The purpose of this study was to determine the value of prophylactic granulocyte transfusions in preventing death from sepsis. An intravenous dose of $10^7$ Escherichia coli was lethal when given to granulocytopenic rats 6 days following irradiation with 750 rads. Only one of 22 irradiated animals survived the sepsis. Although normal (nonirradiated) animals experienced a transient leukopenia from this dose of organisms, it was less than LD_{10} for the normal host. There were no deaths in a group of animals receiving irradiation only. A group of 14 irradiated animals was given a single granulocyte transfusion 2 hr before the septic inoculum, and 57% of these animals survived ($p < 0.01$). No antibiotic therapy was administered to any of these animals. Irradiated animals who received granulocytes and recovered from sepsis had earlier granulocyte reconstitution than animals irradiated but not given the septic challenge. Platelet reconstitution was the same in both groups. In the rat model, prophylactic granulocyte support of septic animals led to improved survival. It was concluded that granulocyte prophylaxis may be of value in selected patients with transient bone marrow failure who are therefore at high risk from sepsis.

INFECTION AND HEMORRHAGE are the major causes of death in patients with impaired bone marrow function. The widespread and successful use of platelet support since the early 1960s has drastically reduced the danger of thrombocytopenic bleeding, and infection now accounts for some 70% of deaths in patients with acute leukemia and 50% in patients with solid tumors. Despite optimal antibiotic therapy, patients continue to die from gram-negative sepsis in the presence of granulocytopenia, although the recent experience with granulocyte support in these patients is encouraging. Several previous studies have suggested that granulocyte transfusions may be effective in the treatment of experimental sepsis in myelosuppressed animals. In addition, there is a recent report indicating the value of prophylactic granulocyte transfusions in dogs rendered leukopenic by cyclophosphamide. The purpose of this study was to determine the efficacy of a single granulocyte transfused given prior to a lethal septic insult.

MATERIALS AND METHODS

Animals

Wistar/Furth rats (Microbiological Research Associates, Bethesda, Md.) weighing 175–250 g were used in all experiments. They were chosen because their tolerance to radiation had been...
studied in our laboratory\textsuperscript{14} and because they were a convenient, inbred strain in whom granulocyte collection by continuous filtration pheresis could be carried out with relative ease. An inbred strain was chosen in order to minimize adverse factors that might affect the function and recovery of the transfused granulocytes. All animals were caged in groups of not more than six, given uniform laboratory chow feeding and ad lib access to water. They were kept in a temperature- and humidity-controlled room.

\textbf{Radiation}

Animals received 750 rads total body irradiation (midline tissue dose) with gamma irradiation from a \textsuperscript{137}Cs Gammacell 40 unit (Atomic Energy of Canada, Ltd.) emitting at 150 rads/min. All animals experienced severe bone marrow failure evidenced by granulocytopenia and thrombocytopenia lasting a minimum of 20 days; no other irradiation toxicity was seen. No antibiotic or other support therapy was given to the animals, and they were treated exactly as normal nonirradiated animals in adjacent cages.

\textbf{Infection}

A single strain of \textit{Escherichia coli} (AS No. 25922) was selected for use in these experiments and was maintained in semisolid nutrient medium in our laboratories at 4°C. On the morning on each experiment, the bacteria were inoculated into a trypticase soy broth and incubated at 37°C for approximately 3 hr. Using a broth blank, the concentration was allowed to reach $2.5 \times 10^9$ organisms/ml by spectrophotometric determination (optical density was read at 600 nm). The estimated concentration was always confirmed by agar pour plating. Recipient animals were chosen for equivalence in weight (mean 200 g) and each animal received a mean inoculum dose of $17.1 \times 10^8$ viable organisms per animal (SE $= \pm 4.6 \times 10^8$). All injections of saline and bacteria were given intravenously under light ether anesthesia.

\textbf{Granulocyte Procurement}

A modification of the continuous-flow filtration leukopheresis method of granulocyte collection initially described by Djerassi et al.\textsuperscript{15} (and miniaturized for experimental rodent work\textsuperscript{16}) was employed. Miniaturized Leukopak filters were constructed of Tygon tubing (Norton Plastics and Synthetics Division, Akron, Ohio) with an outside diameter of 1 cm and a length of 4.5 cm, filled with 1.1 g of nylon wool (Fenwal Laboratories, Morton Grove, Ill.). Each filter was rinsed with saline and primed with normal heparinized rat blood. Animals were anesthetized with intraperitoneal pentobarbital sodium 55 mg/kg. Incisions were made in the neck, and the left common carotid artery and right external jugular vein were isolated. Both of these vessels were cannulated using sterile PE Intramedic tubing (Clay Adams, Parsippany, N.J.) filled with saline but clamped off at either end. Anticoagulation was performed using 0.2 ml (200 units) of heparin sodium (Weddel Pharmaceuticals, London, England) via the jugular venous cannula. The filter was connected, the clamps removed, and the blood allowed to circulate through the filter for 2 hr.

At the end of 2 hr, the donors were sacrificed by injection of a lethal dose of pentobarbital sodium, and the granulocytes were eluted from the filters by flushing with a 50% rat plasma-saline solution as previously described.\textsuperscript{16}

\textbf{Experimental Design}

In pilot studies, an inoculum dose of $10^9$ organisms per animal was found to be LD\textsubscript{10} for normal animals, but LD\textsubscript{100} for animals irradiated 6 days previously and therefore at the granulocytopenic nadir (Fig. 1). Since this dose led to a clear difference in mortality between normal and irradiated animals, it was chosen for all subsequent experiments. As it was likely that granulocytopenia was the explanation for lack of tolerance in the irradiated recipient, the following studies were performed to determine if improved survival could be achieved with transfusion of granulocytes. Four groups of animals were investigated: group 1—22 animals who had received irradiation only; group 2—17 normal animals who had received no x-irradiation were given the inoculum of \textit{E. coli}; group 3—14 animals who had received irradiation and were
Fig. 1. Toxicity of varying numbers of viable \( E. coli \) organisms to normal and irradiated animals. Each dot represents one animal challenged with intravenous \( E. coli \). Inocula up to \( 10^6 \) organisms were uniformly non-lethal to normal animals; \( 10^6 \) organisms were LD\(_{10}\) to normal animals but LD\(_{100}\) to irradiated animals.

then treated on day 6 with a single granulocyte transfusion. 2 hr later, the \( E. coli \) inoculum was given; group 4—18 animals who had received irradiation and were then treated on day 6 with the \( E. coli \) inoculum, no granulocyte transfusions were given to this group.

Sham injections with normal saline were carried out in the groups receiving no granulocyte support. Baseline counts (total and differential leukocyte counts and platelet counts) were performed prior to irradiation and again on day 6 following irradiation, prior to the experimental manipulation. Further counts were performed 2 hr after the granulocyte transfusion (group 3), 30 min after the challenge with \( E. coli \), and at frequent intervals thereafter in all groups. Five separate experiments were performed and the results pooled. The day of death was noted, and autopsies were performed on all dead animals.

RESULTS

Granulocyte Procurement

Granulocytes obtained from four donors were pooled and administered to two recipients. Up to eight experimental leukophereses could be achieved during a single 4-hr period. An average of \( 1.54 \times 10^8 \) granulocytes per donor (SE = \( \pm 0.1 \times 10^8 \)) was eluted from the filters, and each animal received \( 3.08 \times 10^7 \) cells (SE = \( \pm 0.16 \times 10^8 \)). The circulating blood volume of donor rats was estimated at 12–14 ml. The procurement efficiency per donor was calculated to be 1.25–1.75 \( \times 10^7 \) cells/ml of donor blood. The yield of granulocytes thus compared favorably with studies in humans using continuous-flow centrifugation over a similar time period.\(^{15}\) It had previously been shown that granulocytes obtained by this method were capable of active phagocytosis.\(^{16}\)

Hematopoietic Recovery

The absolute granulocyte counts following the septic challenge are shown in Fig. 2. Animals that survived had a prompt granulocyte recovery, developing granulocyte counts well into the normal range the day after the challenge. This finding could not be accounted for by the granulocyte transfusion per se since the average granulocyte increment 2 hr after the granulocyte transfusion was 775 cells/cu mm (SE = \( \pm 142 \) cells/cu mm). Based on a circulating blood volume of 12–14 ml, this represented a recovery efficiency of 3.5%. Animals that died rapidly (within 4 days) had a marked granulocytopenia without recovery (not shown). Normal animals inoculated with \( E. coli \) had a profound but transient leukopenia due mainly to decreased levels of circulating lymphocytes. In these animals, full recovery of circulating counts occurred within 24 hr following the inoculum. There was no difference in the rate of recovery of circulating platelets in the surviving animals who received irradiation plus \( E. coli \) and granulocytes, compared to animals who received irradiation alone. In
normal animals, there was a significant but transient thrombocytopenia in response to the E. coli inoculum.

**Survival**

Since animals receiving granulocytes also received plasma eluted from the filter, an additional eight animals were studied to determine if there was any protective effect from this plasma. The additional animals were irradiated and received E. coli as above preceded by a transfusion (0.5 ml) of cell-free supernatant from plasma–saline eluate just prior to the injection of bacteria. Seven of these eight animals died within 4 days.

**4-day survival.** Figure 3 shows short-term survival of animals in groups 1-4. All deaths in septic animals not receiving granulocyte support (group 4) occurred on or before day 4. In group 3, two animals that succumbed had extension of life past 4 days; one death occurred on day 5 and one on day 7. Thus, 10 of 14 animals given a granulocyte transfusion were alive on day 4, whereas only 1 of 18 unprotected animals survived 4 days. The differences in 4-day survival between groups 3 and 4 were highly significant (p < 0.001 by chi-square test with Yates' correction).

**Long-term survival.** Figure 4 shows per cent long-term survival of septic animals receiving prophylactic granulocytes, compared with animals receiving
the septic challenge without granulocyte support. Only 1 of 18 irradiated animals (no prophylactic granulocytes) survived to 30 days, whereas 8 of 14 animals receiving granulocyte support survived. The difference in long-term survival between the two groups was statistically significant ($p < 0.01$ by chi-square test with Yates' correction).

DISCUSSION

The risk of sepsis in the myelosuppressed patient is inversely related to the depth of granulocytopenia.\textsuperscript{18} Granulocyte transfusion in such patients appears to be a valuable adjunct to therapy.\textsuperscript{6,9-11} Previous observations in myelosuppressed animals have confirmed that granulocyte transfusion given after established septicemia can lengthen survival.\textsuperscript{12,13,22} The studies of Debelak et al.\textsuperscript{23} also support the beneficial effects of granulocyte prophylaxis in the treatment of spontaneous infection occurring in leukopenic dogs. In the present study, a single prophylactic transfusion of granulocytes improved short-term survival (i.e., survival to 4 days) from 5.6\% to 71\% ($p < 0.001$) and cure rate from 5.6\% to 57\% ($p < 0.01$). It should be noted that none of these animals received antibiotics.

The rapid hematopoietic recovery of all surviving animals was probably not due to circulation of the exogenous transfused granulocytes since the increment at 2 hr (immediately before the septic challenge) was small. It is possible that sequestration of these granulocytes took place and that the higher granulocyte level observed at 24 hr was, in part, due to release of these cells. However, in canine studies, Herzig et al. showed that the rate of disappearance of radio-labeled transfused granulocytes from the blood was biphasic with a maximum at 3 hr.\textsuperscript{19} At 24 hr, only 3\% of the transfused granulocytes were present in the circulation. Previous studies have confirmed that endotoxin and other bacterial products can stimulate neutrophil release as well as proliferation.\textsuperscript{20-22} It appears that this mechanism was at least partially responsible for the rapid granulocyte recovery in surviving animals. Although long-term survivors were not expected from this study, the prophylactic transfusion appeared to be adequate for the support of animals through the crucial period, after which spontaneous and/or endotoxin-stimulated leukocytosis was sufficient to effect cure in most cases. It should be noted that the dose of granulocytes trans-
fused was greater than that ordinarily given to patients with sepsis.\textsuperscript{6,10,15}
This dose calculated on the basis of relative blood volume would represent a transfusion of $1 \times 10^{11}$ granulocytes in the average adult.

The results of the present study demonstrate that a single granulocyte transfusion can significantly improve survival from experimentally induced sepsis. This study suggests that there may be a possible role for prophylactic granulocyte transfusion therapy in man, especially in patients with cancer where the risk of infection is high following intensive chemotherapy or radiation therapy.

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\textbf{REFERENCES}


Prophylactic granulocyte support in experimental septicemia

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