Cardiotoxicity of Human Recombinant Interleukin-2 in Rats
A Morphological Study

Jun Zhang, MD; Zu-Xi Yu, MD, PhD; Stephen L. Hilbert, PhD; Maria Yamaguchi, BS; Douglas P. Chadwick, BS; Eugene H. Herman, PhD; and Victor J. Ferrans, MD, PhD

Background. One of the side effects of interleukin 2 (IL-2) cancer immunotherapy in humans is the vascular leak syndrome, which is frequently associated with depression of myocardial function, myocarditis, and myocardial necrosis.

Methods and Results. To investigate this cardiotoxicity, IL-2 (three doses of $5 \times 10^8$ Cetus units/day i.p.) was given to rats for 2, 3, or 5 days. Heart, lung, liver, spleen, and kidney tissues were studied by light and electron microscopy and with immunoperoxidase techniques. Cardiac changes consisted of focal lymphocytic and eosinophilic infiltration, myocyte vacuolization, myofibrillar loss, and necrosis. Ultrastructural alterations included swelling of endothelial cells, with dissociation of intercellular junctions, migration of lymphocytes into the interstitium, and interstitial hemorrhage and edema. Close contact between infiltrating lymphocytes, particularly large granular lymphocytes, and cardiac myocytes was often observed in areas of tissue damage. All lesions were more severe on day 5 than on days 2 and 3. Immunoperoxidase stains demonstrated asialo GM, ganglioside antibody-positive, granular lymphocytes to be much more frequent in myocardium of IL-2-treated rats than in that of control rats.

Conclusions. Although we cannot exclude the possibility of a direct toxic effect of IL-2 on myocytes, our observations suggest that the myocardial damage produced by this agent is triggered by IL-2-activated lymphocytes that exert cytolytic effects, first on endothelial cells and then on cardiac myocytes, thus producing lesions that involve both the cardiac microcirculation and the muscle cells. (Circulation 1993;87:1340-1353)

Key Words • myocarditis • myocardial necrosis • lymphocytes • cells, endothelial

The most frequently recognized cardiovascular complication of cancer immunotherapy with interleukin-2 (IL-2) is the vascular leak syndrome (VLS), which is thought to result, at least in part, from the interaction between IL-2-activated lymphocytes (lymphokine-activated killer [LAK] cells) and endothelial cells.1-4 VLS is characterized by an increase in vascular permeability, with fluid retention, peripheral edema, ascites, pleural effusion, and pulmonary edema.5,6 IL-2 therapy also can result in myocardial toxicity. Cardiac arrhythmias have been reported in 14-21% of patients undergoing IL-2 therapy.6,7 Other major cardiotoxic effects reported include myocardial infarction,6-12 myocarditis,11-14 and cardiomyopathy.15,16 Myocardial infarction has occurred in 1.2%,9 2%,6 and 4%7 of patients treated with IL-2. At necropsy, only some of these patients have had anatomic evidence of preexisting atherosclerotic coronary artery disease.12

Lymphocytic myocarditis and lymphocytic/eosinophilic myocarditis after IL-2 administration have been detected by endomyocardial biopsy or postmortem evaluation.11-14 More recently, two patients were reported to have developed cardiomyopathy after high-dose IL-2 therapy. Endomyocardial biopsy in one of these patients showed marked interstitial edema and myocyte degeneration without myocardial cellular infiltration.15 The other patient had cardiac dilatation but no evidence of myocardial infarction.16

The pathogenesis and the ultrastructural morphology of the cardiac lesions induced by IL-2 have not been investigated. Studies in rabbits,17 rats,18 and mice19 have shown that IL-2 induces extensive infiltration of the myocardium by lymphocytes. The present report describes an animal model in which cardiac morphological changes, including myocarditis and myocardial necrosis, developed in rats after treatment with IL-2 and postulates mechanisms for this cardiotoxicity.

Methods

Sixteen female Sprague-Dawley rats weighing 180-200 g were divided into four groups of four animals each. IL-2 was kindly provided by the Cetus Corp. (Emeryville, Calif.). Three daily doses of $5 \times 10^8$ Cetus units i.p. were given for 2, 3, and 5 days (groups 1, 2, and 3, respectively). Control rats (group 4) were treated with excipient. All rats were killed by intraperitoneal injec-
tions of pentobarbital 12 hours after the last IL-2 injection. Necropsies were performed, and the weights of the hearts and major organs were recorded. Portions of heart, lung, liver, kidney, lymph nodes, and thymus were removed immediately, fixed with 10% formalin, and embedded in paraffin. Sections were stained with hematoxylin and eosin.

Immunohistochemical Studies

The characteristics of the antibodies19–26 used in the immunohistochemical studies described below are listed in Table 1. For these studies, portions of the left ventricles were frozen rapidly, sectioned at a thickness of 5 μm, and air-dried for 20 minutes at room temperature. For the demonstration of asialo GM₁ (neutral glycolipid ganglio-N-tetrasylceramide) surface marker (herein referred to as asialo GM₁ ganglioside antibody), sections were then fixed for 5 minutes in cold acetone and washed in cold phosphate-buffered saline (PBS), pH 7.4, three times for 5 minutes at 4°C. Endogenous peroxidase activity was then blocked by incubation with 3% hydrogen peroxide in methanol for 10 minutes at 4°C. After three washes with cold PBS, nonspecific uptake of immunoglobulins was suppressed by incubation with 10% normal goat serum in PBS for 10 minutes at 4°C. The sections were then washed three times with cold PBS and incubated in a moist chamber for 1 hour at 4°C with a polyclonal antibody, asialo GM₁ ganglioside antibody (Wako Chemicals USA, Inc., Richmond, Va.), diluted 1:1,000 with PBS containing 1% bovine serum albumin and 0.1% sodium azide. After washing with PBS, the sections were incubated with biotinylated goat-derived anti-rabbit IgG (ABC Kit, Vector Laboratories, Burlingame, Calif.) at a 1:200 dilution in a moist chamber for 1 hour at 4°C. After washing with PBS, the sections were incubated with avidin-biotinylated horse-radish peroxidase complex (Vector Laboratories) for 1 hour at 4°C. The sections were then washed with two changes of PBS and one change of 0.1 mol/L Tris-HCl buffer, pH 7.6. The peroxidase reaction was then carried out with 0.05% 3,3′-diaminobenzidine tetrahydrochloride in 0.1 mol/L Tris-HCl buffer and 0.01% hydrogen peroxide for 10 minutes at room temperature, followed by washing in tap water, counterstaining with methyl green, and mounting in Permount. Negative controls included omission of primary or secondary antibodies.

For immunostaining with OX 8, OX 6, and W3/25 monoclonal antibodies, the indirect immunoperoxidase procedure was used.25 Cryostat sections 5 μm thick were air-dried for 20 minutes at room temperature, fixed in absolute ethanol for 10 minutes at 4°C, and washed three times in PBS, pH 7.4. The sections were incubated with a 1:40 to 1:100 dilution of primary antibodies (Accurate Chemical and Scientific Corp., San Diego, Calif.) for 1 hour at 4°C. After three washes with PBS, the sections were incubated with peroxidase-conjugated rabbit anti-mouse IgG (DAKO, Santa Barbara, Calif.) diluted 1:20 in PBS with 5% inactivated normal rat serum for 1 hour at 4°C. Development of color with diaminobenzidine/H₂O₂, counterstaining with methyl green, dehydration, and mounting were carried out as described above. For negative control, an irrelevant monoclonal antibody, W6/32 (Accurate Chemical and Scientific Corp.), was used for testing the specificity of the immunoperoxidase staining. This antibody reacts with human HLA-A, B, and C antigens but not with rat tissue components.26 Omission at the incubation step with the first antibody was also used as a negative control.

For electron microscopic study, the tissues (heart, lung, liver, spleen, and kidney) were fixed with 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer, pH 7.2, washed with several changes of 0.1 mol/L phosphate buffer, postfixed with 1% OsO₄ in phosphate buffer, dehydrated with ethanol and propylene oxide, and embedded in Polybed 812. Sections 1 μm thick were cut, stained with alkaline toluidine blue, and examined with a light microscope to select areas for ultrathin sectioning. Ultrathin sections were stained with uranyl acetate and lead citrate.

Results

Gross Examination

In the IL-2–treated rats, especially after 5 days of administration, the hearts were dilated, the pericardial cavity contained clear, straw-colored fluid, and the pleural cavities contained clear yellowish or bloody fluid. The lungs had hemorrhagic areas. The lymph nodes were enlarged.

Effect of IL-2 on Organ Weight

There were no significant differences between the weights of the hearts, livers, and kidneys in control and experimental animals on days 2, 3, or 5. However, lung weights of experimental animals on days 2 and 5 were greater than those of control animals.

Table 1. Antibodies Used in the Immunohistochemical Studies and Their Reactivity With Rat Tissue Components

<table>
<thead>
<tr>
<th>Name of antibody</th>
<th>Type</th>
<th>Reactivity</th>
<th>Staining method</th>
<th>Dilution</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit anti-asialo GM₁ ganglioside</td>
<td>P</td>
<td>NK cells, activated macrophages, subsets of LAK cells</td>
<td>ABC</td>
<td>1:1,000</td>
<td>19, 20</td>
</tr>
<tr>
<td>OX 8</td>
<td>M</td>
<td>CD8⁺ T-cytotoxic/suppressor lymphocytes, NK cells</td>
<td>I-IPR</td>
<td>1:40–100</td>
<td>22</td>
</tr>
<tr>
<td>W3/25</td>
<td>M</td>
<td>CD4⁺ T-helper lymphocytes and macrophages</td>
<td>I-IPR</td>
<td>1:40–100</td>
<td>24</td>
</tr>
<tr>
<td>OX 6</td>
<td>M</td>
<td>Dendritic cells, B-lymphocytes, and macrophages</td>
<td>I-IPR</td>
<td>1:40–100</td>
<td>23</td>
</tr>
<tr>
<td>W6/32</td>
<td>M</td>
<td>Irrelevant antibody used as control</td>
<td>I-IPR</td>
<td>1:40–100</td>
<td>26</td>
</tr>
</tbody>
</table>

P, polyclonal; M, monoclonal; NK, natural killer; LAK, lymphokine-activated killer; ABC, avidin-biotin complex; I-IPR, indirect immunoperoxidase.
Histological Observations

Lymphocytic infiltration and vascular alterations were observed in the noncardiac tissues examined, and all lesions were more severe on day 5 than on days 2 and 3. Pulmonary alveolar septa were markedly thickened and contained numerous lymphocytes, eosinophils, and macrophages. Electron microscopy revealed that the endothelial cells were swollen. Cytoplasmic processes of lymphocytes in the lumina of the pulmonary venules protruded into the cytoplasm of the endothelial cell. Other lymphocytes appeared to be passing through the endothelial layer into the perivascular spaces. In liver tissues, large numbers of lymphocytes, eosinophils, and macrophages were present, mainly within the periportal areas and the perivascular spaces of the central veins. Foci of hepatocyte necrosis were associated with infiltrates of lymphocytes and macrophages. The sinusoidal lumina were often occluded by lymphocytes. Pseudopods of lymphocytes in the perisinusoidal recesses protruded into the cytoplasm of hepatocytes. In kidney tissue, focal interstitial lymphocytic infiltrates were found in the cortex and the medulla. Thymic cortical depletion of lymphocytes and hyperplasia of lymph nodes and splenic red pulp were evident.

Examination of the myocardium of control rats showed normal morphological features, with no cellular infiltrates. Venules were recognized by their thin walls and by their diameters, which were larger than those of capillaries (Figures 1A and 1B).27,28 Margination of neutrophils or lymphocytes was not found in capillaries or venules.

After administration of IL-2 for 2 days, the changes observed consisted mainly of 1) focal areas of attachment of lymphocytes and neutrophils to the luminal surfaces of endothelial cells in small venules (Figures 1C and 1D) and capillaries (Figure 1E); 2) focal infiltration of the myocardial interstitium by lymphocytes (Figure 1F); and 3) interstitial hemorrhage and myocyte necrosis (Figure 1G) (Table 2). After 3 and 5 days of treatment with IL-2, these changes became more severe and widespread and consisted of infiltration of lymphocytes (Figure 2A), eosinophils, and neutrophils, interstitial hemorrhage, edema, myofibrillar loss, vacuolization of myocytes, and myocardial necrosis. In addition to the lymphocytic infiltrate found in myocardial interstitium, the lumina of myocardial capillaries often were filled with lymphocytes. A few foci of lymphocytic infiltrates were also found in the endocardium and subepicardium. These infiltrates also contained some eosinophils, neutrophils, and macrophages. The lymphocytes infiltrating the myocardial interstitium were in close contact with cardiac myocytes (Figure 2B), which often showed evidence of damage or necrosis (Figures 2C and 2D). This was confirmed by electron microscopic examination (see below).

Most of the infiltrating lymphocytes had large nuclei, prominent nucleoli, and abundant cytoplasm. A few lymphocytes had little cytoplasm and small nuclei. Lymphocytes undergoing mitosis were encountered rather frequently in the myocardial interstitium.

Myocardial necrosis was found primarily in the left and right ventricles. The necrosis was observed in the subepicardium, myocardium, and subendocardium. Necrosis was not observed in the atria. Myocyte necrosis was characterized by cytoplasmic fragmentation and hypereosinophilic staining, without calcific deposit. A few macrophages and eosinophils were present in the necrotic foci. At days 2 and 3, myocardial necrosis occurred in only one of four animals and was minimal. At day 5, necrosis was found in two of four animals; it was more severe and was associated with hemorrhage (Table 2). Myofibrillar loss, vacuolization, interstitial edema, hemorrhage, and congestion of capillaries also were more severe on day 5 than on days 2 and 3. The two animals that had myocardial necrosis on day 5 also showed more severe lymphocytic infiltrations and microcirculatory damage than did the other animals in this group.

Immunohistochemical Study

Study of immunoperoxidase-stained preparations showed that many of the lymphocytes found in the interstitium (Figure 2E) and in the lumina of the capillaries were asialo GM, ganglioside antibody–positive. Cells showing this type of immunoreactivity were rarely found in the myocardium of control animals; these cells were located in the interstitium. Immunohistochemical control preparations gave negative results.

In asialo GM, ganglioside antibody–stained preparations, the distinguishing features of the immunoreactive cells were that 1) their morphology was characterized by large round or elongated shapes; 2) their contacts with myocytes and endothelial cells were frequent; 3) their numbers greatly increased as the treatment continued; and 4) their distribution became extensive, involving predominantly pericardium, subepicardium, and myocardium.

In the hearts of control animals, very few OX 8 antibody–positive T-cytotoxic/suppressor lymphocytes were scattered in the interstitium of the myocardium. These immunoreactive cells increased in number with the increased time of treatment with IL-2. They were found singly (Figure 2F) or in small groups of two or three cells distributed in the myocardial interstitium, subepicardium, and subendocardium. Close contacts between OX 8 antibody–positive cells and myocytes or endothelial cells in capillaries and venules were found only occasionally. In the vicinity of vessels, OX 8 antibody–positive T-cytotoxic/suppressor lymphocytes were also present.

OX 6 antibody–positive dendritic cells, W3/25 antibody–positive T-helper lymphocytes, and W3/25 antibody–positive macrophages were found to increase with the duration of IL-2 treatment. These cell types were distributed individually. No clustering of dendritic cells with T-helper lymphocytes and no aggregates of dendritic cells were found.

Electron Microscopic Observations

The myocardium of control rats was ultrastructurally normal. The capillary endothelial cells showed their usual normal features, with thin walls, well-organized intercellular junctions, and moderate numbers of pinocytotic vesicles. Venules were recognized by the fact that they were larger than capillaries and were composed of an endothelial cell layer and an investing layer that consisted of only a very few, discontinuously arranged smooth muscle cells and/or pericytes.27,28 This investing layer was poorly developed in postcapillary
FIGURE 1. Light micrographs of myocardium from control rats (panels A and B) and rats treated with IL-2 for 2 days (panels C–G). All sections were stained with toluidine blue. Panel A: Normal postcapillary venule has large luminal diameter and thin, flat endothelial cells. Note pericyte (arrow). Magnification, ×630. Panel B: Normal muscular venule has thin wall composed of layer of endothelial cells and single layer of smooth muscle cells. Magnification, ×630. Panel C: A lymphocyte is in close contact with an endothelial cell of a postpapillary venule. Magnification, ×630. Panel D: A neutrophil is in close contact with an endothelial cell of a postcapillary venule. Magnification, ×630. Panel E: A lymphocyte is in contact with a capillary endothelial cell. Magnification, ×630. Panel F: Focal infiltration of the myocardial interstitium by some lymphocytes and monocytes. Magnification, ×630. Panel G: Small area of necrosis with contraction bands is seen in myocardium. Magnification, ×400.
venules. In control hearts, no areas of extensive, close approximation between endothelial cells and lymphocytes or neutrophils were found, although these cells occasionally filled the lumina of capillaries and contacted small areas of their walls.

After 2 days of IL-2 treatment, small numbers of lymphocytes were in close contact with the endothelial cells of capillaries and venules and in the interstitial spaces. These lymphocytes had oval nuclei with abundant chromatin in their periphery. Free ribosomes were frequently scattered throughout the cytoplasm. Several mitochondria, a few profiles of rough-surfaced endoplasmic reticulum, and variable numbers of granules of high electron density were present in the cytoplasm, usually on one side of the cell. These granules were round or oval, measured from 0.2 to 0.7 μm in diameter, were limited by single, trilaminar membranes, and were filled with amorphous, electron-dense material. They imparted a characteristic appearance to the lymphocytes ("large granular lymphocytes"). Both small and large agranular lymphocytes, lacking the characteristic cytoplasmic granules just described, were also present in the interstitium. Such cells were only occasionally found in contact with endothelial cells. The agranular lymphocytes displayed a round nucleus containing dense heterochromatin and some euchromatin. Their cytoplasm contained a few free ribosomes and polyribosomes. Mitochondria, cisterns of endoplasmic reticulum, Golgi complexes, and centrioles were rarely observed.

After 2 days but especially after 3 and 5 days of IL-2 treatment, the granular lymphocytes were in close contact with the endothelial cells in capillaries and venules and with cardiac myocytes. Two types of contacts between lymphocytes and endothelial cells were observed: 1) contacts in which the apposed areas of the two plasma membranes were relatively smooth (Figure 3A) and 2) contacts in which the apposed membranes, particularly those of the endothelial cells, formed extensive interdigitations (Figure 3B). Contacts between neutrophils and endothelial cells were of the first type just described (Figure 3C). Contacts of the second type often were associated with what appeared to be progressive surrounding of the lymphocytes by cytoplasmic processes of the endothelial cells (Figure 3B).

The morphology of the lymphocytes contacting the myocytes was characterized by an oval or triangular cell shape, often with long or short spikelike projections that extended to the sarcolemma of the apposed myocytes. The basement membranes of myocytes were focally damaged or absent at points of contact with lymphocytes, thus allowing the plasma membranes of the two cells to become directly contiguous (Figure 3D). Vesicles that measured from 0.16 to 1.1 μm in diameter and had a lucent content were frequently present in the cytoplasm of the myocytes, just subjacent to the sarcolemma, in areas of contact with lymphocytes (Figure 4A). Mild, focal myofilament loss, myelin figures, cytoplasmic edema, and dilatation of sarcoplasmic reticulum and T-system were noted in some of these myocytes.

Lysis of the myocytes was characterized by loss of the myofilaments and was associated with the accumulation of lysosome-like cytoplasmic structures (Figure 4B). These ranged from 0.25 to 1.75 μm in diameter and contained electron-dense concentric lamellae (myelin figures) (Figure 4B). In the interstitium, lymphocytes were in close contact with fibroblast-like cells that had many cisterns of rough endoplasmic reticulum.

At 2 days and thereafter, some endothelial cells showed cytoplasmic swelling and had increased numbers of pinocytotic vesicles and myelin figures (Figure 5A). Some endothelial cells showed nuclear pyknosis and extensive separation of the basement membrane from the plasma membrane (Figure 5B). The lumina of many capillaries were completely occupied by lymphocytes. The junctions of some capillary endothelial cells were widely dissociated. Some damaged capillaries contained aggregates of platelets, some of which had expanding cytoplasmic processes that appeared to be extending into the interstitium (Figure 5C). Lymphocytes undergoing mitosis were frequently found in the interstitium. The nuclei of endothelial cells in areas of tissue damage became enlarged, and numerous cytoplasmic processes projected into the lumina of the capillaries and small venules (Figure 5D).

Myofilament loss and dilatation of both the sarcoplasmic reticulum and the T-system also were more evident on days 3 and 5 than on day 2. Myocardial necrosis involved small groups of myocytes and was characterized by hypercontraction bands, fragmentation of myofilaments, and intramitochondrial amorphous or flocculent densities.

Some myocytes showed unusual alterations consisting of shrunken, elongated, highly dense nuclei, as well as increased density of mitochondria and myofibrils. Such nuclei contained highly condensed heterochromatin almost exclusively. The interstitium surrounding these myocytes was edematous. Cells showing these changes appeared very dark in light microscopic preparation stained with toluidine blue and hypereosinophilic in histological sections stained with hematoxylin and eosin.

The nuclei of necrotic myocytes showed margination of chromatin followed by karyolysis (Figure 6A).

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**Table 2. Cardiac Histological Findings in Rats Given Three Daily Doses of 5×10^6 Cetus Units of IL-2 for 2, 3, and 5 Days**

<table>
<thead>
<tr>
<th>Duration of treatment</th>
<th>Lymphocytic infiltration</th>
<th>Interstitial hemorrhage</th>
<th>Myocardial necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2 (n=4)</td>
<td>Mild, n=4</td>
<td>Mild, n=3; moderate, n=1</td>
<td>Mild, n=1; absent, n=3</td>
</tr>
<tr>
<td>Day 3 (n=4)</td>
<td>Mild, n=3; moderate, n=1</td>
<td>Mild, n=4</td>
<td>Mild, n=1; absent, n=3</td>
</tr>
<tr>
<td>Day 5 (n=4)</td>
<td>Moderate, n=1; severe, n=3</td>
<td>Moderate, n=3; severe, n=1</td>
<td>Moderate, n=1; severe, n=1; absent, n=2</td>
</tr>
</tbody>
</table>

Control animals were normal.
Figure 3. Electron micrographs of cellular contacts in myocardium of rats treated with IL-2 for 5 days. Panel A: Large granular lymphocyte has area of smooth contact with capillary endothelial cell. Magnification, x10,000. Panel B: An area of interdigitating contact (arrows) between a lymphocyte (Ly) and an endothelial cell. Magnification, x10,000. Panel C: A neutrophil makes smooth contact with an endothelial cell of a postcapillary venule. Magnification, x5,000. Panel D: The basement membrane of a myocyte is focally absent at two points (arrows) of contact with two lymphocytes (Ly). Magnification, x8,000.
FIGURE 4. Electron micrographs of myocyte alterations in myocardium of rats treated with IL-2 for 5 days (panel A) and 2 days (panel B). Panel A: The cytoplasmic granules (arrowheads) and mitochondria of a large granular lymphocyte are concentrated on the areas adjacent to the myocyte. Some vesicles (arrows) are present in the cytoplasm of the myocyte near the area of contact with a lymphocyte. Slight myofibrillar loss, interstitial edema, and erythrocyte extravasation are also seen. The basement membrane of the myocyte is not interrupted. Magnification, ×10,000. Panel B: Loss of myofilament accumulation of lysosome-like granules and myelin figures are noted in necrotic myocyte. Magnification, ×8,000.
Figure 5. Electron micrographs of endothelial alterations in myocardium of rats treated with IL-2 for 2 days (panels A and C) and 5 days (panels B and D). Panel A: A capillary endothelial cell has cytoplasmic swelling, increased numbers of pinocytotic vesicles, and myelin figures. Magnification, ×10,000. Panel B: A capillary endothelial cell shows nuclear pyknosis and extensive separation of the basement membrane (arrows) from the plasma membrane. Magnification, ×18,000. Panel C: Expanding cytoplasmic processes of two platelets in the lumen of a damaged postcapillary venule extend into the interstitium. Magnification, ×10,000. Panel D: Two hypertrophied endothelial cells of a capillary show large nuclei and numerous cytoplasmic projections that extend into the lumen. Magnification, ×8,000.
Whorls of membranous structures were found in the cytoplasm of necrotic myocytes; the mitochondria had lost their cristae and contained amorphous inclusions. The smooth-muscle cells in arterioles and the fibroblast-like cells adjacent to necrotic myocytes showed nuclear pyknosis, which involved shrinkage of the nuclei and condensation of the chromatin (Figure 6B). Myocyte necrosis was accompanied by accumulations of platelets, fibrin, red blood cells, lymphocytes, eosinophils, and neutrophils in the surrounding interstitium.

Some macrophages were also in close contact with myocytes that had focally interrupted basement membranes but were not necrotic. Other conspicuous features of lymphocytes were deeply indented nuclei, well-developed Golgi complexes, and numerous cytoplasmic granules. The eosinophils found in the lumina of capillaries and small veins had bilobate nuclei. Most granules in the cytoplasm showed the structural features typical of normal, mature eosinophil granules (Figure 6C). In necrotic areas, however, the eosinophils in the interstitium showed variable decreases in the intensity of the staining of the central, dense cores of granules (Figure 6D).

Discussion

The morphological features of the cardiotoxicity observed in the present study are similar to those described in humans undergoing cancer immunotherapy with IL-2.11-15 These features include lymphocytic and eosinophilic infiltration, various endothelial alterations, interstitial edema, hemorrhage, myocyte vacuolization, myofibrillar loss, and myocardial necrosis. The extra-cardiac lesions also were similar to those found in humans11 and rats18 receiving IL-2. Thus, we consider that the rat is a useful animal model for the study of the cardiotoxicity of IL-2.

The mechanisms of IL-2-induced cardiotoxicity are not well understood. Whether IL-2 directly or indirectly damages cardiac muscle remains controversial.8,10,29,30 Several studies have suggested that this toxicity is mediated through multiple indirect effects on different cellular components of the heart rather than by a direct effect on cardiac myocytes.29,30 In the context of observations reported previously,1-4,31-38 we interpret our findings as indicating that the cardiac lesions induced by IL-2 develop according to the following sequence of events: 1) activation of lymphocytes to differentiate into LAK cells; 2) activation of endothelial cells leading to the expression of endothelial-cell-leukocyte adhesion molecules; 3) interaction between activated lymphocytes and activated endothelial cells, resulting in increased adherence of lymphocytes to endothelium, particularly in capillaries and postcapillary venules; 4) progressive but focal damage to the microcirculation, with disruption of endothelial junctions, capillary leaks, and plugging of vessels with lymphocytes; 5) migration of lymphocytes and other inflammatory cells into myocardial interstitium; and 6) contacts between lymphocytes and cardiac myocytes, resulting in cytotoxic damage and necrosis of myocytes.

Activation of Lymphocytes by IL-2

The ability to induce the differentiation of LAK cells is a fundamental property of IL-2 and constitutes the basis for its use in cancer immunotherapy.6,31-33 LAK cells are identified on the basis of their cytolytic activity and not because of any unique morphological or immunohistochemical characteristics.39-41 In rats, the morphology of many of the LAK cells corresponded to that of large granular lymphocytes, and many of these cells had immunoreactivity with asialo GM1 ganglioside antibody.18,39,41 The cytoplasmic granules in these lymphocytes contain perforin and various serine esterases,42-44 which are important mediators of the cytotoxicity of these cells. However, other LAK cells appeared to correspond to smaller, cytotoxic/suppressor T-lymphocytes, which express OX 8 surface antigen.39,41 These cells can aggravate the myocardial injury produced by the large granular LAK cells.

Other types of infiltrating cells, such as neutrophils, eosinophils, and macrophages, also can induce or aggravate myocyte damage in IL-2-treated rats. Infiltration of eosinophils is a consistent feature of IL-2 myocarditis in humans.11-14 In rats, IL-2 induces eosinophilic infiltration in various organs.18,45 The reasons for this infiltration are not known. We found evidence of partial degranulation of eosinophils. This change is similar to that described in the hyper-eosinophilic syndrome.46 It seems likely that eosinophils contribute to the myocardial damage produced by IL-2, because of the toxic components of their granules.47 In the present study, we observed increases in the numbers of macrophages, dendritic cells, and T-helper lymphocytes. The functional interrelations among these cells remain the subject of future research.

Activation and Damage of Endothelial Cells

IL-2 increases leukocyte-endothelial-cell adhesion and vascular permeability in the skin of rabbits.48 Endogenous synthesis of cytokines (interleukin-1 [IL-1], tumor necrosis factor [TNF], lymphotoxin, and interferon-γ [IFN-γ]) induced by IL-2 is considered to be an important cause of endothelial cell activation.1,38 In baboons, TNF and IFN-γ induce endothelial activation and adhesion of leukocytes in the small vessels of the skin.49 Activation of endothelial cells in the skin of patients treated with IL-2 results in expression of endothelial-cell-leukocyte adhesion molecule (ELAM-1), which is not detectable in normal skin, and in increased expression of intracellular adhesion molecule-1 (ICAM-1).1 The activation of endothelial cells by cytokines involves a series of events that often lead to endothelial damage. It is difficult to identify the point of transition from activation to damage of endothelial cells.38 Cultured endothelial cells are not directly activated by IL-2, as they are by IL-1, IFN-γ, and TNF.38 Both IL-1 and TNF are able to produce a VLS, which appears to be dependent on increased adherence of neutrophils to endothelial cells of venules and capillaries in subcutaneous tissue.30 Thus, the in vivo activation of endothelial cells by IL-2 is an indirect effect resulting from IL-2-mediated synthesis and release of these other cytokines by lymphocytes and other inflammatory cells.38 The local and systemic administration of IL-2 also produces increased adherence of neutrophils, vascular leakage of macromolecules, and endothelial alterations in the cremaster muscle of the rat.50 Conversely, another study showed that IL-2 does not cause direct damage to cultured rat cardiac myocytes.52 This is in accord with the finding that, in the absence of blood
cells, the perfusion of isolated rat hearts with IL-2 alone has no adverse effects on cardiac function.

The ultrastructural changes found in endothelial cells in our study were similar to those described in other reports of the effect of IL-2.54,55 Such alterations occurred earlier than those involving the cardiac myocytes. Endothelial hypertrophy, similar to that found in the present study, has been induced by TNF.55 The mechanism of this hypertrophy is unknown. The early endothelial changes observed in our study progressed to damage to the microcirculation, with separation of intercellular junctions, disruption of the capillary network, and obstruction of capillaries by masses of lymphocytes and aggregates of platelets. We believe that this damage contributes to the development of myocardial necrosis.

Myocyte Damage and Necrosis

Our ultrastructural observations suggest that two major mechanisms are involved in the pathogenesis of the myocardial necrosis in IL-2-treated rats: 1) ischemic necrosis, related to the microcirculatory disturbances just cited, and 2) cytotoxic/cytolytic necrosis, related to the contacts between IL-2-activated lymphocytes and cardiac myocytes. It is extremely difficult to assess the relative importance of these two factors. Circulating myocardial depressant factors, oxygen free radicals, vasoactive substances, thromboxane, prostaglandins, and secondarily released cytokines (IFN, IL-1, TNF) also may have contributed to the myocardial damage.15,56

Three ultrastructural observations support the concept that the contacts between lymphocytes and myocytes were biologically significant, rather than resulting simply from the topographical approximation caused by the infiltration of lymphocytes into myocardial interstitium: 1) the formation of cytoplasmic processes that extended from the main cytoplasmic mass of the lymphocytes toward the myocytes; 2) the focal loss of the basement membranes of myocytes in the regions of these contacts; and 3) the pattern of organization of the cytoplasmic organelles of the contacting lymphocytes, which became concentrated in the areas adjacent to the myocytes. Thesefindings also are in accord with the concept that the cytolytic effects of lymphocytes are mediated by direct contact with the corresponding target cells.42,57

Myocyte necrosis associated with infiltration of lymphocytes containing large amounts of perforin has been noted very early in the course of Coxsackieviral myocarditis in mice.58 This necrosis is associated with lymphocyte–myocyte contacts that are ultrastructurally similar to those described in the present study.59 Perforin induces the formation of porelike holes in the membrane of the target cell, which results in loss of the barrier function of the membrane, with penetration of Ca2+ ions and various toxic products and leakage of critical cytoplasmic components. These events may lead either to reparative processes or to cell death by cytolysis.

Most of the ultrastructural features of the myocyte necrosis observed in the present study correspond to those of necrosis with contraction bands.60 This type of necrosis occurs in a variety of toxic injuries, but it also develops in myocardial ischemia that causes only temporary or partial reduction in blood flow. It develops when membrane damage permits the entry of excessive amounts of calcium into the myocytes, causing hypercontraction of myocytes. Necrosis with contraction bands is found in peripheral regions of myocardial infarcts. In contrast, coagulation necrosis is characterized by features similar to those of autolysis and typically is observed in central regions of infarcts, in which necrosis develops while tissue perfusion is completely interrupted. The membrane damage and the excessive influx of Ca2+ leading to the contraction band necrosis observed in the present study could have resulted either from the cytolytic effects of lymphocytes or from ischemic damage caused by alterations in the microcirculation. The morphology of the myocyte necrosis observed in the present study differed in two respects from that usually found in contraction band necrosis: 1) the focal accumulations of vesicles subjacent to the sarcolemma in areas of myocyte–lymphocyte contacts and 2) the absence of intramitochondrial calcific deposits. The vesicles may have represented attempts at decreasing cell injury by endocytic uptake (internalization) of damaged areas of membranes. The lack of calcium deposition may have been caused by microcirculatory abnormalities severely limiting the amount of tissue perfusion necessary to bring Ca2+ ions into damaged myocytes.

In summary, the present study shows that the rat is a useful animal model to study the features of the myocardial damage produced by the administration of large doses of IL-2. Myocarditis and myocardial necrosis develop as eventual consequences of a series of events that are triggered by the activation of lymphocytes and endothelial cells, with subsequent damage to the microcirculation and infiltration of LAK cells into myocardial interstitium, where they exert cytolytic effects upon the cardiac myocytes.

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T-lymphocytes

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