Purification and characterization of recombinant human interleukin 4

Biological activities, receptor binding and the generation of monoclonal antibodies

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A synthetic gene coding for human interleukin 4 (IL-4) was cloned and expressed in *Saccharomyces cerevisiae* (baker's yeast) as a C-terminal fusion protein with the yeast prepro α -mating factor sequence, resulting in secretion of mature IL-4 into the culture medium (0.6–0.8 μ g/ml). A protocol was developed for purification of this protein. Crude cell-free conditioned medium was passed over a concanavalin A–Sepharose affinity column; bound proteins were eluted and further purified by S-Sepharose Fast Flow cation exchange and C₁₈ reverse-phase h.p.l.c. Highly purified IL-4 was obtained by this method (0.3–0.4 mg per litre of culture) with a recovery of 51 %. Thermospray liquid chromatography–mass spectrometry showed the C-terminal N-glycosylation site to be largely unmodified, and also showed that the N-terminus of the purified recombinant IL-4 (rIL-4) was authentic. Thiol titration revealed no free cysteine residues, implying that there are three disulphide groups, the positions of which remain to be determined. We have characterized the biological activities of the purified rIL-4. This material is active in B-cell co-stimulator assays, T-cell proliferation assays and in the induction of cell-surface expression of CD23 (the low-affinity receptor for IgE) on tonsillar B-cells. Half-maximal biological activity of the rIL-4 was achieved at a concentration of 120 pM. We have radioiodinated rIL-4 without loss of biological activity and performed equilibrium binding studies on Raji cells, a human B-cell line. The ¹²⁵I-rIL-4 bound specifically to a single class of binding site with high affinity (K_d = 100 pM) and revealed 1100 receptors per cell. Receptor–ligand cross-linking studies demonstrated a single cell-surface receptor with an apparent molecular mass of 124 kDa. Two monoclonal antibodies have been raised to the human rIL-4, one of which blocks both the biological activity of rIL-4 and binding to its receptor.

INTRODUCTION

Interleukin 4 (IL-4), previously called 'B-cell stimulatory factor-1' ('BSF-1'), has been shown to be a product of activated T-cells and mast cells and to influence the growth and differentiation of a wide spectrum of haematopoietic cell lineages. Originally defined by its ability to induce DNA synthesis in resting murine B-cells when co-stimulated with anti-IgM antibodies, it has subsequently been shown to possess multiple biological activities. IL-4 acts on resting murine B-cells to increase the expression of class II major histocompatibility complex (MHC) molecules and CD23, the low-affinity receptor for IgE (Fc ϵ R2). IL-4 will also induce isotype switching in lipopolysaccharide (LPS) activated murine B-cells, leading to enhanced production of IgG₁ and IgE [1–3]. The activity of IL-4 is not restricted to B-cells, since it can induce the proliferation of mast cells and activated T-cells [4]. Similar activities have also been reported in the human; IL-4 will induce CD23 expression and promote B-cell growth in combination with anti-IgM antibodies [5,6]. Human IL-4

(hIL-4) will also enhance IgE production by human peripheral-blood lymphocytes *in vitro* [7], but as yet no clear role in isotype switching has been demonstrated.

On the basis of a published cDNA sequence, hIL-4 is a 14991 Da protein [8], although it has not yet been purified from non-recombinant sources. The protein has six cysteine residues, which are thought, by analogy with the murine molecule, to form three disulphide bonds [1]. To date, recombinant human IL-4 has been expressed, refolded and purified from *Escherichia coli* [9]. We report here the expression, purification and partial biochemical characterization of recombinant human IL-4 (rhIL-4) from yeast transformed with an α -factor fusion secretion vector [10] which obviates the need for refolding. Isolated pure protein was found to be hyperglycosylated, but nevertheless retained full biological activity in B-cell co-stimulator assays, T-cell proliferation assays and in the induction of CD23 on tonsillar B-cells. We have radiolabelled the rhIL-4 to high specific activity without loss of biological activity in order to characterize cell-surface receptors for hIL-4. Finally, we have raised two monoclonal antibodies to *E. coli* derived rhIL-4, one of

Abbreviations used: hIL-4, human interleukin 4; rhIL-4, recombinant human interleukin 4; PHA, phytohaemagglutinin; r.p.-h.p.l.c., reversed-phase h.p.l.c.; Con A, concanavalin A; PAGE, polyacrylamide-gel electrophoresis; TFA, trifluoroacetic acid; i.e.c., ion-exchange chromatography; t.s.-l.c.-m.s., thermospray liquid chromatography–mass spectrometry; MHC, major histocompatibility complex; LPS, lipopolysaccharide; (HI)FCS, (heat-inactivated) foetal-calf serum; DTT, dithiothreitol; AET, 2-aminoethylisothiuronium; SBRC, sheep red blood cells; MEM, minimal essential medium; PBL, peripheral-blood mononuclear cells; DMSO, dimethylsulphoxide; DSS, disuccinimidyl suberate.

which blocks both the binding and biological activity of rhIL-4.

MATERIALS AND METHODS

Materials

rhIL-2 was produced and purified as previously described [11]. Con A-Sepharose, S-Sepharose Fast Flow, Phast-gel media, M_r and pI markers and Ficoll/Hypaque were purchased from Pharmacia. CaCl_2 , α -methyl D-glucoside and 5,5'-dithiobis-(2-nitrobenzoate) were AnalaR grade and obtained from Sigma, Poole, Dorset, U.K. Tosylphenylalanylchloromethane ('TPCK')-treated trypsin and pepsin were obtained from Cooper Biomedical, and N-glycanase and Genzyme recombinant human IL-4 were from Koch-Light. Amino acid standards, phenyl isothiocyanate and TFA (Sequanal grade) were from Pierce Chemical Co., Rockford, IL, U.S.A. Phytohaemagglutinin (PHA) purified grade was from Wellcome Diagnostics. RPMI 1640, foetal-calf serum (FCS), L-glutamine, penicillin and streptomycin were obtained from Flow Laboratories, Irvine, Ayrshire, Scotland, U.K. [^3H]Methylthymidine was from Amersham International. Acetonitrile (h.p.l.c. grade S) was obtained from Rathburn Chemicals, Walkerburn, Peeblesshire, Scotland, U.K. All other chemicals were from BDH, Poole, Dorset, U.K. Water was glass-distilled and Milli-Q-purified.

Cell culture

The human B-cell line Raji was obtained from the American Type Culture Collection and was grown in complete medium: RPMI 1640 with 10% heat-inactivated FCS (HIFCS), L-glutamine (2 mM), penicillin (100 units/ml) and streptomycin (100 $\mu\text{g}/\text{ml}$).

Molecular genetics

DNA methods. Plasmid preparation and manipulations were carried out using standard techniques [12]. Oligonucleotide-directed mutagenesis *in vitro* was performed using a kit from Amersham International (code RPN 2322). DNA sequencing was carried out using the dideoxy chain-termination method [13].

Yeast methods. Yeast transformations were carried out as described by Beggs [14]. Transformants were stored at -70°C in 20 μl aliquots in 15% (v/v) glycerol plus YMM (0.67% Difco nitrogen base, 2% glucose, 40 mg of L-tyrosine/l, 20 mg/l each of L-glutamic acid, L-phenylalanine, L-aspartic acid, adenine and uracil, 10 mg/l each of L-tryptophan, L-histidine, L-aspartic acid, L-methionine, L-isoleucine, L-lysine, L-valine, L-serine and L-threonine, pH 7.0). For batch culture a single aliquot was thawed, used to inoculate a YMM plate (solidified with 2% agar) and incubated at 30°C . Colonies were scraped off into YMM medium and grown at 30°C , and at 300 rev./min to a density of 5×10^7 cells/ml. This was diluted 1:100 into YEPdE (1% Difco yeast extract, 2% Difco bacto-peptone, 0.2% glucose and 2% ethanol) and grown at 30°C and at 150 rev./min to a density of $(1-2) \times 10^8$ cells/ml. The yeast medium containing soluble rhIL-4 was clarified by centrifugation at 5000 g_{av} for 10 min in a Beckman J2-21 centrifuge with a JA10 rotor.

Strains. The *Escherichia coli* strain used was DH1 F $^-$, end A2, hsd R17, ($V_{-k_{mk}^+}$) supE44, thi 1, rec A1, gyr A96, rel A1 [15]. The *S. cerevisiae* strain used was SLP-1B α , set 2-1, pep 4-3, leu 2, ura 1.

Plasmic constructions. The multicopy episomal plasmid pJDB207 [14] was used as a cloning vector. The ADH1 transcription terminator ADHE [16] was inserted into the unique *Hind*III-*Bam*HI sites. An *Eco*RI-*Hind*III fragment containing the MF α 1 promoter and prepro leader [10] was inserted upstream from the terminator. Yeast α -mating factor is naturally processed from its preproleader by the product of the KEX2 gene, leaving multiple copies of the acidic amino acid pair glutamic acid-alanine attached to the mature protein. These are subsequently removed by the STE 13 gene product. When proteins are overproduced from this leader, the STE 13 gene product becomes limiting and the glutamic acid-alanine residues are not removed from the mature protein [17-19]. To eliminate this problem, a *Hind*III site was inserted upstream of the STE 13-processing sites by mutagenesis *in vitro* (Fig. 1a). A synthetic IL-4 gene was designed (originally for expression in *E. coli*) as three portions. These were cloned sequentially from the 3' end into M13 and the sequences verified by di-deoxy sequencing (Fig. 1b). The entire IL-4 gene, together with sequence to rebuild the 3' end of the α -factor up to and including the KEX2 processing site, was subcloned into the yeast expression vector (Fig. 1a).

Purification of rhIL-4

Clarified yeast medium (9 litres) was loaded at a flow rate of 50 ml/min on to a 10 cm $\times$ 5 cm (200 ml) column of Con A-Sepharose equilibrated in 2 mM- CaCl_2 /0.5 M- NaCl /20 mM-Tris buffer, pH 7.4, at 4°C . After a 1.4-litre wash, bound protein was eluted with 1.2 litres of the same buffer containing 0.5 M- α -methyl D-glucoside (the column was regenerated after each run by washing with 500 ml of 1 M- α -methyl D-glucoside/20 mM-phosphate/citrate buffer, pH 3.6, followed by 4 litres of equilibration buffer). The Con A eluate (1200 ml) was diluted 5-fold with 20 mM-phosphate/citrate buffer, pH 5, and loaded at 25 ml/min on to a 2.5 cm $\times$ 5 cm (50 ml) column of S-Sepharose Fast-Flow equilibrated with 20 mM-phosphate/citrate buffer, pH 5. After a 200 ml wash, proteins were eluted with a linear gradient of 0-1 M- NaCl in the same buffer at 8 ml/min. Fractions (4 ml) were collected, and those containing rhIL-4 identified (the column was regenerated after each run by washing with 200 ml of 0.1 M- NaOH , followed by 1 litre of equilibration buffer). The active pool from S-Sepharose was finally purified on a 25 cm $\times$ 1 cm Synchropak RP-P C_{18} column (Anachem) using a Varian 5020 liquid chromatograph and a UV-100 detector. The pool was loaded at 10 ml/min, washed with 60 ml of 0.1% TFA and bound proteins were eluted with the following gradient, where A = 0.1% TFA and B = 65% acetonitrile/0.1% TFA: 100% A-100% B over 32.5 min, with 100% B held for 5 min using a flow rate of 3 ml/min. During the purification, fractions were tested for biological activity using the PHA-activated T-cell blast-proliferation assay described below.

Deglycosylation of rhIL-4

Hyperglycosylated rhIL-4 in h.p.l.c. solvent was freeze-dried and redissolved in 200 mM-sodium phosphate

Fig. 1. Sequence of hIL-4 cDNA and expression vector

(a) Expression vector pMWIL-4; the derivation of this plasmid is described in the text. Restriction-enzyme sites are marked: E, *EcoRI*; P, *PstI*; H, *HindIII*; X, *XbaI*; B, *BamHI*. This expression vector contains a synthetic hIL-4 gene (hIL-4) flanked by the yeast mating factor *MLα1* promoter and prepro leader and the *ADH1* transcription terminator (*ADHt*). The plasmid also contains the 2 μ m origin of replication (2 μ ori), the ampicillin-resistance gene (*AMP^r*) and the selectable marker gene *LEU2*.
(b) The synthetic human IL-4 gene cloned on to a modified yeast prepro- α -factor sequence. Codons for this gene had been optimized for *E. coli*. The KEX2 proteinase cleavage site is marked with an arrow (∇) and stop codons by asterisks. Restriction sites introduced into the human IL-4 sequence which enable the stepwise cloning in M13 are marked.

buffer, pH 8.5, so that the protein concentration was 1–2 mg/ml. *N*-Glycanase (2 μ l of 250 units/ml per 10 μ l of protein solution) was added and the mixture incubated at 37 °C for 16 h.

Amino acid analysis

Amino acid analysis was performed with a Waters PICO-TAG system as described by the manufacturer. Freeze-dried samples (5–10 μ g) were hydrolysed in the vapour phase with HCl and the phenylisothiocarbamyl derivatives separated by r.p.-h.p.l.c. and detected at 254 nm. Aminobutyric acid was used as an internal standard, and quantification was based on the aspartic acid/asparagine content.

M.s.

For t.s.-l.c.-m.s. analysis, 150 μ g of rIL-4 was freeze-dried and dissolved in 150 μ l of 50 mM-NH₄HCO₃, pH 8.4, and digested for 16 h at 37 °C with 12 μ g of tosylphenylalanylchloromethane-treated trypsin. The digest was then reduced with 10 mM-DTT for 2 h at 37 °C and peptides separated by r.p.-h.p.l.c. with u.v. detection using a Brownlee Aquapore (250 mm $\times$ 4.6 mm) C₁₈ column (Anachem) and an acetonitrile/TFA gradient; the total h.p.l.c. eluate was introduced into a Finnegan-MAT 4600 quadrupole mass spectrometer via a thermospray ion source. This method allows matching of u.v. peaks with molecular ions [20,21].

Biological assays

T-cell depletion. T-cell depletion was performed by 2-aminoethylisothiuronium bromide-treated sheep red blood cells (AET-SRBC) rosetting. For AET treatment of SRBC, 25 ml of 50% SRBC in Alsever's solution (Gibco) were washed three times (600 g_{av} , 10 min) in minimal essential medium (MEM) containing 5% HIFCS. A 1.7 g portion of AET was dissolved in 44 ml of water and adjusted to pH 9.0. This solution was sterile-filtered and added to the pelleted SRBC. The cells were incubated at 37 °C for 15 min, then washed five times in medium and finally resuspended at 4%. The AET-SRBC were stored at 4 °C and used within 1 week of preparation. Ficoll/Hypaque-separated peripheral-blood mononuclear cells (PBL) or tonsillar-cell suspensions (prepared as described below) were resuspended at 10⁷/ml in MEM/FCS. Equal volumes of PBL or tonsillar cells, 4% AET-SRBC and MEM/FCS were mixed together in 20 ml tubes. Additional FCS was added to bring its final concentration to 15%. The cells were centrifuged for 10 min at 200 g_{av} , then incubated as a pellet on ice for 1 h. The pellet was then gently resuspended by slowly turning the tube and the cell suspension layered over an equal volume of Ficoll/Hypaque. After centrifugation for 20 min at 800 g_{av} , the non-rosette-forming cells were collected from the interface and washed. These cells were found to be enriched for B-cells as determined by indirect

immunofluorescence with anti-T-cell and anti-monocyte antibodies. This routinely produced cell preparations containing less than 5% T-cells and less than 0.6% monocytes; for simplicity such preparations will be referred to as 'B-cells'.

B-cell costimulator assay. Tonsils were obtained from patients with chronic tonsillitis (Mount Vernon Hospital, Pinner, Middx., U.K.). A single-cell suspension was prepared from the tissue by gently teasing it apart and pushing through a fine meshed sieve with a syringe plunger. Large clumps were allowed to settle out, then the cells were washed twice (200 g_{av} , 10 min) in fresh medium (RPMI 1640 + 10% HIFCS). B-cells were then prepared as described above.

B cells (10^5 per well) were cultured alone or with dilutions of rhIL-4 in a final volume of 200 μ l in 96-U-well plates. To one set of replicates, beads coated with rabbit antibody to human IgM (Bio-Rad, Richmond, CA, U.S.A.) were also added to a final antibody concentration of 10 μ g/ml. Proliferation was assessed on the third day of culture by [3 H]thymidine incorporation over the last 16 h of culture.

CD23 expression. To examine the induction of CD23 expression, tonsillar or peripheral-blood B-cells (5×10^5) were cultured in 1 ml of medium in tissue-culture tubes. In some experiments, rabbit anti-(human IgM)-coated beads were added to the cells at a final antibody concentration of 10 μ g/ml. rhIL-4 (2–1000 units/ml) was added to the cells with and without anti-IgM-coated beads. After culturing for 48 h, the cells were washed in MEM containing 5% HIFCS and 0.05% NaN_3 , then stained for CD23 with the monoclonal antibody B6 (Coulter), followed by fluorescein isothiocyanate-labelled goat anti-mouse Ig (Becton–Dickinson) and analysed by flow cytometry (FACS analyser; Becton–Dickinson).

PHA blast assay. T-cell proliferation assays were performed on human PHA blasts prepared by culturing unseparated mononuclear cells from tonsils or peripheral blood at 10^6 /ml in the presence of 1 μ g of PHA/ml for 3 days. The cells were then washed three times and recultured at 1×10^4 per cell in 96-U-well plates in a final volume of 100 μ l of complete medium. Dilutions of rIL-4 or test column fractions were added to triplicate cultures and proliferation assessed by [3 H]thymidine incorporation over the last 16 h of a 72 h culture.

Radioiodination of rIL-4

Purified rIL-4 from yeast was labelled with 125 I using Iodogen as previously described [22]. Both the hyperglycosylated rIL-4 and deglycosylated rIL-4 were labelled using this technique. Briefly, 2–5 μ g of rIL-4 were incubated for 15 min on ice with 37 MBq of carrier-free $Na^{125}I$ in a glass tube coated with 5 μ g of Iodogen. The labelled protein was separated from free ^{125}I by using a 10 ml column of P6DG (Bio-Rad) equilibrated in PBS containing 0.1% ovalbumin. Purity of the preparation was assessed by C_{18} reverse-phase h.p.l.c. and SDS/PAGE, followed by autoradiography. The specific radioactivity of the ^{125}I -labelled rIL-4 was estimated by mock iodination experiments in which 2–5 μ g of rIL-4 spiked with ^{125}I -IL-4 was put through an iodination protocol, and the recovery of rIL-4 quantified by measuring the ^{125}I -IL-4 recovery. This specific radio-

activity was confirmed by quantifying the concentration of unlabelled rIL-4 required to give 50% inhibition of ^{125}I -rIL-4 binding to Raji cells. The biological activity of the ^{125}I -rIL-4 was determined in a PHA blast proliferation assay. Specific radioactivities in the region of 500–1000 Ci/mmol are routinely obtained without loss of biological activity.

Binding studies

Raji cells were incubated at 4×10^6 /ml in RPMI 1640 (containing 2% BSA and 0.2% NaN_3) with various concentrations of ^{125}I -rIL-4. Binding to the cells was performed at 37 °C for 45 min, in either the absence or presence of a 100-fold molar excess of unlabelled rIL-4 to determine non-specific binding. Under these conditions, binding of the ^{125}I -rIL-4 to the cells had reached equilibrium. The reaction was terminated by centrifugation of the cells and removal of the supernatant. The cell pellet was washed, re-centrifuged and the radioactivity bound to the cells counted. Mathematical transformations of the binding data were performed as described in [23]. To determine the capacity of monoclonal antibodies raised against rIL-4 to block binding of ^{125}I -rIL-4 to Raji cells, a protocol identical with the above-described one was used with the addition of doubling dilutions of ascites to the incubation medium.

Cross-linking to intact cells

Cross-linking of ^{125}I -rIL-4 to its surface receptor on Raji cells was performed by using yeast deglycosylated ^{125}I -rIL-4. Cells (2×10^6) were washed twice in ice-cold PBS and incubated with 1–5 nM- ^{125}I -rIL-4 for 60 min on ice. The cells were washed and finally resuspended in 0.5 ml of ice-cold PBS. Disuccinimidyl suberate (DSS; 5 μ l of 50 mM stock in DMSO) was then added and the cells incubated for 20 min on ice. The cells were centrifuged and the cell pellet resuspended in 50 μ l of lysis buffer (2% Triton X-100, 1 mM-benzamidine, 150 mM-NaCl, 5 mM-EDTA, 50 mM-Tris/HCl, pH 7.4, 100 μ M-phenylmethanesulphonyl fluoride and 5 μ g/ml each of antipain, leupeptin and pepstatin). The cell lysate was incubated for 30 min on ice, followed by centrifugation to remove insoluble material. To the supernatant was added 25 μ l of four-times-concentrated SDS/PAGE sample buffer, followed by electrophoresis on a 5–13%-(w/v)-polyacrylamide gradient gel. In order to determine specificity of cross-linking, control experiments were performed in which a 100-fold molar excess of unlabelled rIL-4, the 4B2-F9 or the MOPC-31 monoclonal antibodies were present in the initial incubation of ^{125}I -IL-4 with the Raji cells.

PAGE

This was carried out as described by Maizel [24], with a 75:2 ratio of acrylamide to bisacrylamide. Vertical slab gels, 1.5 mm thick, with 5–13%-polyacrylamide gradients were used for analytical purposes. For autoradiography, gels were dried and exposed to Kodak XAR-5 film at -70 °C.

Generation of monoclonal antibodies to human rIL-4

BALB/c mice were immunized subcutaneously with 1 μ g of purified *E. coli*-derived rhIL-4 in Freund's complete adjuvant (Difco). Booster immunizations in Freund's incomplete adjuvant were given intraperitoneally at 3 weeks and 6 weeks after initial immunization.

At 4 days before fusion, a final boost of 3 μ g of rIL-4 in PBS was given intravenously. Spleen cells were fused with the mouse plasmacytoma NS-1 (Ag8) by using a modification of the method of Galfre *et al.* [25]. The fusion mixture was plated out in 4 $\times$ 96-well plates in 10% FCS/DMEM/HAT (hypoxanthine/aminopterin/thymidine) (Gibco-BRL) with 5×10^4 – 1×10^5 spleen-cell feeders per well. After 7 days, the culture supernatants were screened by direct-binding e.l.i.s.a., and positive wells were cloned by limiting dilution at 0.3 cell/well. A minimum of two rounds of subcloning were undertaken to ensure monoclonality. Ascitic fluid was generated in BALB/c mice which had been primed 10 days previously with 0.5 ml of Pristane. Cells, 10^7 of each type, were injected intraperitoneally and ascitic fluid removed after 5–15 days. At all stages, anti-IL-4 activity was measured by direct-binding e.l.i.s.a., which was performed in 96-well Immunoplates (Nunc). rIL-4 was coated overnight at 4 °C at 100 ng per well in 0.1 M-carbonate buffer, pH 9.6. Sites that had not reacted were blocked with 2% BSA in PBS. Culture supernatants or ascites were incubated for 1 h at 37 °C, and the presence of anti-IL-4 antibodies detected by β -galactosidase-conjugated anti-mouse Ig (Amersham) with *o*-nitrophenyl galactoside as substrate. Colour development was monitored on a Flow Titertek Multiscan plate reader at 405 nm. This protocol yielded two monoclonal antibodies, namely 4B2-F9 and 4B2-H12. Subclass analysis revealed the 4B2-F9 to be an IgG₁ and the 4B2-H12 to be an IgG₂.

RESULTS

Gene expression

Heterologous genes have been expressed in *S. cerevisiae* at all levels from barely detectable to 100 μ g/ml of culture. The synthetic gene used here was originally designed for expression in *E. coli* using codons favoured in the chloramphenicol acetyltransferase gene. These were modified to introduce restriction sites and eliminate secondary structures, and the 5' end was modified to rebuild the 3' end of the α -factor prepro leader. The codon usage is very different from that predicted for highly expressed *S. cerevisiae* genes [26,27]. There is some concern that the presence of rare codons in heterologous genes may reduce expression levels [28,29]. A number of eukaryotic genes have now been expressed at high levels in *S. cerevisiae* [19,30] using their natural codon bias, but these are less divergent from *S. cerevisiae* highly expressed genes than from those of *E. coli*. We estimate our expression level to be 0.67 μ g/ml of culture, showing that rhIL-4 is reasonably well expressed in spite of the apparently unfavourable codon bias of the gene.

Purification of rhIL-4

Hyperglycosylation is a frequently encountered feature of heterologous proteins expressed in *S. cerevisiae*, owing to the repeated addition of mannose units at *N*-linked sites [31]. Because there are two such *N*-linked glycosylation sites in hIL-4, cell-free supernatant from transfected yeast culture was applied to a Con A-Sepharose column, which specifically adsorbs carbohydrate bearing α -D-mannopyranoside and α -D-glucopyranoside residues. The IL-4 biological activity was found to be largely removed by Con A-Sepharose, and this gel was therefore employed for the first

adsorption/purification step. A 9-litre batch of clarified supernatant was loaded in 3 h, and bound protein was subsequently eluted with α -methyl D-glucoside. Even though only about 100 mg of total protein was retained by the column, 200 ml of gel (typical capacity 5–10 mg/ml of gel) was required to prevent flow-through of rIL-4, suggesting that the column was becoming saturated at lower-than-normal loads. This may have been due to the high molar percentage of mannose that is probably present in the hyperglycosylated protein (see below). Batch elution with 0.5 M- α -methyl D-glucoside gave good recovery and was efficient, with no detectable rhIL-4 eluted in subsequent acid washes.

The Con A-Sepharose eluate was applied to an S-Sepharose Fast-Flow cation-exchange column. Binding

Fig. 2. Purification of rIL-4

(a) I.e.c.: 1.2 litres of Con A-Sepharose eluate was adjusted to pH 5 and loaded on to a 2.5 cm $\times$ 5 cm S-Sepharose Fast Flow column equilibrated at the same pH. The column was developed in an NaCl gradient, and rhIL-4 was eluted as a peak centred on 0.7 M-NaCl. (b) R.p.-h.p.l.c. The rIL-4 peak from the preceding ion exchange step was loaded on to a C₁₈ 1 cm $\times$ 25 cm r.p.-h.p.l.c. column at 3 ml/min and bound proteins were eluted with acetonitrile/0.1%/TFA. The rIL-4 peak was judged to be > 95% pure by SDS/PAGE.

Table 1. Purification of rIL-4 from yeast

Stage	Total protein (mg)*	Volume ml	rIL-4 (mg)	Purity (%)	Purification (fold)	Recovery† (%)
Clarified medium	1395	9000	6.0	0.4	1	100
Con A-Sepharose	182	1200	4.8	2.6	6.5	80
S-Sepharose i.e.c.	9.4	180	3.6	38	95	60
R.p.-h.p.l.c.	3.1	6	3.1	95	238	51

* Protein was estimated by the Bradford assay, except for purified rIL-4, which was estimated using $A_{280}^{1\text{cm}} = 0.52$ for a 1 mg/ml solution, a value calculated from the tryptophan/tyrosine content.

† Recoveries were estimated by biological activity.

was poor above pH 6, which is surprising, considering that the nominal pI of hIL-4 is 9.7, based on the sequence and the expected pK_a values for the individual residue side chains [32]. Deglycosylation of the rIL-4 does in fact produce a species of pI > 9 as judged by isoelectric focusing (results not shown). The decrease in pI is unlikely to be due to sialylation, since both *N*- and *O*-linked glycans in *S. cerevisiae* are reportedly only of the high-mannose type [31]. It is possible that surface basic residues on the protein are being masked by the carbohydrate. For purification purposes, cation-exchange chromatography was run at pH 5 with NaCl gradient elution (Fig. 2a). IL-4 was eluted at 0.7 M-NaCl, as determined by both SDS/PAGE and biological activity, resulting in a 15-fold purification and a 75% recovery. Two minor peaks of biological activity not associated with the rhIL-4 protein (as judged by SDS/PAGE) were also noted. These remain unassigned and are presumed to be due to yeast protein or carbohydrate causing non-specific stimulation of the T-cell blasts in the bioassay.

The ion-exchange eluate was loaded on to a semi-preparative C_{18} r.p.-h.p.l.c. column, which was developed with an acetonitrile/0.1% TFA gradient (Fig. 2b). A peak of highly purified rhIL-4 was eluted at about 37% acetonitrile. This protein was stored at -70°C in acetonitrile/TFA for several months without loss of activity, or freeze-dried at 4°C . The purification scheme is summarized in Table 1.

Characterization of rIL-4

Each step of the purification procedure was analysed by biological activity in a PHA blast proliferation assay and by SDS/PAGE with silver staining. The rhIL-4 shows molecular-mass heterogeneity and appears as a smear on SDS/PAGE between 45 and 95 kDa, which is centred around 70 kDa. This mass is similar to that previously reported for rhIL-4 produced in yeast [33]. *N*-Glycanase, which cleaves at the asparagine linkage to give free oligosaccharide and aspartic acid, gave rise to a 15 kDa band, as expected for the unglycosylated protein, and a 27 kDa intermediate, which could be digested down to 15 kDa with double the concentration of *N*-glycanase (results not shown). This shows the presence of large carbohydrate chain(s) at either or both of the Asn-Xaa-Ser/Thr glycosylation sites in IL-4, namely residues 38–40 and 105–107 [8]. Presumably the intermediate digestion product is due to a population of shorter *N*-linked glycan on one of the sites which is more refractory to digestion than a longer chain on the other site.

During mass-spectrometric investigations aimed at

identifying the *N*-terminus, strong molecular ions (MH^+) were observed at m/z 1086 in peptic digests and at m/z 1550 in tryptic digests, corresponding respectively to residues 100–109 and 103–115 in the rhIL-4 sequence and therefore containing the second *N*-glycosylation site. The presence of appropriate molecular ions in both peptide and tryptic digests makes wrong assignment unlikely. Thus it seems that, to a large extent, this second site is not utilized by the host, although the *N*-glycanase digest intermediate would imply that a small amount of glycosylation occurs at this site. Such incomplete glycosylation has been observed for other heterologous proteins in yeast and may be a consequence of overexpression [31,34]. Molecular ions for peptides containing the *N*-terminal glycosylation site were not detected, presumably because, in contrast with the 105–107 site, the 38–40 site is predominantly or wholly modified by glycan.

Purity of the rIL-4 preparations could be estimated by silver-staining SDS/PAGE, with and without *N*-glycanase digestion (results not shown). A high degree of purity was indicated and confirmed by the good agreement of the experimental amino acid composition with that expected from the sequence (Table 2). The agreement is acceptable within error, except for the isoleucine value, which is too high, presumably because of residual contamination.

Murine IL-4 has been shown to contain no free thiol groups and, by inference, since there are six cysteine residues in the molecule, it is assumed to have three disulphide groups [1]. Human IL-4 also has six cysteine residues, five of which occupy the same positions as in the mouse sequence [1]. In order to determine the disulphide groups in IL-4, our yeast rhIL-4 was dissolved in 6 M-guanidium chloride and thiol groups titrated colorimetrically with 5,5'-dithiobis-(2-nitrobenzoate) [35]. This method demonstrated the absence of any free cysteine residues in the recombinant molecule (results not shown). Pairings of the six cysteine in disulphide groups remains to be determined.

Confirmation of the *N*-terminus of any recombinant protein is important, particularly in this case, since the molecule must be cleaved from a prepro α -factor fusion polypeptide by the KEX2 gene product. This is a thiol endoprotease which specifically cleaves C-terminal to paired basic residues [36] and therefore should release IL-4 with the authentic *N*-terminus (histidine). Correct processing was indicated by the experimental value of 4.8 mol of histidine/mol of rhIL-4 in the amino acid composition (Table 2). This interpretation was confirmed

Table 2. Amino acid composition of purified rhIL-4

Purified hyperglycosylated rhIL-4 was hydrolysed in HCl, and phenylthiohydantoin derivatives were quantified as described in the text. Tryptophan and cysteine are destroyed under the conditions used.

Amino acid residue	Composition (mol/mol of rhIL-4)	
	Experimental	Predicted from sequence
D/N	10.1	10
E/Q	17.9	17
S	8.5	8
G	3.7	3
H	4.8	5
R	8.8	9
T	14.1	15
A	8.9	8
P	1.1	1
Y	1.5	2
V	3.2	3
M	0.7	1
I	4.9	1
L	15.9	16
F	5.9	6
K	12.0	12

by mass spectrometry. T.s.-l.c.-m.s. of reduced tryptic digests showed MH^+ at m/z 1440, corresponding to residues 1–12 (HKCDITLQEIHK), and at m/z 1175, corresponding to residues 3–12. The molecular-ion counts and u.v. peaks corresponding to these peptides were roughly equal, showing either that there was incomplete tryptic cleavage after Lys-2 or that aberrant processing had occurred. The latter possibility is effectively ruled out by the presence of an MH^+ at m/z 829 in the peptic digest corresponding to residues 1–7, but the absence of a peak at m/z 564 for peptide 3–7. Thus the *N*-terminus is correct for the bulk of the protein, although low levels of aberrant cleavage may not be detected using this methodology.

Biological activities of rIL-4

The first described activity of IL-4 was its ability to stimulate resting B-cells to proliferate in the presence of antibodies to surface IgM. Fig. 3(a) demonstrates such a co-stimulator proliferation assay using purified rIL-4. Tonsillar B-cells were cultured in increasing concentrations of rhIL-4, with or without antibodies to IgM (anti-IgM). B-cells cultured with rhIL-4 alone or anti-IgM alone showed no increase in proliferation above control levels. However, in the presence of both rIL-4 and anti-IgM, there was a dose-dependent enhancement of proliferation.

The purified rIL-4 was also tested for its ability to stimulate the proliferation of activated T-cells. PHA-activated T-cell blasts, prepared from human tonsils or peripheral blood, were assayed for their ability to proliferate in response to rhIL-4. Fig. 3(b) shows such a dose-response curve, demonstrating a half-maximal proliferative response at an rhIL-4 concentration of 120 pM.

The purified rhIL-4 was further tested for its ability to upregulate CD23 expression on B-cells, which is a well-documented response of resting B-cells to IL-4 stimu-

Fig. 3. Biological activities

(a) Co-stimulator assay. Tonsillar B-cells were cultured at 10^5 cells per well in the presence of rhIL-4 alone (○) or rhIL-4 and anti-IgM-coated beads together (●). Proliferation was assessed by $[^3H]$ thymidine incorporation over the last 16 h of a 72 h culture. Controls were cultured in medium alone (577 ± 43 d.p.m.) or medium + anti-IgM alone (2005 ± 97 d.p.m.). Each point represents the mean $\pm$ S.E.M. for triplicate cultures. (b) Activated T-cell proliferation assay. Tonsillar lymphocytes prepared as described in the Materials and methods section were stimulated with PHA for 72 h. They were subsequently washed and cultured at 1×10^4 cells per well, either alone or with various dilutions of rhIL-4. Proliferation was assessed by $[^3H]$ thymidine incorporation over the last 16 h of a 72 h culture. Each point represents the mean for triplicate cultures. Half-maximal biological response was attained at an rhIL-4 concentration of 120 pM. (c) Surface CD23 upregulation. Tonsillar B-cells were cultured for 48 h at 5×10^5 per tube (A) alone, (B) with rhIL-4 at 68 pM, (C) with anti-IgM at $10 \mu g/ml$ or (D) with rhIL-4 and anti-IgM together. The cells were subsequently stained with an antibody to CD23, followed by a fluorescein isothiocyanate-labelled antibody. Cell staining was quantified by flow cytometry. Panels represent histograms of the fluorescence intensity on a logarithmic scale, and the hatched areas correspond to the positive staining, defined as the staining above the level of the background control.

lation. Tonsillar B-cells were cultured for 48 h in the presence of rhIL-4 alone or rhIL-4 and anti-IgM. Surface CD23 was detected by incubating the cells with an anti-CD23 monoclonal antibody (B6), followed by fluorescein isothiocyanate-conjugated second antibody, and the

fluorescence measured by flow cytometry. Fig. 3(c) shows the results of a representative experiment. Control B-cells, incubated in medium alone, did not express detectable surface CD23 (panel A). Addition of rhIL-4 at a final concentration of 68 pM (100 units/ml) induced an upregulation of surface CD23 expression (panel B), which was slightly enhanced by the addition of anti-IgM (panel D). Anti-IgM alone did not enhance surface expression of CD23 (panel C). The rhIL-4 in the presence of anti-IgM was effective in CD23 upregulation at concentrations as low as 1.7 pM (2.5 units/ml). At higher concentrations of rhIL-4 there was an increase in the staining intensity, but little further increase in the percentage of positive cells (results not shown).

Radioiodination of rIL-4 and receptor-binding studies

Purified rhIL-4 derived from yeast was radioiodinated as described above. Analysis of the ^{125}I -labelled protein by SDS/PAGE followed by autoradiography revealed a single broad band with an apparent molecular mass of 55–70 kDa (Fig. 4). Complete digestion of this protein with *N*-glycanase reduced the broad band to a single sharp band with an apparent molecular mass of 15 kDa, confirming that both the high molecular mass and the mass heterogeneity of the purified rhIL-4 was due to *N*-linked glycosylation in the yeast. Both the hyperglycosylated and the deglycosylated forms of the rhIL-4 were biologically active; however, radioiodination of both forms demonstrated that the hyperglycosylated rIL-4 gave more reproducible results in receptor-binding studies (results not shown). Consequently, in all binding studies reported here, hyperglycosylated ^{125}I -rhIL-4 was used.

Fig. 5(a) shows a typical experiment involving equilibrium binding of ^{125}I -rhIL-4 to azide-treated Raji cells at 37 °C. Binding was saturable, and Scatchard plots of the data demonstrate that the ligand binds to a single class of binding site with an equilibrium dissociation constant (K_d) of 100 pM and a maximum number of binding sites per cell of 1100 ± 200 (mean of four experiments).

The inhibition of ^{125}I -rhIL-4 binding to Raji cells by unlabelled rhIL-4 is shown in Fig. 5(b). The labelled rhIL-4 was present at a final concentration of 140 pM and binding was determined in the presence of various concentrations of unlabelled rhIL-4. The curve passing through the points is consistent with the presence of a single class of binding site, and 50 % inhibition of binding was achieved at 150 pM unlabelled rhIL-4.

Monoclonal antibodies to rhIL-4

We have raised a panel of monoclonal antibodies to rhIL-4, one of which (4B2-F9) is capable of blocking the rhIL-4-induced proliferation of PHA blasts as described above. To determine antibody specificity, PHA blasts were induced to proliferate by the addition of rhIL-4 (136 pM, 200 units/ml) or rIL-2 (50 units/ml). Various concentrations of 4B2-F9 were also added to the cultures and the subsequent proliferation measured. Fig. 6(a) shows that the 4B2-F9 antibody can specifically inhibit the rhIL-4-induced proliferation of PHA blasts while having no effect on rIL-2-induced proliferation.

In addition, we tested the two anti-IL-4 monoclonal antibodies 4B2-F9 and 4B2-H12 for their ability to inhibit binding of ^{125}I -rhIL-4 to Raji cells (Fig. 6b). The 4B2-H12 antibody showed no inhibitory activity, being

Fig. 4. Radioiodination of rhIL-4

Yeast-derived rhIL-4 was radioiodinated using the Iodogen reagent without loss of biological activity, as determined in an activated T-cell proliferation assay. Specific radioactivities in the range of 500–1000 Ci/mmol were routinely obtained. The yeast-derived protein is hyperglycosylated and has an apparent molecular mass of 55–70 kDa, as shown by this SDS/PAGE run under reducing conditions. Treatment of the 55–70 kDa glycoprotein with *N*-glycanase reduces the apparent molecular mass to 15 kDa.

comparable with the irrelevant control antibody MOPC-31. However, 4B2-F9 was able to block binding in a dose-dependent fashion.

Receptor cross-linking studies

In order to characterize the molecular mass of the cell-surface receptor for IL-4, cross-linking studies were performed using deglycosylated ^{125}I -rhIL-4 and Raji cells (Fig. 7). Deglycosylated ^{125}I -rhIL-4 was used in preference to the hyperglycosylated form, since the latter displays marked mass heterogeneity on SDS/PAGE, which renders the interpretation of receptor cross-linking experiments difficult. The treatment of cell-surface-bound ^{125}I -rhIL-4 with the homobifunctional cross-linking reagent DSS resulted in the generation of an ^{125}I -labelled complex with an apparent molecular mass of 139 kDa. Since the molecular mass of the deglycosylated rhIL-4 is 15 kDa, one can deduce that the mass of the surface molecule to which it has been cross-linked is

Fig. 5. Equilibrium-binding studies

(a) Binding of ^{125}I -rhIL-4 to Raji cells was performed at 37°C in the presence of NaN_3 . Cells (10^6) were incubated with increasing concentrations of ^{125}I -rhIL-4 for 45 min. Binding was determined as described in the Materials and methods section, and non-specific binding was defined by measuring ^{125}I -rhIL-4 binding in the presence of a 100-fold molar excess of unlabelled rhIL-4. Scatchard transformations of the saturation binding data (see the inset) reveal a single class of binding site with a K_d of 100 ± 18 pM and 1100 ± 200 sites per cell (means $\pm$ S.D. for four experiments). (b) Inhibition of ^{125}I -rhIL-4 binding. Binding of ^{125}I -rhIL-4 to Raji cells is inhibitable by unlabelled rhIL-4. 10^6 cells were incubated with 140 pM ^{125}I -rhIL-4 for 45 min at 37°C in the presence of increasing concentrations of unlabelled rhIL-4. Binding was determined as described in the Materials and methods section. The data were corrected for non-specific binding, which was defined as the radioactivity bound in the presence of 12 nM unlabelled rhIL-4. The curve passing through the points is characteristic of a receptor-ligand interaction with a reversible bimolecular interaction which obeys mass action law; 50% inhibition of binding was achieved at an unlabelled-rIL-4 concentration of 150 pM.

Fig. 6. Effect of monoclonal antibodies on rhIL-4 binding and activity

(a) Activity. Human PBL were activated by culturing for 3 days with PHA ($1 \mu\text{g}/\text{ml}$). The cells were then harvested and washed three times. T-cell blasts were cultured at $10^4/\text{well}$ with rhIL-4 (30 units/ml; $\bullet$) or rIL-2 (30 units/ml; $\circ$) alone or with dilutions of purified 4B2-F9 antibody as shown. Proliferation of the cells was assessed by $[^3\text{H}]$ thymidine incorporation over the last 8 h of a 72 h culture. Points are means for triplicate cultures, with the vertical lines representing the S.D. (proliferation control = 309 ± 73 c.p.m., which represents cells cultured in medium alone). (b) Binding of ^{125}I -rhIL-4 to Raji cells is inhibited by 4B2-F9. 10^6 cells were incubated with 100 pM ^{125}I -rhIL-4 for 45 min at 37°C in the presence of various dilutions of 4B2-F9 ($\circ$), 4B2-H12 ($\bullet$) and MOPC-31 ($\blacksquare$) ascites. Binding was determined as described in the Materials and methods section. The 4B2-F9 ascites decreases binding almost to background levels (650 c.p.m.), whereas the 4B2-H12 and the irrelevant control MOPC-31 had no significant effect.

124 ± 11 kDa (mean $\pm$ S.D.; $n = 5$). Control experiments in the presence of an excess of unlabelled rhIL-4 or in the presence of the blocking antibody 4B2-F9 revealed no cross-linked protein. However, in the presence of the irrelevant antibody, MOPC-31, the 141 kDa cross-linked complex could clearly be seen.

DISCUSSION

IL-4 has been shown to play a key role in murine B-cell growth and differentiation, particularly in class switching to IgG1 and IgE [1-3,37,38]. In humans, IL-4 has a similar activity profile, including the promotion of IgE

Fig. 7. Receptor cross-linking studies

Raji cells (2×10^6) were incubated with 1 nM deglycosylated ^{125}I -rhIL-4 for 60 min on ice, either alone (lane 1) or in the presence of the blocking anti-IL-4 antibody 4B2-F9 (lane 2), in the presence of the irrelevant antibody MOPC-31 (lane 3) or in the presence of a 100-fold molar excess of unlabelled rhIL-4 (lane 4). Cross-linking of the receptor–ligand complex with DSS generated a 139 kDa band ($\blacktriangleright$, lanes 1 and 3). Formation of the complex was inhibited by unlabelled rhIL-4 (lane 4) and by 4B2-F9 (lane 2), but not by MOPC-31 (lane 3).

synthesis, although class switching has not been demonstrated [5–7]. In order to characterize further these processes and to attempt to understand their molecular mechanisms, we have generated a synthetic gene coding for human IL-4 which was cloned and expressed in *S. cerevisiae* as a C-terminal fusion protein with the yeast prepro α -mating factor sequence. This expression system resulted in the secretion of rhIL-4 into the culture medium; a purification protocol was developed to recover this protein. The purified rhIL-4 was hyperglycosylated in the yeast and had a molecular-mass range of 45–95 kDa, which could be reduced to a single sharp band of 15 kDa by treatment with *N*-glycanase. The hyperglycosylated and *N*-glycanase-treated rhIL-4 have comparable biological activities, an observation which is consistent with previous studies using rIL-4 produced in yeast [33]. In our biological studies we have consequently made use of both the hyperglycosylated and deglycosylated forms of rhIL-4.

We have validated our recombinant molecule by both protein chemistry and biological activity. The purity and composition of the rhIL-4 was confirmed by the good agreement between the experimentally determined amino

acid sequence and the predicted sequence. Correct processing of the prepro α -factor/IL-4 fusion protein to authentic IL-4 was confirmed by t.s.-l.c.-m.s. Using this technique we demonstrate that residues 1–12 of the *N*-terminus correspond to the expected *N*-terminus of IL-4. Titration of thiol groups with 5,5'-dithiobis-(2-nitrobenzoate) revealed that the rhIL-4 had no free thiol groups. This agrees with the findings for rhIL-4 from *E. coli*, which was refolded/oxidized using a glutathione redox buffer [9], and for murine IL-4, which also has three disulphide groups [1].

Having established the authenticity of our rhIL-4, we went on to demonstrate that our preparation of purified rhIL-4 displayed the expected range of biological activities. To this end, we first demonstrated that our rhIL-4 preparation could induce the proliferation of human tonsillar B-cells when co-stimulated with anti-IgM. Secondly, IL-4 has been shown to induce B-cell and monocyte activation, as demonstrated by its ability to upregulate surface expression of the activation marker CD23 [39–41]. The data presented here show that our rhIL-4 preparation also had this effect on resting tonsillar B-cells. The third biological activity to be tested was the induction of activated T-cell proliferation. Our rhIL-4 preparation was able to cause a proliferative response in a dose-dependent fashion, with a half-maximal biological response at a concentration of 120 pM. This is slightly higher than, but comparable with, the value of 40 pM which has been reported as the rhIL-4 concentration required to give a half-maximal response in Jijoye cells (as measured by the induction of surface CD23) [39]. On the basis of this panel of biological assays, we concluded that our rhIL-4 preparation was valid and would be a suitable ligand to use in IL-4-receptor-binding studies.

The rhIL-4 was radioiodinated using the Iodogen reagent without loss of biological activity, as determined in PHA blast proliferation assays. The ^{125}I -rhIL-4 bound to Raji cells in a saturable fashion, and Scatchard analysis of the equilibrium binding data revealed a single class of binding site with a K_d of 100 pM and 1100 ± 200 binding sites per cell. These values are comparable with previously published values of 40–70 pM with 2200 receptors per cell [39] and 164 pM with 2160 ± 530 receptors per cell [33]. It is noteworthy that the K_d for receptor–ligand interaction (100 pM) corresponds to the rhIL-4 concentration required to give half-maximal biological activity in the PHA blast proliferation assay (120 pM). This finding, together with the observation that ^{125}I -rhIL-4 binding to cells was inhibited by equimolar concentrations of unlabelled rhIL-4, confirm that the iodination procedure alters neither the biological response to rhIL-4 nor the ability to bind to its receptor. The close relationship between K_d and half-maximal biological response has previously been reported for both human [39] and mouse IL-4 [42].

In order to determine the molecular mass of the cell-surface receptor for IL-4, cross-linking studies were performed using the deglycosylated ^{125}I -rhIL-4. We have demonstrated that the rhIL-4 ligand can be cross-linked to a cell-surface protein in a specific fashion. The cross-linked complex has a molecular mass of 139 kDa, and subtracting the molecular mass of the deglycosylated rhIL-4 ligand results in an estimate of ~ 124 kDa for the putative IL-4 receptor. Previous cross-linking studies on the murine receptor have shown it to be a 75 kDa protein [43,44], whereas the human receptor has been reported

to be a 139 kDa protein [33]. These workers attributed the differences between murine and human receptors to several potential causes, one of which was receptor proteolysis. Recently, however, three cell-surface proteins with molecular masses of 130, 75 and 65 kDa have been shown to cross-link with rhIL-4 and were also down-regulated in response to IL-4 [45]. The suggestion from those studies is that the human IL-4 receptor is a complex of three molecular components which can all bind IL-4 and which are co-ordinately downregulated. However, analysis of equilibrium-binding studies, both in our hands and from published sources [33,39], indicates that IL-4 binds to a single class of binding site on the cell surface; this would not appear to be compatible with a multicomponent receptor. In addition, our cross-linking studies reveal only a single receptor with a molecular mass of ~124 kDa, in agreement with the findings of Park *et al.* [33].

In our attempt to characterize the biological activities and receptor-binding properties of IL-4, we have raised a panel of monoclonal antibodies to rhIL-4. We describe two of these antibodies here, namely 4B2-F9 and 4B2-H12. Whereas both antibodies are capable of immunoprecipitating ¹²⁵I-rhIL-4, only 4B2-F9 blocks binding of ¹²⁵I-rhIL-4 to Raji cells. One would predict from such an observation that the 4B2-F9 antibody should also specifically block the biological activity of rhIL-4. We have shown that this antibody is indeed capable of abrogating the rhIL-4-induced proliferation of activated human T-cells while having no effect of rIL-2-induced proliferation. Consequently, 4B2-F9 may be of use in the subsequent mapping of IL-4 epitopes involved in interactions with its receptor.

Further studies are needed to characterize more fully the molecular nature of the IL-4 receptor and how it functions in the complex mechanisms of B-cell activation and differentiation.

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