Mechanisms of Interleukin-2-induced Hepatic Toxicity

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Abstract

Interleukin 2 (IL-2) mediates the regression of metastatic cancer, but its clinical use is limited by associated toxicities including hepatic dysfunction. To determine the mechanism for IL-2-induced hepatic dysfunction, we hypothesized that IL-2 activation of Kupffer cells causes leukocyte-endothelial adhesion and decreases hepatic sinusoidal blood flow. C57BL/6 mice were given injections of latex particles and prepared for intravital hepatic microscopy 2 h after i.p. IL-2 administration. Liver tissue was also prepared to quantitate hepatic tumor necrosis factor (TNF) mRNA and processed for light and electron microscopy. Phagocytosing Kupffer cells and leukocytes adherent to the endothelium were counted, and surface sinusoidal blood flow was quantitated. Kupffer cell activity was quantitated as the ratio of phagocytosing Kupffer cells to sinusoidal blood flow. IL-2 significantly increased Kupffer cell activity (0.56 ± 0.05 for controls versus 0.84 ± 0.05 for IL-2), significantly caused leukocyte-endothelial adhesion (26.7 ± 7.9 for controls versus 87.0 ± 27.6 for IL-2), WBC/mm² endothelial surface), and significantly decreased the number of sinusoids containing blood flow per microscopic field (6.66 ± 0.15 for controls versus 5.79 ± 0.13 for IL-2) without causing changes in systemic hemodynamic parameters. In IL-2-treated livers, light and electron microscopy showed the constriction of sinusoids associated with swollen or ruptured mitochondria, which was consistent with hypoxic deterioration near central venules. Adherent platelets, neutrophils, and lymphocytes within sinusoids and central venules were also observed. PCR revealed that IL-2 significantly induced TNF mRNA expression in the liver. These data suggest that IL-2 activates Kupffer cells in association with the release of monokines including TNF, which causes activation of circulating leukocytes as well as hepatic sinusoidal endothelial cells. The resultant leukocyte and platelet adhesion to the endothelium may then physically impede the sinusoidal microcirculation, resulting in microscopic areas of hepatic ischemia and explaining the mechanism of IL-2-induced hepatic dysfunction.

Introduction

IL-2 mediates the regression of certain human malignancies. Clinical trials demonstrate that response rates with IL-2 therapy for melanoma and renal cell carcinoma are comparable, or superior, to the best regimens of conventional chemotherapeutic agents (1). However, IL-2 therapy causes serious toxicities that limit its clinical application. Liver dysfunction is a major toxicity, and increases in serum transaminases and bilirubin are commonly observed in patients who receive high-dose IL-2 therapy (2, 3). We previously reported that the i.p. injection of IL-2 increased serum transaminases and caused lymphocyte infiltration in the livers of C57BL/6 mice (4). However, the mechanism for this IL-2-induced liver dysfunction remains unknown. The purpose of the present study was to evaluate a potential mechanism of IL-2-mediated liver toxicity. Because IL-2 is known to activate immune effector cells, cell-mediated effects of Kupffer cells are a potential mechanism of IL-2-mediated liver toxicity. Since proposed mechanisms of IL-2-mediated liver toxicity may involve events occurring at the level of the microcirculation in the liver, we used intravital hepatic microscopy to directly observe and quantitate the acute effects of IL-2. We hypothesized that IL-2 would induce secondary cytokines though Kupffer cell activation and would thereby alter leukocyte-endothelial interactions that would ultimately impede the hepatic microcirculation.

Materials and Methods

Animals. Male weaning C57BL/6 mice weighing 20–30 g (Charles River Breeding Laboratory, Wilmington, MA) were individually housed in cages, fed standard laboratory food and water ad libitum, and were used for experiments. Approval for this study was obtained from the University of Louisville Animal Care and Use Committee and was in compliance with the guidelines established by the NIH.

Experimental Protocols. Human recombinant IL-2 (Chiron Therapeutics, Emeryville, CA) at a dosage of 300,000 IU/mouse or vehicle (5% dextrose containing 0.1% endotoxin-free bovine serum albumin) was administrated i.p. This dose of IL-2, when administered i.p. by 15 injections over 5 days three times a day, is known to cause a significant increase in serum glutamic pyruvic transaminase and total bilirubin. Two h after the dose was given, the animal underwent either intravital microscopic study or light and electron microscopic study.

Intravital Microscopy. Each animal was anesthetized i.p. with pentobarbital (75 mg/kg), and its air patency was maintained by a tracheostomy and a tracheal stent. The carotid artery was cannulated, and blood pressure was monitored continuously using a DIGI-MED blood pressure analyzer (MICROMED, Inc., Louisville, KY). The heart rate was calculated from the frequency of the pressure pulse, and respiratory rates and body temperatures were monitored continuously using a WorkBench PC (Kent Scientific Corp., Litchfield, CT).

The liver was gently exteriorized through a subcostal abdominal incision and positioned over a window of cover glass in a specially designed microscope stage that provided for drainage of irrigating fluid. The liver was covered with Saran Wrap (Dow Chemical Co., Midland, MI) to hold it in position and limit any movements induced by respiration. Homeostasis was maintained by constant irrigation of a modified Krebs solution at 37°C with bubbling CO2 and N2 to achieve a pH of 7.40 ± 0.05. The rectal temperature was maintained by a heat lamp at 36 ± 1°C. The stage was positioned under a fluorescent microscope (Leitz KG. E, Hamburg, Germany) so that the liver could be observed by transmitted light or fluorescent microscopy. A closed circuit television system with a videotape recorder was used to monitor and record each experiment for subsequent analysis.

Kupffer cell function was assessed by observing the phagocytosis of 1-μm fluorescent latex particles (Polyscience, Inc., Warrington, PA) by individual cells. The latex was diluted with sterile saline (1:15) and administered i.v. in the tail of each mouse at a dose of 0.1 ml (3 × 10⁶ particles). To assess the number of phagocytosed Kupffer cells, the number of cells that phagocytosed latex were counted in seven periportal and seven centrilobular microscopic fields 15 min after each mouse received the latex solution. In addition, the relative adequacy of blood perfusion through the sinusoids was evaluated by quantifying the number of sinusoids containing blood flow in the same seven periportal and seven centrilobular microscopic fields. Since reduced perfusion...
of individual sinusoids can reduce the delivery of the latex particles to Kupffer cells in these vessels, the ratio of Kupffer cells that phagocytosed latex to sinusoids containing blood flow was used as a measure of Kupffer cell activity.

Three central venules (20–40 μm in diameter, 200–300 μm in length) for each mouse were identified for counting leukocytes that adhered to the endothelial surface. Adherent leukocytes were defined as those which did not move for 30 s during the observation period. They were standardized per endothelial surface area (π × diameter × length of vessel segment observed).

The diameters and lengths of vessels were measured using a video caliper (Microcirculation Research Institute, Texas A & M University, College Station, TX), and the magnification of the system was determined with a stage micrometer. After the intravital microscopic measurement, blood was drawn for hematomatological and blood chemical analyses, and the livers were resected for quantitation of TNF mRNA. The number of animals for the intravital microscopy study was seven for each control and seven for each IL-2 group.

Quantitation of TNF mRNA by Differential PCR. Differential PCR has been used previously to quantitate mRNA in various tissues (5). Total liver RNA was extracted according to the method described by Chomczynski and Sacchi (6), and TNF and β-actin mRNA were reverse transcribed to cDNA using the GeneAmp RNA PCR kit (Perkin Elmer/Cetus, Norwalk, CT) and the primers (Stratagene, La Jolla, CA). After electrophoresis and staining with ethidium bromide, densitometry was carried out to calculate mRNA for each individual specimen. mRNA was quantitated as the ratio of TNF: β-actin and expressed as a percentage of β-actin.

Light and Electron Microscopic Study. After the left ventricle cannulation, saline was perfused through the cannula to wash out blood at an 8 ml/min flow rate for 1 min, then a fixative of 2.5% glutaraldehyde in 50 mM sodium cacodylate buffer (pH 7.40) was perfused for 5 min at the same flow rate. The liver was studied using both light and electron microscopy. Fourteen microscopic areas were selected randomly, and the numbers of closed and open sinusoids in the area were counted in double-blinded fashion using a magnification ×400. Closed sinusoids were defined as either totally collapsed or smaller than the diameter of the RBCs remaining in the tissue.

Statistics. All values were expressed as mean ± SE. A two-tailed t test was performed, and differences were considered significant at P < 0.05.

Results

The mean arterial blood pressure, pulse rate, respiratory rate, rectal temperature, and body weight showed no significant difference between the control and IL-2 groups during the observation period (Table 1).

Two h after the administration of IL-2, the number of the surface sinusoids containing blood flow per microscopic field significantly decreased in the perportal area, centrilobular area, and in whole lobules (Fig. 1A). In both the control and IL-2 groups, the number of phagocytosing cells predominantly existed in the perportal area when compared with the centrilobular area. After the administration of IL-2, the number of phagocytosing cells increased significantly in the perportal area and in whole lobules by 38.5% and 31.2%, respectively, but was not significant in the centrilobular area (Fig. 1B). The number of phagocytosing cells per the number of sinusoids containing blood flow significantly increased in the perportal area and in whole lobules by 59.2% and 51.2%, respectively (Fig. 1C).

Two h after the IL-2 administration, the number of adhering leukocytes in the postsinusoid venules increased significantly compared with controls (26.7 ± 7.9 for controls versus 87.0 ± 27.6 for IL-2, WBC/mm² endothelial surface).

Table 1 Effects of IL-2 on vital signsa

<table>
<thead>
<tr>
<th>Vital signs</th>
<th>Controls (n = 7)</th>
<th>IL-2 (n = 7)</th>
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<tbody>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>81.1 ± 3.25</td>
<td>81.6 ± 3.25</td>
</tr>
<tr>
<td>Pulse rate (min)</td>
<td>373.1 ± 13.5</td>
<td>360.1 ± 14.3</td>
</tr>
<tr>
<td>Respiratory rate (min)</td>
<td>118.5 ± 7.99</td>
<td>120.3 ± 5.00</td>
</tr>
<tr>
<td>Rectal temperature (°C)</td>
<td>36.0 ± 0.33</td>
<td>36.2 ± 0.16</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>23.7 ± 0.89</td>
<td>23.6 ± 0.94</td>
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a Data are expressed as mean ± SE. There was no significant difference between the control and IL-2 group.

Although this administration of IL-2 did not affect the number of leukocytes in the blood acutely, the number of polymorphonuclear leukocytes increased and the number of lymphocytes decreased (Table 2). While glutamic pyruvic transaminase and total bilirubin in the blood did not change within 2 h after IL-2 was administered, γ-glutamyl transpeptidase increased slightly but significantly. PCR revealed that TNF mRNA expression in the livers of IL-2-treated mice...
significantly increased compared with controls (4.94 ± 0.95% for controls versus 9.56 ± 1.57% for IL-2).

The light microscopy showed that IL-2 treatment significantly increased the proportion of closed sinusoids to open sinusoids (17.1 ± 5.5% for controls versus 62.2 ± 4.5% for IL-2). The areas of IL-2-treated livers that had constricted or collapsed sinusoids also had swollen or ruptured mitochondria near the central venules, suggesting hypoxic deterioration (Fig. 2, A versus B). Adherent platelets, neutrophils, and lymphocytes were observed in the sinusoids of the livers of IL-2-treated animals (Fig. 2, C versus D). Degranulated and star-shaped platelets were also observed in the IL-2-treated liver, demonstrating conformational changes commonly seen prior to thrombosis. In both groups, all phagocytosing cells in the livers were identified as Kupffer cells (Fig. 2, B and C).

Discussion

Intravital hepatic microscopy demonstrated a significant increase in the number of firmly adherent leukocytes to central venules within 2 h after IL-2 administration, in association with a decrease in blood flow in sinusoids (Fig. 1A). This in vivo observation was also supported by both light and electron microscopic studies showing adherent lymphocytes, neutrophils, and platelets in hepatic sinusoids with collapsed sinusoids. Blood analysis revealed that IL-2 significantly induced not only slight liver damage, but also decreased the number of lymphocytes in blood within 2 h. These results suggest that IL-2 acutely alters interactions between leukocytes and platelets with endothelial cells, which may then impede blood flow in sinusoids and decrease blood flow in the liver. Since swollen or ruptured mitochondria in hepatocytes, suggesting hypoxic deterioration, were observed in the areas with constricted or collapsed sinusoids, this decrease in blood flow in the sinusoids may be, at least in part, a cause of liver dysfunction due to IL-2 administration.

Kupffer cells, the resident macrophages of the liver, are one of the cellular components of the sinusoids and are the largest macrophage population in the mammalian body (7). It is also known that Kupffer cells are a major part of the reticuloendothelial system (8) and express IL-2 receptors on their surface (9). In the present study, IL-2 acutely increased the number of phagocytosing cells (Fig. 1B) and the phagocytosing activity of the liver (Fig. 1C), consistent with the known IL-2 activation of Kupffer cells.

Activated Kupffer cells produce a variety of mediators such as active oxygen radicals, TNF, IL-1, IL-6, and several eicosanoids (9–11). Although in vivo evidence has also shown that IL-2 induces the production of other cytokines that are potentially important in mediating IL-2 toxicities (12), these toxicities are similar to those observed after the infusion of recombinant TNF (13, 14). Moreover,
IL-2-induced systemic toxicities are remarkably similar to the pathological states of the acute respiratory distress syndrome and multisystem organ failure, as seen in patients who are thermally injured, traumatically injured, and severely infected. Both IL-2 therapy and multisystem organ failure secondary to systemic infection are associated with elevated plasma levels of TNF (15). TNF is known to be a major mediator produced by Kupffer cells after contact with endotoxin or virus (10,11), and recent experiments have strongly implicated TNF in the pathogenesis of septic organ failure (9,14,16–18).

In vitro studies have shown that IL-2 induces seclusions of TNF from monocytes and macrophages, as well as TNF mRNA expression (19). It has been also reported that the i.p. administration of IL-2 into mice induces TNF in ascites and blood and mRNA TNF expression in ascites cells, mainly peritoneal macrophages (20). Elevated circulating levels of TNF have been detected in patients treated with IL-2, with a peak elevation at 2 h after the administration (12,21). Mier et al. (21) have demonstrated that patients treated with IL-2 have increased circulating levels of TNF temporally consistent with the onset of fever and other toxic manifestations of IL-2. We previously reported that pentoxifylline, which mediates suppression of TNF production, inhibited IL-2-induced liver toxicity (4). Fraker et al. (22) have reported that the administration of high levels of neutralizing anti-TNF antibody improves the survival rate after high-dose IL-2 treatment of mice. In this study, TNF mRNA expression in the liver was induced within 2 h after IL-2 was administered. This suggests that some of the liver toxicity of IL-2 therapy may be due to the in vivo induction of TNF in the liver.

Furthermore, TNF is also known to induce cell adhesion molecules, such as ICAM-1, on vascular endothelial cells and to cause leukocyte adhesion (23). It has been reported that ICAM-1 mRNA is strongly induced in nonparenchymal cells in the liver of septic mice associated with TNF production by Kupffer cells (9). Using intravital hepatic microscopy, McCuskey (24) has shown that the administration of TNF or endotoxin results in decreased microcirculation of hepatic sinusoids and activation of Kupffer cells, suggesting that the responses of the hepatic microcirculation to endotoxin or sepsis can be correlated with the state of activation and numbers of Kupffer cells within the hepatic lobule. Collectively, it appears that IL-2 activates Kupffer cells with the release of monokines including TNF, which activate circulating leukocytes as well as hepatic sinusoidal endothelial cells and induce the expression of adhesion molecules. The resultant leukocyte adhesion to the endothelium may then physically impede the sinusoidal microcirculation resulting in microscopic areas of hepatic ischemia as a mechanism of IL-2-induced hepatic dysfunction.

Acknowledgments

We thank Carla Shelton and Sherri Matthews for their secretarial assistance.

References

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