Granulocyte Transfusion Therapy of Experimental *Pseudomonas* Pneumonia

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ABSTRACT Pseudomonas pneumonia was produced in dogs with radiation-induced leukopenia. Treatment of this infection with either gentamicin alone or gentamicin plus daily granulocyte transfusion was compared in a randomized controlled trial. The dogs receiving granulocytes plus gentamicin survived significantly longer than those treated with gentamicin alone ($P < 0.05$). The *Pseudomonas* immunotype which was inoculated into the dogs were recovered at autopsy from none of the granulocyte-transfused dogs, whereas seven or eight of the dogs treated with gentamicin alone had the inoculated *Pseudomonas* immunotype in the area of induced pneumonia at autopsy. As measured by the limulus test, the granulocyte-transfused dogs also did not have endotoxemia as frequently as the dogs given only gentamicin ($P < 0.05$). This controlled study establishes that transfused granulocytes can favorably alter the course of experimental *Pseudomonas* pneumonia and suggests that granulocyte transfusion may be a useful therapy in serious bacterial infections of leukopenic subjects.

INTRODUCTION

It is well known that patients with granulocytopenia are predisposed to bacterial infections. Despite the development of new antibiotics, treatment of infections in granulocytopenic patients is often ineffective (1, 2). *Pseudomonas aeruginosa* infections are particularly common in these patients (3), occurring in up to 25% of the subjects at risk (4).

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Granulocyte transfusion therapy is a direct approach to reconstitution of the granulocyte-deficient host. With the recent refinement of methods for obtaining granulocytes, it is now possible to collect sufficient cells to evaluate their use in treatment of infections. Several recent clinical studies suggest that granulocyte therapy is effective, but these have not been well-controlled trials (5–7). Experimental studies of granulocyte transfusions in leukopenic dogs have shown that transfused cells can alter the course of induced bacteremias (8, 9), but these studies do not indicate if granulocyte transfusions are effective therapy for an established tissue infection.

Because the complicated circumstances of the infections of granulocytopenic patients make controlled trials of granulocyte transfusion therapy in man difficult to perform, we have developed a model of *Pseudomonas* pneumonia in leukopenic dogs and studied granulocyte transfusion in this experimental setting. In a randomized controlled trial, we have established that granulocyte transfusions are an effective therapy for this model infection.

METHODS

Dogs. The dogs infected were 6–12-mo-old beagles, weighing 7–12 kg. They were chosen because of previous experience with the effects of total body irradiation on these dogs (10) and because dogs of this size could be easily handled. The dogs used as leukocyte and platelet donors were English-American foxhounds, weighing 20–30 kg. These animals, entirely unrelated to beagles, were chosen as blood product donors because of their large size and docile temperament. Beagles were not used as blood donors because their small size precluded repeated phlebotomy or leukaphoresis. All dogs were individually caged in a temperature-controlled room, and given uniform feeding and ad lib access to water.

Radiation. The dogs to be infected received 350 rads (midline tissue dose) of total body gamma irradiation from

bilateral opposing 60-cobalt sources at 46 rads/min.[1] This radiation dose produced bone marrow failure with severe leukopenia and thrombocytopenia without other serious toxicity (10). Previous studies established that this radiation dose was lethal in animals supported with only parenteral fluids and platelets and that the dogs uniformly died with pneumonia. However, if the dogs were adequately supported with fluids and platelets and given ampicillin and gentamicin, marrow recovery could occur after 3–4 wk (10).

Infection. One strain of *Pseudomonas aeruginosa* (Fisher immunotype II [no. 05142], obtained from Parke, Davis & Co., Detroit, Mich.) was used to infect all animals. The strain was maintained in semisolid nutrient media at room temperature. The antibiotic sensitivity pattern and biochemical characteristics of the organisms were constant during the 6-mo course of this study.[2] The *Pseudomonas* strain was sensitive to gentamicin at 3 μg/ml and carbenicillin at 50–100 μg/ml but resistant to most other antibiotics. The bacteria for inoculation were grown overnight in trypticase soy broth at 37°C. The morning of their use they were washed and centrifuged 2–3 times in 0.85% saline, and the washed pellet was resuspended to a concentration of 5×10^8 bacteria/ml as determined spectrophotometrically (OD at 620 nm) and confirmed by quantitative agar pour plating.

The dogs were lightly anesthetized with intravenous sodium thiamylal (Surital, Parke, Davis & Co.), and one main stem bronchus was intubated with a sterile cuffed endobronchial tube with a radiopaque tip (Metras bronchographic catheter, 19 F, Rusch, Inc., New York). Through this tube, a sterile radiopaque catheter (polyethylene tubing, 0.037 cm internal diameter, no. 6572, Becton Dickinson & Co., Rutherford, N. J.) was passed beyond the tip of the endobronchial tube as far distal in the lung as possible with gentle pressure. The exact position of the tube and catheter was immediately determined with anterior-posterior and lateral chest X rays.

After determining the tube and catheter placement, 1 ml of the bacterial suspension, containing 5×10^8 bacteria, was injected through the catheter, which was then flushed with 30 ml of air. The time for the intubation and inoculation was less than 15 min, and the duration of anesthesia less than 30 min.

Experimental design. To establish the infection model, dogs were inoculated with bacteria 4, 5, 6, and 7 days after irradiation and without prior irradiation. As controls, dogs were sham infected 6 days after irradiation by instilling 1 ml of phosphate-buffered saline without bacteria. In this series of experiments, pairs of dogs were simultaneously irradiated, and both members of a pair were inoculated with bacteria on the same day. One member of each pair received gentamicin starting 24 h after infection as described below; the other member received no antibiotic therapy.

In a second series of experiments, the effects of granulocyte transfusions were studied in a controlled randomized trial. Pairs of dogs were simultaneously irradiated, infected at 6 days post-irradiation, and identically handled until 24 h after infection. At this time, the animals were assigned by random allocation to receive either gentamicin without leukocyte support or gentamicin plus daily transfusions of at least 5×10^9 leukocytes for at least the next 7 consecutive days.

Fluids. Beginning the day of irradiation, the dogs received 500 ml of Ringer's lactate solution (Abbott Laboratories, North Chicago, Ill.) daily by subcutaneous clysis.

Antibiotics. Beginning 24 h after infection, gentamicin sulfate (1.7 mg/kg/8 h) (Schering Corp., Bloomfield, N. J.) was administered intramuscularly until death or hematologic recovery. This gentamicin dose gave mean serum levels of 9.6 μg/ml at 30 min and 5.8 μg/ml 3 h after injection, levels comparable to those observed in previous studies of gentamicin kinetics in dogs (11).

Blood products. Platelets were obtained from fresh acid-citrate-dextrose (ACD)[3] anticoagulated type A negative blood from the foxhound donors. The blood was centrifuged at 1,500 *g* for 4 min at 20°C and the platelet rich plasma removed. The platelets were concentrated by centrifuging the plasma for 10 min at 1,000 *g* at 20°C and the excess plasma discarded. All dogs were given 50–100 ml of platelet concentrate daily to keep peripheral platelet counts above 25,000/mm³. Moribund animals, however, often had lower counts despite transfusions in the last 24–36 h of life.

Leukocytes were obtained by continuous flow centrifugation as previously described (12). Indwelling arteriovenous shunts were inserted and the dogs leukaphoresed to obtain 100 ml of leukocyte-rich ACD anticoagulated suspension containing at least 5×10^9 leukocytes (3×10^9 granulocytes) by using a cell separator (NCI-IBM cell separator, Endicott, N. Y.). The donors were used for 3–7 days of repeated leukaphoresis. Before transfusion, the leukocytes and the platelet preparations were irradiated with 2,500 rads of gamma radiation from a ^{137}cesium source (Gammator M, Kewaunee Scientific Equipment Corp., Adrian, Mich.). The cells were irradiated to eliminate the possibility that they might cause graft versus host disease. Under these circumstances, although other types of white blood cells were given in the leukocyte transfusions, the granulocytes were regarded as the predominant functional cells transferred.

Clinical observations. Rectal temperatures of the dogs were measured each morning for several days before infection, at 4 h after infection and each morning thereafter. Anterior-posterior chest X rays were taken daily for the first 4 days after inoculation and at 2–3 day intervals thereafter. The animals were examined at least every 8 h to assess their general condition, to administer antibiotics, and to record deaths.

Blood cell counts. Total and differential leukocyte counts were measured before radiation and daily thereafter. Counts were also done 4 h after *Pseudomonas* inoculation and 1 h after each leukocyte transfusion. From these counts, the percent recovery of the transfused cells was calculated by multiplying the 1-h post-transfusion increments (posttransfusion count minus pretransfusion count) by the blood volume and dividing by the number of cells transfused. Leukocyte counts were not systematically studied at any other posttransfusion interval. Counts of less than 1,500–2,000 cells/mm³ were done by direct visual counting with a hemocytometer; higher counts were measured with an electronic particle counter (model Fn, Coulter Electronics, Inc., Hialeah, Fla.). Differential counts were made on air-dried Wright's stained smears. Daily platelet counts were done by phase microscopy.

[1] Radiation facilities were provided by the Armed Forces Radiobiology Research Institutes, Bethesda, Md.

[2] Kindly performed by Dr. E. Ryschenkow, Microbiology Section, Department of Clinical Pathology, NIH, Bethesda, Md.

[3] *Abbreviations used in this paper:* ACD, acid-citrate-dextrose; CML, chronic myelogenous leukemia.

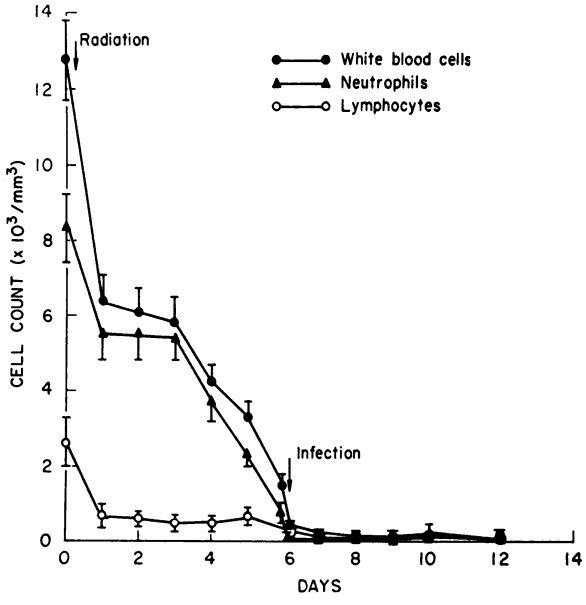

FIGURE 1 The effect of 350 rads of total body irradiation and the endobronchial inoculation of *Pseudomonas* on the total blood leukocyte, neutrophil, and lymphocyte counts. The counts shown are mean values±1 SEM for eight dogs.

Cultures, limulus tests, autopsies. Blood cultures were obtained before and 4 h after infection and daily thereafter. 5 ml of blood were inoculated into 50 ml of brain-heart infusion liquid media and the bacteria identified by standard methods. Simultaneous plasma samples were assayed for endotoxin by the limulus test (13). At autopsy, cultures of heart blood, tracheal fluid, and lung tissue were obtained. From the autopsy cultures, the *Pseudomonas* isolated were identified with type-specific rabbit antisera.[4] At autopsy, tissue from all major organs also was obtained and hematoxylin and eosin stained sections examined.

[4] Anti-immunotype *Pseudomonas* antisera were kindly supplied by Dr. Henry B. Devlin, Research and Development Division, Parke, Davis & Co. *Pseudomonas* immunotyping was kindly performed by Dr. C. H. Zierdt and Mr. Willard Williams, Microbiology Section, Department of Clinical Pathology, NIH, Bethesda, Md.

Antibody titers. Pseudomonas antibody titers were measured before and after *Pseudomonas* infection and for a group of the leukocyte and platelet donor animals. The antibody titer was determined by a bentonite flocculation method in which purified lipopolysaccharide, acid extracted from the type II *Pseudomonas aeruginosa,* was used to coat bentonite particles (14).

Statistical methods. The study was designed primarily to determine the effect of granulocyte transfusions on survival of the infected dogs. The length of survival in the transfused animals was compared to that in the controls by the sequential signed-rank test of Miller (15). This method depends on the outcome in each pair of dogs, namely, which dog survived longer and the difference in the lengths of survival. The study was ended when a significant difference ($P \leq 0.05$, one-tailed test) between the groups was established. Other differences between the groups were compared by using chi-square and two-sample Student's t tests. All summarized data are presented as arithmetic means±1 SEM.

RESULTS

Infection model. Dogs which were irradiated with 350 rads became abruptly lymphopenic and gradually granulocytopenic (Fig. 1). To study the relationship of irradiation and the blood granulocyte count to susceptibility to *Pseudomonas* infection, 11 dogs were irradiated and inoculated with bacteria 4–7 days post-irradiation. Four unirradiated dogs were also inoculated. Two dogs were sham inoculated at 6 days post-irradiation. 9 of these 17 dogs received gentamicin; the others received no antibiotics. In this small trial, there was no apparent difference in the course of the infections of the dogs given or not given gentamicin. There was also no apparent effect of gentamicin on the clearance of *Pseudomonas* from the lung, a finding confirmed in the controlled trial to be discussed below.

Dogs which did not have prior irradiation developed fever (mean temperature increase = 1.4°C) and granulocytosis (Table I) at 4 h after *Pseudomonas* inoculation. They did not have roentgenographically detectable pneumonia or pulmonary symptoms, and all survived (Fig. 2).

TABLE I

Leukocyte and Neutrophil Counts before and after Pseudomonas Inoculation for Various Intervals between Irradiation and Inoculation

Irradiation to inoculation interval	No. dogs	White blood cells per mm³		Neutrophils per mm³	
		Preinfection	4 h post-infection	Preinfection	4 h post-infection
days					
None	4	12,600±1,200	18,700±1,450	6,680±200	15,400±1,000
4	2	3,400±900	4,050±1,250	3,200±950	3,800±1,250
5	2	3,900±1,450	3,100±1,900	2,700±1,000	2,800±1,950
6	5	2,450±850	550±80	1,475±525	150±40
7	2	900±200	500±150	375±175	100±25

Values shown are arithmetic mean±1 SEM.

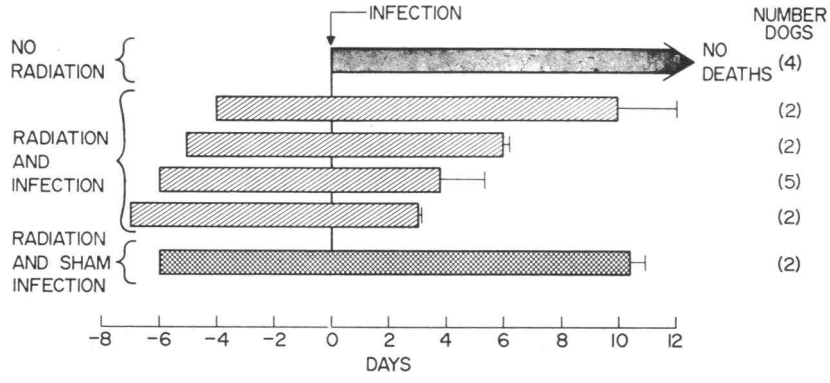

FIGURE 2 The relationship of survival after *Pseudomonas* infection to the time between radiation and infection. The left ends of the bars indicate the time of irradiation. The right ends of the bars for the lower two groups indicate mean survival (±1 SEM) for the number of animals shown.

The number of days the irradiated dogs survived after inoculation was inversely related to the interval between radiation and infection and directly related to the granulocyte counts at the time of infection (Fig. 2 and Table I). In four dogs infected 4–5 days post-irradiation (mean blood granulocytes = 2,950/mm^3), four dogs had fever (mean temperature increase 1.5°C), and three had very modest increases in their blood granulocyte counts (mean increase = 350/mm^3) at 4 h post-infection. Two had pulmonary infiltrates within 48 h. At the time of autopsy, the inoculated *Pseudomonas* immunotype was recovered from only one of these dogs. The seven dogs infected 6 and 7 days after irradiation showed a greater susceptibility to development of lethal *Pseudomonas* infection (Fig. 2). All had fever at 4 h after infection (mean temperature increase 1.2°C), and the fever lasted until their deaths. Seven had decreases in the blood granulocyte counts with infection (Table I), and six had pulmonary infiltrates within 48 h. Five of these dogs had the inoculated *Pseudomonas* immunotype isolated from the lungs at autopsy. The two dogs which were sham infected at 6 days post-irradiation did not develop fever for at least 2 days after the saline inoculation. They did not develop pulmonary infiltrates after the saline inoculation, and they survived 6 days longer than the dogs infected with *Pseudomonas* at the same interval post-irradiation (Fig. 2).

Autopsies showed that all of these irradiated dogs died with pneumonia. In the six dogs from which the inoculated *Pseudomonas* was recovered at autopsy, the area of most dense consolidation corresponded to the site of *Pseudomonas* inoculation. In the dogs from which *Pseudomonas* was not isolated, there was a diffuse bronchopneumonia from which a variety of gram-negative organisms was isolated. Histologically, these leukopenic dogs had necrosis, large clumps of bacteria, and edema in the area of pneumonia with a sparse mononuclear inflammatory response.

Preliminary to the study of the therapeutic effects of granulocyte transfusions, a dog infected at 6 days post-irradiation was given 5×9^9 leukocytes for 3 days, sacrificed, and autopsied at 2 h after the last transfusion. Dense collections of granulocytes were found in the site where the *Pseudomonas* had been inoculated but were not found in the opposite lung.

On the basis of the information from the study of these 17 dogs, it was concluded that *Pseudomonas* pneumonia could be established regularly in leukopenic but not in normal dogs. The infection seemed to be more easily established at progressively lower blood granulocyte counts. To study granulocyte transfusion therapy, infection at 6 days after radiation was chosen, since this was the earliest time when there was clearly a fall, instead of a rise, in the granulocyte counts at 4 h after infection. It appeared to be also a time when the dogs would probably survive for at least 2–3 days after infection, which would allow enough time to evaluate the effects of the treatment regimens.

Controlled trial of granulocyte transfusions. The results of eight pairs of dogs treated with gentamicin or gentamicin plus transfusions indicated that the granulocyte-transfused dogs survived longer (Fig. 3). The difference in the survival of the pairs is statistically significant ($P < 0.05$, sequential signed-rank test). In the one instance in which the dog of the granulocyte transfused group died first, the dog was thrombocytopenic, presumably because he had become alloimmunized to his platelet donor. He died from an intestinal hemorrhage. This was the only death in either group due to bleeding. There were two long-term survivors, both in the transfused group, living to full hematopoietic recovery. Three dogs in the gentamicin alone group died

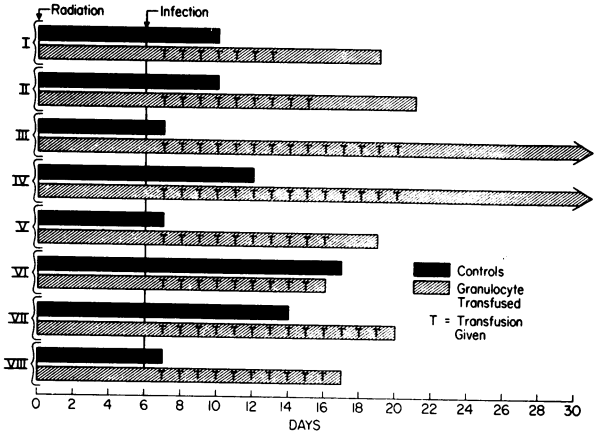

FIGURE 3 The effect of granulocyte transfusions on survival for pairs of dogs simultaneously infected with *Pseudomonas*. The right end of the bars indicate time of death, the two arrows indicate indefinite survival. The "T" indicates days transfusions were given.

the day after infection; two of these died before granulocyte transfusions were actually begun in the other member of the pair. If these two pairs are excluded from the analysis of the data, the difference between the groups is still significant ($P = 0.05$, sequential signed-rank test).

Pseudomonas bacteremia was, in general, not a consequence of this pulmonary infection. Only 1 of 16 blood cultures obtained at 4 h after infection was positive for *Pseudomonas*. 14 of a total of 183 daily blood cultures (7.6%) were positive for *Pseudomonas*. Eight dogs had no blood cultures positive for *Pseudomonas*. For all blood cultures, a total of 83 of 183 cultures (45%) were positive for some bacterial growth and often for more than one organism. The frequency of certain organisms were: *Clostridia* 28%, *Pseudomonas* 7.6%, *alpha streptococci* 6.5%, *Escherichia coli* 4.4%, *Staphylococcus epidermidis* 3.8%, and other aerobic gram-negative rods 3.3%. Undoubtedly, some of the blood cultures were contaminated by skin bacteria. The overall frequency of blood cultures positive for any bacterial growth was not different in the two treatment groups ($P > 0.05$, X^2 test).

The limulus test was negative before infection in all dogs and positive in only 1 of 16 dogs at 4 h after infection. For the period after infection that both dogs of each pair were alive, when a direct comparison of the effects of the treatments on this test could be made, 11 of 28 daily limulus tests were positive in the gentamicin alone group, whereas none of 28 tests were positive in the granulocyte-transfused group ($P < 0.05$, X^2 test). For this same time period, there were three blood cultures positive for *Pseudomonas* in the gentamicin alone group and none in the transfused group. As previously mentioned, six of the eight transfused dogs eventually died, five of these developed positive limulus tests before death. The circumstances of their deaths will be discussed below.

At autopsy, the inoculated *Pseudomonas* immunotype was recovered from none of the transfused dogs, whereas the *Pseudomonas* was grown from the lungs and tracheas of seven of eight of the dogs receiving gentamicin alone. Each of these seven dogs had pneumonia in the area in which the *Pseudomonas* had been inoculated. The one dog receiving gentamicin alone which did not have *Pseudomonas* at autopsy had an *Escherichia coli* pneumonia. In the granulocyte transfused group, three dogs died 3–6 days after granulocytes were arbitrarily discontinued; all three had pneumonia attributable to gram-negative organisms other than *Pseudomonas*. In two other dogs, deaths occurred at a time when the granulocyte recipients may have become sensitized to the granulocytes of the unrelated donors, as indicated by the absence of an increase in the white blood cell count after the leukocyte transfusions. The other transfused dogs which died had an intestinal hemorrhage as already mentioned. Histologic examination of autopsy tissues regularly showed extensive necrotizing bacterial pneumonia to be the anatomic course of death. Acute passive congestion of the liver and submucosal edema of the small intestine were also frequently observed.

Comparisons of the temperatures, white blood cell counts, and platelet counts of the two groups showed no significant differences before infection or 4 h after infection (Table II) ($P > 0.05$, Student's t tests). Interestingly, the two early deaths in the control group mentioned previously were the dogs with the lowest leukocyte counts both before and after infection. Comparisons

TABLE II

Comparison of Leukocyte Counts, Platelet Counts for the Two Treatment Groups and Temperatures

	Gentamicin	Gentamicin and granulocyte transfusion
Blood leukocytes, *per mm*^{3}		
Preinfection	1,425±288	2,252±372
4 h post-infection	519±110	781±193
Platelets, *per mm*^{3}		
Preinfection	243,000±36,800	192,000±28,900
24 h post-infection	96,000±21,500	98,900±14,500
Mean (infection to death)	69,800±24,700	97,800±13,000
Temperatures, °C		
Preinfection	38.6±0.14	38.5±0.13
4 h post-infection	40.8±0.27	40.2±0.20
Mean (infection to death)	39.8±0.18	39.5±0.14

* Values are arithmetic mean±1 SEM.

of the temperatures and platelet counts of the two groups were not different to the time of death ($P > 0.05$, Student's t tests) (Table II).

Four dogs in each group clearly developed roentgenographically visible pulmonary infiltrates. Two of the transfused dogs cleared their infiltrates while receiving cells, whereas in none of the dogs given gentamicin alone did the infiltrates disappear.

The geometric mean titers of agglutinating antibodies for the type II *Pseudomonas* were the same before and after infection (mean before 1:7.5, range 1:2–1:16, mean after 1:8.3, range 1:2–1:32). This same mean antibody titer was also found in a group of the leukocyte donor animals.

Granulocyte transfusions. In these dogs, there were no obvious adverse effects of repeated granulocyte transfusions. The dogs did not developed pulmonary reactions manifested by cough or tachypnea. Pre- and posttransfusion rectal temperatures were usually the same, and X ray examination in several dogs showed no acute roentgenographyically detected effect of the transfusions.

86 transfusions were administered; an average of $(12.0\pm0.6) \times 10^9$ (mean±1 SEM) leukocytes ($[5.6\pm 0.4] \times 10^9$ granulocytes) was given. The mean increase in the recipient blood leukocyte count at 1 h post-transfusion was 830±100 cells/mm^3. The corresponding granulocyte count increase was 580±80 cells/mm^3. Expressed as a percent recovery (12), an average of 4.8% of the total leukocytes transfused and 7.2% of the granulocytes transfused were present in the circulation at 1 h after transfusion.

DISCUSSION

The possibility of using granulocyte transfusions to treat infections in granulocyte-deficient subjects has been considered for many years (16, 17). The chief problem was the lack of techniques for obtaining sufficient cells from the blood of normal individuals to substantially raise the blood granulocyte counts (18). This problem led numerous investigators to study the use of patients with chronic myelogenous leukemia (CML) as leukocyte donors (19–21). Clinical trials suggested that leukocytes could reduce fever and favorably influence the course of infections in leukopenic subjects. It was also shown that CML leukocytes localized at sites of infections (22). The possibility that these transfusions might lead to transplantation of the leukemia (23) and transfer of occult infections prompted development of methods for obtaining granulocytes from normal subjects (24). Although recent clinical studies have suggested that repeated transfusions of normal granulocytes will increase survival of leukopenic patients with infections (5–7), definitive controlled trials in man have not yet been performed.

This study was undertaken to determine if granulocyte transfusions have a clearly beneficial role in treating an experimental infection. *Pseudomonas* pneumonia was chosen as the model infection for study in order to simulate a commonly encountered clinical problem. The results of this randomized controlled trial indicated that granulocyte transfusions significantly prolonged survival, promoted clearance of inoculated *Pseudomonas*, and prevented or delayed the development of endotoxemia as measured by the limulus test. These results strongly suggest that granulocyte transfusions may be generally useful for treating infections in the setting of severe granulocytopenia.

In this study, it was decided arbitrarily to give at least 1 wk of daily granulocytes and to study primarily the effects of the transfusions on the acute infection acquired at the onset of severe leukopenia. Although granulocytes plus gentamicin clearly altered the clearance of the inoculated *Pseudomonas* and prolonged life, three dogs died while still receiving granulocyte transfusions. There are several possible reasons for these deaths. First, the number of granulocytes given these dogs was only about 20% of the number normally utilized by dogs of this size each day (25). Only an average of 7.2% of these transfused cells were present in the circulation at 1 h after transfusion. Thus the number of cells given may have been only marginally adequate to permit the dogs to survive the prolonged period of leukopenia. Second, since unmatched donors and recipients were used, it is also possible that the recipients became alloimmunized to the donor leukocytes and that the transfusions given late in the course of therapy were less effective than those given shortly after infection. Finally, since gentamicin was the only antibiotic administered, the infections from which the dogs died may have been caused by bacteria resistant to gentamicin. Unfortunately, antibiotic sensitivities for the bacterial isolates were not done.

These studies showed that granulocyte transfusions prevented or delayed the development of a positive limulus test, probably by delaying the development of endotoxemia. Granulocytes (26) and buffy coat cells (27) are known to concentrate endotoxin and degrade it (28). They appear to be more effective for detoxifying endotoxin than macrophages (29). The effects of the granulocyte transfusions on the limulus tests could be attributable either to a local effect of the granulocytes on tissue bacteria which prevents endotoxin from reaching the blood or the cells could serve to concentrate and degrade the blood endotoxin. In either case, this effect of granulocytes in preventing endotoxemia may

be a critical reason for the prolonged survival of the transfused animals.

In this trial, therapy of *Pseudomonas* infection with gentamicin and gentamicin plus granulocytes was compared by using a *Pseudomonas* organism which was sensitive to gentamicin at antibiotic concentrations which are readily attainable in serum. Gentamicin alone was clearly an ineffective treatment, as indicated by the recovery at autopsy of the inoculated *Pseudomonas* from seven of eight dogs given this therapy. It has been suggested that antibiotics acting against bacterial cell walls, e.g. carbinicillin or ampicillin, are more effective than the antibiotics which inhibit protein synthesis, e.g. the aminoglycosides, for treatment bacterial infections in granulocytopenic subjects (1, 30). From this suggestion, it follows that granulocyte transfusion therapy should be compared with other antibiotic regimens. Furthermore, other antibiotics should be tried in concert with granulocyte therapy in order to evaluate more precisely how effective granulocyte therapy may be. Studies to compare such treatment regimens in this animal model are now in progress.

This model of *Pseudomonas* pneumonia in leukopenic dogs was developed in order to study the therapy of this infection in a controlled fashion. It is hoped that this clear demonstration of an effective role of granulocyte transfusion therapy in an animal model will prompt further clinical studies to define the specific circumstances in which granulocyte transfusions are of benefit in man.

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