Toxicity of Subcutaneously Administered Recombinant Human Interleukin-2 in Rats

Grushenka H. I. Wolfgang, Rene D. McCabe, and Dale E. Johnson

Department of Toxicology, Chiron Corporation, 4560 Horton Street, Emeryville, California 94608

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Recombinant human interleukin-2 (rIL-2) was administered subcutaneously to rats at doses of 0.3–10 mg/kg/day in a range-finding study and 0.03–0.3 mg/kg/day in a 4-week toxicity study. Treatment-related effects were assessed by hematology, clinical chemistry, anti-rIL-2 antibody production, and gross and histopathologic evaluations. Doses of 1 mg/kg/day or above were not tolerated, resulting in death or moribund termination by Day 7. Slight decreases in red blood cell counts (including hemoglobin) were observed at ≥ 0.1 mg/kg/day. White blood cells counts increased in a dose-dependent manner; increases were primarily due to increases in lymphocytes and eosinophils. Hepatic abnormalities, including increases in aspartate aminotransferase and bilirubin, were noted at 0.3 mg/kg/day. Histologic findings were evident primarily in the spleen, liver, lung, and injection sites, with dose-related increases in inflammatory cell foci/infiltrates noted in these sites. Findings in the liver also included biliary hyperplasia, hepatocellular degeneration, necrosis, vascular mural thickening in the portal triads, and fibrosis. Red and white pulp hyperplasia and capsular fibrosis occurred in the spleen. Most clinical and histopathologic findings were reversible within 4 weeks after termination of treatment. Anti-rIL-2 antibodies were detected beginning on Day 19 and were still present on Day 56. The pharmacological and toxicological effects associated with subcutaneous administration of rIL-2 are comparable to those reported after intravenous administration, indicating that subcutaneous dosing may be an alternative to the current clinical iv regimen.

Key Words: recombinant human interleukin-2; IL-2 toxicity; rat; subcutaneous administration; antibody formation; lymphocytosis.

Human recombinant interleukin-2 (rIL-2) is a lymphokine which is active as a immunomodulator. rIL-2 mimics the activity of naturally occurring IL-2 that is produced by antigen- or mitogen-activated T lymphocytes (Rosenberg et al., 1984; Doyle et al., 1985). IL-2 has been shown to stimulate the proliferation and differentiation of both T and B cells, the activation and proliferation of nonspecific cytolytic effector cells including natural killer and lymphokine-activated killer cells, and the activation of monocytes and macrophages (Anderson et al., 1993). IL-2 is probably the best studied of the cytokines; numerous reviews of its biology have been published (Anderson, 1992; Winkelhake and Gauny, 1990; Smith, 1988; Whittingdon and Faulds, 1993; Mertelsmann and Wetter, 1986).

Clinical use of rIL-2 initially focused on its tumor therapy potential, and it is currently marketed for use in metastatic renal cell cancer. rIL-2 continues to be investigated for human tumor immunotherapy, as immunotherapy in immunodeficiency diseases, and as a vaccine adjuvant (Anderson, 1992; Whittingdon and Faulds, 1993; Kovacs et al., 1996). Early clinical trials in which high doses of rIL-2 were administered by bolus iv or continuous iv infusion were plagued by dose-limiting toxicities including "vascular leak syndrome," characterized by weight gain, pulmonary edema, pleural effusions and acites, and hypotension (Vial and Descotes, 1992; Siegel and Puri, 1991; Margolin et al., 1989; Lotze et al., 1986; Rosenberg et al., 1989). This early experience led to careful investigations of the dose, schedule, and route of rIL-2 administration.

Unlike many recombinant proteins, human rIL-2 has biological activity in a variety of species including mice, rats, cats, dogs, and sheep (Anderson, 1992). Mice and rats given appropriate dosing regimens of rIL-2 exhibit many of the toxicities associated with high-dose therapy in humans including vascular leak syndrome, hematological effects (anemia, lymphocytosis, and eosinophilia), hepatotoxicity, and infiltration of multiple tissues with lymphocytes and eosinophils (Anderson et al., 1988; Anderson and Hayes, 1989). Rodent models can therefore be used to test specific dosing regimens with the aim of reducing toxicity while maintaining pharmacological activity.

The majority of nonclinical studies have been conducted by the iv route. Toxicity has been shown to be dose-related and a function of systemic rIL-2 exposure (Anderson and Sorenson, 1994). Intravenous dosing results in higher peak plasma levels and greater systemic exposure than subcutaneous dosing. Although rIL-2 is only approximately 30–35% bioavailable by the sc route (Investigator's Brochure, 1996) systemic exposure
is of longer duration and lymph exposure to rIL-2 is higher (Anderson, 1994; unpublished data). As many functions of rIL-2 occur through secondary lymphoid tissues, similar or lower sc doses may produce comparable biological effects to iv doses. Subcutaneous administration has been selected for clinical trials due to the difference in exposure, a possible decrease in side-effect profile, and the relative ease of use over intravenous administration methods (Whittingdon and Faulds, 1993; Anderson and Sorenson, 1994).

The purpose of these preclinical studies was to determine the maximum tolerated dose that could be administered subcutaneously to rats (range-finding study) and to characterize the toxicity profile of subcutaneous administration of rIL-2 in rats in a 4-week toxicity study.

**METHODS**

Male and female Sprague-Dawley rats (Charles River UK Ltd., Margate, Kent) weighing approximately 140–210 g for the range-finding study and approximately 160–220 g for the 4-week study were housed in suspended polystyrene/stainless steel grid cages and maintained in an environmentally controlled room on a 12-h light/dark cycle. Animals had ad libitum access to food (Special Diets Services Ltd., Rat and Mouse No. 1 Expanded SQC Diet) and water throughout the study. Studies were conducted at Inveresk Research International Limited, Tranent, EH33 2NE, Scotland, and conformed to the Animals (Scientific Procedures) Act of 1986; the 4-week study was GLP compliant.

rIL-2 (Proleukin, aldesleukin) is a purified protein (approximately 15,300 MW) produced by Chiron Corporation in Escherichia coli carrying an expression plasmid for a modified recombinant IL-2 (Doyle et al., 1985). IL-2 is isolated, purified, and formulated with mannitol, adjusted to pH of 7.5, sterilized, and lyophilized. The biological potency of rIL-2 is 18 X 10^8 IU of activity per 1 mg protein. A single lot of lyophilized rIL-2 was used for both studies. Sterile water for injection was used for reconstitution and dilution of the drug.

**Dose range-finding study.** Five rats/sex/group were dosed with 0 (placebo), 0.3, 3, or 10 mg/kg/day and an additional 3/sex/group were dosed with 1 mg/kg of rIL-2. The length of dosing depended upon the degree of toxicity that developed. The 0 and 0.3 mg/kg groups were dosed for 21 days; the 1 mg/kg group for 7 days; the 3 mg/kg group for 6 days; and the 10 mg/kg/day for 4 days. The dose volume was 10 ml/kg, divided equally among four subcutaneous sites on the dorsal region. Criteria evaluated for treatment-related effects were body weight, clinical signs, food and water consumption, hematology, selected organ weights, and gross pathology.

**Four-week study.** Ten rats/sex/group were dosed with nominal levels of 0 (placebo) 0.03, 0.1, and 0.3 mg/kg rIL-2 daily for 4 weeks. An additional 3/sex/group were included in the 0 and 0.3 mg/kg groups and were retained for an additional 4-week recovery period. The dose volume was 0.3 ml/kg, administered subcutaneously in a single dorsal site. Animals were sacrificed at 4 and 8 weeks. In addition to those parameters detailed for the range-finding study, criteria evaluated for treatment-related effects included serum chemistry, urinalysis, and full histopathology on all animals. Serum chemistry profiles included urea, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), sodium, potassium, chloride, total protein, albumin, albumin/globulin ratio, cholesterol, alkaline phosphatase, calcium, creatinine, triglycerides, inorganic phosphate, total bilirubin, and γ-glutamyl transferase. Urinalysis included volume, pH, specific gravity, protein, glucose, ketones, bilirubin, urobilinogen, and microscopic analysis for epithelial cells, crystals, white blood cells, erythrocytes, organisms, casts, and abnormal constituents.

Satellite animals, 3/sex, were dosed at 0.03, 0.1, and 0.3 mg/kg for antibody determinations. Blood samples were obtained on days 1, 10, 19, 28, and 56. An ELISA procedure was utilized to measure rat IgG antibody against rIL-2 in rat plasma. The coat antigen was rIL-2 (Proleukin) and the conjugate was peroxidase-conjugated affinity-purified goat anti-rat IgG.

**Data analysis.** Data were expressed as means ± standard deviation (SD) or standard error (SE). Body weight, clinical chemistry, and urinalysis data were analyzed using a parametric ANOVA and individual between-group comparisons were made using Fisher's F-protected LSD method via Student's t-test. Differences were considered statistically significant at the p < 0.05 level.

**RESULTS**

**Range-Finding Study**

All animals receiving 3 or 10 mg/kg rIL-2 were found dead or were killed on Day 5/6 due to debilitated condition (Table 1). Animals given 1 mg/kg were killed on Day 7, after displaying clinical signs of scruffy coats, hunched posture, and yellow tray paper staining. All of the 0.3 mg/kg animals survived until termination on Day 22. Decreased body weight gain/body weight loss and decreased food consumption were evident during Week 1 in animals given ≥1 mg/kg. Effects on these parameters were minimal at 0.3 mg/kg. At 0.3 mg/kg decreases in RBC parameters (counts, hemoglobin, and hematocrit) and increases in WBC parameters (counts, all cell types) were observed on Days 14 and 21 (Table 2). At necropsy, thickened/reddened injection sites were observed in many control and treated animals. Enlarged spleens were noted at all dose levels, correlating with increased spleen weights (Table 3). Significant increases in liver and lung weights were also observed in 0.3 mg/kg females. At doses ≥3 mg/kg animals also had enlarged and reddened lymph nodes and yellow intestinal contents. As subcutaneous doses ≥1 mg/kg were not tolerated for more than 7 days of dosing, doses of 0.03, 0.1, and 0.3 were selected for the subsequent 4-week study.

**Four-Week Study**

Clinical signs were limited to thickening of the skin at the subcutaneous dosing site at 0.1 and 0.3 mg/kg and yellow (urine) staining of cage paper at the high dose. There was little

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**TABLE 1**

### rIL-2-Induced Mortality in Rats

<table>
<thead>
<tr>
<th>Route</th>
<th>Dose (mg/kg)</th>
<th>Number of daily doses</th>
<th>Mortality incidence</th>
<th>Mortality (%)</th>
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<tbody>
<tr>
<td>sc</td>
<td>0.03</td>
<td>28</td>
<td>0/20</td>
<td>0</td>
</tr>
<tr>
<td>sc</td>
<td>0.1</td>
<td>28</td>
<td>0/20</td>
<td>0</td>
</tr>
<tr>
<td>sc</td>
<td>0.3</td>
<td>21-28</td>
<td>0/30</td>
<td>0</td>
</tr>
<tr>
<td>sc</td>
<td>1</td>
<td>7</td>
<td>10/10^a</td>
<td>100^a</td>
</tr>
<tr>
<td>sc</td>
<td>3</td>
<td>5-6</td>
<td>10/10^a</td>
<td>100^a</td>
</tr>
<tr>
<td>sc</td>
<td>10</td>
<td>4</td>
<td>10/10^a</td>
<td>100^a</td>
</tr>
</tbody>
</table>

* Animals killed prematurely, animals with clinical signs but not yet moribund.
* Includes moribund terminations.
TABLE 2
Hematological Values in Rats Treated with rIL-2 Subcutaneously for 2 or 3 Weeks

<table>
<thead>
<tr>
<th>Dose mg/kg</th>
<th>Sex</th>
<th>Week</th>
<th>WBC × 10⁹/L</th>
<th>Neutrophil count × 10⁹/L</th>
<th>Lymphocyte count × 10⁹/L</th>
<th>Monocyte count × 10⁹/L</th>
<th>Eosinophil count × 10⁹/L</th>
<th>Basophil count × 10⁹/L</th>
<th>LUC × 10⁹/L</th>
<th>RBC × 10¹²/L</th>
<th>Hematocrit %</th>
<th>Hemoglobin g/dL</th>
<th>Reticulocyte count %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 M</td>
<td>2</td>
<td>10.99 ± 1.02</td>
<td>1.27 ± 0.10</td>
<td>9.12 ± 0.95</td>
<td>0.32 ± 0.05</td>
<td>0.14 ± 0.03</td>
<td>0.03 ± 0.01</td>
<td>0.11 ± 0.02</td>
<td>7.04 ± 0.35</td>
<td>0.426 ± 0.014</td>
<td>14.2 ± 0.5</td>
<td>2.6 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>0.3 M</td>
<td>2</td>
<td>19.50 ± 5.51</td>
<td>3.22 ± 1.38</td>
<td>13.50 ± 2.94*</td>
<td>0.60 ± 0.33</td>
<td>1.57 ± 0.68*</td>
<td>0.10 ± 0.04*</td>
<td>0.52 ± 0.32*</td>
<td>5.58 ± 0.47*</td>
<td>0.323 ± 0.035</td>
<td>10.9 ± 1.0*</td>
<td>6.5 ± 2.6*</td>
<td></td>
</tr>
<tr>
<td>0 F</td>
<td>2</td>
<td>8.74 ± 2.04</td>
<td>0.89 ± 0.31</td>
<td>7.43 ± 2.01</td>
<td>0.19 ± 0.04</td>
<td>0.13 ± 0.06</td>
<td>0.02 ± 0.01</td>
<td>0.07 ± 0.03</td>
<td>6.32 ± 1.11</td>
<td>0.363 ± 0.072</td>
<td>13.0 ± 1.2</td>
<td>1.6 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>0.3 F</td>
<td>2</td>
<td>20.57 ± 7.57</td>
<td>3.66 ± 0.48*</td>
<td>13.51 ± 5.95</td>
<td>0.49 ± 0.32*</td>
<td>2.21 ± 0.80*</td>
<td>0.12 ± 0.10*</td>
<td>0.58 ± 0.55*</td>
<td>5.09 ± 0.18</td>
<td>0.299 ± 0.010</td>
<td>10.4 ± 0.4*</td>
<td>8.6 ± 2.7*</td>
<td></td>
</tr>
<tr>
<td>0 M</td>
<td>3</td>
<td>11.87 ± 1.97</td>
<td>1.14 ± 0.32</td>
<td>9.99 ± 1.65</td>
<td>0.31 ± 0.06</td>
<td>0.13 ± 0.04</td>
<td>0.03 ± 0.01</td>
<td>0.27 ± 0.08</td>
<td>7.37 ± 0.45</td>
<td>0.430 ± 0.016</td>
<td>14.4 ± 0.6</td>
<td>2.8 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>0.3 M</td>
<td>3</td>
<td>31.30 ± 7.77*</td>
<td>5.01 ± 1.27*</td>
<td>20.12 ± 4.30*</td>
<td>0.93 ± 0.38*</td>
<td>3.78 ± 1.46*</td>
<td>0.23 ± 0.08*</td>
<td>1.23 ± 0.51*</td>
<td>5.46 ± 0.24*</td>
<td>0.316 ± 0.017*</td>
<td>10.6 ± 0.6*</td>
<td>7.8 ± 2.5*</td>
<td></td>
</tr>
<tr>
<td>0 F</td>
<td>3</td>
<td>10.29 ± 1.89</td>
<td>0.69 ± 0.06</td>
<td>9.00 ± 1.72</td>
<td>0.22 ± 0.10</td>
<td>0.17 ± 0.07</td>
<td>0.04 ± 0.03</td>
<td>0.18 ± 0.09</td>
<td>7.33 ± 0.32</td>
<td>0.419 ± 0.016</td>
<td>14.6 ± 0.6</td>
<td>2.8 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>0.3 F</td>
<td>3</td>
<td>23.18 ± 10.94*</td>
<td>4.49 ± 2.68*</td>
<td>14.40 ± 6.71</td>
<td>0.56 ± 0.45</td>
<td>2.86 ± 1.46*</td>
<td>0.15 ± 0.13*</td>
<td>0.71 ± 0.71*</td>
<td>5.67 ± 0.37*</td>
<td>0.341 ± 0.022*</td>
<td>11.0 ± 1.3</td>
<td>6.4 ± 2.4*</td>
<td></td>
</tr>
</tbody>
</table>

Note. Data are expressed as means ± SD, N = 5. Blood was collected on Days 14 or 22. LUC, large unclassified cell count.
* Significantly different from respective control, p < 0.05.
animals given 0.1 mg/kg and most animals given 0.3 mg/kg. Focal necrosis was noted in one female at 0.1 mg/kg and one female at 0.3 mg/kg. Liver fibrosis characterized by linear fibrous tracks bridging central veins and portal triads was seen in several males and females in the high-dose group. In the lungs there was a small increase in incidence of pneumonitis (mixed infiltrates) in the high-dose group. There was a treatment- and usually dose-related increase in inflammatory cell foci/infiltrates in numerous organs. Effects were most profound in the liver (periportal), the lungs (perivascular), and at the injection sites (subcutaneous). Organs such as the kidney and pancreas were affected to a lesser extent.

Most histopathological findings were reduced in incidence and severity in recovery animals, but some spleen and liver findings were still evident, particularly splenic capsule fibrosis and liver fibrosis.

Antibodies against rIL-2 were not detected on Days 1 and 10, but beginning on Day 19 and continuing through Day 56, antibodies were observed in all dose groups except low-dose males (Table 6). As the dose increased, the incidence and the titers increased. Antibody responses were generally greater in females than in males at each time point. Antibody titers were maintained during the 4-week reversal period.

**DISCUSSION**

The use of rIL-2 as a therapeutic depends upon the host's pharmacodynamic response to produce a "drug" effect. Studies in animal models and clinical trials have shown that the rIL-2 toxicity/side effect profile is directly or secondarily related to its pharmacological activity. The pharmacological and toxicological findings are dose-related in incidence and sever-
ity. Effort is being made to separate efficacy from toxicity by manipulating the schedule and the route of rIL-2 treatments.

The most consistent pharmacological findings observed in rats have been leukocytosis, lymphocytosis, and eosinophilia. These findings were observed at sc doses as low as 0.03 mg/kg for 4 weeks in females and at >0.1 mg/kg in both sexes. Similar WBC effects have been seen in previous iv studies, with dose-dependent changes observed in rats given 0.005–0.5 mg/kg for 4 weeks (Harada and Yahara, 1993) or 0.2–10 mg/kg for 2 weeks (Anderson and Hayes, 1989). In contrast to the anemia previously reported for iv studies, only minor decreases in RBC parameters were evident following sc administration and no thrombocytopenia was observed.

Correlating with the hematology findings in this study, histopathology revealed inflammatory cell infiltrates (lymphocytes and eosinophils) in liver and lung, with fewer infiltrates in other organs. Increases in lung and liver weights can be attributed to cellular infiltrates, while increased spleen weights were attributed to increased red and white pulp (hematopoiesis). As sc doses of rIL-2 are increased, lymphocytic infiltrates were observed in a greater number of organs.

High doses of rIL-2, whether given by the sc or the iv route, cause an increase in hepatic transaminases and hyperbilirubinemia. In addition, hypoalbuminemia and increased γ-glutamyltransferase have been noted after iv administration (Anderson et al., 1993). Animals given high doses by either route appear icteric and often have bright yellow urine. Lymphoid infiltration and hepatocellular necrosis are reported to be the most likely cause of death following iv administration (Anderson et al., 1993). While histopathology was not conducted on dead or moribund animals in the sc range-finding study, hepatic effects were likely the cause of death. In the 4-week sc study, liver pathology was evident, but was not life-threatening. In a recent clinical trial using continuous iv infusions of low doses of rIL-2 (≤ 18 million IU, 5 days/cycle), the side effect profile mimicked sc dosing in rats in that the most common laboratory abnormalities were elevations in bilirubin and alkaline phosphatase (Kovacs et al., 1996).

While liver toxicity appears to be the primary toxicity and cause of death in rats, in other species, including humans, pulmonary complications are the most prominent finding. Vascular leak syndrome is the dose-limiting side effect of high-dose therapy in humans (Rosenberg et al., 1989); a similar syndrome is produced in mice (0.2 mg/kg, ip) and cynomolgus monkeys (>0.15 mg/kg, iv; Anderson et al., 1993). In rats given doses of 0.3 mg/kg rIL-2 subcutaneously, inflammatory cell infiltrates and a small number of animals with pneumonitis were noted, but the sc route and dosing regimen was not associated with vascular leak. In rats given rIL-2 by the iv route, pathologic changes at high doses (4 mg/kg) included plural effusions, mild perivascular hemorrhages, and increases in lung weights (Anderson and Hayes, 1989). A vascular leak syndrome has been produced in rats by the ip route at doses of 0.5 mg/kg given once or twice a day for 2 weeks (Anderson and Hayes, 1989) or 500,000 cetus units 3X/day for up to 5 days (Zhang et al., 1995). In rats, subcutaneously administered r-IL2 was not associated with severe pulmonary findings, even at high doses (1–10 mg/kg) which caused death. Interestingly, multiple daily dose regimens and ip administration appeared to increase the incidence of pulmonary vascular leak.

The reduction in dose-limiting toxicities following sc administration may be related to two phenomena: the difference in biodistribution between iv and sc routes and biolocalization differences of IL-2 receptor-positive leukocyte populations that produce many of the known mediators of IL-2 toxicity. It has been shown by Bocci et al., (1986)
that absorption of molecules like IL-2 can be targeted to the lymphatic system by sc dosing. Even though serum concentrations would be lower after sc dosing due to bioavailability, lymph concentrations would be approximately equal after iv or sc dosing. Studies in pigs have demonstrated that the lymph to plasma AUC ratio following iv administration was 0.75, whereas the ratio was approximately 4.0 following sc administration, indicating that rIL-2 is preferentially absorbed by lymphatic processes after sc administration (Wolfgang et al., 1997). High- and intermediate-affinity IL-2 receptors are located on NK cells, neutrophils, monocytes, macrophages, and T and B lymphocytes (Caligiuri, 1993). In the body, most of the NK cells, neutrophils, and monocytes are in the blood, while the majority of the T and B lymphocytes are in the lymphatic system (Stites et al., 1994). NK cells and neutrophils produce mediators (interferon-γ, TNF-α, reactive oxygen intermediates) which are considered involved in producing IL-2 toxicities. In contrast, IL-2 receptor-positive T cells thought to be associated with efficacy reside largely in lymphoid organs. The preferential absorption in the lymph compartment following sc administration possibly reduces exposure to NK cells and neutrophils and thus toxic side effects while maintaining efficacy with high exposure to lymphocyte populations.

Anti-rIL-2 antibodies of the IgG class were detected in the 4-week study. An assay for neutralizing antibody was not performed, but based on positive clinical and histopathological findings at 4 weeks the antibodies did not appear to be neutralizing. However, a slight attenuation of effects was noted when comparing responses at 3 weeks (range-finding study) and 4 weeks. Harada and Yahara (1993) found neutralizing antibodies in rats receiving intravenous rIL-2 at doses of >5

## TABLE 5
**Incidence of Pathological Findings in Selected Organs Following rIL-2 Treatment in Rats for 4 Weeks**

<table>
<thead>
<tr>
<th>Injection site</th>
<th>Histopathological finding</th>
<th>Dose (mg/kg)</th>
<th>0 mg/kg</th>
<th>0.03 mg/kg</th>
<th>0.1 mg/kg</th>
<th>0.3 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>sc inflammatory cell infiltrate</td>
<td>0/20</td>
<td>18/20</td>
<td>18/20</td>
<td>17/20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sc fibrosis</td>
<td>0/20</td>
<td>3/20</td>
<td>6/20</td>
<td>4/20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>periportal inflammatory cell infiltrate</td>
<td>0/20</td>
<td>6/20</td>
<td>16/20</td>
<td>20/20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>biliary hyperplasia</td>
<td>0/20</td>
<td>0/20</td>
<td>4/20</td>
<td>14/20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>vascular mural thickening in portal triad</td>
<td>0/20</td>
<td>0/20</td>
<td>7/20</td>
<td>15/20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>hepatocellular degeneration/regeneration</td>
<td>0/20</td>
<td>0/20</td>
<td>5/20</td>
<td>9/20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>focal necrosis</td>
<td>1/20</td>
<td>0/20</td>
<td>1/20</td>
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<tr>
<td></td>
<td>fibrosis</td>
<td>0/20</td>
<td>0/20</td>
<td>0/20</td>
<td>5/20</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>perivascular inflammatory cell infiltrate</td>
<td>0/20</td>
<td>7/20</td>
<td>18/20</td>
<td>18/20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pneumonitis (mixed infiltrates)</td>
<td>1/20</td>
<td>1/20</td>
<td>2/20</td>
<td>7/20</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>capsular fibrosis</td>
<td>0/20</td>
<td>3/20</td>
<td>10/20</td>
<td>18/20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>red pulp hyperplasia</td>
<td>0/20</td>
<td>2/20</td>
<td>5/20</td>
<td>15/20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>white pulp hyperplasia</td>
<td>0/20</td>
<td>0/30</td>
<td>3/20</td>
<td>3/20</td>
<td></td>
</tr>
</tbody>
</table>

**Note.** Results are reported as combined incidence, number of findings/total number of animals (10 males and 10 females).

## TABLE 6
**Antibody Titers for Individual Animals Following rIL-2 Administration**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Sex</th>
<th>Day</th>
<th>1</th>
<th>10</th>
<th>19</th>
<th>28</th>
<th>56</th>
</tr>
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<tbody>
<tr>
<td>0.03</td>
<td>M</td>
<td>&lt;1/50</td>
<td>&lt;1/50</td>
<td>&lt;1/50</td>
<td>&lt;1/50</td>
<td>&lt;1/50</td>
<td>&lt;1/50</td>
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<td>0.03</td>
<td>M</td>
<td>&lt;1/50</td>
<td>&lt;1/50</td>
<td>&lt;1/50</td>
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**Note.** nd, not determined.

* Dilution required for optical density of 0.5 by ELISA assay; 1/50 dilution was used for negative control.
µg/kg/day for 4 weeks or ≥1 µg/kg/day for 13 weeks. Antibodies were considered neutralizing as helper T cell levels were stimulated early in the 13-week study, but declined to predose levels by the middle of the study. Yet, Anderson and Hayes (1989) found no antibodies after 4 weeks of intravenous administration of rIL-2 to rats. No neutralizing antibody against rIL-2 was detected in cynomolgus monkeys (Harada and Yahara, 1993) in studies up to 40 weeks. It may be that route and/or formulation of the product (some formulations contained HSA) play a part in the formation and neutralizing effects of antibodies.

In conclusion, subcutaneous rIL-2 administration in rats at doses ≥1 mg/kg was not tolerated for more than 1 week. Daily doses of ≤0.3 mg/kg were tolerated for as long as 4 weeks but were associated with a dose-related increase in lymphocytes and eosinophils and with infiltrates in the spleen, liver, and lung. The 0.3 mg/kg dose was also associated with hepatic abnormalities. These sc findings are similar in nature to those observed previously with iv administration of rIL-2, with toxicological effects occurring at slightly lower doses by the iv route. Over a 4-week period the formation of antibodies did not appear to neutralize the pharmacological effects of rIL-2. The subcutaneous route is thus a possible route of clinical administration which may be associated with fewer dose-limiting side effects than iv dosing.

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REFERENCES


