Thrombocytopenia in Normal and Sublethally Irradiated Dogs: Response to Human Interleukin-6

By Samuel A. Burstein, Tamyra Downs, Paul Friese, Sheila Lynam, Stacy Anderson, James Henthorn, Robert B. Epstein, and Kathy Savage

The response of megakaryocytes and platelets to the administration of recombinant human interleukin-6 (IL-6) was investigated in normal and sublethally irradiated dogs. IL-6 was administered for 2 weeks at doses of 10 to 160 µg/kg/d to normal animals to assess dose-response and toxicity. Subsequently, 40, 80, or 160 µg/kg/d for 2 weeks was administered to animals treated with 200 cG total body irradiation. Analysis of normal dogs showed a significant increment in the platelet count detectable ~11 days after initiation of IL-6 at all administered doses. Large platelets greater than 6.3 µm in diameter were observed 1 day after beginning IL-6, progressively increasing to as many as 19.1% of the total circulating platelets by day 10. The ploidy distribution of the marrow megakaryocytes did not differ from the normal at doses of ≤80 µg/kg/d, but at 160 µg/kg/d, a shift toward higher ploidy cells was noted. No change in total white count was noted; however, a decrease in hematocrit was seen at all doses. In the irradiated animals, the platelet count recovered earlier than in the IL-6–treated dogs than in the controls, but no consistent change in the ploidy distribution was observed irrespective of dose. Large platelets were also noted in the treated animals, comprising up to 6.9% of the total platelet count. Fibrinogen levels were elevated to greater than 4 times normal. A significant decrease in hematocrit was seen in all animals, while no consistent change was noted in the white count. Elevations in serum cholesterol, triglycerides, and alkaline phosphatase, together with a decline in serum albumin were observed in all the treated animals (both normal and irradiated), but clinical symptoms were observed only in the dogs receiving ≥80 µg/kg/d. The data show that IL-6 alone is capable of enhancing platelet recovery in dogs with bone marrow suppression.

© 1992 by The American Society of Hematology.

MATERIALS AND METHODS

Animals. Beagles (Hazleton Research Products, Cumberland, VA) or random source mongrel dogs (8 to 13 kg) were obtained and housed according to the regulations of the Institutional Animal Care and Use Committee of the University of Oklahoma Health Sciences Center (accredited by the American Association for Accreditation of Laboratory Animal Care). Blood was obtained via a cephalic vein. Rectal temperatures and weights were obtained at least once per week. Less quantifiable clinical side effects (lethargy, appetite, dehydration, vomiting, etc) were recorded.

Irradiation. Dogs were anesthetized with 25 mg/kg sodium pentobarbital (Abbott Laboratories, Chicago, IL). Two hundred centiGray total body irradiation (TBI) prescribed to the midline via right and left lateral opposing ports was delivered with a Kelekt-Barnes cobalt-60 unit at a dose rate of 4 to 5 cGy/min. Thermoluminescent dosimeters (TLD-100) were used to verify the delivered dose (±5%).

Bone marrow aspiration. After the administration of 25 mg/kg sodium pentobarbital anesthesia, 5 mL of bone marrow was aspirated from the humerus using an anticoagulant mixture as described previously.19

Expression and purification of recombinant human IL-6. The human IL-6 cDNA (purchased from Beckman Instruments, Fullerton, CA) was modified for expression using the T7 expression system of Studier et al.20 IL-6 was synthesized as insoluble inclusion bodies, and purified by repetitive detergent washes and C4 reverse phase chromatography. The final product was sterile-filtered and frozen until use. The IL-6 was diluted to the required concentration in 100 mmol/L NaCl, 50 mmol/L NaHCO3, pH 8.2. Concentration and purity were assessed by the IL-6–responsive B9 cell bioassay21 and sodium dodecyl sulfate-polyacrylamide gel
electrophoresis (SDS-PAGE). Endotoxin levels were measured using the limulus amebocyte lysate test at Endosafe Inc (Charlestown, SC). The lower limit of endotoxin detection was 0.03 EU/mL.

**Blood cell counts.** Hematocrit (Hct), total white blood cell (WBC) count, and platelet count were monitored with a Baker Series 9000 whole blood counter (Serono-Baker, Allentown, PA), with discriminators adjusted for canine cells. The mean platelet count of over 50 normal dogs was 275,000/µL, with a range of 130 to 480,000 platelets/µL.

**Serum IL-6 levels.** The concentration of administered IL-6 was determined by bioassay using the IL-6-responsive B9 cell line (provided by Dr Lucien Aarden, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service) as previously described. The specificity of the assay for human IL-6 was determined by preincubating the tested specimens with rabbit antihuman IL-6. This reagent was prepared by immunizing New Zealand White rabbits with three weekly injections of IL-6 prepared as described above, followed by isolation of the IgG fraction from the rabbit serum by protein G chromatography. The specificity of our antibody to human IL-6 was verified with commercially available anti-IL-6 antibody (Genzyme Corp, Cambridge, MA).

**Serum analyses.** An automated chemistry analysis (SMA-18) was performed on serum using an Ektachem 700 XR Analyzer (Kodak, Rochester, NY). For unclear reasons, the canine serum bilirubin and uric acid levels were below detection limits using the reagents standardized for human use, and are not reported. Due to occasional hemolysis related to blood withdrawal, the lactate dehydrogenase values were considered to be unreliable and are not reported.

**Fibrinogen levels.** Fibrinogen levels were measured in the plasma of irradiated dogs using the IL Test PT-fibrinogen (ACL System; Instrumentation Laboratory, Lexington, MA). This device measures the change in light scatter due to fibrin clot formation after the addition of a rabbit brain thromboplastin to plasma.

**Megakaryocyte ploidy analysis.** Unfractionated dog megakaryocytes were analyzed by flow cytometry as previously described, using the platelet/megakaryocyte-specific MoAb 2F9 to identify the megakaryocytes, and propidium iodide to determine the relative DNA content.

**Platelet size.** Platelet size was determined in some animals by flow cytometry. To avoid platelet selection, whole blood was drawn into EDTA and diluted 1:100 in buffered saline-glucose-citrate (0.1 mol/L NaCl, 8.564 mmol/L Na₂HPO₄, 1.6 mmol/L KH₂PO₄, 13.6 mmol/L Na citrate, 11.1 mmol/L glucose, pH 6.5) and was labeled directly with 2F9-fluorescein isothiocyanate (FITC). Initially, platelet size was assessed by pulse processing of the forward light scatter (FSC) width using a FACStar® flow cytometer (Becton Dickinson, Mountain View, CA), and converting the channel number into microns by measuring FSC width of polystyrene standard sizing beads. Linearity of sizing was observed to as low as 4 µm. After standardization, platelets were compared with red blood cell (RBC) diameter, measured to be 6.3 to 9 µm. Because the RBC population is easily located by flow cytometry, the percentage of 2F9-positive cells exceeding the lower threshold of RBC diameter was determined. Platelet clumping or binding to other cell types was ruled out by direct examination of blood films. No doublets or greater aggregates were observed in blood drawn into EDTA, nor were any time-related alterations in platelet size seen after 2 hours in EDTA solution.

**Experimental protocol—normal dogs.** One to 2 weeks before treatment, bone marrow was aspirated in most animals to ascertain the ploidy distribution, while blood was obtained for serum chemistry studies and IL-6 levels. On days 1 through 14 of the protocol, one animal was administered 10, 20, or 160 µg/kg/d of IL-6, while four animals were administered 80 µg/kg/d of the cytokine in three divided subcutaneous doses. Three control animals were administered the IL-6 diluent (50 mmol/L NaHCO₃, 100 mmol/L NaCl, pH 8.2) on the same schedule. Blood was obtained three to five times per week for blood counts, chemistry analysis, and IL-6 levels. Bone marrow was aspirated between days 7 and 12 and between days 31 and 35 in most dogs to evaluate the megakaryocyte ploidy distribution.

**Experimental protocol—irradiated dogs.** One to 2 weeks after marrow and blood testing, animals were administered 200 cGy irradiation after pentobarbital anesthesia. One experimental and one control animal were irradiated simultaneously if they were small enough to fit in the irradiation field, and sequentially if larger. The day of irradiation was designated day 0. Immediately after irradiation, IL-6 was administered at 40 (n = 2), 80 (n = 4), or 160 (n = 3) µg/kg/d in two divided doses subcutaneously for 14 days (preliminary data indicated no difference in response between two or three divided doses per day). Controls (n = 6) received the IL-6 diluent buffer on the same schedule. Blood was obtained up to three times per week for blood counts, platelet sizing, blood chemistries, and IL-6 levels. Bone marrow was aspirated between days 7 and 12 and 31 and 35 for evaluation of the megakaryocyte ploidy distribution.

**RESULTS**

**Production of IL-6.** About 100 to 150 mg IL-6/L of bacterial culture was recovered. The specific activity of the purified product was 2 to 5 x 10⁸ U/mg protein assessed by B9 assay and had an endotoxin level of less than 2 EU/mg. One batch of IL-6 was administered to two dogs before recognition that it was contaminated with 1,000 EU/mg of endotoxin.

**General effects of IL-6.** No significant change in temperature or weight was observed in animals administered 10 (n = 1) or 20 (n = 1) µg/kg/d. In contrast, 12% ± 5% and 16% of body weight was lost in animals administered 80 (n = 4) and 160 (n = 3) µg/kg/d, respectively, and a 1 to 2°C increment in baseline body temperature was observed that declined to normal after 3 days. In these latter animals, loss of appetite and lethargy manifest by decreased mobility was observed, with the dog receiving 160 µg/kg/d requiring forced feeding.

**Effects of IL-6 on serum chemistry analysis.** By SMA-18 analysis, no IL-6-related abnormalities were noted for the following serum components: electrolytes (Na, K, Cl, CO₂), glucose, blood urea nitrogen, creatinine, total protein, glutamic/oxaloacetic transaminase, Ca, P, and creatine phosphokinase. In contrast, an increase in cholesterol, triglycerides, and alkaline phosphatase, and a decrease in albumin was observed. The cholesterol increased by 25% to 130% of the pre-IL-6 level (pre-IL-6 range, 172 to 248 mg/dL; IL-6-treated range, 216 to 483 mg/dL). The triglycerides increased by 10% to 60% of the pre-IL-6 level (pre-IL-6, 32 to 58 mg/dL; IL-6-treated, 51 to 72 mg/dL). The alkaline phosphatase decreased by 9% to 225% of the pre-IL-6 level (pre-IL-6, 32 to 58 mg/dL; IL-6-treated, 51 to 72 mg/dL). The pre-IL-6 level (pre-IL-6, 2.9 to 3.6 g/dL; IL-6-treated, 2.2 to 2.7 g/dL) was observed.
IL-6 augments the platelet count in normal dogs. At each of the tested doses, IL-6 augmented the baseline platelet count of normal dogs, with the greatest increment observed with the highest dose used (Fig 1). In the seven treated animals, an early decrease in platelets to as much as 31% of the initial count was noted, but in no case did the count decrease below normal levels. The increment in count was observed 4 to 9 days after the initiation of the IL-6, was usually maximal by day 11, and gradually declined after the IL-6 was discontinued at day 14. The maximal increment in count observed was in the dog receiving 160 μg/kg/d. In this animal, the initial count of \(284 \times 10^9\) platelets/L increased to \(680 \times 10^9\) platelets/L by day 11, and remained elevated on the last day tested (\(389 \times 10^9\) at day 35).

IL-6 augments platelet size. In three dogs administered 80 μg/kg/d of IL-6, flow cytometric measurements of platelet size were determined on a daily basis for 10 days (Fig 2). Before IL-6 administration, the percentage of platelets exceeding 6.3 μm (determined with standard sizing beads; this diameter is approximately the lower diameter threshold for RBCs) was less than 0.1%. At day 1 after beginning IL-6, this increased significantly (0.5% to 1.5% in the treated dogs vs <0.1% in controls; \(P < 0.01\)), and was as high as 19.1% of the total platelet population by day 10. In the control dog, the percentage of these large platelets never exceeded 0.6%. The appearance of the platelets is shown before (Fig 3A) and at day 10 (Fig 3B) in a dog receiving 80 μg/mg/d of the cytokine.

![Fig 1. The effect of IL-6 on the platelet count in normal dogs.](image)

![Fig 2. Flow cytometric analysis of platelet size in IL-6-treated normal dogs (80 μg/kg/d).](image)
Influence of IL-6 on megakaryocyte ploidy. Megakaryocyte DNA content was evaluated 1 to 2 weeks before and between days 7 and 12 and 31 and 35 after the outset of treatment. The ploidy distribution of normal dogs (n = 24) was: 2N, 6 ± 4; 4N, 5 ± 4; 8N, 19 ± 5; 16N, 57 ± 9; 32N, 14 ± 5; and 64N, 0.1 ± 0.2. In dogs receiving 10, 20, or 80 μg/kg IL-6/d, no effect on the ploidy distribution was observed at day 14, despite an increment in the platelet count. In contrast, 28% 32N and 8% 64N cells were noted at 160 μg/kg/d (Fig 4). Ploidy levels were normal in all dogs by day 35 (data not shown).

Effect of IL-6 on the WBC and Hct. The total WBC ranged from 7.2 to 32.2 × 10^9/L in the IL-6–treated dogs and from 8.4 to 14.8 × 10^9/L in the controls. No significant differences were observed in the WBCs of control and treated animals (P > .05). In contrast, IL-6 administration resulted in a consistent decrement in the Hct of all animals consistent with a previous report. The Hct decrease ranged from 17% to 29% in the IL-6–treated dogs and was dose-related. The decline in Hct usually began within the first few days of IL-6 administration, and appeared to be independent of the state of hydration of the dogs.

Serum IL-6 levels. In three dogs, the levels of serum IL-6 were determined at various times after the subcutaneous administration of the cytokine. Figure 5 shows serum IL-6 levels over a 16-day period in dogs administered 20, 80, and 160 mg/kg/d. The data show that the levels of serum IL-6 declined after 9 days of administration, despite no alteration of the administered dose.

Irradiated dogs—clinical effects of IL-6. In a manner similar to that observed in normal dogs, the most significant clinical side effects were noted at 160 μg/kg/d. At this dose, a 29% body weight loss was noted in the dog administered 160 μg/kg/d of endotoxin-free IL-6. A fever of up to 2°C over baseline was noted in that dog and in one dog administered 80 μg/kg/d. These findings were accompanied by lethargy and decreased appetite. In contrast, at 40 μg/kg/d, no differences between treated and control animals were noted.

Serum chemistry analysis. Similar abnormalities were noted in the irradiated IL-6–treated dogs as were observed in the normal animals. Significant increments in cholesterol (control, 180 ± 15 mg/dL vs IL-6–treated, 398 ± 88; P < .01); triglycerides (control, 36 ± 8 mg/dL vs IL-6–treated, 20 μg/kg/d; (n) 80 μg/kg/d; (m) 160 μg/kg/d.
treated, $85 \pm 27$ mg/dL; $P < .01$); and alkaline phosphatase (control, $97 \pm 27$ U/L vs IL-6–treated, $444 \pm 322$; $P < .01$); and a significant decrease in albumin (control, $3.25 \pm 0.4$ g/dL vs IL-6–treated, $2.4 \pm 0.2$ g/dL; $P < .01$) were noted.

**Platelet recovery.** The nadir of the platelet count usually occurred between 12 and 14 days after 200 cGy TBI, with a range of 7 to 17 days (Fig 6). In seven of nine dogs receiving IL-6, an enhanced rate of platelet recovery was observed. Figure 6A shows the time course of platelet recovery in one dog receiving 40 μg/kg/d of IL-6 compared with its concurrent control, while the mean counts of all dogs, including two that did not respond to IL-6, are shown in Fig 6B. Figure 7 shows the number of days required to achieve 50, 100, and $150 \times 10^9$ platelets/L for each of the responding animals. In two animals receiving 160 μg/kg/d of IL-6, no increment in the platelet count was noted. These animals received an IL-6 batch that was contaminated with endotoxin, and it is estimated that each of these dogs received 2,000 to 2,300 EU/day for each of the last 10 days of IL-6 administration. In the responding animals, a 2- to 7-day improvement in the time to achieve $50 \times 10^9$ platelets/L was observed. In general, the rate of platelet recovery was dose-related. From the nadir platelet count through day 28, the rate of platelet recovery in the IL-6–treated dogs significantly exceeded that of controls (12,800 $\pm 6,000$ vs 7,100 $\pm 1,700$ platelets/μL/d, respectively; $P < .02$).

**Platelet size.** When analyzed flow cytometrically using 2F9 to mark the platelets, large platelets were observed in both treated and control irradiated dogs, although the percentage of these large platelets was significantly greater after IL-6 treatment. At day 3, 3.3% of the platelets in the treated dog were large, compared with 1.6% of control cells. A day-7 analysis of platelets in control irradiated and IL-6–treated irradiated animals showed 1.7% versus 6.9% of platelets $\geq 6.3$ μm, respectively. The percentage of large platelets in the IL-6–treated dogs declined after day 7, was 3.4% at day 19, and was 0.4% on day 26, the last day analyzed. The control irradiated dog platelets increased to a maximum of 2.9% on day 19, and declined to 0.1% on day 26.

**Analysis of megakaryocyte ploidy.** Table 1 shows the ploidy distribution of irradiated dogs at day 12. No significant differences were observed among control animals and

---

**Figure 6.** Platelet recovery in irradiated dogs. (A) Time course of recovery of a dog receiving 40 μg/kg/d of IL-6 for 14 days compared with its concurrently irradiated control receiving IL-6 buffer. The nadir of the platelet count was noted at day 14. Although the platelet counts of both dogs recovered after this time, the rate of recovery of the IL-6–treated dog was more rapid, and at day 21, the platelet count was $150 \times 10^9$/L in the treated versus $60 \times 10^9$/L in the control dog. (B) Platelet recovery in IL-6–treated dogs (n = 9). Although two of the treated dogs had no increment in platelet count, a statistically significant increase in the rate of platelet recovery was still observed in the treated animals ($P < .02$). The error bars represent the SEM. (■) IL-6–treated; (○) control.

**Figure 7.** Platelet recovery in irradiated IL-6–treated dogs. The bars represent the number of days after irradiation required to achieve platelet counts of 50, 100, and $150 \times 10^9$/L, respectively. A significant decrease in the time to recovery to each of these levels was noted for all groups compared to control ($P < .01$). (□) control; (■) 40 μg/kg/d; (■) 80 μg/kg/d; (■) 160 μg/kg/d.
IL-6 AND THROMBOCYTOPOIESIS IN DOGS

Table 1. Ploidy Distribution of Irradiated Dogs on Day 12

<table>
<thead>
<tr>
<th>IL-6 Dose (µg/kg/d)</th>
<th>n</th>
<th>2N</th>
<th>4N</th>
<th>8N</th>
<th>16N</th>
<th>32N</th>
<th>64N</th>
</tr>
</thead>
<tbody>
<tr>
<td>controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>2</td>
<td>12</td>
<td>2</td>
<td>9</td>
<td>16</td>
<td>4</td>
<td>38</td>
</tr>
<tr>
<td>80</td>
<td>3</td>
<td>16</td>
<td>9</td>
<td>8</td>
<td>32</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>160 (endotoxin free)</td>
<td>1</td>
<td>62</td>
<td>25</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Before irradiation, the ploidy distribution of both treated and control animals was: 2N, 5 ± 4 (% ± 1 SD); 4N, 5 ± 5; 8N, 22 ± 4; 16N, 57 ± 9; 32N, 12 ± 6; 64N, 0.1 ± 0.1. On days 31 through 35, the ploidy distribution of the control irradiated dogs was: 2N, 5 ± 3; 4N, 6 ± 6; BN, 22 ± 4; 16N, 49 ± 12; 32N, 18 ± 2; 64N, 0.2 ± 0.5, while the ploidy distribution of the IL-6-treated irradiated dogs was: 2N, 9 ± 7; 4N, 11 ± 10; 8N, 18 ± 5; 16N, 43 ± 16; 32N, 20 ± 4; 64N, 1 ± 2. These ploidy distributions were not significantly different than that observed in normal canine marrow.

Recovery rate was not significantly different in control versus treated animals. However, as observed in the normal dogs, a significant decline in the Hct was observed in all animals receiving IL-6 (Fig 8B). The Hct did not decline to less than 25%, fell most rapidly during the initial 10 days after radiation, and stabilized thereafter.

Serum IL-6 levels. Serum IL-6 levels in the irradiated dogs were similar but more variable than those observed in normal animals. The serum IL-6 levels progressively declined with time, but the differences were not statistically significant through day 12 (P > .1; Fig 9).

Serum IL-6 levels in the irradiated dogs were similar but more variable than those observed in normal animals. The serum IL-6 levels progressively declined with time, but the differences were not statistically significant through day 12 (P > .1; Fig 9).

**DISCUSSION**

IL-6 is a broad spectrum cytokine that exhibits potent effects on megakaryocytic maturation. When used in combination with the early acting cytokine IL-3, IL-6 is synergistic, promoting increased growth of megakaryocytic and early hematopoietic progenitor cells. In vivo administration of human IL-6 results in an increment in the platelet count in both mice and primates and, as shown in this study, dogs. IL-6 may be acting at the level of a precursor population directly, or acting indirectly via other cytokine(s). Alternatively or concurrently, the factor may be acting upon mature megakaryocytes, but at a rate that is dependent on the recruitment of sufficient megakaryocytes into an IL-6-responsive state. If the cytokine acts primarily by augmenting the rate of maturation of megakaryocytes, a delay in platelet recovery of irradiated animals dependent on marrow repopulation with immature, IL-6-responsive megakaryocytes might be expected. The present experiments suggest this may be the case, because the nadir of the platelet counts in both IL-6-treated and control dogs is concordant, but the subsequent rate of recovery is greater in the treated animals. The decrease in the platelet count seen soon after administration of IL-6 might have some effect on the rate of subsequent thrombocytopoiesis, but the degree to which that may occur is unclear, because the decrement never reached thrombocytopenic levels in the normal animals. The etiology of this decrease is unknown, but may be due to redistribution or transient consumption of platelets.

Although the cytometric measurement of platelet width may not be identical to absolute in vivo platelet size (because size will vary according to the choice of anticoagu-
the cytokine (directly or indirectly) modifies terminal maturation of megakaryocytes. This finding may be contrasted with the lack of increment in the mean platelet volume of IL-6-treated mice reported by others, although analysis of mean platelet volume is dissimilar to flow cytometric analysis of individual platelet width.\textsuperscript{12,14} The substantial proportion of platelets that achieve extremely large size (up to 19.1\%) in three normal dogs administered 80\( \mu \)g/kg/d of IL-6 may produce erroneous platelet counts and mean platelet volumes, because electronic volume platelet measurements would not include these cells within the size discrimination boundaries for platelets (indeed, we observed a distinct peak at the lower end of the WBC histogram that may reflect platelets). Thus, the reported platelet counts in this study (and perhaps others) may be underestimates of the true counts, which, in this situation, can only be assessed accurately by enumerating platelets identified with specific markers. The significance of these large platelets is unknown. They might be analogous to shift reticulocytes produced in response to a marked erythropoietin stress.\textsuperscript{30} It is not known if these platelets are hemostatically superior to the nonenlarged platelets, or if platelets from IL-6-treated animals (irrespective of their size) are hemostatically more effective than platelets from normal animals.\textsuperscript{31}

Analysis of the relative DNA content of megakaryocytes in the normal animals showed that at doses of IL-6 less than 160\( \mu \)g/kg/d, no changes in ploidy were observed, despite an increment in the platelet count. However, at the highest doses of the cytokine, a rightward shift in the ploidy was seen. A shift in ploidy after IL-6 administration to normal animals has been reported for both mice and primates.\textsuperscript{14,17} One interpretation of our observations is that shifts in ploidy are a relatively insensitive indicator of impending augmentation of the platelet count in the dog; conversely, an observed shift may reflect a strong stimulus to platelet production. In the irradiated animals, no obvious trend in the ploidy distribution was observed in IL-6-treated animals compared with controls. This might be expected in this complex physiologic situation, with the competing demands for megakaryocyte proliferation and differentiation. The leftward shift in the dog receiving 160\( \mu \)g/kg/d might reflect a relatively increased rate of input from precursor cells that exceeds the rate of endoreduplication, but this interpretation is limited by the fact that only one animal administered this dose was evaluable. Because bone marrow aspirates were not performed more than once weekly, it is also possible that alterations in ploidy were early or transient and were missed.

The serum IL-6 levels in normal and irradiated dogs roughly paralleled the dose administered. Although values appear to be more variable in the irradiated animals, the schedule of IL-6 administration was different (twice a day), and thus the serum concentrations obtained immediately before the next administered dose cannot be compared directly with the serum concentrations in normal animals. A decline in IL-6 levels was observed after days 9 to 12 of IL-6 administration, perhaps indicating the development of antibodies. Thus, further administration of human IL-6 to dogs beyond this time may not be effective. Whether injection of canine IL-6 would induce more rapid recovery or a quantitatively greater increase in the platelet count is unknown.

It has previously been reported that IL-6 administration augments the neutrophil and monocyte counts in irradiated mice.\textsuperscript{18} Although the total WBC count did not change in our study, we did not perform differential counts, and it is possible that elevations in the neutrophil or monocyte counts may have occurred. The decrease in Hct, observed in both the normal and irradiated animals, has been previously noted, and its etiology is difficult to explain in view of other reports suggesting that IL-6 augments erythropoiesis.\textsuperscript{9,32,34} The rapid decline in Hct suggests either an acute expansion of blood volume or hemolysis, with the former not clinically evident, and no obvious manifestations of the latter (no hemoglobinuria or pink serum). Further studies will be necessary to establish the pathophysiology of the IL-6-related anemia.

At doses under 80\( \mu \)g/kg/d, the incidence of ascertainable clinical side effects was minimal. At higher doses, lethargy, weight loss, and a low-grade fever were observed. Several of these side effects were similar to what had previously been described in normal primates.\textsuperscript{9} It was not surprising to observe a marked increment in the fibrinogen levels of these animals, because IL-6 has been shown to be a potent hepatocyte stimulatory factor, raising the concentrations of fibrinogen and other acute-phase reactants.\textsuperscript{16,35,36} Albumin, on the other hand, declines during the acute-phase response, an effect observed in our study and a previous report.\textsuperscript{9,36} The reasons for the increase in alkaline phosphatase, cholesterol, and triglycerides are unclear. In a previous primate study, cholesterol was reported to be decreased.\textsuperscript{9} These abnormalities were generally dose-related, but gradually normalized after discontinuation of the cytokine.

This study shows that IL-6 moderately augments platelet recovery in most animals after irradiation. The reasons for the failure to observe an increment in two of the dogs is
uncertain, but a likely possibility is concurrent platelet consumption related to excessive endotoxin administration.\textsuperscript{37} This interpretation is supported by the observation that two of three dogs receiving 160 \(\mu\)g/kg/d in the irradiated group received the largest amount of endotoxin, while the normal dog receiving this same dose (but endotoxin-free) had the greatest increment in platelet count.

The observed platelet increments occurred relatively late in the clinical course and at times when the platelet count is generally no longer considered to be at a dangerously low level in humans, although the findings in dogs may not be extrapolated directly to humans. Because IL-6 also appears to produce an early decrease in platelet count before augmenting the count, it is possible that potential bleeding might be exacerbated early after administration of the cytokine. Administration of IL-6 together with, or subsequent to, other cytokines that act on the megakaryocytic or earlier progenitor cell level (eg, IL-3, granulocyte-macrophage colony-stimulating factor [GM-CSF], c-kit ligand, or novel megakaryocyte colony-stimulating factors), or on a different schedule than used here may promote a more rapid platelet recovery that might prove to be clinically more useful.

Two other growth factors, leukemia inhibitory factor (LIF) and IL-11, are capable of enhancing megakaryocytic maturation in vitro and, in the case of LIF, the platelet count in vivo.\textsuperscript{38-40} It is presently unknown if the toxicity profile of these growth factors would render them more suitable for clinical use than IL-6, if they would prove to be more potent than IL-6, or if a combination of one or more of these factors with IL-6 would display greater efficacy.

Acknowledgment

The authors appreciate the excellent assistance of Ed Moehlenbrock.

References


