Clearance rates and systemic effects of intravenously administered interleukin 2 (IL-2) containing preparations in human subjects

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Summary  The present study was designed to examine the feasibility of in vivo administration of interleukin 2 (IL-2) to induce cytotoxic cell activity against tumours in human subjects. IL-2 was prepared from blood leukocytes stimulated with phytohaemagglutinin (PHA) and partially purified by membrane chromatography to exclude PHA. Administration of different amounts of IL-2 in vivo to 2 patients with melanoma revealed that the initial level of IL-2 in the circulation was related to the dose given and had a half-life of ~22.5 minutes. The initial and subsequent levels of IL-2 were lower than that expected to occur from equilibration in plasma and extracellular fluid. This was not apparently due to inactivation by serum factors because fresh human serum had little effect in vitro on the induction of mitogenic or cytotoxic activity by IL-2. Spontaneous division of lymphocytes was increased following IL-2 administration and it is suggested that clearance of IL-2 in vivo may reflect, in part, absorption by activated lymphocytes in the circulation. Side effects noted shortly after administration of the partially-purified IL-2 preparations included transient pyrexia, hypoglycaemia, increased cortisol levels, lymphocytopenia and signs of mild intravascular coagulation. No long-term effects were noted. These initial results suggest that systemic injection of purified preparations of IL-2 may be a feasible approach to induce cytotoxic T cells in vivo.

Previous studies have shown that the lymphokine, interleukin 2 (IL-2), appears necessary as a second signal for the generation of cytotoxic T cells (Lafferty et al., 1980; Wagner et al., 1980a). Addition of IL-2 from mitogen-stimulated lymphocytes to cultures of lymphocytes and tumour cells was shown to induce cytotoxic activity against syngeneic animal tumours (Mills & Paetkau, 1980; Warren et al., 1978, 1979; Gillis & Watson, 1981) and against autologous human malignancies such as leukaemia (Zarling & Bach, 1979), melanoma (Lotze et al., 1980) and various carcinomas (Vose & Bonnard, 1982; Vose & Moore, 1981). IL-2 was shown previously to act on activated T cells rather than resting T cells (Lafferty et al., 1980) so that these results suggested T cells activated against melanoma antigens were present in the circulation of patients with melanoma.

In view of these findings it seemed possible that injection of IL-2 in vivo may lead to the induction of cytotoxic T cells against melanoma in patients analogous to the induction of cytotoxic T cells against allografts in nude mice by systemic administration of IL-2 (Wagner et al., 1980b). With this in mind the present study was carried out to determine whether IL-2 could be detected in vivo after systemic injection in human subjects, and if so, to estimate the rate of clearance of IL-2 activity from the circulation. The results indicate that IL-2 can be detected by biological assays in the circulation for ~1h after intravenous injection. The side effects noted were relatively mild and may be further reduced by administration of more purified preparations of IL-2.

Materials and methods

Patients

These were 2 subjects with advanced melanoma.
Patient 1 was a 26-year old male who presented with extensive pulmonary and pleural metastases 41 years after the removal of ocular melanoma. He failed to respond to treatment with several courses of DTIC (Dacarbazincine) and progression in the size and number of pulmonary deposits occurred in the 3 months after initial diagnosis of recurrent melanoma. Further chemotherapy had been refused. Tumour growth was relatively static for 2 months prior to administration of IL-2 and there was no clinical evidence of metastases elsewhere. He was treated as an outpatient. Seven months after presentation bone metastases were detected on bone scans and only palliative treatment was continued.

The second patient was a 64-year old male with history of removal of a primary melanoma from his right arm 31 years previously. Local recurrences had been removed surgically 13 and 17 months after the primary. Subsequently chemotherapy was given for extensive local recurrences. This proved ineffective and eventually a forequarter amputation of his right arm was carried out 30 months after removal of the primary. Further recurrences around the amputation site were detected 6–10 months after amputation and were removed surgically. At the time of IL-2 injection subcutaneous metastases were present on scalp, left knee and chest wall. Multiple courses of chemotherapy had been given over the course of his illness. Studies on the patient were terminated when cerebral metastases were detected. The experimental nature of IL-2 administration and the need for repeated blood sampling after administration to obtain information as to its effects were fully explained to both patients and their relatives.

**Production of IL-2**

IL-2 was produced from mononuclear cells collected from relatives or friends by leukopheresis on an Amicon celltrifuge. The yield obtained after purification on Ficoll:Hyphaque mixture (SG 1.078) was \( \sim 10^{10} \) lymphocytes. These were cultured at 5 x 10⁶/ml in 1 litre plastic bags (Tuta Laboratories, Lane Cove, N.S.W.) with 1% phytohaemagglutinin (PHA) (Code HA15, Wellcome Pharmaceuticals, Concord, N.S.W.) in RPMI 1640 (Flow Laboratories) culture medium (without foetal calf serum) for 24–36 h. Further details of the procedures are described elsewhere (Hersey et al., 1981). After membrane chromatography on YM30 membranes (Amicon) in 300-ml capacity Amicon diaflo cells to remove PHA, the volume of the supernatant was reduced to 60–100 ml by ultrafiltration on YM5 (Amicon) membranes. This procedure was shown previously to remove PHA from the preparations as determined in mitogenic assays. Each batch was filtered through Millipore 0.45 μm diameter filters and sterility checked by culture for bacteria and fungi. Assays for IL-2 activity, levels of interferon (IFN) and endotoxin were conducted on each batch.

**IL-2 assays**

Assays of IL-2 in supernatants were carried out as previously described (Hersey et al., 1981). Normal blood lymphocytes which had been maintained in IL-2 for 7 days after stimulation by PHA for 3 days were used as the target cell populations. The assay cells were washed free of IL-2 and resuspended in RPMI 1640 supplemented with 10% foetal calf serum (FCS) (CSL batch N. 249.3). Cells (10⁵ in 100 µl) were placed in triplicate 70 x 10 mm round-bottomed tubes then serially diluted with the IL-2 sample (100 µl) to be assayed. Cultures were incubated for 24 h in a humidified atmosphere of 7% CO₂ in air at 37°C with the addition of 2 μCi of radiolabelled iododeoxyuridine \(^{125}\)IUDR (New England Nuclear, Boston, Mass.) for the final 4 h of culture. Cells were harvested by washing 3 x in saline and once in 5% trichloroacetic acid (TCA) and counted in a gamma counter. \(^{125}\)IUDR incorporation data were plotted against log₂ of IL-2 dilution to give a dose-response curve. The x-axis dilution co-ordinate of the control sample which crossed this curve at the 50% maximum \(^{125}\)IUDR uptake (y-axis co-ordinate) was defined as that value which corresponded to 1 unit of IL-2 activity. Aliquots of the control sample of IL-2 were repeatedly used to standardize the activity of individual batches.

Assay of IL-2 activity in sera from the patients was carried out as follows: The assay cells as above were washed free of IL-2 and resuspended in RPMI 1640 + 10% FCS at 7.1 x 10⁵/ml. 10⁵ cells in 140 µl were placed in triplicate 70 x 10 mm round-bottomed tubes followed by 60 µl of the serum sample to be assayed. (Final serum concentration 30%). Cultures were incubated for 24 h in a humidified atmosphere of 7% CO₂ in air at 37°C with the addition of 2 μCi of radiolabelled iododeoxyuridine \(^{125}\)IUDR (New England Nuclear, Boston, Mass.) for the final 4 h of culture. Cells were harvested by washing 3 x in saline and once in 5% trichloroacetic acid (TCA) and counts per minute (c.p.m.) measured in a γ counter.

Six control human sera were used to give a baseline of cell turnover in the absence of IL-2 but in the presence of 30% human serum. Units/ml (u/ml⁻¹) of IL-2 activity in serum were measured from a standard curve prepared by addition of 40 µl of different dilutions of the infused IL-2 in RPMI to 60 µl of the patient's undiluted serum obtained.
before administration of IL-2. Aliquots (100 μl) of these dilutions were then added to 100 μl of cells in RPMI as described above for assay of IL-2 in serum samples. (Final concentration of serum, 30%). A plot of μml⁻¹ for these samples against 125IUDR incorporation enabled the construction of a standard curve from which the μml⁻¹ in the patient’s serum samples after infusion of IL-2 could be calculated by reading from the graph against the appropriate 125IUDR incorporation value.

Endotoxin assays

The supernatant was assayed for endotoxin content by the Limulus Amebocyte Lysate (LAL) assay (Sigma E Toxate Kit). One hundred-μl of the supernatant and 10-fold dilutions of the supernatant were mixed with 100 μl of the LAL and incubated for 1 h. This was compared with the coagulation seen in 10-fold dilutions of the 2 μg ml⁻¹ standard endotoxin supplied in the kit.

Electrophoresis in polyacrylamide gels in sodium dodecylsulphate (PAGE-SDS)

Samples of IL-2 were analysed by PAGE-SDS using a Pharmacia PAA 4/30 gradient gel (Pharmacia, Uppsala, Sweden). The electrophoresis buffer used was 0.04 M Tris; 0.02 M sodium acetate; pH 7.4 with 2 mM EDTA and 0.2% SDS. Samples were heated at 90°C for 5 min and diluted 1:1 before electrophoresis with a solution of 20 mM Tris-HCI pH 8.0, 2 mM EDTA 5.0% SDS, 10% metcaptoethanol, 15% sucrose and 0.01% bromphenol blue. Pre-electrophoresis (without samples) was for 1 h at 70 V. Twenty-μl of the diluted samples were applied and electrophoresed for 3–5 h at 100 V.

Interferon assays

IFN assays were based on the cytopathic effect of Encephalomyocarditis (EMC) virus on Vero cells. The endpoint was taken as the amount of interferon (in units/ml) to give 50% protection of the cell layer. Full details of the assay are described elsewhere (Hersey et al., 1982). IFN titres were determined relative to a leukocyte international standard (G-023-901-527 from the Antiviral Substances Programme of the National Institute of Allergy and Infectious Diseases, NIH Bethesda, Maryland 20014, U.S.A.) or relative to a laboratory standard previously related to an international standard.

Cytotoxic assays

The 51Cr release assays and target cells used in studies on the effect of fresh serum on IL-2 induced cytotoxic activity are fully described elsewhere (Hersey et al., 1981).

Results

Effect of PHA and IL-2 on unstimulated blood mononuclear cells and IL-2 dependent cells

As shown in Table I, 1% PHA had little stimulating activity on the IL-2 dependent cells used in the assay. The table also shows that attempts to remove PHA from the crude IL-2 preparations by filtration through YM30 membranes and then concentration on YM5 membranes was effective in removing mitogenic activity for normal unstimulated blood lymphocytes.

IL-2 assays

Assay of the IL-2 activity in the partially purified supernatants given to the patients is shown in Figure 1. An IL-2 preparation prepared from spleen cells from a patient with melanoma

| Final dilution in assay | Unstimulated blood lymphocytes* 3-day incubation (mean c.p.m. ± s.d.) | IL-2 dependent lymphocytes 2-day incubation (mean c.p.m. ± s.d.) |
|------------------------|-------------------------------------------------|-------------------------------------------------|
|                        | 1/2                                             | 1                                               | 1/2                                             |
| PHA 1%                 | 48,798 ± 596                                   | 19,488 ± 3155                                   | 2,958 ± 346                                     |
| IL-2 prefiltration     | 46,822 ± 3610                                  | 11,125 ± 2050                                   | 144,938 ± 3747                                  |
| IL-2 postfiltration    | 2,792 ± 303                                    | 1,495 ± 251                                     | 92,978 ± 110                                    |

*From normal volunteers.

Table I: Effect of PHA and IL-2 preparations (before and after membrane chromatography) on unstimulated lymphocytes and IL-2 dependent lymphocytes.
Influence of activity experiments melanoma cells alone examined. A constant amount activity final preparation. Reduction of percentage lymphocytes lymphocytes 25%. The effect patients studies. Relative to the standard, the IL-2 given to Patient 1 had 100 \( \text{u ml}^{-1} \) and 81 \( \text{u ml}^{-1} \). The IL-2 given to Patient 2 had 132 \( \text{u ml}^{-1} \) and 566 \( \text{u ml}^{-1} \). Values indicated are mean ± 1 s.d.

Containing 100 \( \text{u ml}^{-1} \) was used as a standard during the studies. Patient 1 (48 kg) received 14,000 and 5,000 units and Patient 2 (75 kg) 17,000 and 67,000 units.

**Analysis of the partially-purified IL-2 containing supernatants**

PAGE-SDS analysis of the IL-2 containing supernatants prepared by membrane chromatography as described revealed fractions with molecular weights (mol. wts.) of ~10, 13–16, 18, 20, 22, 24, 28, 32 and 67 Kilodaltons (Kd). The predominant fraction was that detected at 13–16 Kd which from previous studies was likely to contain IL-2 (Mier & Gallo, 1980). The 28 Kd fraction may have been a contaminant of PHA in that when the same amount of PHA as used for IL-2 production was processed as for IL-2 production and analysed by PAGE-SDS, a 28 Kd fraction was detected. (Before membrane chromatography the major fraction in the PHA had a mol. wt. of ~32 Kd. Minor fractions were detected with mol. wts. of ~28 and 12 Kd). The 67 Kd fraction in the IL-2 preparations may have been human serum albumin non-specifically absorbed to the human lymphocytes used for IL-2 preparation. (FCS was not added to the leukocytes during preparation of IL-2).

**Endotoxin and interferon content of the partially purified supernatants**

The limit of detection of the endotoxin standard with the LAL was 0.2 ng. By comparison with the standard endotoxin preparation in the kit, the IL-2 preparations had <2 ng/ml of endotoxin.

The IFN levels were <2.5 \( \text{u ml}^{-1} \) in the injected preparations.

**Clearance of IL-2 from the circulation**

After i.v. injection of IL-2, blood samples were taken at regular intervals. Serum from these samples were assayed for IL-2 activity as described. The results in Figures 2 and 3 indicate an exponential clearance of IL-2 from the circulation over 1 h. A plot of the log of the counts against time revealed that clearance of half the IL-2 activity from the circulation (T1/2) took 22.5 min on both occasions in Patient 1. The equivalent T1/2 times in Patient 2 were 20 and 25 min respectively. A dose-response curve for the infused IL-2 in a 30% dilution of the patient's pre-treatment serum was constructed as described to determine the units of IL-2 activity in the serum of the 2 recipients. The initial \( \text{u ml}^{-1} \) obtained in the serum related to the dose given in that particular patient e.g. as shown in Figure 2, in Patient 1 injection of 14,000 units gave an initial level of 3 u \( \text{ml}^{-1} \) whereas 5,000 units gave an initial level of 1 \( \text{u ml}^{-1} \). In Patient 2 the injection of 17,000 and 67,000 units gave initial
Table II  Effect of serum concentration on lymphocyte stimulation (\(^{125}\text{IUDR}\) incorporation) by IL-2

| % Serum | 75    | 50    | 25    | 12.5  | 6.25  | 0     |
|---------|-------|-------|-------|-------|-------|-------|
| c.p.m. with 3u/ml IL-2 ± s.d. | 17,559±1864 | 18,522±1129 | 26,176±1888 | 27,232±2217 | 26,105±823 | 28,504±251 |
| c.p.m. in absence of IL-2 ± s.d. | 4,985±344 | 5,367±440 | 7,043±309 | 7,568±375 | 6,595±430 | 6,755±388 |
| % Inhibition of IUDR uptake caused by presence of serum | 50.3 | 45.9 | 10.7 | 5.8 | 11.0 | 0 |

Mean values ± s.d. for \(^{125}\text{IUDR}\) uptake by 10^5 cells.

Table III  Effect of presence of 10% autologous serum on generation of cytotoxic T cells and \(^{125}\text{IUDR}\) incorporation in 6-day cultures

|                | Cell-mediated cytotoxicity (% \(^{51}\text{Cr}\) release above baseline) | \(^{125}\text{IUDR}\) incorporation c.p.m. |
|----------------|--------------------------------------------------------------------------------|-------------------------------------|
|                | Autol. MM200 MM96 Chang K562 MCF-7                                           |                                     |
| **PATIENT 1**  |                                                                                  |                                     |
| Day 0          | —                                                                              | 7±2 9±0.5 4±1.5 26±1 3±1            | 1,928±159                        |
| Day 6 ±10% FCS | —                                                                              | 36±1 70±1 65±2 64 35±1             | 16,098±1215                     |
| Day 6 ±10% aut. serum | —                              | 36±1 58±0.5 (17) 64±2 59 29±4 (17) | 21,737±4163                    |
| **PATIENT 2**  |                                                                                  |                                     |
| Day 0          | n.t.                                                                           | 8±1.5 10 2 15 4±1                 | 1,917±107                       |
| Day 6 ±10% FCS | 31                                                                              | 34 48±3 60±0.5 60±2 29±3          | 8,801±768                      |
| Day 6 ±10% aut. serum | 26                               | 28±2 (18) 38±3 (21) 43±3 (28) 62±1 (21) 23±1 (21) | 16,719±1817                   |

Mean values ± s.d. Effector:target cell ratios 100:1 except for K562 = 30:1. MM200 and MM96 = melanoma cell lines; MCF-7 = breast carcinoma line. K562 = myeloid cell line. Chang = liver cell line. Figures in parentheses indicate percentage inhibition of cytotoxicity in presence of autologous serum.
Figure 2  Clearance of IL-activity from the circulation of Patient 1 after i.v. injection of 14,000 units and 27 days later 5,000 units. The dashed line indicates the mean $^{125}$IUDR c.p.m. of IL-2 dependent lymphocytes in the serum of 6 individual normal subjects (mean ± s.d., 3915 ± 550 c.p.m. The $^{125}$IUDR c.p.m. corresponding to 0.25 to 3 u/ml of the standard IL-2 assayed in 30% serum of the patient is shown on the right of the ordinate. T1/2 for clearance of injected IL-2 was 22.5 min.

Figure 3  Clearance of IL-2 activity from the circulation of Patient 2 after i.v. injection of 17,000 units and 67,000 units 27 days after the first injection. The dashed line indicates the mean IL-2 activity in 6 sera from normal subjects (mean ± s.d., 4056 ± 305 c.p.m.) T1/2 for clearance of IL-2 was 20 min and 25 min respectively.

levels of ~1.25 and 6 units respectively (Figure 3).

The levels of IL-2 obtained with a given dose of IL-2 varied between the 2 patients e.g. in Patient 1 a dose of 14,000 u gave ~3 u/ml in the circulation whereas a dose of 17,000 u in Patient 2 gave ~1 u/ml. The body weight of Patient 1 was 48 kg and of Patient 2, 75 kg. (Estimated plasma volume for Patient 1 was 1,921. For Patient 2, 2,921. Estimated total extracellular fluid for Patient 1 was 9,121 for Patient 2, 13,871). The difference in IL-2 activity obtained in the serum between the 2 patients for a given dose was therefore not merely due to the difference in body weight. This aspect is discussed further below.

$^{125}$IUDR incorporation into blood mononuclear cells after IL-2 injection

As shown in Figure 4, in both patients the incorporation of $^{125}$IUDR into blood
mononuclears was increased in blood samples taken immediately after IL-2 injection and returned to baseline levels by 24h. In Patient 1 there was no significant increase in $^{125}$IUDR uptake in samples taken from Day 1 after the first or second injection of IL-2. This also applied to Patient 2 after the first injection but there was a significant increase in $^{135}$IUDR incorporation in leukocytes taken 6, 7, 9 and 13 days after the second IL-2 injection.

**Haematological changes after i.v. injection of the partially purified IL-2 containing supernatants**

The haematological data obtained from sequential studies of the 2 patients after IL-2 administration is recorded in Table IV. The results can be summarized as follows: (1) No significant changes occurred in haemoglobin (Hb) levels in Patient 1 (who had metastases in bone marrow). In Patient 2 there was a slight increase in Hb at Days 7–12 after both injections. This increase coincided with an increase in platelet counts, and myelocytes and metamyelocytes. (2) The total white blood cell (WBC) count and the neutrophil (neut) count showed a marked increase 2–4h after the injections but returned to normal by Day 1 (1d) or Day 2 (2d). (3) Lymphocyte (lym) counts were reduced immediately after the injections. They returned to pre-treatment values 1 day after the injections in Patient 1. In Patient 2 the lymphocyte count tended
Table IV Haematological changes after injection of supernatants containing IL-2

| Patient 1 | 14,000 units | 5,000 units |
|-----------|--------------|-------------|
|           | 0 4h 1d 2d 3d 7d 13d 25d | 0 4h 1d 2d 3d 7d 8d 12d 15d |
| Hb.       | 11.9 13.0 11.6 11.4 11.5 11.2 11.0 11.0 | 11.0 10.6 10.6 10.6 10.1 10.7 |
| WBC       | 7.1 14.7 7.6 5.6 6.8 6.1 5.2 5.7 | 5.6 15.4 8.4 7.8 5.2 6.3 5.0 |
| Neut.     | 5.6 13.2 6.0 3.9 5.8 4.9 4.2 4.0 | 4.2 14.3 7.2 5.8 4.0 5.2 4.0 |
| Lymph.    | 0.7 0.4 0.9 1.1 0.5 0.48 0.57 1.2 | 0.7 0.46 0.9 1.3 0.7 0.6 0.6 |
| Mono.     | 0.8 1.0 0.45 0.4 0.47 0.6 0.3 0.3 | 0.6 0.6 0.2 0.6 0.5 0.4 0.4 |
| Platelets | 326 289 347 317 332 412 439 341 | 360 345 381 404 445 451 339 |

| Patient 2 | 17,000 units | 67,000 units |
|-----------|--------------|-------------|
|           | 0 4h 1d 2d 3d 7d 9d 12d | 0 4h 1d 2d 3d 7d 8d 9d 12d 16d 21d 23d |
| Hb.       | 12.9 13.3 12.2 12.8 13.2 13.8 13.6 | 13.4 12.3 12.6 11.9 12.6 13.5 14.2 14.0 13.3 13.5 13.0 13.0 |
| WBC       | 9.6 20.9 11.6 8.1 9.4 9.3 8.5 | 12.2 23.5 32.6 11.9 8.7 10.1 10.4 12.6 10.6 11.5 8.0 8.4 |
| Neut.     | 7.1 19.6 10.3 7.0 7.5 7.9 7.4 9.5 22.8 30.0 10.5 7.5 7.3 8.8 10.8 9.1 10.5 6.1 6.9 |
| Lymph.    | 2.1 0.63 1.04 0.81 1.4 1.3 0.77 | 1.59 0.5 1.3 0.8 1.2 2.2 0.9 1.1 0.8 0.7 1.5 1.2 |
| Mono.     | 0.3 0.4 0.3 0.3 0.5 0.3 | 0.8 0.2 0.9 0.6 0.1 0.4 0.6 0.5 0.6 0.3 0.3 0.3 |
| MM & Myl. | -- -- -- -- -- -- | -- -- occ. 1 occ. -- -- -- |
| Platelets | 328 295 312 324 404 472 471 | 443 383 377 337 371 430 518 508 483 477 369 373 |

1g/dl.  
2 Leukocyte and platelet counts × 10\(^{-9}\).  
3 Occasional atypical lymphocytes noted.  
4 Occasional hypersegmented neutrophils.

to remain below the initial pre-treatment values. In Patient 1 there was a secondary reduction of lymphocyte numbers after the first injection on Days 3, 7 and 13. In Patient 2 there also appeared to be secondary reduction in lymphocyte count on Day 2 following both injections and Days 12 and 16 after the second injection. (4) Monocyte (mono) counts showed no significant changes. (5) Platelet counts showed a reduction of 5–14% of pre-treatment counts by 4h after the injections. This appeared related to the dose of IL-2 injected and persisted for 1–2 days.

Clinical observations

The following changes were observed clinically after i.v. injection of the IL-2 containing supernatants. Rigors developed ~10–15min following injection and lasted for ~1h. This was followed by pyrexia at 38–39°C lasting from 2–6h. The duration of the pyrexia was related to the number of IL-2 units injected. After 5,000 units the pyrexia lasted for ~2h and after 67,000 units for 6h. Tachycardia of 100–120 was noted during the pyrexia and a transient drop of blood pressure of 5–15mmHg diastolic occurred at the beginning of the pyrexia. The first patient developed nausea and vomiting 15min after the second injection of 5,000 units. The second patient developed transient nausea 10–15min after the second injection of 67,000 units. Patient 1 was ambulant and attended for treatment as an outpatient. Patient 2 was an inpatient for stabilization of anti-coagulant therapy (given for a deep calf vein thrombosis which developed after his last surgical treatment) and for treatment of an ulcer over the lateral aspect of his left ankle. No clinically apparent adverse effects were noted in the days subsequent to the injections.

Immunoglobulin levels No significant change in immunoglobulins A, G, or M levels were detected in serum samples from the 2 patients taken at weekly intervals over the study period (8–10 weeks).

Auto-antibodies Auto-antibodies to red blood cells, anti-nuclear factors, parietal cells, smooth muscle cells, thyroid tissue and mitochondria were not detected in pre-treatment serum samples or in
samples taken ~3 weeks after each treatment. Rheumatoid factor was detected by the latex agglutination test both before and after treatment in Patient 2.

Cortisol levels An increase in cortisol levels were observed within 2 h of the injections. The degree of the rise appeared related to the dose of IL-2 given. In Patient 2 after injection of 17,000 units peak cortisol levels of 720 nM/l were seen at 4 h. After 67,000 units peak levels of 1780 nM/l were seen at 4 h. Pre-injection values were 280 and 270 nM/l respectively. The levels had returned to within the normal range 20 h after the injections (270 and 415 nM/l respectively. Normal range 280–830 nM/l).

Liver enzymes and blood glucose levels No significant change in the enzyme alkaline phosphatase, lactic dehydrogenase, glutamyl pyruvate, glutamyl oxalacetic and γ-glutamyl transaminases were recorded at 1, 4 and 24 h after the IL-2 injection. The serum glucose level in Patient 1 before and at 1, 4 and 24 h after injection of 14,000 units was 4.4, 3.3, 6.5 and 5.1 mM 1−1 respectively. (Normal venous fasting levels 3.9–6.1 mM 1−1). In Patient 2 levels of blood glucose before and at 1, 4 and 24 h after injection of 67,000 units was 5.3, 3.3, 5.3 and 5.8 respectively.

Effect of IL-2 on the coagulation system Patient 2 was on anti-coagulants and was not studied. At 45 min after injection of 14,000 units in Patient 1 there was significant prolongation of the thrombin time from 14 to 30 sec and of the partial thromboplastin time with kaolin (PTTK) of 27 to 35 sec. These had returned to normal by 4 h. Moderate levels of fibrinogen degradation products (FDPs) were detected at 45 min (40–80 ng ml−1) and fibrinogen levels were also low 45 min after the injections (15 mg ml−1) (Normal range, 200–400 mg/ml). The latter had returned to normal by 4 h but 10–40 ng ml−1 of FDPs were still present at this time.

IFN levels in serum following administration of IL-2 containing supernatants No significant levels of IFN (<1 unit/ml) were detected in serum samples taken at 1 and 4 h or at the daily intervals shown in Figures 2 and 3 after injection of 14,000 units in Patient 1 and 67,000 units in Patient 2.

Complement levels No significant changes occurred in the C₃ or C₄ levels after the injections in either patient.

Discussion

The dose of IL-2 given to the subjects in this study was selected so that an initial level of at least 2 u/ml in plasma would be obtained as this was the level used in vitro for induction of cytotoxic T cell activity (Hersey et al., 1981). Based on an expected plasma volume of ~4% of body weight and assuming no destruction or absorption of the IL-2 it would be expected that 14,000 u in Patient 1 would give an initial value of 7.3 u ml−1 (calculated plasma volume 1.92 l) and 17,000 u in Patient 2 an initial value of 5.8 u ml−1 (calculated plasma volume 2.92 l). Observed values were approximately 3 u/ml and 1 u/ml respectively. Similarly on the assumption that IL-2 equilibrated in total extracellular fluid (TEF) and that this was 19% of body weight it would be expected that the final level in Patient 1 would be 1.5 u.ml−1 (calculated TEF = 9.12 l) and 1.22 u.ml in Patient 2 (calculated TEF = 13.87 l). In fact zero levels were seen at 1 h. [Equivalent values for the second injection in both patients were Patient 1, expected initial 2.6 u ml−1 (observed approximately 1.25 u/ml, Patient 2 expected initial 23 u/ml, observed approximately 6 u ml−1)].

The discrepancy between the expected and observed IL-2 values in the circulation may be the result of several factors. One possibility is that the IL-2 may be inactivated by factors in serum as reported to occur in mice (Wagner et al., 1980a). Similar inactivation of IL-2 by human serum was not detected in the present studies. The mitogenic activity of IL-2 on activated T cells in vitro was only inhibited by high concentrations of fresh human serum and the induction of cytotoxic cells in vitro by IL-2 in autologous serum was comparable or only moderately reduced compared to that in FCS. We cannot exclude that factors inhibiting mitogenesis may have been released into the serum in vivo following IL-2 administration and that these may have influenced the clearance rates by their effect on the bioassay used in the study.

A second explanation for loss of IL-2 activity is that it was absorbed by activated T cells in vivo as shown to occur in vitro. (Smith et al., 1979; Watson & Mochizuki, 1980). At high cell concentrations in vitro 70% of IL-2 activity was found to be absorbed in 30 min (Smith et al., 1979) which is comparable to the clearance rate from the patient’s serum found in the present study. Absorption of IL-2 by activated lymphocytes may explain why different levels of IL-2 were seen in the 2 patients for a given dose of IL-2. Patient 1 had a lower lymphocyte count than Patient 2 and hence less absorption of IL-2 and relatively higher serum levels of IL-2 may have occurred.

The latter explanation was supported by evidence
of increased division of cells in the circulation after injection of the IL-2 containing preparations. This was consistent with absorption to and activation of cells in the circulation which expressed IL-2 receptors. Recent studies suggest the latter cells are activated into the G1 phase of the cell cycle by interaction with antigen but require IL-2 for subsequent cell division (Kristensen et al., 1982).

A number of clinical and biochemical effects were observed in the recipients of the IL-2 containing preparations which may have reflected the activity of contaminating monokines and lymphokines in the preparation, e.g. pyrexia may have resulted from interleukin I (IL-1) in the supernatants as the latter is believed to be synonymous with endogenous pyrogen (Murphy et al., 1980). Similarly the transient hypoglycaemia and increase in FDPs may indicate the activity of such factors as glucocorticoid antagonizing factor (Moore et al., 1978) and thromboplastins (Geczy & Hopper, 1981) released from monocytes in the blood leukocyte preparations during IL-2 production. The increased platelet count, haemoglobin and metamyelocytes noted at 7 days may indicate the presence of colony stimulating factors in the preparations.

Elevation of glucocorticoids in the circulation was previously noted after injection of lymphokine preparations in rodents (Besedovsky et al., 1981) and may have been responsible in part for the neutrophilia and lymphocytopenia noted hours after the injections. Both the latter are well known systemic effects of corticosteroids. The systemic effects noted in these studies were similar to those reported after administration of a lymphokine preparation (Dumonde et al., 1982) and recombinant IFN except that neutrophilia rather than neutrophilia was noted after the latter (Gutterman et al., 1982). IFN was not detected in the IL-2 containing supernatants in the present study and it seems likely that the side effects common to these procedures are in part due to a stress response.

These results highlight the need for administration of purified preparations of IL-2 to allow the effects of IL-2 in vivo to be clearly defined. The present study, however, provides some encouragement that systemic administration of IL-2 may be a feasible approach for the activation of T cells in vivo. Such an approach would be technically much simpler than injection of cytotoxic T cells grown in vitro in the presence of IL-2 as described by previous authors (Mills et al., 1980; Warren et al., 1979; Gillis & Watson, 1981) and may allow activation of lymphocytes at the site of tumour growth. Although the half-life of 20–25 min for IL-2 in the circulation appears relatively short this does not appear to be due to inactivation by serum factors. If subsequent studies confirm that clearance is at least in part due to absorption to activated T cells this may be sufficient to trigger these cells into cytotoxic activity despite the subsequent absence of detectable IL-2 in the circulation. Further studies with purified IL-2 preparations are required to answer these questions.

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