Characterization and Pharmacokinetic Parameters of Recombinant Soluble Interleukin-4 Receptor

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The interleukin-4 receptor (IL-4R) is expressed as a 140-Kd membrane glycoprotein that binds IL-4 with high affinity. Recently, cDNA clones for the murine IL-4R have been isolated. One clone encodes an integral membrane protein, while another encodes a protein in which translation is terminated before the transmembrane region, thus producing a soluble form of the IL-4R (sIL-4R). HeLa cell clones overexpressing sIL-4R were isolated using a novel filter-overlay and 125I-IL-4 ligand binding technique. Quantitative analysis demonstrated that the kinetics and affinity of IL-4 binding to the recombinant sIL-4R were similar to the native membrane-bound IL-4R. As low doses of sIL-4R specifically inhibited IL-4–induced proliferative responses in vitro, sIL-4R biodistribution and elimination parameters were evaluated to assess the pharmacokinetic potential of sIL-4R as a therapeutic agent. Pharmacokinetic studies demonstrated that radiolabeled sIL-4R had a distribution half-life of 9 minutes and an elimination half-life of 2.3 hours following intravenous (IV) administration. When administered by intraperitoneal or subcutaneous (SC) injection, the elimination half-lives were prolonged to 4.2 hours and 6.2 hours, respectively. Although the initial blood level of sIL-4R was reduced if administered by SC injection, the bioavailability was comparable with IV administration. The main sites of sIL-4R elimination were the liver and kidney.

INTERLEUKIN-4 (IL-4) is a T-cell product initially described as a factor that could stimulate the proliferation of activated B cells in vitro.1 In addition, IL-4 has been shown to induce the expression of class II major histocompatibility complex determinants,2,3 enhance both the expression and secretion of IgG1 and IgE molecules by resting B cells,4,5 and regulate the expression of low-affinity Fc receptors for IgE.6,7 Activities associated with IL-4 are mediated following binding to high-affinity cell-surface receptors. Although results from several laboratories indicated that the IL-4 receptor (IL-4R) was a surface glycoprotein of 60 to 75 Kd,8,9 subsequent studies have demonstrated that the receptor is a 140-Kd molecule that is sensitive to proteolytic degradation, thereby explaining the previously underestimated molecular weight.10,11

Recently, cDNA clones encoding several forms of the murine IL-4R have been isolated.12,13 The full-length cDNA encodes an 810 amino acid polypeptide that contains a 208 amino acid extracellular region and a 553 amino acid cytoplasmic domain. A second IL-4R cDNA was found to contain a 114-base pair insertion in the third amino acid sequence coding for the extracellular region. This insertion originated due to alternate splicing of mRNA transcribed from a single exon within the IL-4R gene (B. Renshaw and B. Davison, personal communication, November 1990), leading to a reading frame alteration. This results in the addition of six new amino acids at the C-terminus, and termination of translation before the transmembrane region. When expressed transiently in COS-7 cells, the putative soluble form of the IL-4R (sIL-4R) could be detected in culture supernatants, and these supernatants were capable of inhibiting IL-4 binding and IL-4–mediated proliferative responses.13

The studies reported here were designed to characterize the binding of IL-4 to the sIL-4R as compared with native cell-associated IL-4R. Results indicated that sIL-4R has the same binding kinetics and affinity for IL-4 as detected for the integral membrane form of the IL-4R. In addition, low concentrations of purified sIL-4R specifically inhibited IL-4–mediated proliferation in vitro, suggesting its utility as a possible therapeutic agent. A complete pharmacokinetic analysis of sIL-4R was performed to determine the blood kinetics and tissue biodistribution following various routes of sIL-4R administration. These pharmacokinetic analyses will allow selection of an administration route and dose regimen that will optimally maintain sIL-4R levels in vivo.

MATERIALS AND METHODS

Construction and expression of the sIL-4R. Recombinant sIL-4R was produced in 653-6 HeLa cells expressing the Epstein-Barr Virus (EBV) nuclear antigen. The vector used to direct the expression of the sIL-4R in HeLa cells was a derivative of pDC201 termed HAV-EO8 that contains the EBV origin of replication. The HAV-EO-NEO plasmid replaces the adenovirus major late promoter of pDC201 with sequences from human immunodeficiency virus-1 (HIV-1) extending from −148 to +76 relative to the CAP site of the viral mRNA and includes the HIV-1 tat gene under the control of the SV40 early promoter. Because HAV-EO-NEO also contains a BglII-SmaI fragment containing the neomycin resistance gene of pSV2neo10 inserted into the BglII and HpaI sites downstream of the SalI cloning site, the resulting vector permits selection of transfected cells for neomycin resistance. The murine IL-4R clone 18 cDNA11 was trimmed with EcoNI and SalI to generate a 760-base pair fragment. This fragment was rendered blunt-ended using T4 polymerase and subcloned into the blunted SalI site of HAV-EO-NEO to generate HAV-EO-NEO–sIL-4R18.

The HAV-EO-NEO–sIL-4R18 plasmid was introduced into 653-6 cells by electroporation as described by Dowar et al,11 except that cells were trypanosed 2 days after transfection and split at a ratio of 1:8 into medium containing 1.0 mg/mL of G418 (GIBCO, Grand Island, NY). Culture medium was changed twice weekly until neomycin-resistant colonies were established. Clones expressing high levels of the sIL-4R were detected by a filter-overlay screening method. This technique is based on the adsorption of secreted protein onto nitrocellulose filters12 followed

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by the detection of sIL-4R by \(^{125}\)I-IL-4 binding. G418-resistant cultures of cells transfected with the mIL-4R18 plasmid were seeded into 10-cm petri dishes at 200 cells/plate and incubated for 2 weeks to allow for colony formation. Growth medium was removed, cells were washed twice with phosphate-buffered saline (PBS), and a sterile Teflon mesh was placed over the cells. A sterile nitrocellulose BA85 filter was subsequently placed over the mesh. Filters were then weighed onto the cells with glass beads and serum-free culture medium was added back to the cells. After an overnight incubation, the filters were removed and soaked in 0.05 mol/L Tris, 0.15 mol/L sodium chloride, 3% bovine serum albumin, pH 7.5 (TBS-3% BSA) to block nonspecific binding. The filters were then incubated with \(4 \times 10^{11}\) mol/L \(^{125}\)I-IL-4 in TBS-3% BSA for 2 hours at 4°C with gentle agitation, washed three times with PBS, dried, and exposed on Kodak X-Omat film (Eastman Kodak, Rochester, NY) overnight. Following development of the X-ray films, cell colonies were aligned with spots on films indicating high levels of IL-4 binding. Colonies were isolated individually, grown in culture, and their supernatants were tested for their ability to inhibit IL-4 binding to CTLL-2 cells. The highest expressing clone, HeLa E3C3, was cloned twice by limiting dilution.

**Purification and characterization of sIL-4R.** Recombinant sIL-4R was purified from HeLa E3C3 culture supernatants as described for the natural IL-4. Briefly, conditioned medium was passed over a 4.0-mL bed volume IL-4 Affigel column, which was then washed with PBS and eluted with 0.1 mol/L acetic acid, 0.15 mol/L sodium chloride, pH 3.0. Eluate fractions were neutralized immediately with 1.0 mol/L HEPES and tested for IL-4-binding activity. Protein purity and concentration were determined by amino acid analysis. N-glycosidase digestion was performed by incubating 500 ng of purified sIL-4R with N-glycosidase according to manufacturer’s suggestions (Genzyme, Boston, MA). Gel electrophoresis was performed as described by Laemmli using 10% acrylamide gels under reducing conditions.

**Radiolabeling of IL-4 and sIL-4R.** Recombinant murine IL-4 was generated in yeast and purified to homogeneity as described previously. IL-4 was radiolabeled with \(^{125}\)I by the Enzymobead technique (Biorad, Richmond, CA) to a specific activity of \(1 \times 10^{10}\) cpm/mmol and stored as a stock of \(3 \times 10^{-4}\) mol/L in binding medium (RPMI 1640 medium, 10% BSA, 0.1% sodium azide, 20 mmol/L sodium chloride, pH 7.4). Recombinant murine sIL-4R was purified as described above and radiolabeled by the same method used for IL-4. The \(^{125}\)I-sIL-4R preparation used in pharmacokinetic studies had a specific activity of \(1 \times 10^{9}\) cpm/mmol and was stored in PBS containing 0.01% mouse serum albumin (BSA) before use.

**Cell lines and culture conditions.** The murine T-cell line CTLL-2 (ATCC TIB214) was maintained in supplemented Dulbecco’s modified Eagle’s medium (DMEM) as described previously. HeLa 65-6 cells and HeLa cells transfected with a plasmid encoding the sIL-4R (HeLa E3C3) were grown in DMEM supplemented with 5% fetal bovine serum as described. Additionally, HeLa E3C3 cells were maintained in 250 \(\mu\)g/mL G418. To collect recombinant sIL-4R, cells were grown to confluence in roller bottles, washed twice with PBS, and incubated for 3 additional days in serum-free DMEM. The culture supernatant was then collected and sIL-4R was purified as described above.

**Assay determination of sIL-4R binding to IL-4.** A solid-phase binding assay was developed similar to that described by Dower et al for the IL-1 receptor. Briefly, 96-well enzyme immunoassay (EIA) plates (Corning Glass Works, Corning, NY) were coated with M2 anti-murine IL-4R antibody at a concentration of 10 \(\mu\)g/mL overnight at 4°C. Plates were washed with PBS, incubated with sIL-4R for 4 hours at 4°C, and washed with PBS before use. Bound sIL-4R was then detected by the level of \(^{125}\)I-IL-4 binding under various conditions as described in the figure legends. Binding data were calculated and graphed using RS1/1 (BBN software products, Cambridge, MA) as previously described.

**Effects of sIL-4R on proliferation of CTLL-2 cells to IL-4.** CTLL-2 cells were washed extensively and cultured in flat-bottom microtiter plates (2 \(\times\) 10\(^5\) cells/well) in 100 \(\mu\)L of DMEM supplemented with 1.0 ng/mL recombinant murine IL-4 or recombinant human IL-2. Purified murine sIL-4R was added to the cultures at the concentrations indicated. Cultures were pulsed with 1 \(\mu\)Ci of \(^{3}H\)-thymidine (Amersham, Arlington Heights, IL; 25 \(\mu\)Ci/mmol, 1.0 mCi/mL) during the last 6 hours of 24 hours of incubation. Incorporation of radioactivity was determined by harvesting cells onto glass fiber filters followed by liquid scintillation spectrophotometry.

**Pharmacokinetics/biodistribution study design.** Experiments were performed using BALB/c mice (The Jackson Laboratory, Bar Harbor, ME), 7 to 9 weeks of age. The mice were maintained in a specific pathogen free animal facility for a minimum of 1 to 2 weeks before use and fed ad libitum. In the first experiment, 30 mice were injected intravenously (IV) via the lateral tail vein with 1.1 \(\mu\)g TCA precipitable, \(^{125}\)I-sIL-4R in a total volume of 150 \(\mu\)L. At various time points from 2 minutes to 24 hours, groups of mice (three mice per group) were bled by cardiac puncture and killed. Blood samples were heparinized, weighed, and the radioactivity was determined using a Packard Cobra Autogamma Scintillation counter (Packard Instruments, Laguna Hills, CA). Plasma was then isolated from an aliquot of the blood and the actual TCA precipitable counts were determined for blood samples. Eight additional tissues (liver, kidney, spleen, lung, heart, intestine, muscle, and brain) were collected, weighed, and the radioactivity was determined as described above. All blood and tissue samples were processed and represented as percent of the injected dose of \(^{125}\)I-sIL-4R per gram of the collected tissue (%ID/gram).

**Pharmacokinetic data analysis.** Pharmacokinetic parameters for the blood kinetics were determined from blood concentration-time profiles for each route of administration. In addition, pharmacokinetic parameters for liver, kidney, and lung were estimated using the tissue concentration-time profiles for each route. The apparent elimination rate constant (K) and the half-life (T\(_{1/2}\)) was calculated using a pharmacokinetics half-life program on a RS/1 system. The log linear portion of the blood (or tissue) concentration-time curve was used to calculate K with T\(_{1/2}\) determined by the formula T\(_{1/2}\) = In2/K. Half-life values are presented as T\(_{1/2}\) ± SE, where SE indicates the error in fitting the log linear line to the data points in calculating the K value. For the IV route of administration, the distribution (T\(_{1/2}\)) and elimination (T\(_{1/2}\)) half-lives were calculated using a biexponential pharmacokinetics program relating the respective log linear concentration-time curves to specific K and T\(_{1/2}\) values. The area under the blood (or tissue) concentration-time curve from time 0 to infinite time (AUC) was determined by conventional trapezoidal summation and extrapolation. The rela-
tive blood bioavailability of sIL-4R administered by IP or SC was estimated by dividing the mean AUC for IP or SC administration by the mean AUC for IV administration. The maximum serum concentration (Cmax) and the time of maximum serum concentration (tmax) were read directly from the plotted data. Clearance (Cl) was calculated by dividing the injected dose by AUC, and the volume of distribution (Vd) was calculated by dividing Cl by K. To evaluate the sIL-4R levels in tissue over time, cumulative tissue distribution values for all tissues were determined by AUC analysis. Cumulative tissue distribution values for the amount of sIL-4R per gram tissue as well as per actual organ weight were calculated.

RESULTS

Expression and purification of the sIL-4R. An HeLa cell-based expression system was used to produce recombinant sIL-4R by inserting the murine IL-4 receptor clone 181 into the HAV-EO-NEO expression plasmid.19 The 653-6 HeLa cells were electroporated with the HAV-EO-NEO IL-4R 18 expression plasmid and G418-resistant colonies were selected for further screening. Cell clones expressing high levels of sIL-4R were detected by a filter-overlay and 125I-IL-4 blotting technique as described in the Materials and Methods. Colonies that represented the highest 125I-IL-4 binding on the filters (and therefore expressing the highest level of sIL-4R) were isolated, and grown in mass culture. Confluent monolayers of HeLa cells were tested for secretion of sIL-4R into the culture supernatant as detected by inhibition of 125I-IL-4 binding to CTLL-2 cells.22 One clone, HeLa E3C3, secreted ~600 ng/mL sIL-4R and was chosen for further characterization. Following cloning of these cells by limiting dilution, and optimization of cell culture and harvest conditions, HeLa E3C3 cells produced between 1 to 3 µg/mL of sIL-4R.

The sIL-4R was purified from HeLa E3C3 culture supernatants by IL-4 Affigel affinity chromatography.19 Gel electrophoresis of the purified sIL-4R showed that it contained two bands of molecular weight (MW) ~37,400 and ~39,800 (Fig 1). No difference in apparent molecular weight was observed on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in the absence of reducing agents (not shown). Digestion of the sIL-4R protein with N-glycanase produced a major band of MW ~25,000, indicating the heterogeneity in size was due to N-linked glycosylation. A minor band apparent at a MW of ~32,000 is likely due to incomplete digestion with N-glycanase. A protein of ~68,000 MW is due to albumin contamination in buffers used in preparing and running gels, and was present in all lanes whether or not they contained sample protein.

Binding properties of the sIL-4R. To test whether the binding capacity of the sIL-4R was similar to the native cell-associated IL-4R, a solid-phase binding assay was developed using the purified sIL-4R and a recently characterized monoclonal antibody (MoAb) against the IL-4R.22 A dose-response curve for 125I-IL-4 binding to microtiter plates was evaluated by coating plates with various concentrations of anti-IL-4R MoAb and purified sIL-4R either alone or in combination (Fig 2A). Binding of 125I-IL-4 was not detected if wells were uncoated or coated with either anti-IL-4R MoAb or sIL-4R alone. Only wells coated first with anti-IL-4R (10 µg/mL) and followed with sIL-4R (100 ng/mL) bound 125I-IL-4. When wells were coated with both anti-IL-4R MoAb and sIL-4R, a dose-response curve for 125I-IL-4 binding was observed even when extremely low levels of sIL-4R were used (Fig 2B). In addition, binding of 125I-IL-4 was specific and could be reduced to background levels in the presence of 100-fold molar excess of unlabeled IL-4 (data not shown). Thus, this assay is a specific and sensitive method for the detection of sIL-4R with the capability of detecting as low as 0.5 ng/mL sIL-4R.

To design conditions suitable for equilibrium binding studies, association kinetic experiments were performed. The binding of 125I-IL-4 to the sIL-4R was relatively rapid, reaching equilibrium in approximately 30 to 60 minutes at 4°C (Fig 3) and was similar to the rate of 125I-IL-4 binding to CTLL-2 or FDCP-2 cells at 37°C, as previously described.19 Based on these results, an incubation period of 2 hours was chosen for solid-phase equilibrium binding experiments. Analysis of data from equilibrium binding experiments demonstrated that a single class of binding sites was detected for the sIL-4R with an affinity of 1.61 x 10⁹ M⁻¹ for IL-4 (Fig 4). This binding constant is approximately the same as that for the full-length IL-4R on CTLL-2 cells (1.63 x 10⁹ M⁻¹),14 or the recombinant full-length (7B9-2) IL-4R expressed transiently in COS-7 cells (1.4 x 10⁹ M⁻¹).16

Inhibition of IL-4 bioactivity by the sIL-4R. Previous studies with COS-7 cells transfected with an expression plasmid containing the sIL-4R showed that conditioned medium blocked the induction of cell proliferation by IL-4.19 The experiments described here were designed to allow direct quantitation of the sIL-4R concentration required to inhibit this response. CTLL-2 is a murine T-cell line that is maintained in long-term culture with IL-2;
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Fig 2. Binding of 125I-IL-4 and sIL-4R to anti-IL-4R coated plates. (A) Specificity of binding. Data are plotted as femtomoles 125I-IL-4 bound to plates coated with: (□) nothing, (△) anti-IL-4R at 10 µg/mL, (□) soluble IL-4R at 100 ng/mL, and (○) anti-IL-4R at 10 µg/mL + sIL-4R at 100 ng/mL. (B) IL-4R dose-response curve. Plates were coated with anti-IL-4R at 10 µg/mL followed by sIL-4R at the doses indicated. Finally, 125I-IL-4 (6.7 x 10^-9 mol/L) was added. Background counts bound in the absence of added sIL-4R have been subtracted from the data.

However, CTLL-2 cells maintain a short-term proliferative response to either IL-2 or IL-4 in a dose-dependent manner.26 When stimulated with a constant mid-range dose of IL-4 (1.0 ng/mL), a dose-dependent inhibition of cell proliferation by the sIL-4R was detected (Fig 5A). Furthermore, no inhibition of IL-2-mediated proliferation of CTLL-2 cells was detected following addition of the sIL-4R. The data indicate that the inhibitory effect of the sIL-4R is specific for IL-4, and not due to toxicity associated with the protein preparation (Fig 5B). Half-maximal inhibition of IL-4-dependent proliferation was obtained with ~10 ng/mL of sIL-4R, which equates to a fivefold molar excess of sIL-4R.

Pharmacokinetics of sIL-4R IV administration. Soluble IL-4R was radiolabeled with Na125I by the Enzymobead technique. The radiolabeled sIL-4R had biologic activity identical to unlabeled sIL-4R (Fig 5A). The distribution half-life (T1/2) for a single bolus, IV injection of 125I-sIL-4R was 9 ± 1 minute (Table 1). Approximately 75% of the initial dose distributed into the tissues during the distribution phase, which was 90% completed within 30 minutes (3.3 half-lives = 90%). During this time, distribution was mainly to the kidney, liver, and lungs (Fig 6). Liver was the primary site of distribution having a Cmin almost twofold greater than that detected in either kidney or lungs (Table 1). Some distribution to spleen and heart was observed and little to no sIL-4R was distributed to intestine, muscle, or brain (data not shown).

The blood elimination half-life (T1/2b) of sIL-4R was 2.3 ± 0.8 hours with the remaining 25% of the initial dose eliminated over 8 to 11 hours (3.3 to 5 half-lives). Rapid elimination was mediated mainly by the liver, which had a T1/2b of 0.4 hours, followed by the kidney, which had a T1/2b of 2.3 hours (Table 1). The T1/2b for lung was 2.0 hours, which
was similar to the $T_{1/2}$ for blood and, thus, probably reflects little to no elimination of sIL-4R by the lungs.

Cumulative tissue distribution levels indicated that significant amounts of sIL-4R were detectable in the circulation (Table 2). On a per gram basis, kidney, liver, and lung were the major organs showing significant cumulative sIL-4R tissue levels. However, on an actual organ weight basis (with blood volume estimated at 8% body weight), the highest cumulative sIL-4R levels occurred in the blood with liver being the major site of elimination.

**Pharmacokinetics of sIL-4R administered via SC or IP routes.** When sIL-4R was administered by IP or SC routes as compared with IV administration, the blood kinetics of sIL-4R showed delayed $T_{max}$ values, decreased $C_{max}$ values, prolonged $T_{1/2}$ values, and larger $V_{d}$ values. The $T_{max}$ values for sIL-4R administered by these routes were 60 minutes as compared with 2 minutes for IV injection. The $C_{max}$ values were similar for IP and SC routes (approximately 0.2 μg/mL), and sixfold lower than the $C_{max}$ value observed following IV injection (1.2 μg/mL). The $T_{1/2}$ values for IP and SC routes were 4.2 hours and 6.2 hours, respectively, compared with 2.3 hours for IV administration. The $V_{d}$ for both IP and SC routes was increased approximately 2.7-fold. The clearance rates were similar for IV and SC administered sIL-4R, and slightly increased for IP injection.

Distribution of sIL-4R to tissues was generally lower in concentration and prolonged over time for IP and SC injection as compared with IV administration (Fig 6). Both IP and SC administration showed a reduction in %ID/gram values for blood, liver, kidney, lungs, spleen, and heart during the initial 2-hour period after injection. Similarly, IP and SC routes delayed liver/kidney $T_{max}$ values to 15 minutes and 60 to 120 minutes, respectively, as compared with 2 to 5 minutes for the IV route. This result may reflect the slower absorption of sIL-4R from IP and SC sites into the blood circulation in contrast to the immediate bolus effect of sIL-4R into the circulation from IV administration.

On an organ weight basis, the cumulative tissue levels for IP or SC administration showed that cumulative sIL-4R levels were highest in the circulation (Table 2). The total blood bioavailability of sIL-4R if administered by IP or SC injection was 79% and 112%, respectively, compared with IV administration. Similar to IV administered sIL-4R, liver was the major site of elimination, with kidney also mediating significant clearance. The slight decrease in bioavailability for the IP route was mainly due to lower $T_{max}$ values, decreased $C_{max}$ values, prolonged $T_{1/2}$ values, and larger $V_{d}$ values. The $T_{max}$ values for blood, liver, kidney, and lungs were collected and processed as described in Materials and Methods.

**Table 1. sIL-4R Pharmacokinetic Parameters**

<table>
<thead>
<tr>
<th>Parameter†</th>
<th>Route of Administration*</th>
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<tr>
<td>Blood Kinetics</td>
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<tr>
<td>$C_{max}$</td>
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<td>$T_{max}$</td>
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<tr>
<td>$T_{1/2}$</td>
<td>9 ± 1 min</td>
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<tr>
<td>$T_{24}$</td>
<td>2.3 ± 0.8 h</td>
</tr>
<tr>
<td>$Cl$</td>
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<tr>
<td>$V_{d}$</td>
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<tr>
<td>Tissue Kinetics</td>
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<tr>
<td>Kidney:</td>
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</tr>
<tr>
<td>$C_{max}$</td>
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<td>$T_{1/2}$</td>
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<tr>
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<tr>
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<td>$T_{1/2}$</td>
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</table>

* Mice were injected with 1.1 μg TCA precipitable, 125I-sIL-4R by IV, IP, or SC injections at time 0. At multiple time points, blood, kidney, liver, and lungs were collected and processed as described in Materials and Methods.

†Pharmacokinetic parameters for blood and tissue kinetics were determined from blood/tissue concentration-time profiles for each route of administration. Various parameters were calculated as described in Materials and Methods.
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Fig 6. Blood and tissue distribution of sIL-4R when administered by IV, IP, and SC routes. Radiolabeled sIL-4R was injected IV, IP, or SC into mice at time 0. At various time points, blood and tissues were collected, weighed, and radioactivity determined. Data are represented as percent of the initially injected %sIL-4R dose per gram of collected tissue (%ID/gm). Each data point is the mean ± SEM for three mice.

DISCUSSION

IL-4 has many biologic activities mediated through binding to the IL-4R, such as generation of antigen-specific cytolytic T lymphocytes (CTL), proliferation of B cells, and regulation of polyclonal IgE responses in mice. Indeed, large doses of a MoAb to IL-4 have been shown to suppress IL-4-dependent induction of IgE in mice. Several cDNA clones have recently been isolated for the IL-4R. One clone encodes a cell-surface membrane IL-4R and another a soluble or secreted version of the receptor. Interestingly, a protein that binds IL-4 with high affinity was recently detected in various murine biologic fluids. Using antibodies directed against the murine IL-4R, the receptor has been detected in medium conditioned by various cell lines as well as in ascites fluid and urine. These results suggest that soluble IL-4-binding proteins may represent a mechanism to regulate the many pleiotropic activities of IL-4 in vivo. Because soluble IL-4 binding proteins such as the recombinant sIL-4R may have an in vivo role as therapeutic agents, we have expressed the recombinant sIL-4R, and characterized its biologic and pharmacokinetic parameters.

To isolate a stable cell line expressing the recombinant sIL-4R, a novel expression and amplification strategy was developed. This strategy allowed for efficient screening and cloning of colonies exhibiting the highest sIL-4R expression. Following optimization of cell culture and harvest conditions, the best producing clone (HeLa E3C3) yielded 1 to 3 µg/mL sIL-4R activity following a 3-day medium-conditioning period. Purification of the sIL-4R from HeLa E3C3 culture supernatants was accomplished by affinity chromatography. Purified recombinant sIL-4R consisted of several MW species that differed in the amount of N-linked glycosylation. By averaging the MW of these sIL-4R species in the preparations, an average ~38,600 was obtained. The sIL-4R preparations contained approximately 38% carbohydrate, but heterogeneity in glycosylation was observed. The predicted MW for the 206 residue sIL-4R is 23,900. N-glycanase-treated sIL-4R migrated at ~25,000 on SDS-PAGE.

A solid-phase IL-4-binding assay was developed that permitted quantitative equilibrium binding analysis of the sIL-4R. Examination of the 125I-IL-4 binding characteristics of purified recombinant sIL-4R showed an affinity for IL-4 similar to the full-length, membrane-bound IL-4R expressed on various B- and T-cell lines, or the recombinant IL-4R expressed in COS-7 cells. The kinetics of the sIL-4R association with 125I-IL-4 were also similar to results previously obtained using cell-based assays.

Because the sIL-4R inhibited IL-4-mediated stimulation
of murine T cells in vitro, the sIL-4R could be an effective inhibitor of IL-4-mediated immune responses in vivo. Results of Maliszewski et al. have shown that the sIL-4R is also a potent inhibitor of B-cell activation in vitro. In these studies, the sIL-4R effectively blocked IL-4-stimulated proliferation, CD23, and IgA expression, as well as IgG1 and IgE secretion in splenic B cells. Furthermore, preliminary results of Fanslow et al. indicate that IP administration of sIL-4R in vivo prolongs allograft survival and reduces the strength of the allogeneic response occurring at the site of allogeneic cell injection. The ability to further evaluate sIL-4R as a potential therapeutic agent in preclinical trials would be enhanced by a thorough understanding of the pharmacokinetic properties of sIL-4R.

The results from the pharmacokinetic studies demonstrate a biphasic pattern for sIL-4R clearance including both distribution (α) and elimination (β) phases when administered as a single IV injection. Distribution of sIL-4R from the circulation into the tissues was accomplished within the first 30 minutes (T1/2α = 9 minutes). The remaining sIL-4R was eliminated during the succeeding 11 hours (T1/2β = 2.3 hours).

When administered in a single IP or SC injection, the maximum blood concentrations of sIL-4R were 10-fold lower than that detected in mice receiving an IV bolus injection. Although initially lower in concentration, the blood levels remained constant with elimination half-lives twofold to threefold longer for IP or SC as compared with IV administered sIL-4R. Distribution to tissues was delayed by 15 minutes and 60 to 120 minutes for IP and SC routes, respectively, as compared with IV injection. The delayed rates of distribution were most likely the result of differential rates of sIL-4R absorption into the circulation from the individual injection sites. In general, similar distribution patterns were observed for IP and SC routes with the exception that less sIL-4R accumulated in the liver when administered by the SC route. Because the SC injection site was at the nape of the neck (which has venous drainage to the superior vena cava), the sIL-4R may not have been subject to a first-pass elimination effect via the liver. In contrast, because the peritoneum partially drains into the portal venous system, some of the IP administered sIL-4R may have been eliminated quickly by the liver in a first-pass effect. This theory would explain the lower blood bioavailability as well as the higher clearance rate observed for the sIL-4R administered via the IP route as compared with the SC and IV routes.

Because the distribution and elimination of a drug depends on its physicochemical properties, the ability of sIL-4R to distribute easily into tissues is a function of its molecular size, isoelectric point, and hydrophobic/hydrophilic properties. The sIL-4R has numerous short hydrophobic stretches (data not shown). However, the isoelectric point of the nascent sIL-4R protein has been calculated to be 5.09. Based on this, it would be anticipated that the sIL-4R would be mostly ionized and, thus, more hydrophilic at a physiologic pH of 7.4. Indeed, these studies showed that sIL-4R did not distribute to brain or muscle, sites where a hydrophobic molecule would have distributed. Drugs are also known to distribute to tissues at different rates depending on the blood flow to that tissue. The rapid, initial appearance of sIL-4R levels in such tissues as spleen, heart, and lungs was most likely an example of the high vascularity of these organs instead of active accumulation or elimination, especially because the T1/2β for lungs and blood were similar. The kidneys have been shown to be major organs for clearance of molecules with MW below 50 to 70,000. Thus, the cumulative distribution of sIL-4R to the kidneys most likely indicates renal clearance. However, based on organ weight the liver was found to be the main site of cumulative sIL-4R tissue levels, an indication of hepatic metabolism.

In summary, a method has been developed that enables us to isolate a stable cell line expressing large quantities of sIL-4R for purification and preclinical studies. The purified sIL-4R has the same binding characteristics as the native membrane IL-4R and inhibits the biologic activity of IL-4 in vitro at low concentrations. If administered as a therapeutic agent, blood concentrations of sIL-4R could be maintained for a longer period of time if administered SC compared with IV administration, although larger doses would be necessary to achieve similar initial blood and tissue concentrations. Because sIL-4R distributed readily to tissues, similar if not greater overall bioavailability was achieved by SC administration. Thus, the binding and pharmacokinetic properties of sIL-4R support the prospect of its use as a novel therapeutic agent for pathologic conditions in which IL-4 is thought to be involved.

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