Hemopathologic Consequences of Protracted Gamma Irradiation: Alterations in Granulocyte Reserves and Granulocyte Mobilization

By T. M. Seed, S. M. Cullen, L. V. Kaspar, D. V. Tolle, and T. E. Fritz

Aplastic anemia and myelogenous leukemia are prominent pathologic effects in beagles exposed to continuous, daily, low-dose gamma irradiation. In the present work, granulocyte reserves and related mobilization functions have been sequentially assessed by the endotoxin stress assay during the preclinical and clinical phases of these hematopoietic disorders. Characteristic patterns of granulocyte reserve mobilization are described that reflect given stages of pathologic progression. For radiation-induced leukemia, a five-stage pattern has been proposed. In contrast, a simple pattern of disorders is observed. Characteristic patterns of granulocyte reserve mobilization are described that reflect given stages of pathologic progression. Sequential change in the reserves and associated mobilization functions between ~200 and 400 days (phase V) following endotoxin stress typify the responses of dogs during the intermediate phase, whereas late preclinical, preleukemic phases (phase IV) are characterized by a further expansion of the reserves and in the mobilization capacities, particularly of the less mature granulocytes. Such late alterations in the pattern of granulocyte mobilization, together with other noted cellular aberrancies in the peripheral blood and marrow, appear to indicate leukemia (phase V) onset.

previous studies have indicated that myeloproliferative disorders (MPD) in beagle dogs are prominent late arising hemopathological consequences of protracted gamma irradiation. Under continuous whole-body irradiation at the dose rate of 10 R/day, approximately one-half of the dogs died of aplastic anemia following ~200 days of exposure, i.e., 2000 R of accumulated exposure, while the other half of the population partially recovered marrow function, survived for prolonged periods, and eventually succumbed to MPD (~80% of the long-term survivors) in a mean time of ~800 days. The high probability that survivors of early acute hemopoietic suppression will eventually develop MPD makes these dogs ideal experimental subjects to study pathologic progression during early preclinical phases. As the predominant terminal pathology is granulocytic leukemia, we have studied the sequential change in function of various granulopoietic compartments of the marrow of individual dogs from the time of initial radiation exposure to the presentation of overt MPD. Our intent has been, and continues to be, to identify the characterize cellular alterations of the granulopoietic system that would have prognostic value. Numerous investigators have examined granulocyte reserve function by measuring the extent of endotoxin-induced granulocyte-toxicosis in a variety of species, including man, under various clinical and experimental conditions.

For this investigation, we have examined the longitudinal change in the granulocyte reserves and granulocyte mobilization capacity of dogs subjected to continuous low-dose gamma irradiation that were in preclinical and clinical phases of septicemia, aplastic anemia, and myeloproliferative disease.

materials and methods

Animals

Outbred beagles included in this study were derived from the closed Argonne National Laboratory colony whose status, origin, and general management have been described in detail elsewhere. Animals utilized are part of a long-range study whose overall aim is to evaluate the genesis of late arising, radiation-induced hemopathologies. Dogs used in this study were of both sexes, were anatomically normal and healthy, and approximately 400 days old at the start of the experiment. Prior to and during the experiment, the 24 test and 6 control dogs were monitored clinically and hematologically.

Irradiation

Experimental dogs were maintained in standard size fiber glass cages within a specially designed irradiation facility equipped with an attenuated 15 Ci 60Co source (Atomic Energy of Canada, LTD, Ottawa). Dogs were continuously irradiated for 22 hr each day at a dose rate of 10 R/day. Dosimetric methods and calculations were outlined in detail in previous studies. During the 2 hr when dogs were not exposed, they were fed, watered, and experimentally manipulated. Nonirradiated control animals were similarly treated and handled, except that they were maintained in cages in a shielded, adjoining anteroom.

Hematology

Hematograms were performed by standard methods on each irradiated animal every 14 days and on each control animal every 28 days. Blood samples were collected, via jugular venipuncture, into evacuated tubes containing EDTA. Erythrocyte and leukocyte counts were performed electronically. Differential white cell deter-
Granulocyte Reserve Assays

Experimental dogs were subjected to the granulocyte reserve assay prior to beginning irradiation and approximately every 100 days thereafter, representing differences in accumulated radiation dose of 1000 R (i.e., 10 R/day for 100 days – 1000 R). On the day of each assay, experimental dogs were removed at 7:00 a.m. from the irradiation facility and placed in the shielded area occupied by the nonirradiated controls. After removal of a blood sample (using 5-ml evacuated tubes containing EDTA), the dog was intravenously injected with 50 ng/kg body weight of purified (phenol extracted) bacterial endotoxin [Salmonella typhimurium lipopolysaccharide (LPS), type no. L-3629, Sigma Chemical Co., St. Louis, Mo.] in approximately 0.1 ml of pyrogen-free saline. Subsequently, 5-ml blood samples were obtained at 1, 2, 4, 6, 8, 10, 12, 14, and 24 hr after injection. To double check endotoxin effects, a limited number of control dogs received 0.1 ml of pyrogen-free saline solution intravenously. For each blood sample collected, total numbers of erythrocytes and leukocytes were determined, blood smears were made and stained with Wright’s-Giemsa, and differential white cell counts were performed.

Data Collection and Treatment

The typical time-dependent granulocyte response to endotoxin injection of an unirradiated control dog is schematically illustrated in Fig. 1. Quantitative features of granulocyte reserve function were derived from the response curves of individual control and test animals. Peripheral blood concentration of granulocytes, at time zero, was taken to represent the size of the circulating granulocyte pool. Blood granulocyte concentration at the peak (plateau region) of the response was considered to be a relative measure of the size of the marrow granulocyte pool held in reserve.4 Non-neutrophilic granulocytes contributed <5% of the total granulocyte numbers at the pre-endotoxin reading and <1% at the peak value. The endotoxin-induced granulocyte increment (ΔG) was calculated from the mean peak value less the pre-endotoxin baseline value.14 15 The slope of initial decline in granulocyte number following LPS injection was considered to be a measure of the rate of granulocyte margination;14 20 the positive slope of the granulocytic response, occurring between 2 and 10 hr post-LPS injection, was assumed to numerically represent a rate function of bone marrow granulocyte reserve mobilization.4 21 22 30 Both slopes were determined by linear regression analysis39 of data obtained from the response curves of individual dogs; average rates and degree of standard error of granulocyte margination and mobilization were calculated and recorded for each group.

RESULTS

Survival Times and Terminal Pathology

Of the 24 irradiated dogs included in this longitudinal study of granulocyte reserve function, 17 have died to date; 2 died of acute bacterial infections at 213 ± 31 days; 13 died of aplastic anemia at 249 ± 80 days of exposure; the other 2 deaths were attributed to myeloproliferative disorders occurring at 406 days (myelomonocytic leukemia) and at 505 days (myelofibrosis with myeloid metaplasia). (A second case of radiation-induced myelomonocytic leukemia, which occurred in another currently running longitudinal study, was also examined by the endotoxin stress assay for granulocyte reserve function and is included in this report.) The time sequence of the development of the hemopathologic endpoints (i.e., septicemia deaths first followed by aplastic anemia, and then the late-rising myeloproliferative syndromes) is characteristic of our previous experience with dogs continuously irradiated at 10 R/day for duration of life.2 3 The seven surviving animals are termed “radioaccommodated” and are presently regarded as being in preclinical phases of leukemia.

Circulating Blood Granulocyte Levels

The sequential changes in the concentrations of circulating blood granulocytes with time-of-irradiation of dogs with various developing pathologies are shown in Fig. 2 A and B. Distinctive granulocytic response patterns are evident for the various types of pathologic endpoints. Dogs succumbing early to systemic bacterial infection showed a progressive, nearly linear decline in blood granulocyte concentration with time of exposure. In dogs with progressing marrow aplasia, the decline was generally somewhat less rapid, and tended to stabilize at low values for
weeks prior to death (Fig. 2A). A large segment of the exposed population survived the hemopoietic crisis which characteristically occurred between 200 and 300 days of irradiation (Figs. 2A and B) and resulted in the early deaths from septicemia and marrow aplasia. As indicated by previous studies, these survivors have a high probability (i.e., \( \approx 80\% \)) of developing MPD, with the most prominent subtype being myelogenous leukemia (Fig. 2B).

Sequential Assessment of Granulocyte Reserves and Granulocyte Mobilizing Capacity

Preirradiation control response. The granulocyte response of the nonirradiated, control dogs remained relatively constant over the 1400 days of testing, despite periodic endotoxin stress. The average response, and its variance, of all control, nonirradiated animals is included in Fig. 3 and 4 for reference. The calculated granulocyte reserves and the rates of granulocyte mobilization generated from these control curves are listed in Table 1. The salient features of the control response include: the high rate of granulocyte mobilization (i.e., \( \approx 1.20 \times 10^9 \) cells/hr); the large circulating and stored granulocyte pools, in an approximate ratio of 1:2; and a quick response time (i.e., rebound time, \( \approx 4.5 \) hr) to the initial result of endotoxin stress (Table 1).

Septicemias and aplastic anemia. Dogs dying earliest of acute bacteremia or others slightly later of aplastic anemia (Fig. 3) exhibited a progressive decline in capacity to mobilize stored granulocytes effectively following endotoxin stress. This decline was correlated with the fall in circulating blood granulocyte levels (Fig. 2A). During the terminal stages of disease (i.e., \( \approx 200 \) days with septicemias and \( \approx 300 \) days with the aplasias), the animals were basically unresponsive; granulocyte reserves and rates of mobilization were calculated to be less than 5% of control values, the ratio of circulating to storage pool values was less than one, and the response time greater than 10 hr (Table 1).

Radioaccommodated, Suspect Preleukemics. Figs. 4A–D show the sequential change in the granulocyte response to endotoxin challenge of the longer surviving irradiated animals. From previous studies, these dogs are expected to be highly prone to develop myeloproliferative disorders. As in all dogs irradiated continuously at 10 R/day, the initial response for the first 200 days of exposure (or \( \approx 2000 \) R accumulated irradiation exposure) was one of granulopoietic decay; the circulating pool contracted to \( \approx 41\% \) of its preirradiation size and the functional reserve pool to \( \approx 21\% \), while the rate of mobilization...
Fig. 4. Sequential change in the pattern of granulocyte mobilization with time of irradiation in the long-lived radioaccommodated dogs. (A) The initial ~200-day decline in the mobilization curves. (B) Increasing rate and magnitude of granulocyte mobilization between 300 and 400 days. (C) The intermediate phase subnormal mobilizations. (D) A second late-arising phase of increased granulocyte mobilization that corresponds in time with other noted preleukemic syndromes.

decayed to ~12% of the control (Table 1). After 200–300 days of exposure, the long-term survivors exhibited noticeable increases in circulating blood levels of granulocytes (Fig. 2A), in granulocyte reserves, and in granulocyte mobilizing capacity (Table 1). The circulating to reserve pool ratio remained unchanged, however, at the nadir (i.e., ~1.0). Despite the stress of continuous irradiation, the various granulopoietic functions measured exhibited marked improvement for several hundred days. Rates of granulocyte mobilization increased some 380% from the nadir at ~200 days of exposure to approximately the 400th day. The capacity of the functional reserves increased some 220% during the same period, resulting in an improved circulating to reserve pool ratio of ~1.6 (Table 1). Prolonged granulocyte mobilizations after endotoxin injection were characteristic during this phase of hemopoietic restoration (Fig. 4B). For an extended period (e.g., between 400 and 800 days) following this initial expansion of reserve function, the mobilization response remained relatively stable, although below control values. Following this period of subnormal hemopoietic equilibrium, the granulocyte reserves, granulocyte mobilization, and
related functions expanded for a second time until preirradiation values were reached after approximately 1200 days of continuous irradiation.

In contrast to the control granulocyte mobilization response, which was dominated by mature segmented neutrophils, the response of these radioaccommodated suspect preleukemic dogs was attributable to a greater extent to immature granulocytes (band forms), which comprised a greater fraction of the marrow reserves (Fig. 5A and B).

Myeloproliferative disorders. Figure 6 and 7 show the sequential change in the granulocyte reserve mobilization response to endotoxin stress of two dogs that developed myeloproliferative disorders. These have been characterized histopathologically as acute myelomonocytic leukemia (Fig. 6) and myelofibrosis with myeloid metaplasia (Fig. 7). The sequential change in the granulocyte mobilizing response with increasing irradiation of the dog with developing leukemia is markedly similar to those of the radioaccommodated dogs. The nadir of the declining mobilization response was reached at 200 days. At 300 days, there was some improvement and, again, sustained secondary mobilization characteristic of this phase. The entry into patent disease took only an additional 100 days so that the prepatent period was only about 400 days. This time is considerably shorter than the mean prepatent period of 800 days observed in

Table 1. The Sequential Change in the Granulocyte Reserves of Dogs in Preclinical and Clinical Phases of Specific, Radiation-Induced Pathologies

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>No. Animals</th>
<th>Exposure* Time</th>
<th>Mobilization Rate† (x 10³/cu mm/hr)</th>
<th>Rebound‡ Time (hr)</th>
<th>Circulating Pool§ (x 10³/cu mm)</th>
<th>Storage Pool∥ (x 10³/cu mm)</th>
<th>Pool ratio</th>
<th>∆G** Ratio (x 10³/cu mm)</th>
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<tr>
<td>Controls</td>
<td>18(54)†††</td>
<td>0</td>
<td>1.20 ± 0.47†††</td>
<td>4.5</td>
<td>5.42 ± 0.26</td>
<td>11.80 ± 0.49</td>
<td>2.18</td>
<td>6.38</td>
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<td>3.7</td>
<td>2.81</td>
<td>6.88</td>
<td>2.92</td>
<td>4.07</td>
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<td></td>
<td>2</td>
<td>200</td>
<td>0.03 ± 0.11</td>
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<td>0.55 ± 0.43</td>
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<td>0.73</td>
<td>0</td>
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<td>Aplastic anemias</td>
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<td>6.5</td>
<td>3.40 ± 0.40</td>
<td>4.89 ± 0.57</td>
<td>1.41</td>
<td>1.43</td>
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<tr>
<td></td>
<td>9</td>
<td>200</td>
<td>0.08 ± 0.11</td>
<td>&gt;10</td>
<td>0.87 ± 0.28</td>
<td>0.78 ± 0.42</td>
<td>0.90</td>
<td>0</td>
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<td>2</td>
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<td>0.06 ± 0.03</td>
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<td>0.75 ± 0.05</td>
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<tr>
<td>4</td>
<td>100</td>
<td>0.72 ± 0.21</td>
<td>5.4</td>
<td>3.7 ± 0.09</td>
<td>6.99 ± 0.78</td>
<td>1.84</td>
<td>3.20</td>
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<td>7</td>
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<td>0.19</td>
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<tr>
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<tr>
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<tr>
<td>6</td>
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<td>5.02 ± 1.41</td>
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<td>6</td>
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<td>5.37 ± 0.62</td>
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<tr>
<td>4</td>
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<tr>
<td>4</td>
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<td>7.0</td>
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<td>6.36 ± 1.29</td>
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<tr>
<td>4</td>
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<td>7.79 ± 2.17</td>
<td>1.66</td>
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<tr>
<td>3</td>
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<td>6.80</td>
<td>0.77</td>
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*Exposure time = total days of irradiation at 10 R/day.
†Mobilization rate = the slope of the endotoxin-induced granulocyte response curve between 2 and 10 hr after injection.
‡Rebound time = the time required to bring peripheral blood granulocyte levels back to baseline preinjection values.
§Circulating pool = blood concentration of granulocytes prior to endotoxin injection.
∥Storage pool = a relative estimate based on peaked blood granulocyte levels (at ~10 hr) following endotoxin injection.
**Pool ratio = mean storage pool value divided by the circulating pool value.
†††All values listed are means ± standard error.
‡‡‡Values listed are means ± standard error.
§§§Second case of myelomonocytic leukemia. This dog initially received 10 R/day for 200 days, after which the radiation was terminated. Patent leukemia was presented after 2041 days.

The entry into patent disease took only an additional 100 days so that the prepatent period was only about 400 days. This time is considerably shorter than the mean prepatent period of ≈800 days observed in

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GRANULOCYTE RESERVES OF IRRADIATED DOGS

Fig. 6. The pattern of granulocyte mobilization of a single dog from the initial period of irradiation exposure to preclinical and clinical phases of acute myelomonocytic leukemia.

Fig. 7. The pattern of granulocyte mobilization of a single dog that died of myelofibrosis.

responded qualitatively similar in manner to the case described above; however, the rate of mobilization was lower, as was the magnitude of the total, as well as the immature granulocyte response (Table 1). The pathologic nature of the mobilization response and the composition of storage pools in the terminally ill leukemia dogs was indicated by the release of immature granulocytes following endotoxin stress (Fig. 8). The latter were mainly band forms, although sizable numbers of myeloblasts and progranulocytes were released as well. Evidence of this change in composition of stored granulocytes released following endotoxin injection was seen as early as 300 days of exposure, nearly 100 days prior to entry into patent

previous studies2,3,6 or the anticipated prepatent periods of the long-lived, suspect leukemias described above. The prolonged period of subnormal hematopoietic equilibrium, which generally lasts several hundred days, appears to have been compressed in time. In the terminal phases of leukemia, the primary absolute granulocyte response (i.e., at between 2 and 10 hr) was greater than the preirradiated control values; total granulocyte reserves approached control values, while the circulating pool was approximately 170% larger. This enlarged circulating pool contributes significantly to the low circulating-to-reserve pool ratio and to the delayed rebound time (Table 1).

The second case of radiation-induced myelomonocytic leukemia tested by the endotoxin stress assay
Fig. 8. The pattern of immature granulocyte mobilization with time of irradiation is shown for the dog that developed myelomonocytic leukemia. The total granulocyte response of this dog is shown in Fig. 7.

Fig. 9. The pattern of immature granulocyte mobilization with time of irradiation is shown for the dog that developed myelofibrosis. The total granulocyte response of this dog is shown in Fig. 7.

phase of leukemia (Fig. 8). Similar shifts in cell composition of the endotoxin-induced response were not evident in the dog developing myelofibrosis (Fig. 9).

DISCUSSION

Numerous attempts have been made to measure granulocyte reserves in a variety of species, including man. A basic approach commonly employed involved the incremental measure of peripheral blood granulocyte response to "inflammatory" agents, e.g., bacterial endotoxin, etiocholanolone, and corticosteroids. Dale et al. compared the capacity of these agents to release marrow neutrophils and found good overall correlation. Deubelbeiss and Roth made a careful quantitative study of the relationship between the size of the postmitotic neutrophil reserve of the marrow and the incremental increase in peripheral blood neutrophils following hydrocortisone injection and concluded that the increment provided a good index of reserve capacity. Joyce and Boggs, who conducted clinical experiments with neutropenic patients using Pseudomonas sp. endotoxin, reported that the size of marrow granulocyte reserves correlated with the magnitude of the induced neutrophilia provided that the segmented and band neutrophil compartment of the marrow comprised ~25% of the granulocytic marrow elements.

The pyrogen-stress assays have been successfully employed to measure the relative size and mobility of the granulocytic reserves in a variety of clinical conditions, including alcohol intoxication, cirrhosis of the liver, kidney disease in patients undergoing hemodialysis, chemically induced myelosuppression, chronic idiopathic granulocytopenia, metastatic carcinomas, and myeloproliferative disorders.

Our results show that the granulocyte reserves and the rate of granulocyte mobilization are altered by protracted, low daily dose gamma irradiation. The pathologic consequences of such ionizing radiation exposure are either early arising aplastic anemia, which is occasionally complicated by systemic bacterial infections, or the late arising myeloproliferative disorders. Myelogenous leukemia is the prevalent pathology within the latter disease complex. With time of irradiation and progression to each of these pathologic endpoints, the pattern of granulocyte mobilization appears to change sequentially and distinctively. In dogs with developing septicemia or marrow aplasia, the rate of endotoxin-induced granulocyte mobilization declined progressively with time of exposure, with barely perceptible responses recorded just prior to death. These low cell mobilizing responses were associated with both extremely depleted circulating pools and stored granulocyte pools; i.e., the increasing severity of the radiation-induced neutropenia corresponded with increasingly depleted marrow granulocyte reserves. As early as 100 days into the experiment, the granulocyte mobilizing capacity of dogs in preclinical phases of marrow aplasia was considerably less than those dogs destined to radiate the marrow (Table 1). The absolute cellularity of biopsied marrow, which appeared fatty and acellular, was consistently reduced to less than 10% of preirradiation values. The remaining granulopoietic elements generally had a less than normal percentage of
bands and mature segmented neutrophils. The stem cell pool committed to granulo-monopoiesis (i.e., GM-CFUa) has also been shown to be severely depleted, indicating radiation-induced lesions in both the proliferative as well as in the storage compartments of the granulopoietic system (Seed et al., unpublished observations).

In marked contrast to the dogs dying early of aplastic anemia, the longer-lived suspected preleukemic animals predictably exhibited, between 200 and 400 days of exposure, a small but significant expansion of the granulocyte reserves and improved reserve mobilization. Such improved function appeared to extend to a marginally expanded granulopoietic stem cell pool. A remarkable feature of this partial recovery is that it occurred during continuous daily irradiation. The hemocytotoxic effects of ionizing radiation, readily observed during the initial period of exposure, became much less apparent as blood elements increased and marrow started to regenerate. The phenomenon of hemopoietic recovery, as it occurs following single or short-term radiation exposure, is well documented in various species under various radiation conditions, but recovery during continuous irradiation, although documented, is far less well understood.

The initial period of hemopoietic recovery has been considered to be the second phase of a five-phase sequence leading to leukemia. The five phases are: (I) the initial radiotoxic phase characterized by the progressive development of severe leukopenia and thrombocytopenia; (II) the partial recovery phase mentioned above; (III) a period of equilibration of subnormal hemopoietic function, which is of variable duration and appears to dictate the length of the prepatent period; (IV) a classical, cytologically defined preleukemic phase; and (V) overt leukemia. In phase III, calculated granulocyte reserve functions (Table 1) remained fairly stable, but below control levels. There was apparent disparity between the increased marrow cellularity and the subnormal mobilization responses to endotoxin, although the slight life-shift in maturational status of the marrow granulocytic elements in biopsied marrow along with the reduced percentage of segmented neutrophils in the marrow would, in part, explain the subnormal mobilizations. The circulating blood concentrations of granulocytes, although still depressed in phase III, were maintained at somewhat artificially high levels seemingly at the expense of severely depleted reserves, as indicated by the inordinately low storage to circulating pool ratios. The latter hematologic accommodation would certainly have survival value.

Secondary granulocyte mobilizations occurring 12–14 hr after endotoxin injection were characteristic responses of radioaccommodated (e.g., phase II–III) dogs. The delayed release of neutrophils from an expanding reserve compartment might indicate functional changes in the marrow stromal elements, considered to be responsive to endotoxin (or to neutrophil releasing factor) and regulatory in releasing stored blood elements. In preliminary studies, we found that the higher injected doses of endotoxin (e.g., 500–1000 ng/kg) resulted in a delayed, but accentuated granulocytosis, comprised to a greater extent of neutrophilic bands than observed at lower endotoxin doses. It is clear that in certain instances the noted secondary mobilization (occurring late in phase III) was attributable, in part, to the increased release of less-than-mature neutrophils, which comprised an enlarged fraction of the marrow granulocyte reserves (e.g., Figs. 6 and 8; 300 days).

The transition from phase III, a period of subnormal hemopoietic equilibrium, to phase IV, a cytologically defined preleukemic phase, was marked by a second expansion of the granulocyte reserve (the first occurring in the initial recovery of phase II). The mobilization curves began to reflect "control-like" patterns. However, unlike the controls, the less-than-mature neutrophilic bands began to comprise a greater proportion of the cell population mobilized. This would indicate a shift, within a time frame of several hundred days, in the composition of the marrow granulocyte reserves. As indicated in previous work and in ongoing studies, marrows biopsied during this phase were generally hypercellular, with moderately increased myeloid:erythroid ratios, and left shifts in maturational status of the granulopoietic elements. The granulopoietic stem cell compartment appeared enlarged with clonal aberrancies, whereas the serum level of the suspect granulopoietin, i.e., colony-stimulating factor, was depressed. Other preleukemic changes, reported in dogs and also in man, include: dyserythropoiesis, circulating nucleated erythrocytes, oscillating platelet values, giant platelets, and atypical granule formation and bizarre nuclear configurations in the neutrophilic series. In one series, 19 of 28 cases (68%) of radiation-induced hemoproliferative disorders in dogs showed such "preleukemic" changes prior to onset of patent disease.

During the acute phase of leukemia in both man and canines, there appears to be considerable variation in the capacity of individuals to mobilize granulocytes following endotoxin stress. Such differences are attributed to either the extent of neoplastic cell involvement in the marrow, which would alter the size and/or the composition of the cellular reserves, to the aberrant nonuniform granulocyte production kinetics known to occur in acute myelogenous and myelomonocytic leukemia, or to changes in the func-
Godwin et al. found a positive correlation between granulocytes and myeloblasts. An abnormal release of similar to the observations made by Srodes et al. and studies, Craddock et al., Thatcher and Smith, and Godwin et al. reported a positive correlation between a low baseline granulocyte count and the patient's subsequent low response to endotoxin. This finding is similar to the observations made by Srodes et al. and Joyce and Boggs of the blood levels of neutrophils and the endotoxin-induced granulocyte mobilization in patients with severe neutropenia. In contrast to the negligible responses found in acute leukemia, Marsh and Perry reported that patients in early stages of chronic myelogenous leukemia or in remission, but not in blast-crisis, had adequate granulocyte mobilization.

In comparison to the above clinical reports, the endotoxin-induced granulocyte responses of the three dogs with radiation-induced myeloproliferative disease have been variable. The case of myelofibrosis exhibited an exceedingly low mobilizing response, whereas the two cases of acute myelomonocytic leukemia exhibited quantitatively greater granulocyte mobilization. However, the response, in each case, had aberrant features; the most notable was the release from the marrow into the peripheral circulation of large numbers of immature granulocytes, including progranulocytes and myeloblasts. An abnormal release of immature cells from the marrow has been observed in human leukemia and probably indicates an impairment of the marrow stromal elements.

In summary, the pattern of endotoxin-induced granulocyte reserve mobilization in beagles was altered as a consequence of continuous exposure to low daily doses of whole-body 137Cs irradiation. The pattern of granulocyte mobilization appeared different for the major pathologic endpoints, namely aplastic anemia and myeloproliferative disease. The granulocyte reserve capacity progressively declined with time of irradiation in dogs with aplastic anemia. In contrast, the dogs that accommodated to irradiation, and therefore prone to develop myeloid leukemia, exhibited two periods of granulocyte reserve expansion, one early and one late. These periods were bridged by a period of fairly stable, but subnormal, hemopoietic function of variable length. In the single case of progressing myelofibrosis, the second expansion phase of granulocyte reserve function was absent.

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