The Effect of Leukocyte Transfusions on Experimental Bacteremia in the Dog

By Robert B. Epstein, Reginald A. Clift and E. Donnell Thomas

Severe granulocytopenia induced by drugs, irradiation or primary disorders of the hemopoietic system frequently results in uncontrollable sepsis and death. Attempts at treating or preventing sepsis in such patients have generally involved the use of antimicrobial drugs and, more recently, the exploration of sophisticated methods of patient isolation and environmental bacterial control.1,2 Despite these efforts, infection remains the most common cause of death in the patient with marrow failure.3,4

Granulocyte replacement therapy offers a rational approach to this problem but has been limited by the difficulties of granulocyte procurement and storage and by the short life span of these cells, particularly in the infected individual. In addition, the potential hazards of homologous leukocyte infusions include production of graft-versus-host disease.5 Despite these limitations, initial efforts at infusing granulocytes into septic leukopenic patients have suggested that temporary control of bacteremia can be achieved.6,7 In view of the complexities of the clinical setting and the technical problems of designing controlled studies of the application of leukocyte infusions in preventing or controlling sepsis, an in vivo animal model system for evaluating the potential usefulness of leukocyte transfusions would be helpful.

The present studies were carried out with the following aims: (1) to develop a standard system for producing gram-negative septicemia in the leukopenic dog, (2) to evaluate the effect of leukocyte transfusions on the course of septicemia using fresh or irradiated leukocytes obtained from normal dogs and (3) to apply the NCI-IBM continuous flow centrifuge as a method of leukocyte procurement from normal donors.

Material and Methods

Hemopoietic failure was produced in dogs by exposure to 1200 r. of whole body irradiation delivered by opposing 60Co sources at a dose rate of 9.2 r./minute, measured at the midline in air.8 The development of hemopoietic failure and its reversibility by success-

From the Department of Medicine, University of Washington School of Medicine and the U.S. Public Health Service Hospital, Seattle, Washington.

This investigation was supported by Research Grants CA 10167 and AM 02215, National Institutes of Health, and RH 00311, National Center for Radiological Health, U.S. Public Health Service.

First submitted April 14, 1969; accepted for publication July 10, 1969.

R. B. Epstein, M.D.: Assistant Professor of Medicine, University of Washington School of Medicine. R. A. Clift: Research Associate, University of Washington School of Medicine. E. D. Thomas, M.D.: Professor of Medicine, University of Washington School of Medicine; supported by Research career program award 1-K6-AI-2425 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U.S. Public Health Service.
ful isogeneic or allogeneic bone marrow grafts have previously been described. By the fourth or fifth day following irradiation leukocytes fell rapidly to below 1000/mm³ while platelets were still above 100,000/mm³. Animals with total leukocyte counts below 1000/mm³ were accepted for study on day 4 or 5. During the postirradiation period, animals routinely received 300 ml. of Ringer’s solution subcutaneously per day as fluid replacement for postirradiation vomiting or diarrhea.

Bacterial challenge was carried out with a strain of E. coli serogroup 06 obtained from a blood culture during the course of a fatal spontaneous septicemia arising in an irradiated dog. The challenge inoculum consisted of a 5 mm. loopful of an overnight broth culture suspended in 1 ml. of sterile isotonic saline. The mean dose of viable organisms inoculated was 32.7 \times 10^6 (S.D. 11.2). Blood cultures were pour-plated with trypticase soy agar and incubated aerobically at 37 C. for 24 hours before counting. The volume of blood cultured was 1 ml. When high bacterial counts were anticipated, appropriate blood dilutions were prepared.

The bacterial inoculum was injected into the right jugular vein. Blood samples were removed from the left jugular vein before and at the following time intervals after bacterial challenge: 2, 10, and 30 minutes; 1, 2, 4, 8 and 24 hours; and twice daily thereafter.

Dogs used as leukocyte donors weighed 8 to 15 Kg. On the day prior to leukocyte procurement, the animals were anesthetized with pentobarbital and an arterial venous shunt was established between the carotid artery and jugular vein by the insertion of indwelling teflon silastic cannulas as previously described. Leukocyte procurement was carried out using the NCI-IBM continuous flow centrifuge. Details of the centrifuge and its operation have been presented elsewhere. Blood entered the centrifuge from the arterial side of the shunt, and following the collection of buffy-coat cells the combined red cell and plasma fractions were returned to the donor through the venous cannula. Flow rate through the centrifuge was maintained at 40 ml./min. Leukapheresis was carried out over a 2-hour period, during which time the donor received 100 i.u./Kg. of sodium heparin. Cell-free plasma was centrifuged at 2000 rpm for 20 minutes before infusion. In studies of irradiated leukocytes, 1000 r. \( ^{60} \text{Co} \) irradiation was delivered at a dose rate of 100 r./min. to the leukocyte concentrates before administration.

Five experimental groups of dogs were studied:

- **Group I**—Five normal unirradiated dogs received the bacterial challenge and were observed for 1-2 weeks.
- **Group II**—Seven dogs were given 1200 r. of whole body irradiation 4 days prior to bacterial challenge.
- **Group III**—Five pairs of dogs received the bacterial challenge 4 days following irradiation. One dog of each pair received an infusion of freshly collected homologous leukocytes 2.5 hours following the injection of bacteria, while leukocytes were withheld from the second member of the pair.
- **Group IV**—Seven dogs were inoculated with bacteria 4 days following irradiation. In this group, freshly collected homologous leukocytes were infused 26 hours after bacterial challenge. Only dogs with demonstrable bacteremia 24 hours after challenge were admitted to this group.
- **Group V**—Five pairs of dogs were irradiated together and injected with bacteria 5 days following irradiation. One dog of each pair received an infusion of irradiated homologous leukocytes 2.5 hours after the bacterial challenge. At the same time, the partner received an infusion of an equal volume of cell-free plasma from the leukocyte donor.

**RESULTS**

Table 1 details the volume and cellular content of the 35 consecutive 2 hour leukocyte collections made for this study. Leukocyte counts in recipient animals showed an average rise of 2,160 mm³ 2 hours following transfusion consisting predominantly of granulocytes. Leukocyte kinetic studies were not performed as part of these experiments. The colony counts obtained in 5 normal dogs
challenged with the bacterial inoculum (Group I) are detailed in Table 2. Clearance of bacteria occurred in all cases within 30 minutes, and no circulating bacteria could be demonstrated during the subsequent 96 hours. The dogs remained free of clinical signs during the period of observation. A transient leukopenia was observed with counts returning to normal within 8 hours.

Thirty of 46 dogs subjected to 1200 r. of irradiation were found suitable for study at 4 days. Eleven dogs were excluded because white cell counts were over 1000/mm³ and 5 dogs died within the 4 day postirradiation period. The colony counts for 7 dogs receiving bacterial challenge 4 days postirradiation (Group II) are shown in Table 2. All dogs had completely cleared the bacteria by 2 hours. Three of 7 dogs required a longer period to remove the bacteria than the dogs of Group I. The subsequent course of events in these dogs is summarized in Figure 1. Dog 543 died in less than 24 hours with sterile blood cultures. At autopsy, the cause of death was considered to be pneumonia, and samples of cardiac blood were sterile. The other 6 dogs died with E. coli septicemia, the blood cultures having become positive by 24 hours in 4 and within 60 hours in 2. The mean survival time after bacterial challenge was

Table 1.—Volume and Cellular Content of 35 Two-hour Collections with the NCI-IBM Centrifuge

<table>
<thead>
<tr>
<th></th>
<th>Range</th>
<th>Mean</th>
<th>S. D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mls.)</td>
<td>80-130</td>
<td>101</td>
<td>13</td>
</tr>
<tr>
<td>RBC volume (mls.)</td>
<td>2.0-12.8</td>
<td>7.6</td>
<td>2.8</td>
</tr>
<tr>
<td>Total leukocytes (× 10⁹)</td>
<td>4.4-36.9</td>
<td>20.7</td>
<td>8.7</td>
</tr>
<tr>
<td>Band forms (× 10⁹)</td>
<td>0-5.0</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Polymorphs (× 10⁹)</td>
<td>1.7-22.5</td>
<td>10.0</td>
<td>6.9</td>
</tr>
<tr>
<td>Lymphocytes (× 10⁹)</td>
<td>1.1-19.8</td>
<td>6.3</td>
<td>3.4</td>
</tr>
<tr>
<td>Monocytes (× 10⁹)</td>
<td>0.3-4.4</td>
<td>1.6</td>
<td>1.0</td>
</tr>
<tr>
<td>Eosinophils (× 10⁹)</td>
<td>0-3.9</td>
<td>1.6</td>
<td>1.0</td>
</tr>
<tr>
<td>Platelets (× 10⁹)</td>
<td>12.5-80.0</td>
<td>43.9</td>
<td>21.2</td>
</tr>
</tbody>
</table>

Table 2.—Clearance of Injected E. coli from the Blood of Normal (Group I) and Irradiated (Group II) Dogs

<table>
<thead>
<tr>
<th>Bacterial Inoculum (× 10⁶)</th>
<th>Colonies per ml. blood</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 minutes</td>
</tr>
<tr>
<td>Group I</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>8,000</td>
</tr>
<tr>
<td>8</td>
<td>1,400</td>
</tr>
<tr>
<td>30</td>
<td>4,990</td>
</tr>
<tr>
<td>30</td>
<td>12,500</td>
</tr>
<tr>
<td>10</td>
<td>1,100</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>7,200</td>
</tr>
<tr>
<td>30</td>
<td>4,000</td>
</tr>
<tr>
<td>30</td>
<td>16,000</td>
</tr>
<tr>
<td>10</td>
<td>3,200</td>
</tr>
<tr>
<td>10</td>
<td>5,200</td>
</tr>
<tr>
<td>58</td>
<td>10,000</td>
</tr>
<tr>
<td>33</td>
<td>22,000</td>
</tr>
</tbody>
</table>
51 hours. Thus, leukopenic irradiated dogs were able to clear injected bacteria from the bloodstream, but a persistent bacteremia subsequently developed, usually within the first 24 hours. Bacteria isolated at this time were of the same serogroup as the inoculum.

The effect of transfusion of homologous leukocytes 2 hours following challenge on the course of bacteremia (Group III) is illustrated in Figure 2. Each treated animal was paired with an untreated control simultaneously challenged with the same bacterial inoculum. In each experiment, the survival of the transfused dog (mean 88 hours) was longer than that of its non-transfused partner (mean 65 hours). The difference in survival times is significant ($p < 0.01$). Dog 599 had a transient bacteremia of 6 organisms per ml. at 24 hours and was the only transfused dog with a positive blood culture during the first 48 hours after bacterial clearance. Subsequent blood cultures from this dog were sterile until 72 hours. The onset of persistent bacteremia in the transfused dogs was much delayed compared with the nontransfused dogs, each of which developed a rapidly increasing bacteremia by 30 hours. The mean time of reappearance of progressive bacteremia in the latter was 18 hours, compared with 74 hours for the transfused dogs, a significant difference ($p < 0.0005$). In 2 leukocyte treated dogs (572 and 577) terminal bacteremia was due to a different serogroup of E. coli than originally inoculated. Figure 2 also shows the number of granulocytes infused into each treated dog, and the results suggest that larger doses of granulocytes are more effective than smaller ones in delaying bacterial recrudescence. The course of events in Dog 599 makes it difficult to draw any firm conclusions on this point.

In the dogs of Group IV, an attempt was made to evaluate the effect of leukocytes infused after the reappearance of bacteremia. Figure 3 details the data obtained in this group of dogs, all of which had demonstrable bacteremia at 24 hours prior to the infusion of leukocytes at 26 hours. Three of 7 dogs cleared the pre-existing bacteremia after leukocyte infusion. The mean survival
Fig. 2.—The course of bacteremia in Group III dogs.

time of Group IV dogs was 84 hours, compared with 60 hours for the 9 dogs in Group II and III which had demonstrable bacteremia at 24 hours and received no leukocytes. Although the difference in these mean survival times is significant at the $p < 0.001$ level, the earlier reappearance of bacteremia in control animals does not permit precise assessment of comparative survival.

An evaluation of the effect of leukocytes exposed in vitro to 1000 r. of irradiation was carried out in Group V animals. This study was done on the fifth postirradiation day, and included a control plasma treated animal for each dog infused with leukocytes. Figure 4 summarizes the findings in the study. In 4 of 5 pairs of animals, bacterial reappearance occurred later, and survival was longer in those animals receiving irradiation leukocytes. A serogroup of E. coli differing from the original challenge organism was responsible for the terminal bacteremia in dogs 688 and 701. Both of these dogs received leukocyte transfusions. The mean time for the appearance of persistent bacteremia was 54 hours for dogs receiving irradiated leukocytes and 25 hours for those receiving plasma. The difference is significant ($p < 0.005$).
**Discussion**

Under ordinary circumstances in the uncompromised host, introduction of small inocula of bacteria into the bloodstream through trauma or surgical procedures may frequently be handled by host defences without the need for medical intervention. The precise mechanism for rapid clearance of bacteria and the factors that lead to the development of clinical sepsis are unknown. The finding that lethally irradiated dogs do not have gross impairment of bacterial clearance conforms to published experience with other animals. Gordon et al. using challenge with Klebsiella pneumoniae in leukopenic rabbits demonstrated that the initial clearance of bacteria was followed by the reappearance of a persistent lethal bacteremia. The present study in the dog also demonstrates a consistent pattern of response to E. coli challenge in leukopenic dogs. The normal dogs in Group I were able to sterilize the bloodstream rapidly and permanently. Irradiated leukopenic animals showed some impairment of initial clearance, but still exhibited a period without bacteremia. This period was followed by the early reappearance of fatal bacteremia. The crucial role of leukocytes in these events was demonstrated by the clear prolongation of life associated with the extended period of blood sterility seen in those animals given a single injection of leukocytes 2 hours after bacterial challenge. In those dogs infused with leukocytes after the
bacteria had reappeared (Group IV), sterility of the blood again occurred in 3 of 7 instances, and overall prolongation of life was suggested. Thus, a beneficial effect was apparent after a single infusion of homologous leukocytes, either during the latent phase, or in the presence of overt sepsis. The mechanisms involved in the development of infections in patients with leukopenia of differing etiologies may vary. However, the present findings lend support to data in man suggesting clinical improvement in infected leukopenic patients following leukocyte infusion. Moreover, they suggest that the quantities of leukocytes obtainable from a hematologically normal donor may be useful, particularly before overt infection occurs. The normal donor animals used in this study showed no ill effects from the procedure.

The dogs of Group V demonstrated the in vivo effectiveness of irradiated leukocytes. The significance of these findings rests on the potential hazards of fresh transfusions in the immunologically suppressed recipient. Proliferation of infused immunologically competent cells may result in a clinical syndrome of the graft-versus-host type. The graft-versus-host reaction following homologous white cell infusions has been described in animals and in man.14,15

Fig. 4.—The course of bacteremia in Group V dogs.
Irradiation of the leukocytes prior to infusion may offer a method of avoiding this complication.\textsuperscript{16}

The canine system described in this report was adequate for the demonstration of the efficacy of leukocyte transfusions in the leukopenic host. Challenges with other bacteria, assessment of antimicrobial drugs, and evaluation of dose and timing of leukocyte infusions could be studied. In addition, leukocyte kinetics and the effectiveness of leukocyte preservation techniques could be evaluated in this in vivo system.

**SUMMARY**

A system was developed in the irradiated dog for evaluating the effectiveness of fresh or irradiated homologous leukocyte infusions during the course of induced bacterial sepsis. Dogs with white cell counts below 1000/mm\textsuperscript{3} 4 or 5 days following 1200 r. whole body irradiation were challenged with an intravenous inoculum of E. coli. Untreated animals showed an initial period of bacterial clearance followed within 24 hours by the reappearance of bacteremia. Dogs given a single transfusion of homologous leukocytes following bacterial challenge had a significantly longer period of blood sterility and subsequent survival. Leukocytes irradiated with 1000 r. were effective in extending the period of blood sterility and in prolonging life. It was concluded that the irradiated leukopenic dog challenged with a bacterial inoculum provides a model for demonstrating the usefulness of homologous leukocyte infusions.

**SUMMARIO IN INTERLINGUA**

Esseva disveloppate un systema in le irradiate can pro evalutar le efficacia de fresc o irradiate infusiones de leucocytos homologe durante le curso de inducite sepse bacterial. Canes con numerationes leucocytic de infra 1000 per mm.\textsuperscript{3} como resultato de irradiation del corpore total con 1200 r. applicate 4 o 5 dies previemente esseva provocate con un inoculo intravenose de Escherichia coli. Canes assi tractate sed non protegite per le infusion de leucocytos monstrava un periodo initial de clearance bacterial sequite intra 24 horas per le reaparition de bacteriemia. Canes additionalmente subjicite a un sol transfusion de leucocytos homologe habeva un significativamente plus longe periodo de sterilitate del sanguine e de superviventia subsequente. Leucocytos irradiate con 1000 r. etiam esseva efficace in extender le periodo del sterilitate de sanguine e in prolongar le vita del animal. Es conclusionate que le irradiate can leucopenic subjicite a un provocation consistente in un inoculo bacterial provide un modello pro demonstratar le utilitate de homologe infusiones leucocytic.

**ACKNOWLEDGMENT**

The authors wish to thank Doctor Marvin C. Turck for advice and assistance in typing the strains of E. coli, and Mr. T. C. Graham, Miss Marilyn T. Murphy and Mrs. P. McKenna for skilled technical assistance.

**REFERENCES**

1. Bodey, G. P., Loftis, J., and Bowen, E.: Protected environment for cancer pa-
2. Levitan, A. A., and Perry, S.: Infec-
The Effect of Leukocyte Transfusions on Experimental Bacteremia in the Dog

ROBERT B. EPSTEIN, REGINALD A. CLIFT and E. DONNALL THOMAS