

The Primary Binding Subunit of the Human Interleukin-4 Receptor Is Also a Component of the Interleukin-13 Receptor*

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Interleukin (IL)-13 elicits a subset of the biological activities of the related IL-4. The basis of this functional similarity is that their specific cell-surface receptors (called IL-13R and IL-4R) are distinct, yet are complex and share a common subunit(s). The IL-4R primary binding subunit (called IL-4R α) does not by itself bind IL-13. We show that the ability of IL-13 to partially compete for IL-4 binding to some human cell types depended on co-expression of IL-4R and IL-13R. However, IL-13 binding was always associated with IL-4 binding. Hyperexpression of IL-4R α on cells expressing both IL-4R and IL-13R decreased their binding affinity for IL-4, abrogated the ability of IL-13 to compete for IL-4 binding, and yet had no effect on IL-13R properties. Anti-human IL-4R α monoclonal antibodies which blocked the biological function and binding of IL-4 also blocked the function and binding of IL-13. These data show that IL-4R α is a secondary component of IL-13R.

Adjacent genes encode the T cell-derived cytokines IL-13 and IL-4 (Morgan *et al.*, 1992; McKenzie *et al.*, 1993a). These proteins are distantly related at the amino acid sequence level and elicit similar proliferative responses and morphological changes on many cell types, including monocytes, B cells, endothelial cells, and natural killer cells. However, unlike IL-4, IL-13 has no effect on T cells (reviewed in Zurawski and de Vries (1994)). The overlap of these biological functions appears to be due to the specific high affinity cell surface receptors for IL-4 and IL-13 being distinct multisubunit complexes which share at least one component. The direct evidence to support this notion is 1) IL-13 can partially compete for IL-4 binding to a human cell line that responds to both IL-13 and IL-4, but cannot compete for IL-4 binding to a human T-cell line or to cells expressing the 130-kDa IL-4R α protein (Zurawski *et al.*, 1993a); and 2) a mutant human IL-4 antagonist protein, which binds to IL-4R α with an affinity equal to that of hIL-4 and binds ~100-fold less well than hIL-4 to IL-4R, also inhibits the

biological action of IL-13 (Zurawski *et al.*, 1993a; Aversa *et al.*, 1993). In addition, the complex nature of IL-4R has been inferred from observations of IL-4R α -associated proteins (Foxwell *et al.*, 1989; Galizzi *et al.*, 1990a; Harada *et al.*, 1990) and heterogeneity in IL-4 binding (Foxwell *et al.*, 1989; Fernandez and Vitetta, 1990, 1991) and activation (Rigley *et al.*, 1991) studies. Recently, the IL-2 receptor γ subunit (IL-2R γ) has been identified as a component of human and mouse IL-4R that functions in signal transduction and slightly enhances the ability of IL-4R α to bind IL-4 (Russell *et al.*, 1993; Kondo *et al.*, 1993, 1994).

In this work, we have further studied the ability of IL-13 to cross-compete for IL-4 binding on human and monkey cell types and utilized for the first time direct IL-13 binding analyses to characterize human IL-13R properties. In addition, we have used anti-human IL-4R α mAbs to show that IL-4R α is a component of IL-13R.

EXPERIMENTAL PROCEDURES

Cell Culture and Reagents—Purified hIL-4 (10⁷ units/mg), produced in *Escherichia coli* (Le *et al.*, 1988), was obtained from Schering-Plough Research (Kenilworth, NJ). Purified hIL-13 and mIL-13 were prepared as described by McKenzie *et al.* (1993b). The soluble extracellular domain of hIL-4R α (called hIL-4R α^s) was derived from Cos-7 cells transfected with a plasmid obtained by *Dra*III digestion of the full-length hIL-4R α cDNA (Galizzi *et al.*, 1990b) and encoded a 200-amino acid protein. hIL-4R α^s was purified from transfected cell culture supernatants by hIL-4-Affi-Gel 10 chromatography as described by Galizzi *et al.* (1990a). Purified phytohemagglutinin (PHA) was from Wellcome (Dartford, Great Britain). The fluorescein isothiocyanate-conjugated F(ab')₂ fragment of goat anti-mouse Ig was from Grub (Vienna, Austria). Phycoerythrin-conjugated streptavidin (streptavidin-PE) was from Becton Dickinson (Mountain View, CA). Jijoye cells, a Burkitt lymphoma; Jy, an Epstein-Barr virus-transformed human B-cell line; Cos-7, a monkey kidney fibroblast cell line; NALM6, a pre-B human cell line (Hurwitz *et al.*, 1979); L cells, a fibroblast cell line; and NS1, a myeloma cell line, were from ATCC. Human monocytes used in the binding studies were prepared as described by de Waal Malefyt *et al.* (1993). Monocytes used for the cell surface expression studies were purified in a standard chamber by counterflow centrifugal elutriation (J2-21 M elutriation system, Beckman Instruments) from peripheral blood mononuclear cells isolated by Ficoll-Hypaque centrifugation. This monocyte preparation was >90–95% pure, as determined by flow cytometry and May Grünwald Giemsa staining. Jijoye, NS1, L cells, tonsil B cells, and monocytes were cultured in complete culture medium RPMI 1640 (Life Technologies, Inc.) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 50 μ g/ml gentamycin (Schering-Plough). Other cells were cultured as described by Zurawski *et al.* (1993a). Geneticin G418 was from Life Technologies, Inc., or from Schering-Plough and was used at 1 mg/ml. Where not specified, chemicals were from Sigma.

Transfection and Fluorescence-activated Sorting of L Cells—The neomycin resistance gene was introduced into a mammalian expression vector, pME18s (Kitamura *et al.*, 1991), containing the hIL-4R α cDNA (pME18s neo hIL-4R). L cells, plated at 10⁶ cells/10-cm plate the previous day, were harvested by trypsinization and washed once with RPMI 1640, 10% fetal calf serum and resuspended at 10⁷ cells/ml in RPMI 1640. 25 μ g of linearized (*Kpn*I endonuclease) plasmid (pME18s

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¹ The abbreviations used are: IL, interleukin; hIL, human interleukin; mIL, mouse interleukin; Ig, immunoglobulin; LPS, lipopolysaccharide; mAb, monoclonal antibody; PHA, phytohemagglutinin; R, receptor; R α , primary binding component of the receptor; PE, phycoerythrin; PBS, phosphate-buffered saline; FACS, fluorescence-activated cell sorting; MHC, major histocompatibility complex.

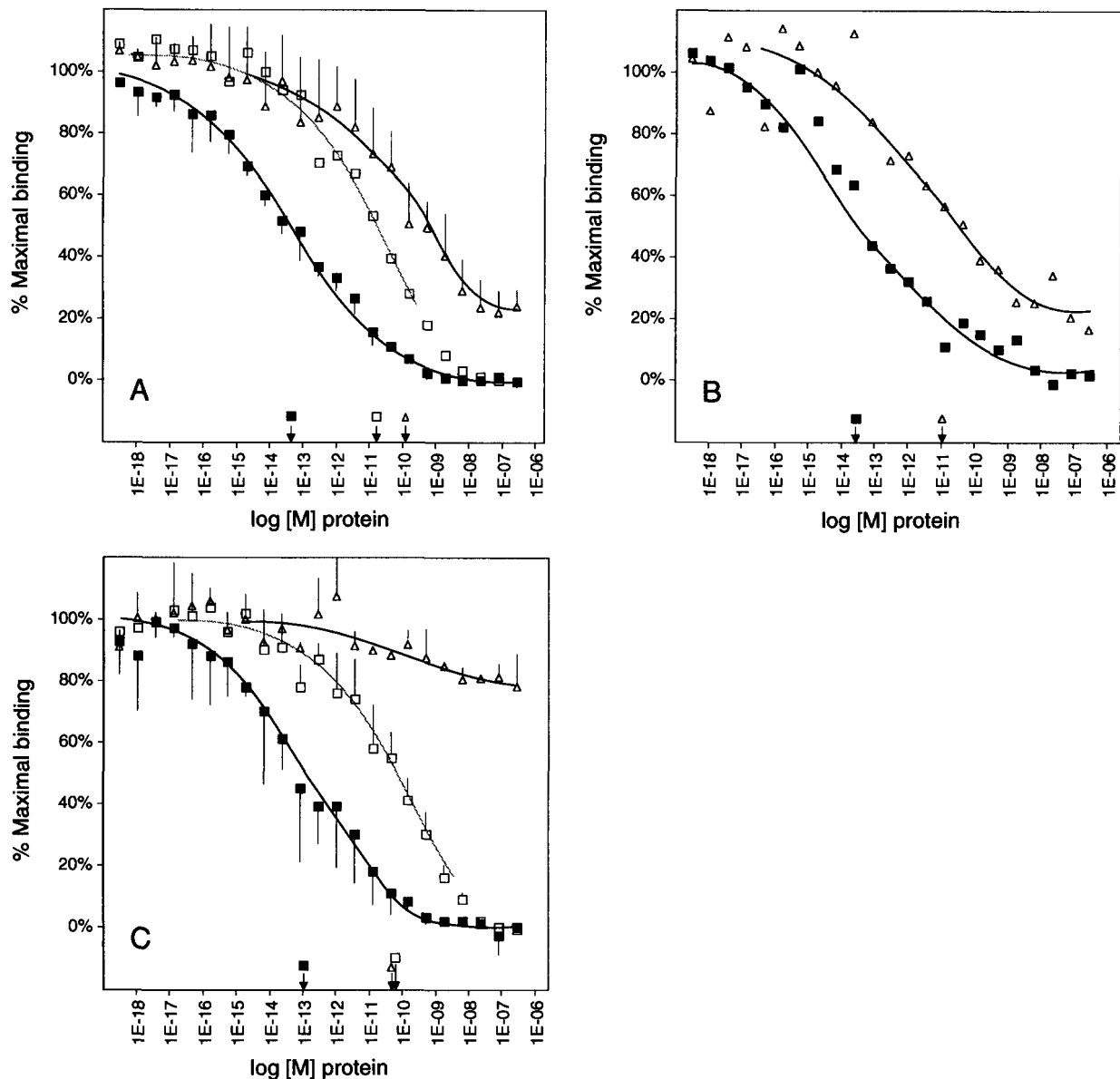


FIG. 1. Competition displacement of ^{125}I -hIL-4 binding to various cell types. Various amounts of unlabeled hIL-4 (■), hIL-4.Y124D (□), and hIL-13 (△) were incubated for 2 h at 4 °C with 2×10^{-11} M ^{125}I -hIL-4; ^{125}I -hIL-4 bound to cells was then determined. Panel A, Cos-7 cells, maximum was 5810 ± 314 cpm, minimum was 93 ± 51 cpm, ~23% of the bound counts were not competed for by hIL-13. Panel B, monocytes, maximum was 702 ± 66 cpm, minimum was 73 ± 16 cpm, ~23% of the bound counts were not competed for by hIL-13. Panel C, Jy cells, maximum was 3790 ± 161 cpm, minimum was 270 ± 25 cpm, ~79% of the bound counts were not competed for by hIL-13. Arrows on the abscissa indicate the dose required to displace 50% of the maximal counts displaced (IC_{50}). Error bars represent standard deviation of the mean (S.D., $n = 2$).

neo hIL-4R or pME18s neo) DNA were added to 0.8 ml of cell suspension (10^7 cells/ml) in a 0.4-cm electroporation cuvette (Bio-Rad). Electroporation was performed using a gene pulser (Bio-Rad) at 960 mF and 250 V. Transfectants were selected with G418. After 1 week of selection, 4×10^7 cells were harvested in phosphate-buffered saline (PBS, 10 mM sodium phosphate, 150 mM NaCl, 3.6 mM KCl, pH 7.4) with 0.5 mM EDTA and washed by centrifugation, and the pellet was resuspended at 10^7 cells/ml in PBS, 0.5 mM EDTA, 0.1% bovine serum albumin (PEB buffer). The cell suspension was incubated (4 °C) with 0.1 μM biotinylated IL-4 in the absence or presence (nonspecific) of 4 μM unlabeled hIL-4. After 90 min of incubation at 4 °C, cells were washed twice by centrifugation with PEB buffer and further incubated with a 1/5 dilution of streptavidin-PE for 30 min at 4 °C (10^7 cells/ml). Then, samples were washed twice by centrifugation and resuspended at 10^6 cells/ml in PEB. Fluorescence-activated cell sorting (FACS) was on a FACS 440 (Becton Dickinson) equipped with a 5-W argon laser running at 488 nm, 0.5 W. The 4% brightest stained cells were collected at each sort and cultured in G418-containing culture medium until the next sort. After four cycles of sorting, cells were cloned at 0.5 cell/well in 96-well plates. One clone (C70), selected among 300, expressed 500,000 sites/cells, as

measured by ^{125}I -hIL-4 binding.

Generation of Monoclonal Anti-IL-4R Antibodies (mAbs)—Four BALB/C mice (Iffa Credo, Les Oncins, France) were immunized intraperitoneally with 10 μg of hIL-4R α in Freund's complete adjuvant, as described previously (Garrone *et al.*, 1991). At 3-week intervals, booster immunizations (2×10 μg , 1×50 μg) were given in Freund's incomplete adjuvant. Immune mouse spleen cells (3×10^8 cells) were fused with NS1 cells (ratio 5:1) using 50% polyethylene glycol 1500 (Boehringer Mannheim) in 50 mM Hepes, pH 7.0. Then 20 96-well plates were seeded at 2×10^5 spleen B cells/well (100 μl) in RPMI 1640 medium containing 0.1 mM hypoxanthine, 58 μM azaserine, 0.15 mg/ml oxaloacetate, 50 μg /ml pyruvate, 0.2 IU/ml insulin, 10% hybridoma cloning efficiency factor (EGS, Interchim), 20% fetal calf serum, 2 mM L-glutamine, and 40 μg /ml gentamycin. Hybridomas secreting antibodies specific for hIL-4R α were screened for their ability to stain L cells transfected with hIL-4R α by FACScan analysis, and cloned by limiting dilution. Positive hybridomas were produced in ascites from pristane-treated BALB/c mice, and mAbs were purified by an anion exchange chromatography (DEAE 5PW, Waters Associates, Millipore Corp., Milford, MA). The mAb isotype was determined with the mouse

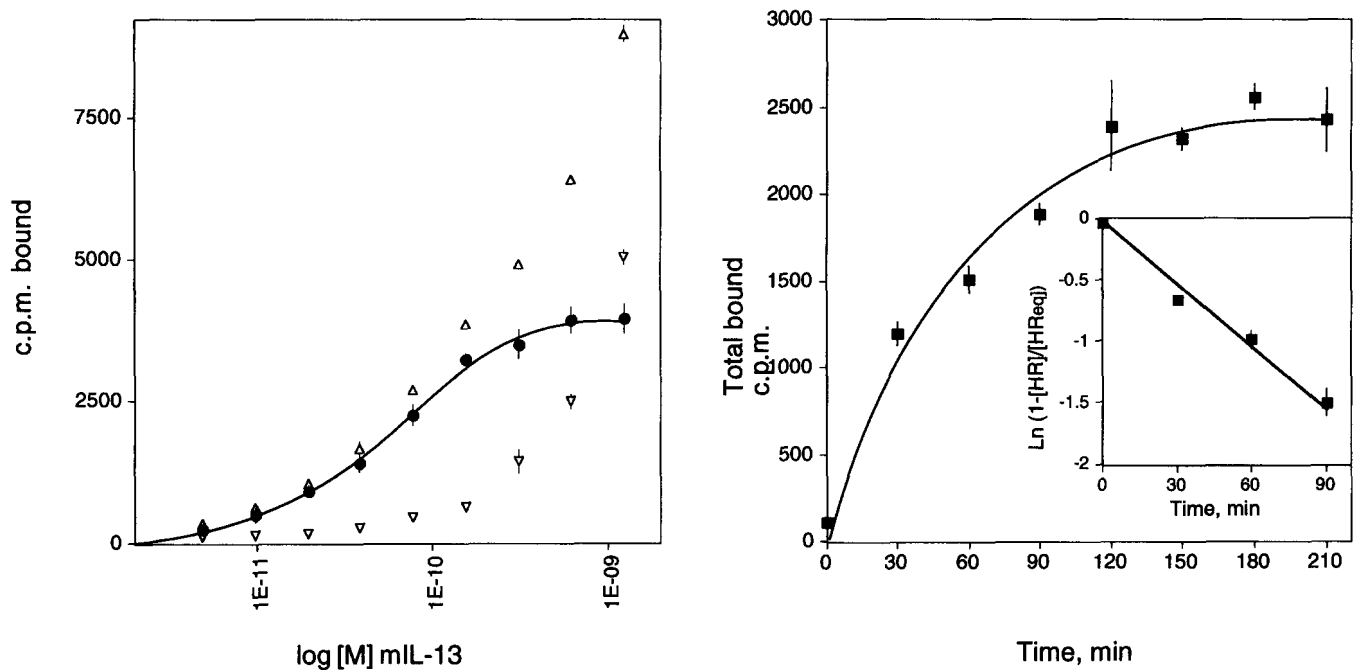


FIG. 2. **Binding of ^{125}I -mIL-13 to Cos-7 cells.** Panel A, Various amounts of labeled mIL-13, together with (▽) or without (△), a 500-fold excess of unlabeled hIL-13 were added to cells and incubated for 2 h at 4 °C; ^{125}I -mIL-13 bound to cells was then determined. Analysis of specific binding (●) by the program Ligand (Munson and Rodbard, 1980) indicated the presence of ~1000 binding sites with a single affinity class of $K_d \sim 1.4 \times 10^{-10}$ M. Panel B, 10^{-10} M ^{125}I -mIL-13 was added to cells at 4 °C and aliquots were removed at various times and ^{125}I -mIL-13 bound to cells was then determined. The inset shows a natural log plot of the data with $[\text{HR}]$, bound ^{125}I -mIL-13 at time t ; $[\text{HReq}]$, bound ^{125}I -mIL-13 at equilibrium.

Typing Isotyping kit (Bio-Rad).

Inhibition of ^{125}I -hIL-4 Binding to Cells—hIL-4 was labeled with Na^{125}I using a Tejedor-Ballesta method at the specific radioactivity of 2000–4000 cpm/mmol, as described earlier (Cabrillat *et al.*, 1987) or as described below for labeling mIL-13. Binding on Jijoye cells and PHA blasts was performed as described (Galizzi *et al.*, 1990a). Briefly, 3×10^6 cells were incubated in RPMI 1640, 1% bovine serum albumin at 4 °C in the presence of increasing concentrations of sera, hybridoma supernatant, or purified mAbs for 30 min. Then, 50 pM of ^{125}I -hIL-4 was added with or without 10 μM cold hIL-4. After 4 h, samples (500 μl total) were washed twice by centrifugation with 2 ml of binding medium at 4 °C and radioactivity of the cell pellet was determined in a gamma counter. Binding on all other cell types was similar, but with the modifications previously (Zurawski and Zurawski, 1992) noted.

Biotinylation and Cross-competition between mAbs for Binding to Cell Lines—1 mg of mAb was biotinylated as described by Bayer (1980). 5×10^5 cells (C70) were incubated with unlabeled mAb in PBS, 1% bovine serum albumin, 0.1% azide (PBA), 15 min at 4 °C. Cells were washed twice with 200 μl of PBA and stained with 200 ng of the biotinylated anti-hIL-4R α mAbs for 15 min. After washing twice, cells were incubated with 100 μl (1/5 dilution in PBA) of streptavidin-PE, washed twice, and analyzed by FACS.

Immunoprecipitation and Western blot Analysis— ^{125}I -labeled hIL-4R α was prepared at a specific radioactivity of 3300 Ci/mmol by incubating 1 μg of hIL-4R α with 0.5 mCi of Na^{125}I and 25 μl of chloramine T (10 $\mu\text{g}/\text{mg}$ protein) in 150 μl of PBS for 5 min. Free ^{125}I was separated from bound by PD10 gel permeation (Pharmacia, Uppsala, Sweden). 10 μg of anti-mouse IgG (Sigma) in 100 μl of PBS was coated on polyvinyl microplates (96 wells, Dynatech) at 4 °C, overnight. Plates were then saturated 1 h at 25 °C with 300 μl of 10% fetal calf serum, PBS. After washing with PBS (four times), wells were filled with 100 μl (10 $\mu\text{g}/\text{ml}$) of anti-hIL-4R α mAbs, 4 h at 4 °C. Wells were incubated with 3 nM ^{125}I -hIL-4R α in PBS, 10% fetal calf serum (100 μl). After 12 h, wells were washed, and radioactivity was extracted with SDS-gel buffer and counted. Samples were also analyzed by electrophoresis to confirm the presence of hIL-4R α .

Proliferation of PHA Blasts—To test the effects of anti-hIL-4R α mAbs on PHA blast proliferation, peripheral blood mononuclear cells purified from normal blood were cultured at 1×10^6 cells/ml with 1 $\mu\text{g}/\text{ml}$ PHA in complete RPMI 1640 medium. After 4 days, proliferation of cells was measured by [^3H]deoxythymidine incorporation as described previously (Garonne *et al.*, 1991).

Biological Assays on B Lymphocytes—Functional properties of anti-hIL-4R α mAbs were also studied on B lymphocytes purified from tonsils as described previously (Defrance *et al.*, 1987). Study of CD23 and surface IgM expression or proliferation assays were previously described (Garonne *et al.*, 1991).

Purification of Proteins and Radiolabeling of mIL-13—*E. coli*-derived hIL-4, hIL-4.Y124D, hIL-13, and mIL-13 were purified as described previously (van Kimmenade *et al.*, 1988; McKenzie *et al.*, 1993b). mIL-13 (5 μg) was radiolabeled to a specific activity of 1525 Ci/mmol with 2.5 mCi of Na^{125}I (IMS30, Amersham) in 100 μl of PBS and 5 μg of Iodogen (Pierce) for 5 min followed by PD-10 gel permeation in RPMI 1640, 2% bovine serum albumin, 0.01% NaN_3 , 20 mM Hepes.

Affinity Labeling of IL-4R and IL-13R Proteins—Chemical cross-linking was performed essentially as described by Kitamura *et al.* (1991). Cells were incubated with 2 nM ^{125}I -hIL-13 or 1 nM ^{125}I -hIL-4 in the presence or absence of a 100-fold excess of unlabeled hIL-13 or hIL-4. After 2 h at 4 °C, the cells were collected by centrifugation, washed once in PBS containing 200 mM bis-sulfosuccinimidyl suberate (BS ^{3} , Pierce), then incubated in this buffer for 30 min at 4 °C. Following centrifugation the cell pellet was solubilized in 1% Triton X-100, 500 mM Na $_3$ EDTA, 100 mM phenylmethylsulfonyl fluoride, 10 $\mu\text{g}/\text{ml}$ leupeptin, 1 $\mu\text{g}/\text{ml}$ pepstatin A, 10 $\mu\text{g}/\text{ml}$ antipain, and analyzed on 7.5% gel SDS-polyacrylamide gel electrophoresis followed by autoradiography.

Determination of hIL-6 Production by Human Monocytes—Monocytes were activated in 96-well plates for 48 h with lipopolysaccharide (LPS, 1 $\mu\text{g}/\text{ml}$), alone or in combination with hIL-4 (0.001–100 units/ml) and/or hIL-13 (0.0001–100 ng/ml), or anti-IL-4R α mAbs (20 $\mu\text{g}/\text{ml}$). After incubation, cell-free supernatants were collected and frozen at -20 °C until IL-6 levels were determined. Levels of hIL-6 were measured by specific enzyme-linked immunosorbent assay (sensitivity: 600 pg/ml) using two rat mAbs (39C3 for IL-6 coating and biotinylated 13A5 for IL-6 detection) kindly provided by Dr. J. S. Abrams (Abrams *et al.*, 1992).

Immunofluorescence Analysis of Monocyte Cell Surface Markers—After 48 h of culture, monocytes (3×10^6 cells/bag) were harvested from Teflon bags. For fluorescence staining, cells were incubated with 10 μl of fluorescein isothiocyanate-labeled mAbs: CD14, HLA-DR, HLA-DQ (Becton Dickinson), or 10 μl of PE-labeled mAb CD23 (Serotec). After washes, the labeled cell samples were analyzed by flow cytometry on FACscan.

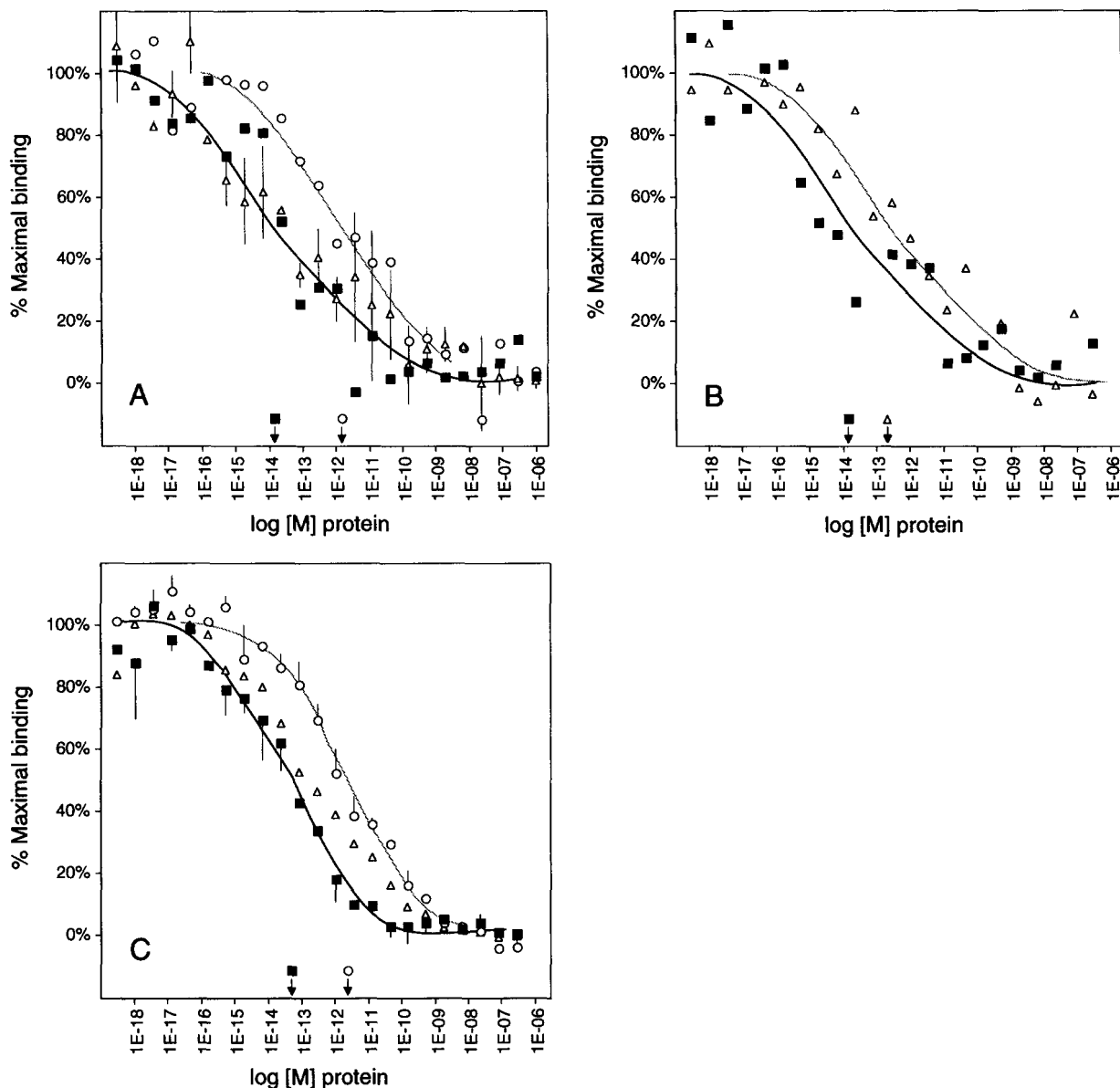


FIG. 3. Competition displacement of ^{125}I -mIL-13 binding to various cell types. Various amounts of unlabeled hIL-4 (■), hIL-13 (△), and mIL-13 (○) were incubated for 2 h at 4 °C with 10^{-10} M ^{125}I -mIL-13; ^{125}I -mIL-13 bound to cells was then determined. Panel A, TF-1 cells, maximum was 496 ± 30 cpm, minimum was 243 ± 20 cpm. Panel B, monocytes, maximum was 202 ± 16 cpm, minimum was 80 ± 12 cpm. Panel C, Cos-7 cells, maximum was 1388 ± 71 cpm, minimum was 367 ± 32 cpm. Using similar conditions, no specific binding to NALM6 cells was detected. Arrows on the abscissa indicate IC_{50} . Error bars represent S.D. ($n = 2$).

RESULTS

Characteristics of IL-4R on Various Cell Types—Competitive ^{125}I -hIL-4 displacement studies have defined three classes of IL-4 binding (Zurawski *et al.*, 1993a). 1) Cells expressing only human IL-4R α bind hIL-4 and the biologically inactive hIL-4 Tyr¹²⁴ → Asp mutant protein (hIL-4.Y124D) with a similar affinity ($K_d \sim 10^{-10}$ M). This type of hIL-4 binding is not competed for by hIL-13. 2) hIL-4-responsive human SP-B21 T cells bind hIL-4.Y124D at this same affinity, but bind hIL-4 ~ 100 -fold more avidly. This type of hIL-4 binding is also not competed for by hIL-13. 3) IL-4R on hIL-4- and IL-13-responsive human pre-myeloid erythroleukemic TF-1 cells are like those on SP-B21 cells, except that hIL-13 can partially (a maximum of $\sim 65\%$) compete for ^{125}I -hIL-4 binding. We have extended these studies to other cell types which are known to bind hIL-4. Fig. 1A shows that IL-4R on monkey kidney Cos-7 cells had properties identical to those described above for TF-1 cells. Human monocytes, which respond similarly to both hIL-4 and

IL-13 (McKenzie *et al.*, 1993b; Minty *et al.*, 1993; de Waal Malefyt *et al.*, 1993; Herbert *et al.*, 1993), had fewer IL-4R, but hIL-13 competed for ^{125}I -hIL-4 binding similarly to that observed for IL-4R on TF-1 and Cos-7 cells (Fig. 1B). The Epstein-Barr virus-transformed human Jy B-cell line had IL-4R with properties identical to SP-B21 cells, except that hIL-13 competed for only $\sim 20\%$ of the bound ^{125}I -hIL-4 (Fig. 1C). Collectively, these hIL-4 binding data establish that high affinity IL-4R on various cell types are similar and that hIL-4.Y124D typically binds to such IL-4R ~ 100 -fold less avidly than hIL-4. In contrast, the ability of hIL-13 to compete for ^{125}I -hIL-4 binding varies dramatically with cell type. Simplistically, these data suggest that there are subtypes of high affinity IL-4R (*i.e.* those which can also bind IL-13 and those which cannot bind IL-13). To explore this notion, we developed a radiolabeling protocol for IL-13 which permitted direct examination of IL-13R.

Properties of ^{125}I -IL-13 Binding to Cos-7 Cells—IL-4R on

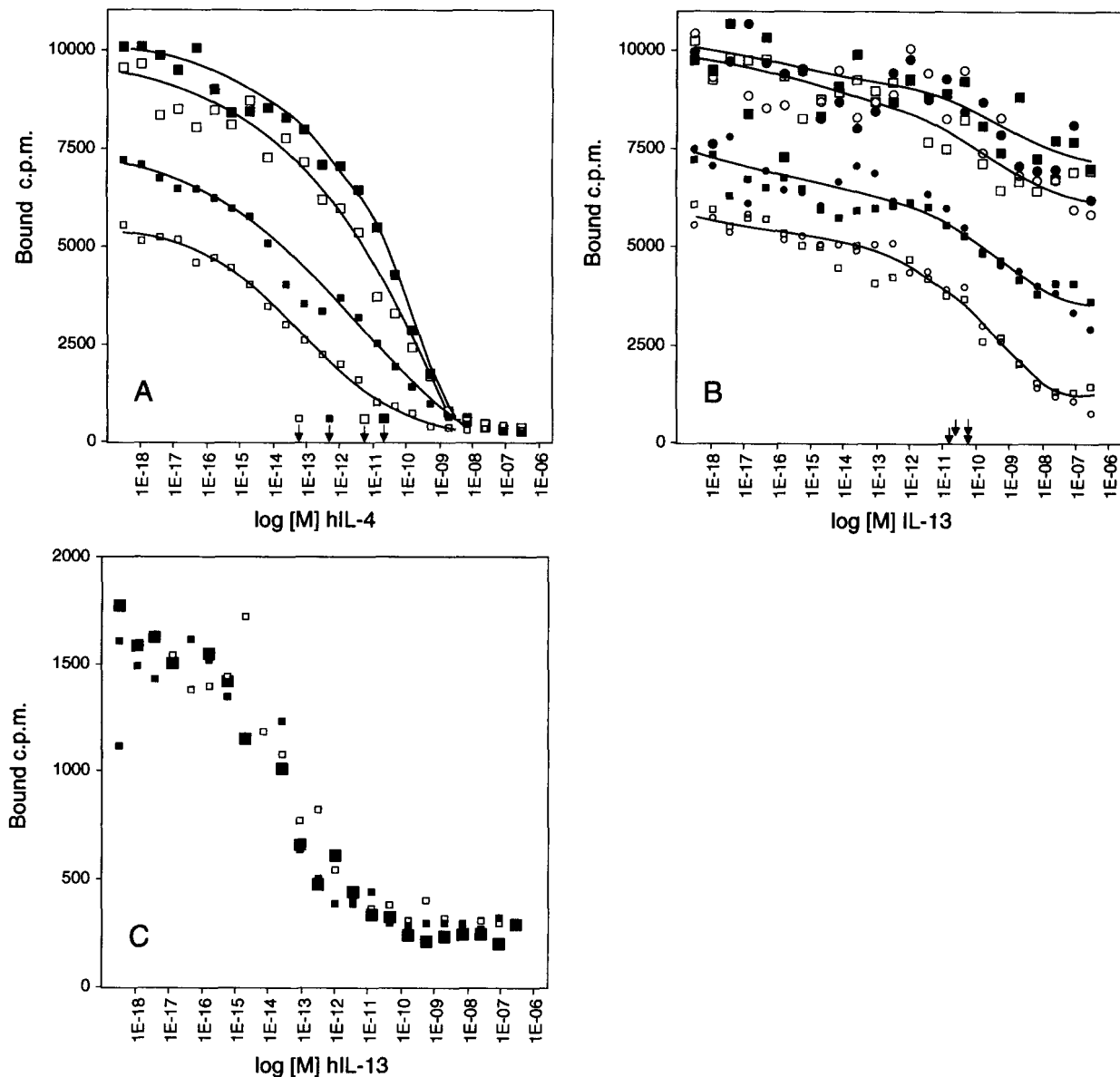


FIG. 4. Effect of hyperexpression of hIL-4R α on the properties of IL-4R and IL-13R on Cos-7 cells. Panel A, various amounts of unlabeled hIL-4 were incubated for 2 h at 4 °C with 10^{-11} M ^{125}I -hIL-4; ^{125}I -hIL-4 bound to 10^6 cells was then determined. Cells (10^7) were untransfected ($\square$) or transfected with 5 μg ($\blacksquare$), 15 μg ($\square$), or 40 μg ($\bullet$) hIL-4R α expression plasmid. Panel B, various amounts of unlabeled mIL-13 (circles) or unlabeled hIL-13 (squares) were incubated with 10^{-11} M ^{125}I -hIL-4, and ^{125}I -hIL-4 bound to cells was then determined. Cells were untransfected ($\square$, $\circ$) or transfected with 5 μg ($\blacksquare$, $\bullet$), 15 μg ($\square$, $\circ$), or 40 μg ($\blacksquare$, $\bullet$) hIL-4R α expression plasmid. Panel C, various amounts of unlabeled hIL-13 were incubated with 10^{-10} M ^{125}I -mIL-13, and ^{125}I -mIL-13 bound to cells was then determined. Cells were untransfected ($\square$) or transfected with 5 μg ($\blacksquare$), or 40 μg ($\bullet$) hIL-4R α expression plasmid. Arrows on the abscissa indicate IC_{50} .

Cos-7 cells are of the type where IL-13 partially competes for ^{125}I -hIL-4 binding, inferring the existence of IL-13R on these cells. This was demonstrated directly by the ability of ^{125}I -mIL-13 to bind specifically to these cells (Fig. 2A). Specific ^{125}I -mIL-13 binding to Cos-7 cells saturated at $\sim 5 \times 10^{-9}$ M (no specific binding was observed above 10^{-9} M ^{125}I -mIL-13 due to high nonspecific binding). Scatchard analysis of the data was most consistent with a single class of ^{125}I -mIL-13 binding site with $K_d \sim 10^{-10}$ M (Fig. 2A). Kinetic analysis revealed that ^{125}I -mIL-13 associated with Cos-7 cells relatively slowly ($t_{1/2} \sim 60$ min, Fig. 2B).

Characteristics of IL-13R on Various Cell Types—Competitive ^{125}I -mIL-13 displacement studies on Cos-7 cells, TF-1 cells, and human monocytes were remarkably similar. These data (Fig. 3, A–C) revealed that hIL-13 binds to these cells slightly more avidly than does mIL-13 ($K_d = 2 \times 10^{-12}$ M versus $6 \times$

10^{-12} M) and that hIL-4 is a potent ($K_d = 1 \times 10^{-12}$ M) and complete competitor of ^{125}I -mIL-13 binding. Cos-7 had ~ 1000 IL-13R/cell, while TF-1 and monocytes had ~ 70 IL-13R/cell. In similar experiments, no specific ^{125}I -mIL-13 binding was detected on NALM6 cells, a human pre B-cell line (Hurwitz *et al.*, 1979) on which ^{125}I -hIL-4 binding was not inhibited by up to 10^{-6} M IL-13 (data not shown). There was an absolute correlation between the existence of the IL-4R subtype which interacts with IL-13 and the presence of IL-13R as detected by IL-13 binding.

Effects of IL-4R α Hyperexpression on IL-4R and IL-13R—To further explore the notion that a subtype of IL-4R can bind IL-13, we took advantage of a transient expression system in Cos-7 cells. Previous studies demonstrated that binding of hIL-4 to Cos-7 cells hyperexpressing hIL-4R α is not competed for by IL-13 (Zurawski *et al.*, 1993a). With the availability of a

direct probe for IL-13 binding, we could investigate the effects of expressing various amounts of hIL-4R α on a cell type which expresses both subtypes of IL-4R. Increasing hIL-4R α expression on Cos-7 cells resulted in an enhanced total bound hIL-4, concomitant with decreased apparent affinity of binding by hIL-4 (Fig. 4A). This result is expected since additional hIL-4R α contributes to binding hIL-4, but its affinity for hIL-4 is markedly less than that of the endogenous IL-4R. Of interest was the observation that these changes in IL-4R were associated with a decreased maximal ability of IL-13 to compete for binding by hIL-4, without concomitant changes in the potency of IL-13 competition or changes in number and affinity of IL-13R (Fig. 4, B and C). These observations are consistent with a model in which the ability of IL-13 to compete for binding of IL-4 to IL-4R is determined by the relative abundance of IL-4R subtypes and show that the IL-4R subtype which interacts with IL-13 on Cos-7 cells is not limited by availability of IL-4R α .

IL-13R Cross-linking Studies—Cross-linking studies with ^{125} I-hIL-4 and various human cell lines reveal a predominant 130–140-kDa hIL-4R α protein (reviewed in Harada *et al.* (1992a)). These same studies suggested that other proteins of ~80 and ~65 kDa could sometimes be found associated with hIL-4R, and other studies show that the 64-kDa human IL-2R γ protein is a component of IL-4R (Russell *et al.*, 1993; Kondo *et al.*, 1993, 1994). We attempted to identify protein components

specific to IL-13R by cross-linking ^{125} I-mIL-13 bound to TF-1 and Cos-7 cells. On both cell types, ^{125} I-mIL-13 affinity-labeled a predominant protein of ~65 kDa (assuming a 1:1 complex with 11.6-kDa mIL-13) and this affinity-labeled species was not detected in the presence of excess unlabeled hIL-13 or hIL-4 (Fig. 5). This property correlates absolutely with the binding properties of IL-13R and contrasts with parallel experiments utilizing ^{125} I-hIL-4 as the affinity probe. On Cos-7 cells, ^{125} I-hIL-4 predominantly labeled a 130–140-kDa species and minor ~65-kDa species which were partially competed for by unlabeled hIL-13 and completely competed for by unlabeled hIL-4 (Fig. 5). On TF-1 cells, ^{125} I-hIL-4 labeled 130–140-kDa, ~95-kDa, and ~65-kDa species which were competed for by both unlabeled hIL-13 and hIL-4 (Fig. 5). Taken together, these data reveal significant differences between IL-4R and IL-13R and tentatively identified a ~65-kDa IL-13R protein(s) as the primary binding protein for IL-13. Further such work with other cell types should reveal whether the 65-kDa protein is specific to IL-13R.

Production and Characterization of Anti-IL-4R α Monoclonal Antibodies—We developed mAbs against hIL-4R α to aid in the further study of IL-4R and IL-13R. A 200-amino acid extracellular portion of hIL-4R α (hIL-4R α^*) was used to immunize mice. We subsequently derived several hybridomas which produced mAbs reactive in FACS analysis to an L cell line stably expressing hIL-4R α (C70), but were not reactive to the parental L cell line. The mAbs varied in their reactivity to hIL-4R α as judged by blotting, precipitation, inhibition of ^{125} I-hIL-4 binding, and cross-competition (Table I). Table II shows that three of the mAbs were effective inhibitors of ^{125} I-hIL-4 binding to monocytic (monocytes), B-cell (Jijoye), and T-cell (PHA blasts) types. As expected from their ability to inhibit binding of hIL-4, these mAbs also abolished IL-4-induced biological effects, such as proliferation of B and T cells and induction on B cells of low affinity IgE receptor (Fc ϵ II) and surface IgM (Fig. 6), as described previously (Defrance *et al.*, 1987; Garonne *et al.*, 1991).

Anti-IL-4R α Monoclonal Antibodies Inhibit IL-13 Binding—The above and previous data show that, although hIL-4R α alone cannot bind IL-13, there appears to be an absolute correlation between IL-4R and IL-13R. We utilized the anti-hIL-4R α mAbs to investigate a possible role for hIL-4R α in IL-13R. Two anti-hIL-4R α mAbs tested were equipotent inhibitors of both ^{125} I-hIL-4 binding and ^{125} I-mIL-13 binding to TF-1 cells (Fig. 7). These data revealed that hIL-4R α , which itself cannot bind IL-13, is an important component for the binding of IL-13 by IL-13R.

Anti-IL-4R α Monoclonal Antibodies Inhibit IL-13 Action—IL-13 and IL-4 elicit diverse effects on monocytic cells (McKenzie *et al.*, 1993b; Herbert *et al.*, 1993; de Waal Malefyt *et al.*, 1993; Doherty *et al.*, 1993) including inhibition of LPS-stimulated synthesis of pro-inflammatory cytokines such as IL-6 and regulation of cell surface marker expression such as

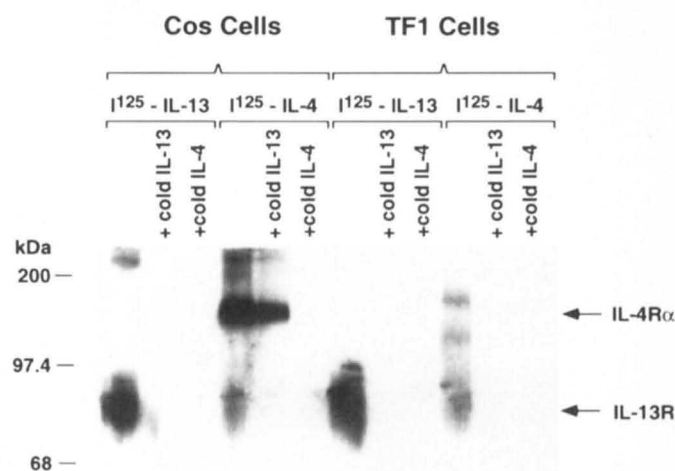


FIG. 5. Affinity labeling of IL-4R and IL-13R proteins on Cos-7 and TF-1 cells. Cells were incubated for 2 h at 4 °C with ^{125} I-hIL-13 or ^{125} I-hIL-4 in the presence or absence of excess unlabeled hIL-13 or hIL-4, then washed and incubated with cross-linking agent. Solubilized cells were then subjected to SDS-polyacrylamide gel electrophoresis and autoradiography. Positions of the cross-linked receptor subunits are indicated by arrows. The high molecular mass band (>200 kDa) may represent a higher order complex.

TABLE I
Properties of anti-hIL-4R α mAbs

mAbs ^a	Reactivity		Inhibition of IL-4 binding	Ability to cross-compete		
	Western	Precipitation		s456	s697	s924
s17	—	—	—	—	—	—
s103	—	+	+	+	+	+
s456	—	+	+	+	+	+
s697	+	+	+	+	+	+
s924	—	+	+	+	+	+
o296	—	+	+	—	—	—
o361	—	—	—	—	+	+
o497	—	—	—	—	+	+
o735	—	—	—	—	+	+

^a All mAbs were IgG1 and methods for their purification, Western blot analysis, immunoprecipitation, inhibition of ^{125} I-hIL-4 binding, and cross-competition are described under "Experimental Procedures."

CD23, CD16, and MHC class II. We first assessed the effect of anti-IL-4R α mAbs on IL-6 production by LPS-activated human monocytes cultured in the presence of IL-13. Fig. 8 shows that the two blocking anti-IL-4R α mAbs tested reversed the hIL-13-induced inhibition of hIL-6 production by monocytes. This effect was not observed with a control mAb, the nonblocking anti-IL-4R α mAb s697 (Fig. 8), or with an anti-IL-4 mAb (data not shown).

We also investigated whether anti-IL-4R α mAbs reversed the hIL-13 effects on the expression of the monocyte cell-surface markers CD23, CD14, and MHC class II. Fig. 9 shows that the anti-IL-4R α mAbs s103 and s924 reversed in a dose-dependent fashion the hIL-13- and hIL-4-induced increase of CD23 expression on monocytes. No inhibition of CD23 induction was seen with the nonblocking anti-IL-4R α mAb s697.

TABLE II
Inhibition of hIL-4 binding to cells by anti-hIL-4R α mAbs

Cell type	mAb EC ₅₀ ^a		
	s103	s456	s924
Jijoye	0.2 (90%)	0.15 nM (100%)	0.1 (100%)
PHA blasts	0.35 (90%)	0.1 (100%)	0.5 (90%)
Monocytes	0.25 (90%)	0.7 (90%)	ND ^b

^a Half-maximal inhibitory concentration (μ g/ml mAb). Number in parentheses is the percentage inhibition observed at 10 μ g/ml mAb.

^b Not determined.

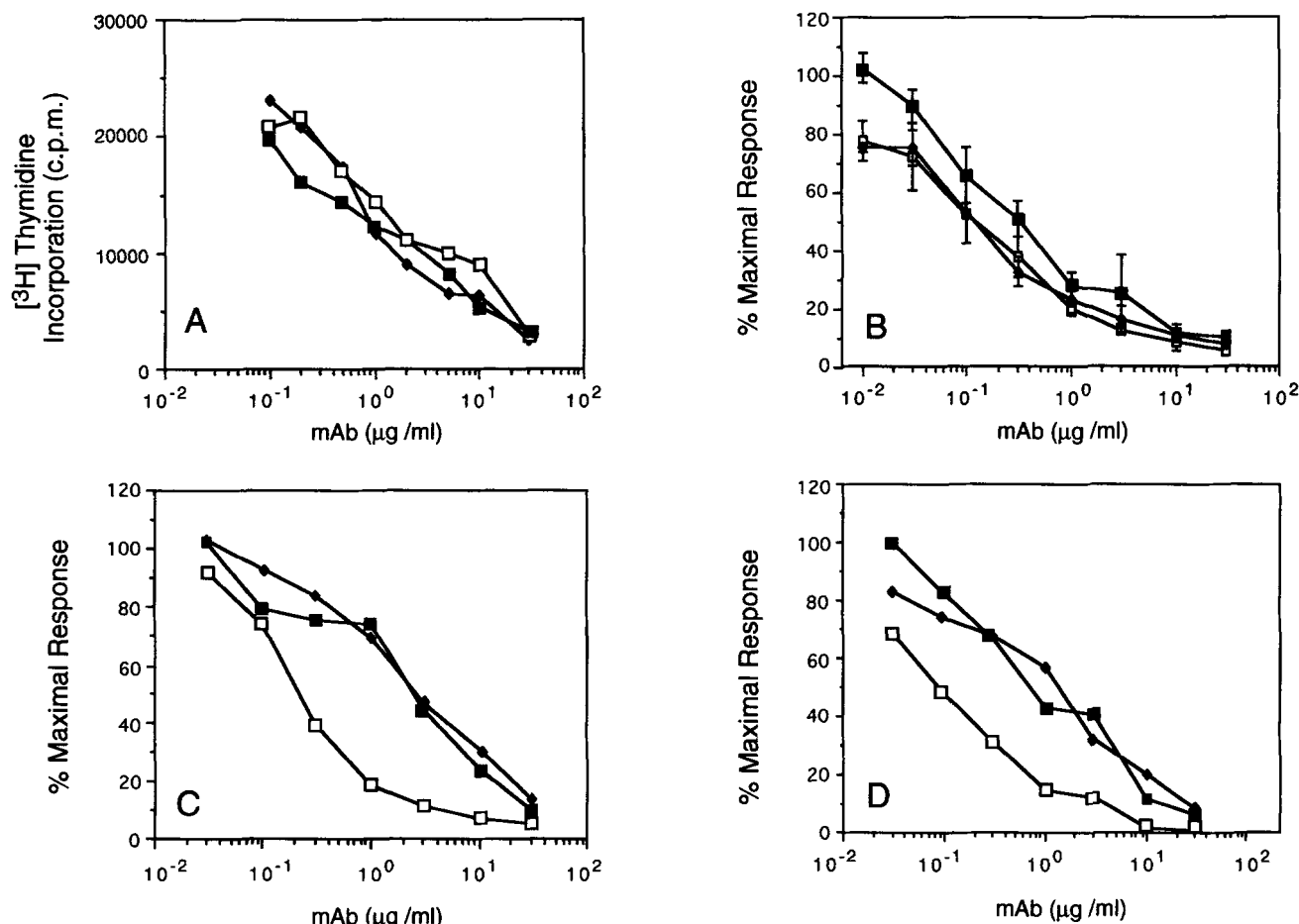


FIG. 6. Inhibition of hIL-4-induced biological effects by anti-hIL-4R α mAbs. Panel A, peripheral blood mononuclear cells were preactivated for 4 days with 1 μ g/ml PHA and viable blasts were recultured for 3 days with 5×10^{-11} M hIL-4 and various concentrations of mAbs s103 (■), s456 (□), and s924 (▲). Panel B, purified tonsil B cells were cultured for 3 days with 5 μ g/ml insolubilized anti-IgM and 5×10^{-11} M hIL-4 and various concentrations of the above mAbs. The response was measured by uptake of [^{3} H]thymidine. Panels C and D, purified tonsil B cells were cultured for 48 h with 2.5×10^{-12} M hIL-4 and various concentrations of the above mAbs. The response was measured by staining cells with specific anti-CD23 (Panel C) or anti-IgM (Panel D) antibodies together with fluorescein isothiocyanate-labeled anti-mouse IgG and FACscan analysis.

Furthermore, an anti-hIL-4 mAb blocked CD23 expression induced by hIL-4 but did not block CD23 expression induced by hIL-13 (data not shown). Similar results were obtained with anti-IL-4R α mAbs when we investigated the expression of CD14 and MHC class II molecules on monocytes in the presence of hIL-4 or hIL-13 (Fig. 10). In addition, we have previously shown that s460 reverses hIL-13 inhibition of the growth of a leukemic human B-cell precursor (Renard *et al.*, 1994). Together, these data show that IL-4R α is a functionally important component of IL-13R.

DISCUSSION

In this work, we have further studied the structures and relatedness of IL-4R and IL-13R. When probed with hIL-4 and the antagonist hIL-4.Y124D, the properties of IL-4R on various human cell types were remarkably similar. In all cases, hIL-4.Y124D bound to IL-4R $\sim$ 100-fold less avidly than did hIL-4. We have previously observed that the cloned hIL-4R α protein alone binds both hIL-4 and hIL-4.Y124D with the lesser affinity characteristic of hIL-4.Y124D binding to IL-4R (Zurawski *et al.*, 1993a). These data imply that an additional subunit associates with hIL-4R α to enhance its affinity for hIL-4 and that this association facilitates signaling and involves interaction with the Y124D residue of hIL-4. The hIL-2R γ subunit appears to associate with hIL-4R α , although co-expression of hIL-4R α and hIL-2R γ did not enhance the affinity of hIL-4R α to the extent expected from the above studies (Russell *et al.*, 1993).

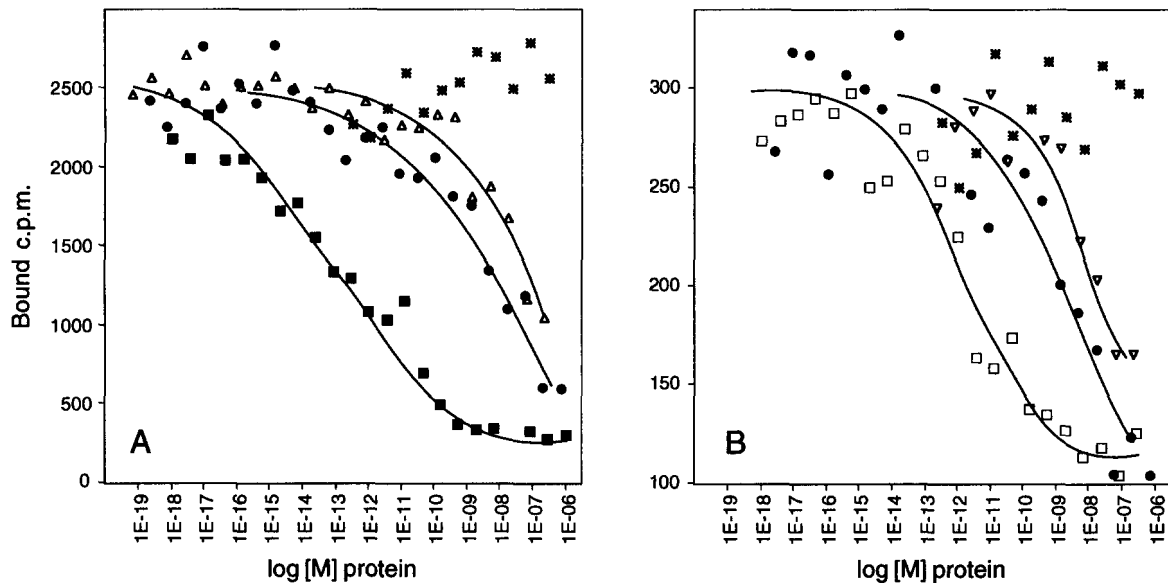


FIG. 7. Displacement by anti-hIL-4R α mAbs of ^{125} I-mIL-13 and ^{125} I-hIL-4 binding to TF-1 cells. Various amounts of unlabeled hIL-4 (■), mIL-13 (□), and mAbs s456 (△), s103 (●), or o361 (*) were incubated for 2 h at 4 °C with Panel A, 10^{-11} M ^{125} I-hIL-4 or Panel B, 10^{-10} M ^{125} I-mIL-13, and radioligand bound to cells was then determined.

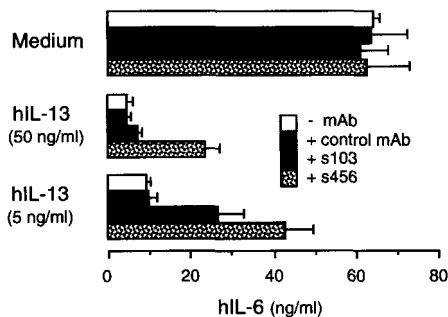


FIG. 8. Reversal by anti-hIL-4R α mAbs of hIL-13 inhibition of monocyte hIL-6 production. LPS-activated human monocytes were incubated alone or with hIL-13 and with and without mAbs for 48 h. Supernatants were then collected and levels of hIL-6 produced were determined. Similar experiments (not shown) established that 5 ng/ml hIL-13 elicits a ~50% maximal response and 50 ng/ml elicits a ~90% maximal response. Also, blocking anti-hIL-4 mAb and non blocking anti-hIL-4R α mAb s697 (both at 20 μ g/ml) had no effect on hIL-13 action (data not shown).

IL-2R γ plays similar affinity-enhancing and signaling roles in IL-4R and IL-2R (where it interacts with IL-2R α or IL-2R β ; reviewed in Minami *et al.* (1993)). Additionally, mutations in similar locations, but different side chains (Gln in IL-2, Tyr in IL-4) within the C-terminal α -helix of IL-2 and IL-4 abrogate interaction with IL-2R γ (Zurawski *et al.*, 1993a, 1993b).

In contrast to the homogeneity of hIL-4R that we observed on various cell types probed with hIL-4 or hIL-4.Y124D, the ability of IL-13 to compete for hIL-4 binding to these same cells varied widely, indicating that this is not a general property of IL-4R. The simplest notion accounting for the above data is the existence of two IL-4R subtypes, only one of which binds IL-13. This concept of two IL-4R subtypes was supported by our observations of IL-13R using radiolabeled mIL-13 as a probe. IL-13R were detected only on those cell types on which IL-13 effectively competed for IL-4 binding. Importantly, IL-13 binding to IL-13R was always fully competed for by hIL-4. These data suggest a model where one type of IL-4R, the complex of IL-4R α + IL-2R γ , cannot bind IL-13, while the other type of IL-4R is IL-13R.

Two studies permitted us to define the composition of IL-

13R. First, a cross-linking study revealed a ~65-kDa protein that was specifically associated with IL-13R. It is most likely that this ~65-kDa affinity-labeled protein is the primary binding subunit of IL-13R (which we shall here refer to as IL-13R α). The ~65-kDa protein is distinct from hIL-2R γ , since hIL-2R γ and IL-4R α are present on cells such as human SP-B21 T cells which do not bind IL-13 (data not shown). Second, we observed that certain anti-hIL-4R α mAbs exhibited equipotent inhibition of IL-4 and IL-13 binding and biological activity. These data show that hIL-4R α is a component of IL-13R. Thus IL-13R is composed of IL-4R α + IL-13R α .

Our characterization of IL-13R accounts for a number of biological and receptor binding observations. Biological studies (reviewed in Zurawski and de Vries (1994)) have revealed that IL-13 elicits only a subset of the responses of IL-4. This occurs since IL-4R (as IL-4R α + IL-2R γ) appears on a large number of cell types, while IL-13R α , whose presence determines the ability of a cell to bind IL-13, has a more restricted distribution. The remarkably similar cellular responses to IL-4 and IL-13 (Zurawski and de Vries, 1994) reflect the action of the intracellular domain of the shared IL-4R α in signaling (Harada *et al.*, 1992b). The hIL-4.Y124D antagonist acts against responses to both IL-4 and IL-13 (Zurawski *et al.*, 1993a) since it binds normally to hIL-4R α , which is a component of both IL-4R and IL-13R. The ability of IL-13 to compete for IL-4 binding varies widely with cell type because the prevalence of IL-4R α , IL-2R γ , and IL-13R α varies. We demonstrated this directly by showing that increasing levels of hIL-4R α decreased the maximal ability, but not the potency, of IL-13 to compete for hIL-4 binding. This and the concomitant decrease in the affinity of hIL-4 binding (due to increased ratio of IL-4R α to IL-2R γ) had no effect on the numbers or binding properties of IL-13R.

It is possible that IL-13R contains IL-2R γ as well as IL-4R α + IL-13R α ; however, recent studies (Matthews *et al.*, 1994) show that IL-13 responses in B cells from two X-SCID patients with characterized IL-2R γ mutations do not respond to IL-2, but respond normally to IL-13. These same B cells respond normally to IL-4, suggesting that IL-4 can signal via binding to IL-13R.

The anti-hIL-4R α mAbs which we developed will be important reagents for further characterizations of IL-4R α abun-

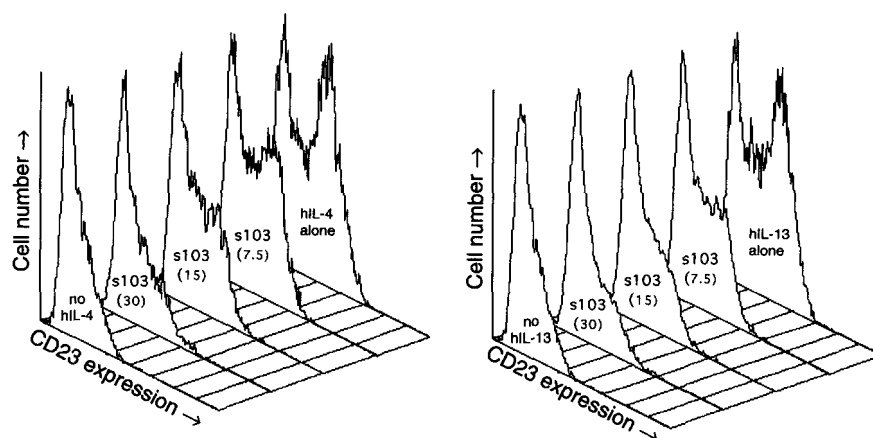


FIG. 9. Reversal by anti-hIL-4R α mAb of hIL-4- and hIL-13-directed enhancement of monocyte CD23 expression. Human monocytes were cultured with LPS in the presence of 50 units/ml hIL-4 or 50 ng/ml hIL-13 with and without various amounts (indicated in micrograms/ml in the parentheses) of the anti-hIL-4R α mAb s103. After 48 h the cells were stained with phycoerythrin-labeled CD23 antibody and analyzed by FACScan flow cytometry. Parallel experiments (not shown) revealed that mAb s924 had similar though slightly less potent effects and that the nonblocking anti-hIL-4R α mAb s697 and blocking anti-hIL-4 mAb had no effect on hIL-13 action.

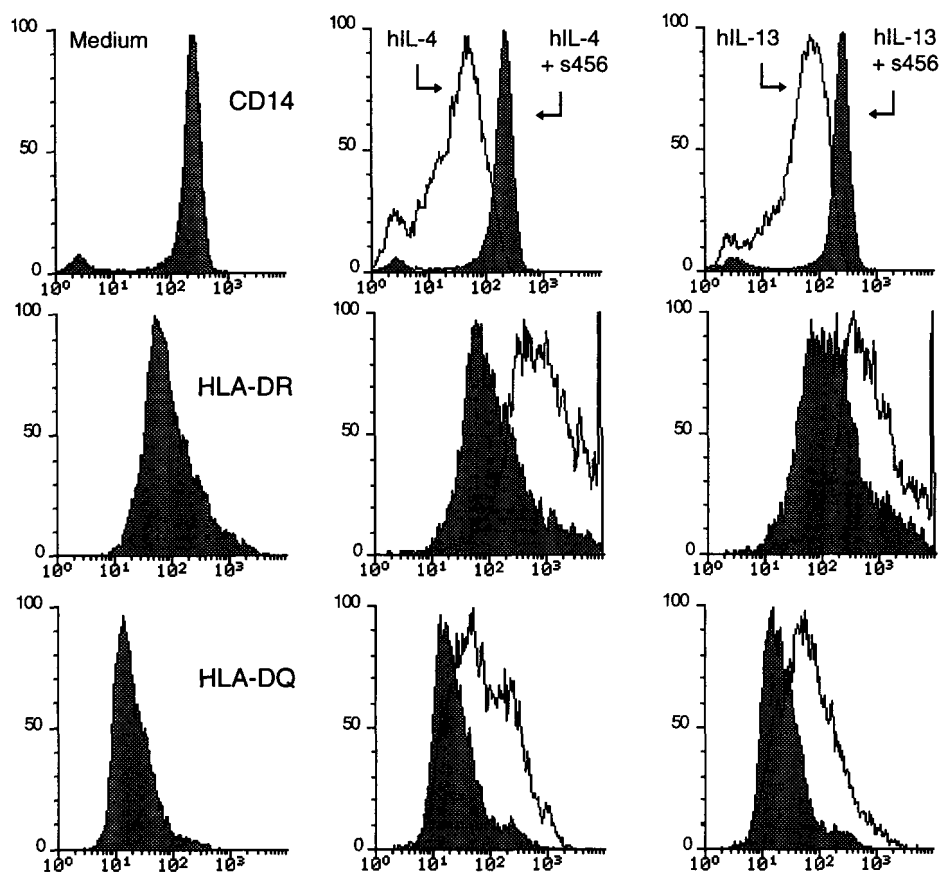


FIG. 10. Reversal by anti-hIL-4R α mAb of hIL-13-directed changes of monocyte CD14, HLA-DR, and HLA-DQ expression. Human monocytes were cultured with LPS in the presence and absence (medium control, left panels) of 50 ng/ml hIL-13 with (shaded histogram in center and right panels) and without (unshaded histograms) 20 μ g/ml of the anti-hIL-4R α mAb s456. After 48 h the cells were stained with fluorescein isothiocyanate-labeled CD14, HLA-DR, and HLA-DQ antibodies and analyzed by FACScan flow cytometry. Parallel experiments (not shown) revealed that mAb s103 had effects similar to those of s456 on CD14 and HLA-DR expression and that these effects were proportionally greater at suboptimal levels of hIL-13.

dance and distribution. Since some recognize distinct epitopes, they can be used in solid phase assays for the detection of hIL-4R α in biological fluids (not shown). Such soluble forms of IL-4R α have been detected in mouse sera (Fernandez and Vitetta, 1990, 1991; Fanslow *et al.*, 1990) and may be indicators of disease states such as allergy and graft rejection.

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