activity of these preparations were  $2.3 \times 10^7$  units/mg for hIL-10 and  $1.6 \times 10^7$  units/mg for mIL-10 as measured by the MC/9 proliferation assay (see below). One unit is defined as the amount of IL-10 needed to stimulate 50% maximal proliferation of 5,000 MC/9 cells in 100  $\mu$ l of assay media in a 48-h period. Recombinant hIL-6 was purchased from Genzyme Corp. (Cambridge, MA). Monoclonal antibodies to IL-10 and IL-5 (16) were provided by J. Abrams (DNAX, Palo Alto, CA).

**Iodination of hIL-10**—Purified hIL-10 protein was labeled using the Enzymobead radioiodination reagent (Bio-Rad), which is an immobilized preparation of lactoperoxidase and glucose oxidase, following the manufacturer's protocols. The purified protein was passed through a PD-10 column (Pharmacia LKB Biotechnology Inc.) to remove free label. Additional samples were also custom-iodinated following the lactoperoxidase method (Du Pont-New England Nuclear). Specific radioactivity obtained was in the range of 100–180  $\mu$ Ci/ $\mu$ g hIL-10. The iodinated material was then passed through a 120-ml Sephadex G-75 column (Pharmacia) with 1.1-ml fractions collected in phosphate-buffered saline (PBS). Trichloroacetic acid precipitation was done by incubating aliquots of the fractions in 10% trichloroacetic acid for 1 h at 4  $^{\circ}$ C. Pellets formed after centrifugation were then counted in Clinigamma counter (Pharmacia).

**MC/9 Proliferation Assay**—Biological activity of hIL-10 was determined using the colorimetric MTT dye-reduction assay (17, 18). Briefly, 5,000 MC/9 cells/well in 100  $\mu$ l of media containing 100 units of mL-4/ml in a 96-microtiter well were treated for 48 h with varying amounts of human IL-10. The hIL-10 standard was used at a maximum of 200 units/100  $\mu$ l and 2-fold serially diluted. Twenty-five microliters of 5 mg/ml MTT was added and incubated for 3–5 h. The cells were then detergent-lysed in 10% SDS with 10 mM HCl, and the plates were read for absorbance at 570 nm.

**Binding Assays and Scatchard Analysis**—Approximately  $5 \times 10^6$  cells for each cell line tested were pelleted by centrifugation at  $200 \times g$  for 10 min, washed in PBS, and resuspended in 200  $\mu$ l of binding buffer (PBS, 10% fetal calf serum, 0.1% NaN<sub>3</sub>) containing iodinated hIL-10 at a concentration of 100–500 pM. After incubation at 4 °C for 2 h in a rotary mixer, the cells were centrifuged at  $200 \times g$  for 10 min, resuspended in 100  $\mu$ l of binding buffer without labeled hIL-10, layered over 200  $\mu$ l of a 1:1 mixture of dibutyl- and dioctylphthalate oils in elongated microcentrifuge tubes, centrifuged at  $400 \times g$  for 5 min at 4 °C, and quickly frozen in liquid nitrogen. The cell pellets were then cut and counted in a Clinigamma 1272 counter (Pharmacia). Nonspecific binding was determined by performing the binding in the presence of 500–1000-fold molar excess unlabeled hIL-10. For saturation binding experiments, 2-fold serial dilutions of approximately 600 pM solution of iodinated hIL-10 were used, with a parallel series done to determine nonspecific binding. The dissociation constants ( $K_d$ ) and maximal concentration of ligand bound to cells ( $B_{max}$ ) were calculated by Scatchard analysis of the saturation binding data using linear regression analysis with the EBDA program (Elsevier-Biosoft, Cambridge, U.K.). The results of Scatchard analysis were replotted with the program Cricket Graph (Computer Associates International, San Diego, CA). Antibody inhibition was performed under the above binding conditions but with the addition of a 100-fold molar excess of each of the indicated monoclonal antibodies. Cytokine specificity was determined under similar conditions but with the addition of 500-fold molar excess of the cytokines indicated.

**Chemical Cross-linking**—About  $2 \times 10^6$  cells were incubated for 4 h at 4 °C in 2 ml of binding medium consisting of PBS, 0.1% NaN<sub>3</sub>, 10% bovine serum albumin, and 200 pM <sup>125</sup>I-hIL-10 with or without 200 nM unlabeled hIL-10. The cells were washed 2 times with PBS and then resuspended in 1 ml of PBS. To the cell suspension, 10  $\mu$ l of 15 M stock 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (Pierce Chemical Co.) was added, and the cells were incubated for 1 h at room temperature with constant rocking. The reaction was stopped by washing the cells twice with cold PBS followed by the addition of 150 mM glycine (pH 7.2) buffered with Tris-HCl. The cells were collected by centrifugation and then lysed by adding 1 ml of the lysis buffer, which contains 10 mM Tris-HCl (pH 7.5), 140 mM NaCl, 2 mM EDTA, 1 mg/ml leupeptin (Sigma), 2 mM Pefabloc SC (Boehringer Mannheim), 2 mM iodoacetamide (Sigma), 2 mM o-phenanthroline (Sigma), and 1% Triton X-100 (Sigma). The lysates were centrifuged at  $10,000 \times g$  for 20 min at 4 °C. The supernatants were harvested and incubated overnight at 4 °C with rabbit anti-hIL-10 polyclonal antiserum (provided by Dr. G. Seelig, SPRI) presorbed to protein G resin (Pierce Chemical Co.). Each sample contained 20  $\mu$ l of the resin. After incubation the resin was washed three times with PBS and finally resuspended in 20  $\mu$ l of the SDS-electrophoresis buffer without reducing agent. Twenty microliters of each sample were then electrophoresed on a 4–15% gradient polyacrylamide gel (Daiichi Chemicals Co., Tokyo) in the presence of SDS according to Laemmli (19) under nonreducing conditions. A set of prestained molecular weight markers (Life Technologies, Inc.) was co-electrophoresed to determine the size of the cross-linked complexes. After electrophoresis, the gel was dried and exposed to a Kodak XAR film for 48 h at –80 °C with two intensifying screens.

## RESULTS

**Lactoperoxidase-labeling Method Retains the Biological Activity of hIL-10**—Purified CHO-derived hIL-10 was iodinated to high specific activity (100–200  $\mu$ Ci/ $\mu$ g protein) using the lactoperoxidase method. Initial attempts to label CHO-derived hIL-10 with the IODO-GEN reagent (Pierce Chemical Co.) resulted in protein of insufficient specific activity to be used in receptor characterization (data not shown). The lactoperoxidase method yielded iodinated hIL-10 with a specific

activity approximately 5-fold higher than that obtained with IODO-GEN.

To determine if the high specific activity-labeled hIL-10 is biologically active, samples were examined for their ability to induce MC/9 cell proliferation (14). Fig. 1 shows the assay result with 50 ng/ml iodinated hIL-10 in comparison with the same concentration of unlabeled protein. The estimated activity for the sample was  $1.5 \times 10^7$  units/mg compared with  $2.32 \times 10^7$  units/mg for the standard, giving a retention of 64% biological activity for this sample. Repetition of biological assay results with other samples of iodinated hIL-10 indicated routinely greater than 50% biological activity retention.

**The Active Form of Radiolabeled hIL-10 Appears to Be a Dimer That Binds Specifically to Its Cellular Receptors**—The labeled protein mixture, when passed through a Sephadex G-75 gel-filtration column, was resolved into three distinct species (Fig. 2A). This fractionation was found to be necessary to reduce background binding to target cells. The largest species was a high molecular weight form, which elutes with the excluded volume. The smallest eluted between the lowest molecular mass standard (13.7 kDa) and the dye marker bromphenol blue. Sizing with molecular mass standards showed the second species to be approximately 37 kDa (Fig. 2B), consistent with the predicted molecular mass for a hIL-10 dimer. Polyacrylamide gel electrophoresis of the three species in the presence of SDS (Fig. 2C) revealed that the high molecular mass form runs between 43 and 200 kDa and most likely represents an aggregated form of human IL-10. The second species, under these conditions, migrated at approximately 18 kDa, while the third species was not observed at all. The radioactivity associated with the large and middle species was trichloroacetic acid-precipitable while that with the small species was not (data not shown). About 47 and 43% of the counts were trichloroacetic acid-precipitable for the large and the middle species, respectively. Less than 1% of the counts was recovered with the smallest species; this degradation product was probably too small to be precipitated.

Based on the observation that the radioiodinated hIL-10 was biologically active, fractionated samples were tested for their ability to bind specifically to candidate cell lines. Because MC/9 cells have been shown to respond to hIL-10 by proliferation (14), they were first used to determine the bind-

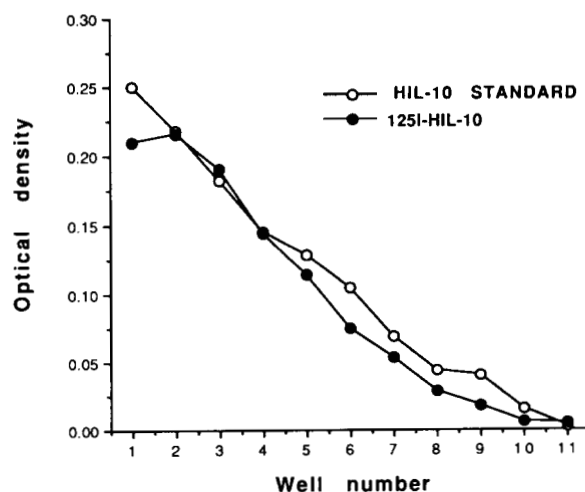
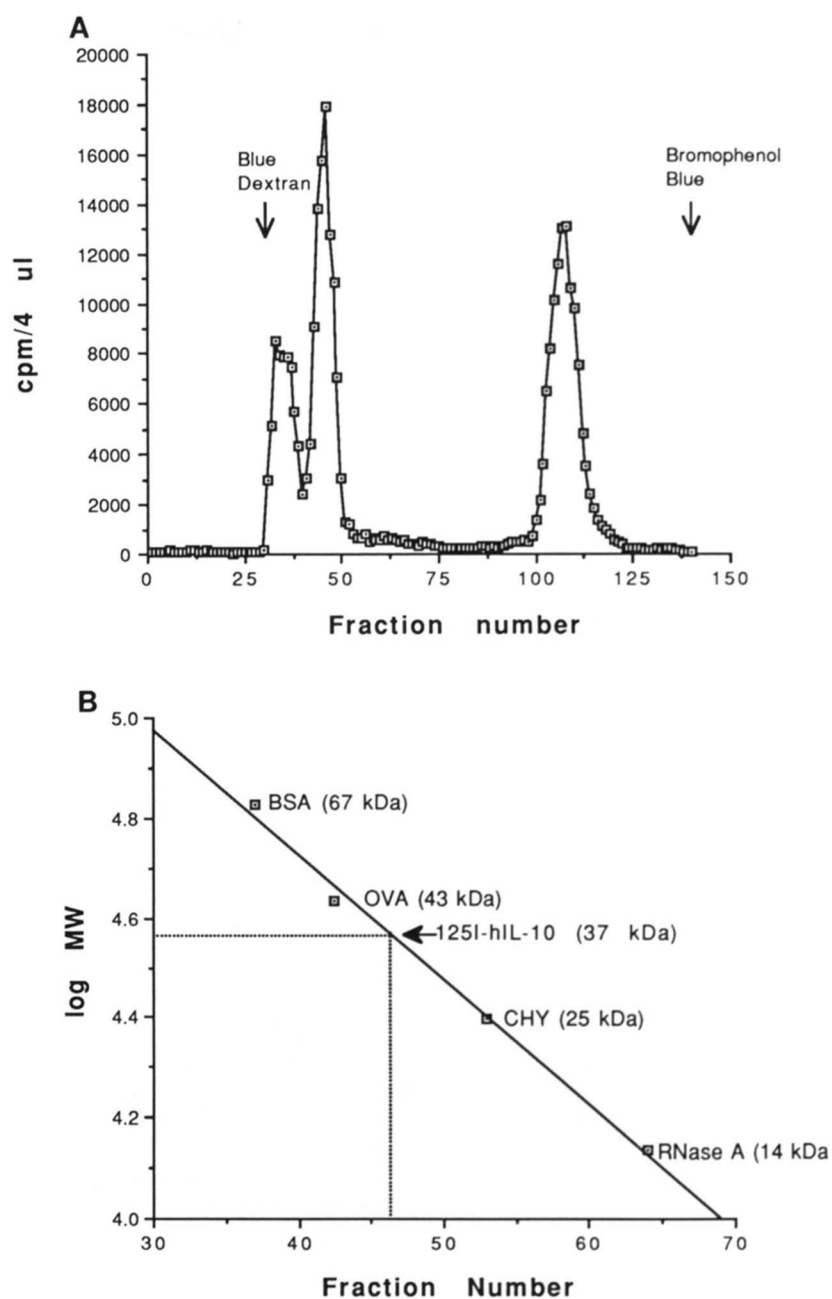


FIG. 1. MC/9 proliferation assay of radioiodinated hIL-10. Unfractionated hIL-10 at a concentration of 50 ng/ml was tested for its proliferative effect on MC/9 cells using the colorimetric MTT assay (18). Unlabeled hIL-10, used also at 50 ng/ml at the highest concentration, was examined in parallel. The samples were 2-fold serially diluted for assay.



**FIG. 2. Gel filtration chromatography of radioactively labeled hIL-10.** **A**, approximately 0.4 ml of lactoperoxidase-iodinated hIL-10 was passed through a 120-ml G75-Sepharose column and collected in 1.1-ml fractions. Radioactivity was determined from fraction aliquots with a Clinigamma Counter. Blue dextran and bromphenol blue were used as markers for the void and total volumes, respectively. **B**, low mass markers were applied to the G75 column, 1.1-ml fractions were collected, and concentrations of these fractions were determined using the BCA protein detection system. The fractions of peak protein concentrations were then plotted against the logarithms of the molecular masses. BSA, bovine serum albumin; OVA, ovalbumin; CHY, chymotrypsin. **C**, approximately 20,000 cpm from the three peak fractions were partitioned in a 15% SDS-polyacrylamide gel electrophoresis under nonreducing conditions. At the completion of the electrophoresis the gel was vacuum-dried on Whatman paper followed by autoradiography using XAR film. Molecular mass markers were visualized by Coomassie Blue staining.

ing specificity of hIL-10. When the three species fractionated from the G-75 column were tested for binding to MC/9 cells, the 37-kDa species, but not the other two, was able to bind to a high degree; moreover a 500-fold molar excess of unlabeled human IL-10 protein could block greater than 90% of the labeled IL-10 binding (data not shown). Although the binding assays were performed using 500–1000-fold molar excess unlabeled hIL-10, in later experiments we found that the binding of radiolabeled hIL-10 to MC/9 cells can be inhibited to a similar extent by the addition of about 150-fold molar excess of unlabeled hIL-10.

To ascertain the specificity of hIL-10 binding to its receptor, other cytokines, as well as monoclonal antibodies to hIL-10, were tested for their ability to inhibit the binding of iodinated hIL-10 to its cell surface receptor. We found that excess hIL-10 was capable of competing with labeled hIL-10 in binding to TF-1 human erythroleukemia cells (data not shown). In contrast to hIL-10, hIL-5, hIL-4, interferon- $\gamma$ , GM-CSF, and hIL-6 were ineffective in competition for binding to TF-1 cells (data not shown). To further demonstrate that the binding of hIL-10 to TF-1 cells was specific, monoclonal antibodies to hIL-10 and hIL-5 were examined for their ability to block binding of iodinated hIL-10 to its receptor. Neutralizing monoclonal antibodies 19F1, 19B1, and 9D7, which were generated against hIL-10, were capable of inhibiting the binding of labeled hIL-10 to TF-1 cells, while an anti-human IL-5 monoclonal antibody was unable to block binding of labeled IL-10 as expected (data not shown).

**Human IL-10 Binds to Cellular Receptors with High Affinities**—After demonstrating the specific binding of fractionated, iodinated hIL-10 to target cell lines and the retention of biological activity of this labeled protein, we sought to determine the binding affinity and estimate the number of binding sites or receptors per cell. Typical saturation binding curves with MC/9 and JY cells are shown in Fig. 3A and 4A, respectively. Maximal binding occurs at approximately 300–400 pM of labeled hIL-10 for both cell lines. Scatchard analyses of representative binding data (Fig. 3B for MC/9 and Fig. 4B for JY) provided linear graphs with slopes yielding a  $K_d$  of approximately 50 pM for the MC/9 cells and 140 pM for the JY cells.  $B_{max}$  values obtained, which represented the maximal concentration of ligand bound to cells, were 4.1 and 7.5 pM for MC/9 and JY cells, respectively. Assuming that one hIL-10 dimer ligand molecule binds one receptor, these results provide an estimate of approximately 100 receptors/cell for MC/9 and 180 receptors/cell for JY. From several independent experiments (data not shown), the human IL-10 binding affinity for JY and MC/9 cells was approximately 50–200 pM, with between 100 and 300 receptors/cell.

**Human IL-10 Binds to Lymphoid, Myeloid, and Mast Cells**—Binding assays with a number of different cell lines indicated that hIL-10 was able to bind to most of these lines to varying extents (Fig. 5). The highest degree of binding was seen with the mouse mast cell line MC/9 and the human B-lymphoma line JY. TF-1 (a human erythroleukemia line) as well as Ramos and BH5 (human B-lymphoma lines) show a reduced level of binding relative to JY and MC/9. Two other B-lymphoma lines, BJAB and Jiyoye, show even lower levels of specific binding. This is also true for the T cell lines C10 and JD, the human promyelocytic leukemia cell line HL-60, as well as the human monocytic lines U937 and THP-1. A binding assay with WEHI 265.1, a mouse monocytic cell line that responds to hIL-10 in proliferation assays, also shows a relatively low level of binding (not shown).

**Mouse IL-10 Receptor Binding Appears to Be Species-specific whereas Human IL-10 Receptor Binding Is Not**—To

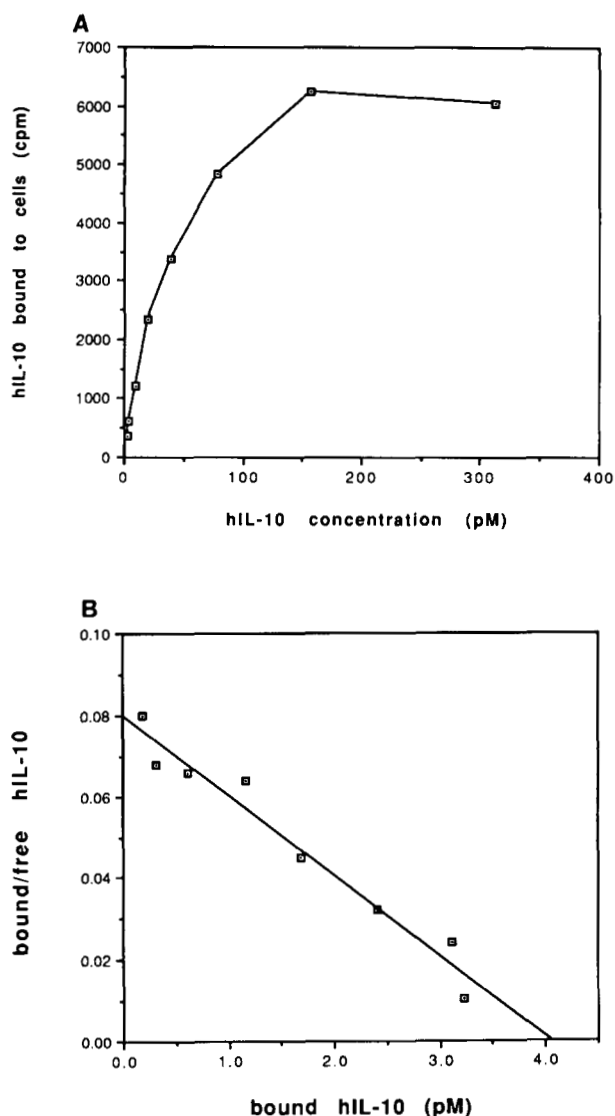
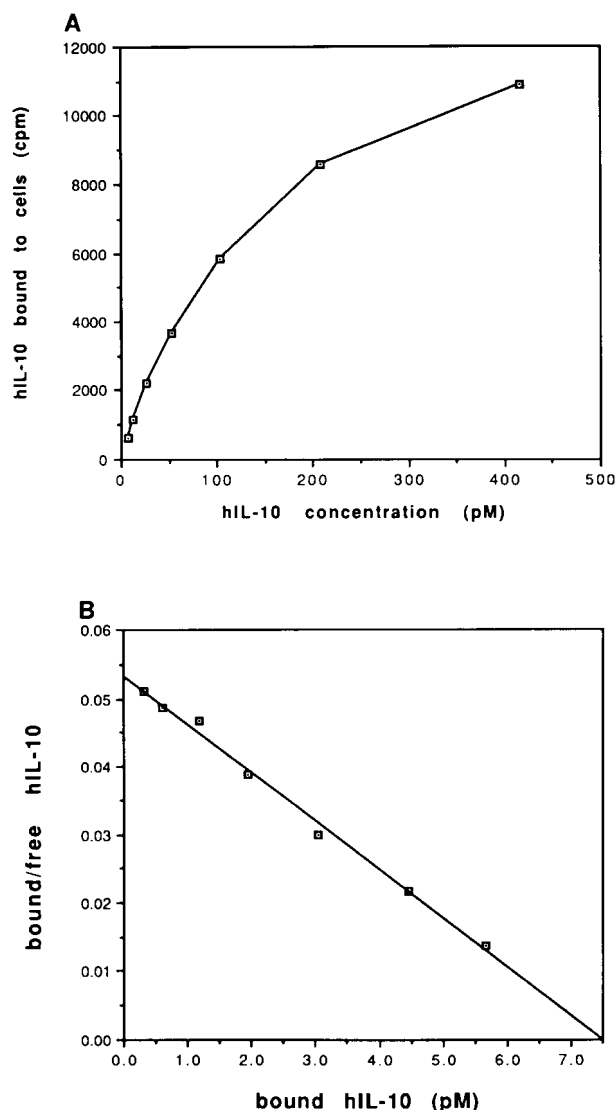


FIG. 3. Saturation binding of MC/9 cells with radioactive hIL-10. A, increasing concentrations of labeled hIL-10 were added to  $5 \times 10^6$  MC/9 cells. Human IL-10 bound to cells was determined by subtracting nonspecific binding from total binding. Points are means of duplicate determinations. B, scatchard analysis of equilibrium binding data.

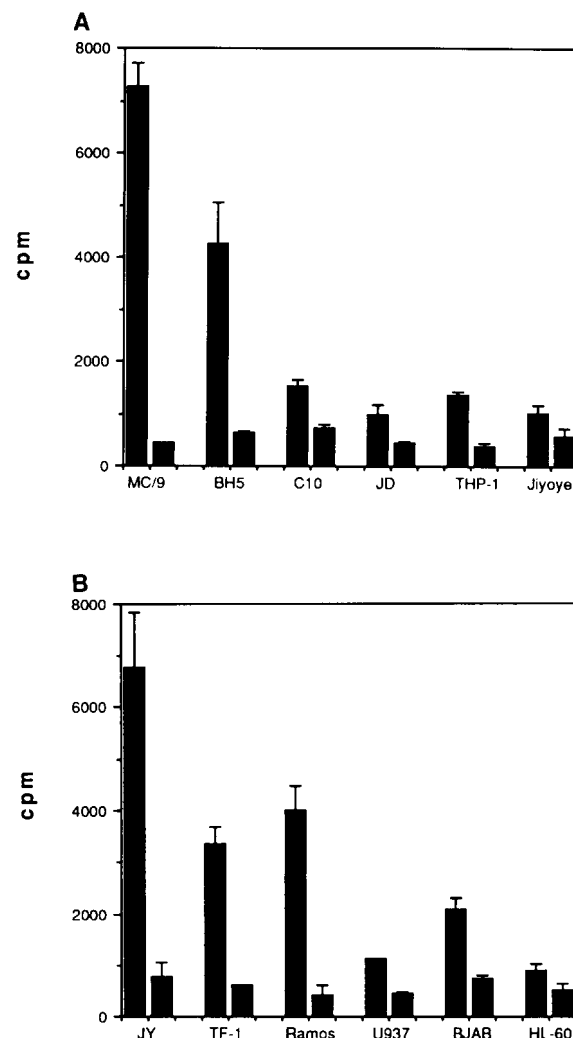
examine the species specificity of receptor binding, the ability of mouse or human IL-10 to compete with labeled human IL-10 for binding to mouse and human cell lines was examined. Fig. 6 shows the results of such competition experiments. Because the specific biological activity of *E. coli*-derived murine IL-10 was 60–70% of human IL-10 as determined by the MC/9 biological assay (see "Materials and Methods"), the concentrations of human and murine IL-10 in the competition experiments were adjusted accordingly. Fig. 6A shows that both mouse and human IL-10 were able to block the binding of labeled hIL-10 to the mouse MC/9 line. In contrast, Fig. 6B shows that human IL-10, but not mouse IL-10, is able to successfully compete with the binding of labeled hIL-10 to the human B-lymphoma line JY.

**Multiple Complexes Were Found after Chemical Cross-linking of Radiolabeled hIL-10 to Its Receptors**—To estimate the size of hIL-10 receptor binding complexes,  $^{125}$ I-hIL-10 was bound to JY and MC/9 cells and treated with bifunctional cross-linker EDC. Because of the low number of hIL-10 receptors with both cell lines tested here, the cell lysates were



**FIG. 4. Saturation binding of JY cells with fractionated radioactive hIL-10.** A, increasing concentrations of labeled hIL-10 were added to  $5 \times 10^6$  JY cells. Human IL-10 bound to cells was determined by subtracting nonspecific binding from total binding. Points are means of duplicate determinations. B, scatchard analysis of equilibrium binding data.

immunoprecipitated after cross-linking with anti-hIL-10 polyclonal antiserum to enrich the binding complexes. The immunoprecipitated proteins were then analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. As shown in Fig. 7, after a 48-h exposure to x-ray film, both JY and MC/9 cells yielded hIL-10-specific binding complexes. The apparent molecular weights were estimated based on co-electrophoresed molecular size standards. A major form of binding complex, estimated about 97 kDa, appeared in both JY and MC/9 cells. JY cells yielded two additional bands at estimated molecular masses of 190 and 210 kDa, respectively. The absence of these two bands in MC/9 cells was apparent with a prolonged exposure of the gel (data not shown), which confirmed that the 190 and 210 kDa protein complexes were specific for JY cells. A few minor bands were also seen migrating between the 68- and 43-kDa markers, which could be degradation products of the higher molecular mass complexes. Cross-linked  $^{125}\text{I}$ -hIL-10 appeared as a band migrating between the 43- and 29-kDa markers (Fig. 7). Formation of all cross-linked complexes was completely inhibited by the



**FIG. 5. Binding of radiolabeled hIL-10 to cell lines.** Approximately  $5 \times 10^6$  cells were used for each sample. A, the two bars of each set indicate binding in the absence or presence of 1000-fold molar excess-labeled hIL-10, respectively, in binding buffer with 500 pM labeled hIL-10. Triplicate binding assays were performed as described under "Materials and Methods." B, two binding experiments using the same preparation of labeled hIL-10 are presented.

presence of 1000-fold molar excess of unlabeled hIL-10 (Fig. 7).

## DISCUSSION

In this report, we show that high specific activity iodinated hIL-10 can be enzymatically prepared and that this labeled ligand can bind in a specific and saturable manner to its receptor in several mouse and human cell lines. MC/9 proliferation assays showed that this labeled protein retains greater than 50% biological activity. The apparent partial loss in activity may result from aggregation and/or degradation, as shown by gel filtration chromatography. Molecular weight sizing of the purified, iodinated protein indicated that the protein exists predominantly as a dimer and in this form is capable of binding specifically to its receptor. The 37-kDa dimer of human IL-10, when examined under reducing conditions, may be dissociated by detergent to a single 18-kDa species. This is consistent with the 37-kDa species representing a noncovalently linked dimer of the cytokine. Because only the 37-kDa species binds to MC/9 cells and radioiodinated hIL-10 retains biological activity, it seems that active

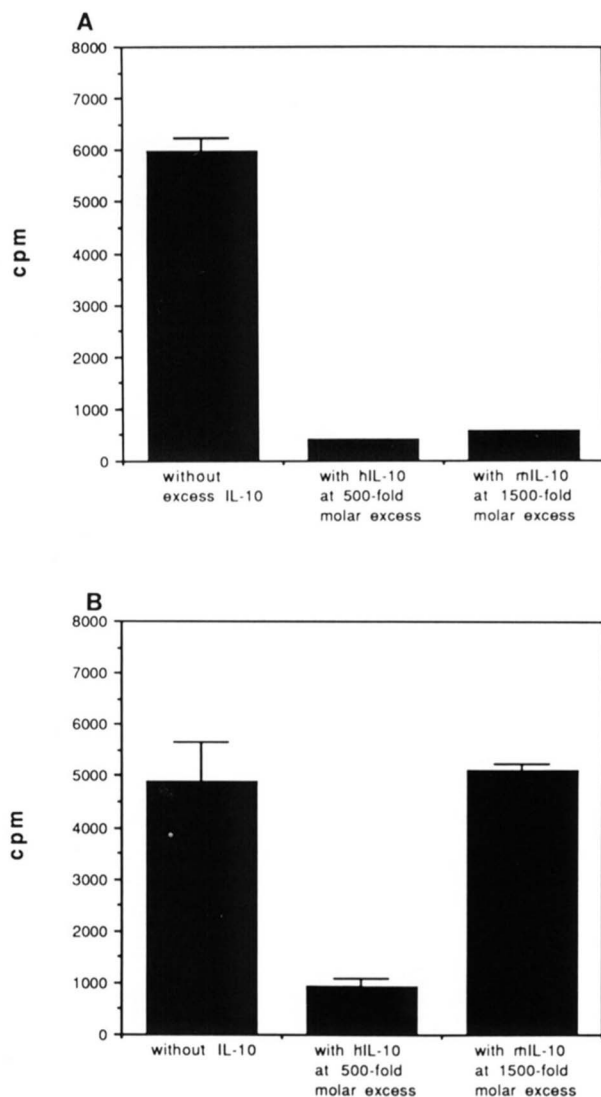


FIG. 6. Human or mouse IL-10 competition with radioiodinated hIL-10 binding to MC/9 or JY cells. A, a 500-fold molar excess of human IL-10 or 1500-fold excess mouse IL-10 was incubated in duplicate with 100 pM iodinated hIL-10 in a binding assay with the mouse mast cell line MC/9. B, same as A except that the human B-lymphoma line JY was used.

hIL-10 is a noncovalently linked dimer.

Screening for specific binding with several cell lines of mouse and human origin indicates that murine mast cell line MC/9 and human B-lymphoma cell line JY have the highest number of unoccupied receptors/cell. Human B cell lines Ramos and BH5, as well as erythroleukemia line TF-1, bind at a reduced level relative to MC/9 and JY. The other human cell lines tested, the EBV<sup>-</sup> B cell lymphoma line BJAB, the EBV<sup>+</sup> B cell lymphoma line Jiyoye, the T cell lymphoma lines C10 and JD, monocytic cell lines THP-1 and U937, as well as the promyelocytic leukemia line HL-60, show reduced and variable levels of specific binding. These cell lines were chosen based on the reported observations that mast cells, macrophage/monocytes, B cells, and T cells respond to IL-10 (3). Perhaps it is not surprising that MC/9 cells demonstrate the highest level of specific binding of labeled hIL-10, because this cell line has been shown previously to respond well to IL-10 in proliferation assays (14). Attempts to demonstrate the possible proliferative effects of hIL-10 on JY cells have been unsuccessful. The TF-1 cell line, originally derived from an erythroleukemic patient, is dependent on IL-3, erythropoietin,

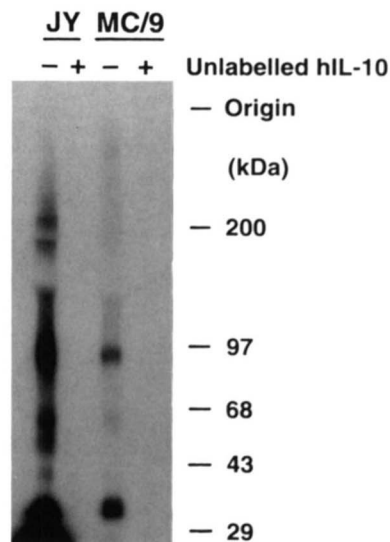


FIG. 7. Characterization of IL-10 receptors by chemical cross-linking. JY or MC/9 cells were cross-linked to <sup>125</sup>I-hIL-10 in the absence or presence of the unlabeled hIL-10 competitor, extracted, immunoprecipitated, electrophoresed, and autoradiographed as described under "Materials and Methods."

or GM-CSF for long term growth (15). The cell line is also responsive to IL-4, IL-5, and IL-6 in proliferation assays (15). Despite the responsiveness of the TF-1 cell line to a variety of cytokines, no proliferative effects on TF-1 cells in response to hIL-10 either alone or in combination with other cytokines could be detected (data not shown).

The  $K_d$  values obtained from Scatchard analysis indicate that hIL-10 binds with relatively high affinity to its receptor on both mouse and human cells and that the receptor is present at the numbers between 100 and 300/cell. Several cytokines and growth factors, for example IL-2 (20), IL-5 (21), and IL-6 (22), possess both high and low affinity binding sites on cell lines. Under the conditions we have used to examine the binding of human IL-10 to cells, we have been unable to detect more than one class of binding site with the JY and MC/9 cell lines. Performing the saturation binding assays with concentrations of <sup>125</sup>I-hIL-10 as high as 1.2 nM yielded similar  $K_d$  values (data not shown). This does not preclude the possibility of the existence of low affinity binding sites on these lines which are not detectable under the binding and washing regimen we employed, or the presence of low affinity binding sites on other cell lines not included in this study. It is also to be noted that our binding analyses only measured the number of unoccupied receptors. It is still possible that there are receptors occupied by IL-10, which may be secreted by the cell lines tested in this work. Indeed, both mast cells (23) and B cells (24) have been reported to express IL-10.

Competition binding assays with human and murine IL-10 on the mouse mast cell line MC/9 and the human cell line JY demonstrated that while the mouse ligand is able to compete with binding of iodinated hIL-10 to the mouse cell line, it cannot do so with the human cell line. One explanation is that under the binding conditions employed, hIL-10 can recognize and bind to both the mouse and the human receptor, while the mouse IL-10 can only recognize the mouse receptor. Our preliminary data using iodinated murine IL-10 show that it is unable to bind to the human cell line JY while still retaining its ability to bind the mouse MC/9 cell line. Supporting this notion of species specificity of the mouse ligand in binding site recognition is the absence of any significant biological cross-reactivity of murine IL-10 on human cells (3).



Examination of the amino acid sequences of human and mouse IL-10 showed that the major differences between the two are in the N-terminal third of the protein (5). This region may contain the sequences responsible for the apparent species specificity of the mouse ligand.

Chemical cross-linking of  $^{125}$ I-hIL-10 to MC/9 and JY cells yielded a common 97-kDa complex (Fig. 7). Because the molecular mass of most reported lymphokine receptors ranges from about 50 to about 140 kDa, for instance IL-1 receptor (25), IL-2 receptor p55 subunit (26), IL-3 receptor  $\alpha$  subunit (27), IL-4 receptor (28), IL-5 receptor  $\alpha$  subunit (29), IL-6 receptor (30), IL-7 receptor (31), IL-8 receptor (32), interferon- $\gamma$  receptor (33), and G-CSF receptor (34), it is reasonable to consider that this 97-kDa complex contains not more than one hIL-10 receptor polypeptide. We then estimated the size of the receptor by subtracting the molecular mass of bound hIL-10 from the 97-kDa complex.

The results of our receptor binding experiments (Fig. 2) showed that the dimer form of hIL-10 is an active binding species. Thus, the 97-kDa cross-linking product observed in the SDS-polyacrylamide gel electrophoresis gel may represent a complex of dimeric hIL-10 (37 kDa) and its receptor of 60 kDa. Cross-linking of fractionated hIL-10 in the absence of cells at 150 mM of EDC showed greater than 70% of the hIL-10 dimer to be effectively cross-linked (data not shown). However, it remains possible that due to inefficient cross-linking in the presence of cells, it may be also composed of a monomer hIL-10 molecule (18 kDa) and one receptor of 79 kDa.

The 190-kDa product from cross-linking with JY cells may be a complex of two hIL-10 dimers and two hIL-10 receptors, while the 210-kDa product may represent a complex containing additionally glycosylated forms of the receptor or other proteins associated with the receptor. Ligand-induced receptor dimerization has been reported for several growth factors, for example interferon- $\gamma$  (35), platelet-derived growth factor (36), and Kit ligand (37). Several lymphokine receptors, for example IL-2 receptor (38), GM-CSF receptor (39), and IL-6 receptor (40), have also been reported to form a multimeric structure upon ligand binding. Thus it is conceivable for the hIL-10 receptor to behave similarly. In the cases of platelet-derived growth factor (36) and Kit ligand (37), receptor dimerization appeared to be important for signal transduction.

The presence of the common 97-kDa band in MC/9 and JY suggests that human and mouse IL-10 receptors are similar in size. In contrast to JY cells, the binding of hIL-10 to MC/9 cells did not form any detectably higher molecular mass complexes other than the one at 97 kDa (Fig. 7), indicating that the mouse IL-10 receptor may not dimerize, at least with the human ligand under the conditions described in this paper. In either case, neither the biological activity nor the affinity of the mouse receptor seems to be dependent on the formation of the higher molecular weight complexes.

In summary, we have detected high affinity hIL-10 receptors in a human B cell line and a mouse mast cell line in binding assays and demonstrated the association of these receptors with the ligand. We have also shown evidence of the formation of higher molecular weight complexes with hIL-10 in JY cells.

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