PURIFICATION OF HUMAN INTERLEUKIN 2 TO APPARENT HOMOGENEITY AND ITS MOLECULAR HETEROGENEITY*

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Interleukin 2 (IL-2, formerly termed T cell growth factor), discovered by Morgan et al. (1) is produced by T lymphocytes after antigen or mitogen stimulation and is required for the proliferation of activated T cells. IL-2 is an essential mediator of the immune response (2, 3), and there is preliminary evidence that it may also be responsible for the abnormal cell proliferation in human lymphoblastic leukemias (4).

Studies of the mechanism of action of IL-2 using unpurified or partially purified preparations have been very difficult, because conditioned media contain other lymphokines and cytokines with potent biological activities. Several groups have reported purification procedures for both murine (5, 6) and human IL-2 (7–9). These IL-2 preparations have permitted an increasingly better definition of IL-2 regulation. However, the purity of the human IL-2 preparations has not always been well documented.

In this study, we report a purification procedure of IL-2 which involves chromatography on blue agarose and chromatography on Procion-red agarose, permitting a rapid purification of human IL-2 to apparent homogeneity. Biochemically, the purified IL-2 appears to be free of any contaminating proteins. Up to three biologically active protein components can be detected in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels. This molecular heterogeneity is dependent on the experimental conditions used for the IL-2 production.

Materials and Methods

Preparation of Lymphocyte Conditioned Medium (Ly-CM, Fraction I). Human lymphocytes (PBL) were obtained from peripheral blood of multiple donors (New York Blood Center, New York).

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Abbreviations used in this paper: BCGF, B cell growth factor; Con A, Concanavalin A; CTLL, cytotoxic T cells; GM-CSF, granulocyte-macrophage colony-stimulating factor; IEF, isoelectrofocusing; [3H]TdR, tritiated thymidine; IL-1, interleukin 1 (lymphocyte-activating factor); IL-2, interleukin 2 (T cell growth factor); Ly-CM, lymphocyte conditioned medium; PEG 6000, polyethylenglycol (6,000 mol wt); PBL, peripheral blood lymphocytes; PBS, phosphate-buffered saline, pH 7.2; PHA, phytohemagglutinin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TRF, T cell-replacing factor.

In a typical procedure, the cells were initially stimulated by Sendai virus (10^4 U/ml) as part of a protocol to induce interferon. 12 h later, the culture medium, rich in α-interferon, was removed by centrifugation (800 g). The cells were resuspended to 4 × 10^6/ml in serum-free RPMI 1640 (Grand Island Biological Co., Grand Island, NY) supplemented with 0.25% bovine serum albumin (Sigma Chemical Co., St. Louis, MO) and 1% phytohemagglutinin-M (PHA-M) (Grand Island Biological Co.) and incubated at 37°C for 48 h. In some preparations irradiated (5,000 rad) Daudi cells (10^6/ml) were added to the medium to increase IL-2 production. At the end of the incubation, cells and cell debris were separated from the conditioned medium by centrifugation (10,000 g, 15 min) and the supernatant was used for purification of IL-2.

Ammonium Sulfate Precipitation (Fraction II). 1683 g of (NH_4)_2SO_4 was added to 3 liters of LCM, to achieve 80% saturation. After gentle stirring overnight at 4°C, the precipitate was spun down (10,000 g, 15 min), dissolved in 0.05 M Tris-HCl, pH 7.8, in a final vol of 100 ml, and subsequently dialyzed against 50 vol of 0.05 M Tris-HCl buffer, pH 7.8, for 48 h with five changes of the dialyzing buffer.

Anion-Exchange Chromatography (Fraction III). The dialyzed concentrate was loaded on a 200-ml column of DEAE-cellulose (DE 52, Whatman, England) which had been previously equilibrated with 0.05 M Tris-HCl, pH 7.8. The column was washed with the same buffer and IL-2 was eluted using a linear gradient of Tris-buffered NaCl (0-0.3 M NaCl) and 5-ml fractions were collected. IL-2-containing fractions were pooled and dialyzed against phosphate-buffered saline (PBS), pH 7.2, containing polyethyleneglycol (6,000 mol wt) (PEG 6000; 50%, wt/vol) to concentrate pooled fractions.

Gel Filtration (Fraction IV). The concentrated DEAE-cellulose preparation was applied to AcA 44 Ultrogel (LKB Products, Inc., Rockland, MD) column (2.5 × 90 cm), previously equilibrated with PBS containing 0.1% PEG 6000. The flow rate was adjusted to 30 ml/h and 6-ml fractions were collected. IL-2-containing fractions were pooled. The column was calibrated with bovine serum albumin (68,000 mol wt), chymotrypsinogen (25,000 mol wt), and ribonuclease A (14,000 mol wt), all obtained from Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, NJ.

Chromatography on Blue Agarose (Fraction V). 200 ml of the active fractions pooled from the AcA 44 Ultrogel column were applied to a blue agarose (BRL, Gaithersburg, MD) column with a bed vol of 40 ml, previously equilibrated with PBS containing 0.1% PEG 6000. The flow rate was adjusted to 30 ml/h and 6-ml fractions were collected. The IL-2-containing fractions were pooled and PEG 6000 added to a final concentration of 0.1% (wt/vol), to stabilize the IL-2.

Chromatography on Procion-Red Agarose (Fraction VI). The pool of active fractions eluted from blue agarose was dialyzed against PBS and loaded on a 10 ml Procion-red agarose (BRL) column, previously equilibrated with PBS. The column was then washed with PBS and bound proteins were eluted by using a stepwise gradient of NaCl in PBS with a starting salt concentration of 0.3 M NaCl and a final concentration of 1.0 M NaCl.

Isoelectrofocusing. 10 ml of the purified IL-2 were supplemented with 20% glycerol (vol/vol) and 2% Ampholines (vol/vol), pH 3.5–10 (LKB Products, Inc.). A 5–60% glycerol density gradient, containing 0.1% PEG 6000 (wt/vol) and 2% Ampholines, pH 3.5–10, was layered into an isoelectrofocusing column (LKB 8100). The IL-2 sample was applied onto the isodense region of the gradient, followed by focusing for 24 hr at 4°C using a constant power supply (LKB 2103). The terminal voltage was 2,000 V and the terminal current 3-4 mA. 5-ml fractions were collected and the pH determined in every fraction. All fractions were dialyzed against PBS containing 0.1% PEG 6000 (wt/vol) to remove the bulk of Ampholines and glycerol. The IL-2-containing fractions were pooled.

Protein Assay. The protein content of samples was measured using the Lowry technique (10). For protein concentrations <5 μg/ml, samples were subjected to SDS-PAGE, the protein bands were visualized by a modification of the silver staining technique (11), and the protein concentration estimated by comparison with known amounts of protein standards (soybean trypsin inhibitor and α-lactalbumin). Serial dilutions (200 ng–2 ng) of these marker proteins were used.

SDS-PAGE. The discontinuous Tris-glycine system of Laemmli (12) was used for 1.5 mm-thick slab gels using a 5–20% gradient or a 15% of acrylamide. The samples were analyzed...
under both, reduced (2% SDS, 5% mercaptoethanol) and nonreduced (2% SDS) conditions. After electrophoresis, gels were stained by a silver nitrate method (11). Apparent molecular weights were determined using protein standards bovine serum albumin (68,000 mol wt), ovalbumin (43,000 mol wt), carbonic anhydrase (30,000 mol wt), soybean trypsin inhibitor (20,000 mol wt), and α-lactalbumin (14,500 mol wt). After electrophoresis, parallel gels were sliced into 1-mm sections and proteins from each slice were eluted in 0.3 ml PBS. After 12-18 h, the eluted materials were assayed for IL-2 activity.

Assay for IL-2 activity. For the IL-2 assay, 4,000 murine IL-2-dependent cytotoxic T lymphocyte cells (CTLL) were grown in the presence of log2 dilutions of putative IL-2-containing medium in 96-well microtiter plates (Costar Data Packaging, Cambridge, MA). The total vol in each well was 0.2 ml. 24 h later, 0.5 μCi of tritiated thymidine [3H]TdR (20 Ci/mmol sp act; New England Nuclear, Boston, MA) was added to each well. After 4 h, the cells were harvested on glass fiber strips and [3H]dT incorporation measured in a liquid scintillation counter (Packard Instrument Co., Downers Grove, IL). The IL-2 concentration in the experimental sample was then calculated by probit analysis (13) using a standard containing 2 U/ml of IL-2. 1 U/ml of IL-2 was defined as the quantity of IL-2 released in 48 h culture medium conditioned by rat spleen cells (1 × 10⁶/ml) stimulated by concanavalin A (Con A; 5 μg/ml) (13).

Results

Purification of IL-2: Evidence for Molecular Heterogeneity Dependent on the Stimuli for Its Production. High levels of IL-2 production were achieved by sequential stimulation of pooled PBL from multiple donors. PBL were first stimulated by Sendai virus for 12 h. After a change of serum-free culture medium, these cells were restimulated by PHA for an additional 48 h.

Sendai virus or PHA alone stimulated the production of IL-2 at 6-10 U/ml level, and sequential stimulation by Sendai virus and PHA increased IL-2 production to 50-100 U/ml. An additional increase in IL-2 production, up to 200 U/ml, was achieved by co-stimulation with Daudi cells.

A 10-fold concentration of proteins from Ly-CM was achieved by precipitation with ammonium sulfate at 80% saturation. The dialyzed (NH₄)₂SO₄ precipitate was placed on a DEAE-cellulose (DE 52) column. IL-2 was eluted with a salt gradient from 0-0.3 M NaCl in 0.05 M Tris-HCl, pH 7.8, buffer. The IL-2 produced in the absence of Daudi cells eluted as a broad peak at low salt concentration (0-0.03 M NaCl), whereas the IL-2 produced by co-stimulation with Daudi cells eluted at salt concentrations between 0.03 and 0.08 M NaCl. The bulk of proteins of this preparation was eluted at higher salt concentrations (0.1-0.3 M NaCl).

The IL-2 eluted from DEAE cellulose was purified further by AcA 44 Ultrogel gel filtration. The IL-2 produced in the absence of Daudi cells was eluted in fractions 42-52 as a single peak corresponding to a 26,000 mol wt. When IL-2 was produced by co-stimulation with Daudi cells, a major peak of activity was eluted on fractions 52-66, corresponding to a 13,000-18,000 mol wt, whereas a minor peak of activity was found at 26,000 mol wt (Fig. 1). The IL-2 activity-containing fractions were pooled.

The final two steps involved chromatography on blue agarose and Procion-red agarose. From the blue agarose column, IL-2 was eluted with 0.5-0.6 M NaCl in PBS and could be clearly separated from α-interferon, which eluted at 0.05-0.4 M NaCl in PBS (Fig. 2). IL-2 was also bound strongly to a Procion-red agarose column and eluted as a broad peak between 0.6 and 0.9 M NaCl in phosphate buffer with peak activity in the 0.7 and 0.8 M NaCl eluate (Fig. 3). This broad elution profile was
suggestive of molecular heterogeneity of IL-2. A majority of the other proteins did not bind to Procion-red agarose, the rest eluted at low salt concentration.

The degree of IL-2 purification achieved with the various steps is listed in Table I. The protein content of the Procion-red agarose preparations was measured by comparing the density of protein bands, visualized by silver staining, with serial dilutions of protein standards of known concentrations. Taking into account the limitation of this measurement, an $\sim 10^6$ U/mg protein sp act and a final purification of 37,191-fold were obtained. The overall recovery of IL-2 was 19%.
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Fig. 3. Chromatography of IL-2 on Procion-red agarose. The IL-2-containing fractions from blue agarose were pooled, and loaded on a Procion-red agarose column. The bound proteins were eluted with a stepwise increase in salt concentration (0.15-1.0 M NaCl in PBS). Absorption at 280 nm (○); IL-2 (U/ml) (▲).

Table I

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<th>Fraction</th>
<th>Total protein (mg)</th>
<th>Total activity (U)</th>
<th>Sp act (U/mg protein)</th>
<th>Purification (fold)</th>
<th>Yield (%)</th>
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<tr>
<td>I Ly-CM*</td>
<td>10,800</td>
<td>297,000</td>
<td>27</td>
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<td>100</td>
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<tr>
<td>II (NH₄)₂SO₄ precipitate</td>
<td>9,000</td>
<td>247,000</td>
<td>27</td>
<td>1</td>
<td>84</td>
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<tr>
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<td>1,356</td>
<td>50</td>
<td>62</td>
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<td>3,625</td>
<td>135</td>
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<tr>
<td>V Blue agarose</td>
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<td>91,333</td>
<td>3,382</td>
<td>30</td>
</tr>
<tr>
<td>VI Procion-red agarose</td>
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<td>55,229</td>
<td>1,004,164</td>
<td>37,191</td>
<td>19</td>
</tr>
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</table>

* The IL-2 activity in the Ly-CM was 100 U/ml.
‡ Protein concentration was determined by comparison of the protein content in the samples with known amounts of protein standards (Material and Methods).

The final preparations obtained after blue agarose chromatography and Procion-red agarose chromatography were analyzed on a 5–20% gradient gel followed by the highly sensitive silver staining method as shown in Fig 4. It is evident that the Procion-red agarose step significantly reduced contaminating proteins (Fig. 4 b and 4 c). In addition, the molecular heterogeneity of IL-2 was apparent, and this heterogeneity was dependent on the stimuli employed for its production. When IL-2 was produced in the absence of Daudi cells, the purified IL-2 showed only two active bands with 16,000 and 17,000 mol wt (Fig. 4 c) under both reducing and nonreducing conditions. When Daudi cells were used as a co-stimulator for IL-2 production, one active protein band with a 14,500 mol wt was observed (Fig. 4 d). It is of interest to note that if suboptimum concentrations of Daudi cells (<5 × 10⁵/ml) were used, three protein bands with 14,500, 16,000, and 17,000 mol wt were found (data not shown). To obtain a better resolution, the purified IL-2 was also analyzed on a 15% acrylamide gel. After staining, a similar molecular weight pattern was obtained as in the gradient gel. A parallel gel was sliced into 1-mm sections, and proteins from each slice were eluted in PBS. IL-2 activity was found to be localized in slice numbers corresponding to...
Fig. 4. SDS-PAGE of blue agarose and Procion-red agarose-purified IL-2. IL-2 was treated with 2% SDS and 5 mM 2-mercaptoethanol and applied to a 5–20% gradient gel. The protein bands were visualized by a silver nitrate method. The following marker proteins (200 ng each) were used: ovalbumin (43,000 mol wt), carbonic anhydrase (30,000 mol wt), soybean trypsin inhibitor (20,000 mol wt) and α-lactalbumin (14,500 mol wt). (a) protein standards; (b) blue agarose-purified IL-2 produced in the absence of Daudi cells; (c) Procion-red agarose-purified IL-2 prepared in the absence of Daudi cells; (d) Procion-red agarose-purified IL-2 obtained by co-stimulation with Daudi cells.

Fig. 5. SDS-PAGE of Procion-red agarose-purified IL-2 produced in the presence or absence of Daudi cells. The IL-2 preparations were treated with 2% SDS and 5 mM 2-mercaptoethanol and applied to a 15% polyacrylamide gel. After electrophoresis, the gel was sliced into 1-mm sections and proteins eluted with 0.3 ml PBS. The eluted material was assayed for IL-2 activity. The arrows indicate the position of the protein standards soybean trypsin inhibitor (20,000) and α-lactalbumin (14,500 mol wt). IL-2 (U/ml) produced in the presence (A) or absence (O) of Daudi cells.

To 16,000 and 17,000 mol wt (Fig. 5) in IL-2 produced in the absence of Daudi cells, and in the slice number corresponding to 14,500 mol wt in IL-2 produced in the presence of Daudi cells (Fig. 5). Re-electrophoresis of the proteins present in each slice with IL-2 activity showed one single band with molecular weight identical to that of the eluted band (data not shown).

The purified IL-2 was subjected to isoelectrofocusing (IEF) analysis using Ampholines with a broad pH range (pH 3.5–10). The IL-2 obtained without Daudi costimulation was focused with an isoelectric point of ~6.7 (Fig. 6). An isoelectric point of ~8.1 was found if PBL were stimulated in the presence of Daudi cells. The yield of IL-2 from the IEF column was ~30%.

The purified IL-2 did not bind either to Con A or wheat germ agglutinin column. Neuraminidase treatment of IL-2 did not affect its biological activity or its molecular weight pattern (data not shown).
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**Figure 6.** Isoelectric focusing of AcA 44 Ultrogel-purified IL-2. An isoelectric focusing column (110 ml) was prepared by loading a 5-60% glycerol density gradient containing 2% Ampholines of pH 3.5–10. Purified IL-2 was supplemented with 2% Ampholines (pH 3.5–10) and 20% glycerol and was layered onto the isodense region of the column gradient. A constant power was then applied to the column with a terminal voltage of 2,000 V and a current of 3–4 mA (24 h, 4°C). pH gradient (△); IL-2 (U/ml) produced in the presence of (○) or absence (△) of Daudi cells.

**Biological Activity of Purified IL-2.** The mitogenicity on normal PBL, the capacity to support the growth of murine and human CTLL, and the presence of other cytokines were studied in the purified IL-2. To test for the presence of PHA, the mitogenicity of the IL-2 preparations was studied on normal PBL. The purified IL-2 was completely free of mitogenic activity. It very actively supported the long-term growth of human and murine CTLL lines. It is of interest that human CTLL appear to require ~10 U/ml purified IL-2 for their optimum growth, whereas murine CTLL are maximally stimulated at 2 U/ml. The purified IL-2 contained no detectable α- or γ-interferon, granulocyte-macrophage colony-stimulating factor (GM-CSF), T cell-replacing factor (TRF), B cell growth factor (BCGF), and thymocyte-differentiating activity.

**Discussion**

Ly-CM harvested after sequential stimulation of PBL with Sendai virus and PHA, and co-stimulation with Daudi cells, showed an IL-2 concentration of 100–200 U/ml. It was possible, therefore, to obtain a high IL-2 concentration in the absence of the commonly used tumor promoter phorbol myristate acetate (14).

The purification procedure presented in this paper introduces the chromatography on blue agarose and on Procion-red agarose as two new purification steps for IL-2. The binding of IL-2 to these dyes is likely a result of the electrostatic or hydrophobic interactions. The use of these two steps permitted a 37,000-fold purification of IL-2 from medium conditioned in the presence of 0.25% bovine serum albumin, with a 19% overall recovery of IL-2 activity. All other purification methods, for both murine and human IL-2, have achieved neither a specific activity nor a yield comparable to those described here. Our purification procedure also avoided time-consuming steps. This has made our procedure very useful for large-scale purification of IL-2.
Lymphokines and other regulator molecules such as interleukin 1 (IL-1, formerly termed lymphocyte-activating factor) (15), α- or γ-interferon (K. Welte, unpublished observation; B. Rubin, personal communication), TRF (K. Welte and P. Ralph, unpublished observation), and GM-CSF (16) have different capabilities of forming hydrophobic interactions. We have exploited these properties to separate IL-2 from other lymphokines and factors, which contaminate most partially purified IL-2 preparations. For example, α-interferon co-purified with IL-2 during ion-exchange chromatography and gel filtration steps, but was clearly separated from IL-2 by blue agarose chromatography (Fig. 2). After chromatography on Procion-red agarose, the IL-2 preparation did not contain any detectable interferon (α or γ), GM-CSF, TRF, BCGF, or thymocyte-differentiating activities. In addition, the Procion-red agarose-purified IL-2 appeared to be free of any contaminating proteins (Fig. 4).

Native IL-2 has been previously shown to exist in several molecular forms (7-9). Here, we show that the stimuli used for IL-2 induction by PBL can be responsible for this heterogeneity. IL-2 produced in the presence or absence of Daudi cells exhibited 14,000 and 26,000 mol wt, respectively, by gel filtration (Fig. 1). Both molecular forms could be obtained by varying the concentration of co-stimulator cells. These results would offer an explanation for the molecular weight differences reported by Mier and Gallo (7) and Gillis et al. (8). These are most likely a result of different methods of IL-2 induction.

After denaturation by SDS, the 26,000-mol wt IL-2 exhibited a molecular weight of 16,000-18,000 mol wt by gel filtration (data not shown). SDS-PAGE of this denatured form demonstrated the presence of two active bands with 16,000 and 17,000 mol wt, respectively. These results together with those of Caplan et al. (17) indicate that after SDS denaturation, human and murine IL-2 exhibit similar molecular weights. The molecular weight of human IL-2 produced in the presence of Daudi cells was not affected by SDS denaturation. It appears that IL-2 activity is present in a polypeptide of 14,000–17,000 mol wt in all species studied thus far (8, 18, 19). It is not certain if the different molecular forms observed in this have different functions, such as preferential stimulation of T cell subsets. It is also not known whether the same T cell will switch its IL-2 synthesis from 26,000 d to 14,000 mol wt upon co-stimulation by Daudi cells, or whether different T cell subsets are indeed responsible for the release of IL-2 in two molecular forms. If two functional T cell subsets are involved, one subset would produce the 26,000-mol wt IL-2 in response to PHA alone, whereas the second subset would require PHA as primary stimulus and co-stimulation by Daudi cells as a second signal for the production of 14,000 mol wt IL-2. Daudi cells express HLA-DR antigens and Fc receptors. Both of these surface molecules have been implicated in the augmentation of the IL-2 response (20, 21). The effect of Daudi cells on the IL-2 response, however, does appear to be more complicated than previously suggested (20, 21) in view of (a) the shift in mol wt of IL-2 from 26,000 to 14,000 induced by Daudi cells, (b) the augmentation of IL-1 independent IL-2 production as seen in the human lymphoblastic cell line Jurkat, and (c) the superinduction of IL-2 in human lymphoblastic leukemic cells by co-stimulation with Daudi cells. The possibility that different T cell subsets are responsible for the production of the two IL-2 forms is supported by recent reports on the molecular weights of IL-2 produced by human leukemic cells. We have previously reported that non-T non-B lymphoblastic leukemias produce the 26,000-mol wt form
of IL-2 after PHA stimulation, whereas Friedman et al. (22) recently reported that cells obtained from a patient with T cell chronic lymphocytic leukemia, stimulated under identical conditions, released the 14,000-mol wt form of IL-2. This molecular, and possibly functional, heterogeneity of IL-2 observed here are not unique because other cytokines, such as GM-CSF, have been shown to exhibit different molecular characteristics, dependent on the producer cell type, and these factors were also shown to exert preferential effects on subsets of the granulocyte-macrophage cell lineage (16).

The reason for the molecular heterogeneity of the SDS denatured and native IL-2 remains to be explored. A variable degree of glycosylation may provide an explanation of this phenomenon (9, 23). Robb et al. (9) have shown that neuraminidase, glycosidases, and inhibitors of glycosylation can reduce the heterogeneity of IL-2 produced by tonsil lymphocytes. However, neuraminidase treatment of IL-2 did not affect its molecular weight. In addition, IL-2 failed to bind to immobilized lectins (Con A and wheat germ agglutinin), which was in agreement with a previous report by Mier and Gallo (7).

The purification steps described in this study produced IL-2 with an $\sim 10^6$ U/mg protein sp act. Because the lowest mol wt of an active IL-2 polypeptide was 14,500, it could be calculated that 1 U/ml of IL-2 was equivalent to a molar concentration of $7 \times 10^{-11}$ M. An IL-2 concentration of $1.4 \times 10^{-11}$ M, or $\sim 4 \times 10^5$ molecules/cell, was required for one-half maximum stimulation of murine CTLL. Similar values have been reported by Mizel and Mizel (15) for IL-1 on T cells.

The highly purified IL-2 is now used in this laboratory to investigate the biological effects of IL-2, both in vitro and in vivo. In preliminary experiments, we were able to restore, in vitro, the response of T cells of a patient with Nezeloff's syndrome in both the allogeneic mixed lymphocyte reaction and in cell-mediated lympholysis by addition of purified IL-2 (N. Flomenberg, K. Welte, and R. Mertelsmann, unpublished observations). In addition, suppressed mitogen response of T cells from several patients with various immunodeficiency syndromes (combined variable immunodeficiencies, Kaposi's sarcoma, and advanced Hodgkin's disease) could be restored, in vitro, to near normal values by addition of purified IL-2 (N. Ciobanu, G. Kruger, and K. Welte, manuscript in preparation). A phase I clinical trial using this purified IL-2 has been initiated. These studies should contribute to the understanding of normal human lymphocyte function, immunodeficiency syndromes, and the pathophysiology of human lymphoblastic leukemias (4).

**Summary**

Interleukin 2 (IL-2), produced with and without co-stimulation by the Burkitt's lymphoma line Daudi, was purified 37,000-fold to apparent homogeneity from lymphocyte conditioned medium by (NH$_4$)$_2$SO$_4$ precipitation, DEAE-cellulose ion-exchange chromatography, gel filtration, and chromatography on blue agarose and on Procion-red agarose. The purified IL-2 showed a $10^6$ U/mg protein sp act. IL-2 produced in the absence of Daudi cells had a mol wt of 26,000 as measured by gel filtration and an isoelectric point of 6.7. This IL-2 showed a 16,000 and 17,000 mol wt in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). IL-2, produced in the presence of Daudi cells ($10^6$/ml), showed a mol wt of $\sim 14,000$, as measured by both gel filtration and SDS-PAGE, and an isoelectric point of 8.1. The purified IL-2 lacked detectable interferon (α and γ), granulocyte-macrophage colony-
stimulating factor, B cell growth factor, T cell-replacing factor, and thymocyte-differentiating activity and was free of any contaminating proteins as judged by silver staining in SDS-PAGE. All three molecular forms of IL-2 were biologically active at concentrations of $10^{-11}$ to $10^{-10}$ M, supporting the growth of human and murine cytotoxic T cell lines.

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