Experimental Candidiasis in Neutropenic Dogs: Tissue Burden of Infection and Granulocyte Transfusion Effects

By Herbert S. Chow, Suleyman C. Sarpel, and Robert B. Epstein

Neutropenic dogs with systemic candidiasis were studied to establish quantitative relationships between tissue burdens of microorganisms and the kinetics of transfused granulocytes. Three groups of dogs rendered neutropenic by chemotherapy were evaluated. Group I consisted of 9 control dogs that were not challenged with Candida albicans; group II, 6 dogs challenged with $10^6$ C. albicans; group III, 7 dogs challenged with $10^7$ C. albicans. Granulocytes obtained from random donors were transfused 24 and 48 hr after fungal challenge in groups II and III and at comparable times in noninfected controls. Serial granulocyte counts obtained following each transfusion were compared for 1-hr increments and half-time disappearance ($T'/2$). At autopsy, 24 hr following the last transfusion, tissues were quantitatively assayed for C. albicans.

Although granulocyte transfusion therapy is used in the management of neutropenic patients, criteria for employment of this blood component remain poorly defined and controversial.\(^1,2\) Difficulties exist in establishing quantitative relationships between tissue antimicrobial effects of transfusions during the course of infections of varying intensity and measurable kinetics of granulocytes, such as half-time disappearance and increments achieved following transfusion. Studies in animal models and in man have suggested that such relationships exist but detailed in vivo data are lacking.\(^3,4\) The utilization and effectiveness of granulocytes in mild and severe infection and the relationship of transfusion dose, blood increments achieved, and tissue microbicidal effects need to be defined.\(^5,6\) Using a model of induced systemic candidiasis in neutropenic canines, Ruthe et al. clearly showed that measurable reductions in tissue infection occurred following a course of granulocyte transfusions.\(^9\) Dogs infected with Candida albicans have also been examined for quantitative transfusion effects in local as well as systemic disease.\(^10\) In the present studies, two challenge inocula differing by tenfold were investigated to determine the utilization of granulocytes as measured by the difference of granulocyte levels before and after transfusion (increments) and half-time survival in the circulation of transfused granulocytes. Quantitative relationships of antimicrobial effects, transfusion requirements, and increments after transfusion were determined.

MATERIALS AND METHODS

Induction of Neutropenia

Mongrel dogs weighing between 7 and 23 kg were dewormed, immunized against distemper and hepatitis, and observed for 2 wk prior to use. They were rendered neutropenic by an intravenous injection of 1,3-bis (2-chloroethyl)-nitrosourea (BCNU), 5 mg/kg body weight, followed by a dose of 50 mg/kg cyclophosphamide 24 hr later. Animals received Ringer's solution during periods of anorexia following drug administration. To prevent intercurrent bacterial infection, penicillin G (10,000 U/kg body weight) and gentamicin (1.5 mg/kg body weight) were administered twice daily.

Preparation of C. albicans Inoculum

A culture of Candida albicans obtained from a patient was maintained at 4°C on blood base agar plates. Twenty-four hours before C. albicans was administered, a single yeast colony was inoculated into each of two tryptic soy broth tubes and incubated at 37°C for 24 hr. The candida suspension was adjusted to $10^6$ or $10^7$/ml as determined by hemocytometer count. The inocula were verified quantitatively by the pour plate technique using Mycobiotic agar (Difco Laboratories, Detroit, Mich.).\(^9\)

Granulocyte Procurement and Transfusion

Granulocytes were procured from normal healthy dogs by intermittent flow leukapheresis (Haemonetics Corp., Natick, Mass.) using trisodium citrate as the anticoagulant and hydroxyethyl starch as the sedimenting agent.\(^11\) Buffy coat cells were collected at a rate of 20 ml/min for 3.5 min. After three periods of collection, an additional separation was performed at 25 ml/min for 6 min to reduce the total fluid volume.\(^13\) Transfusions were administered within 10 min.

Experimental Design

Five days following drug administration, neutropenia (granulocytes <500/cu mm) was observed in all dogs. Three groups of...
animals were studied. Group I consisted of 8 control neutropenic dogs receiving no C. albicans challenge; group II, 6 dogs challenged with $10^6$ C. albicans i.v.; group III, 7 dogs challenged with $10^7$ C. albicans. Granulocytes collected from random donors were infused at 24 and 48 hr after fungal challenge in groups II and III and at comparable times in group I.

Granulocyte increments were determined by blood sampling at 1, 3, 6, 8, and 24 hr after transfusion and were expressed as differences between granulocyte counts/cu mm before and after infusion. One-hour granulocyte increments adjusted for numbers of granulocytes transfused/kg were expressed as:

Corrected granulocyte increment/cu mm

\[
= \frac{\text{1 hr Granulocyte increments/cu mm}}{\text{Granulocytes transfused} \times 10^8/\text{kg}}
\]

Quantitation of C. albicans From Tissues

Twenty-four hours after the second transfusion, all dogs were autopsied. Tissue sections from the kidney, liver, and spleen were obtained for culture and processed as previously described. Between 0.5 and 1 g of each tissue was homogenized and diluted with 10 ml of 0.9% NaCl solution. The candida were quantitated by the pour plate method using Mycobiotic agar. Cultures were carried out at dilutions of 1:50 to 1:1000 and results expressed as the mean colony count of duplicate plates. The coefficient of variation between duplicate plates was 11%. Colonies isolated on agar plates were randomly identified by germ tube formation in normal dog serum and stained for the distinct morphology of candida by Gram’s method.

Statistical Analysis

Comparisons of circulating half-time and increments of granulocytes were analyzed by Student’s group t test. Logarithmic regression analysis (one-tailed) was employed to study the quantitative relationship between the number of yeasts recovered per gram tissue and the posttransfusion increments or the number of granulocytes transfused.

RESULTS

Granulocyte Kinetics

The granulocyte counts of the dogs in the study were consistently less than 50/cu mm prior to transfusion. Table I summarizes the 1-hr granulocyte increments observed following transfusion in the three groups of dogs. When increments were expressed either as numbers/cu mm or corrected for numbers of cells transfused, no significant difference was found between groups I and II. A significant decrease ($p < 0.05$) was observed for increments achieved following transfusions to the dogs challenged with $10^7$ organisms (group III). Changes in circulating granulocyte counts following transfusion are shown in Fig. 1 and the disappearance of granulocytes from the circulation plotted in Fig. 2 as a percentage of the 1-hr increments. While the increments and circulating half-time of granulocytes in groups I and II dogs are similar, group III dogs show significant reductions ($p < 0.05$) in both these measurements (increment = $218 \pm 46$/cu mm, T'/2 = $2.1 \pm 0.3$ hr).

Bacteriologic Data

The relationships between the tissue burden of C. albicans and transfusion therapy are shown in Figs. 3 and 4. Each point represents the Candida albicans colony count per gram tissue expressed as the arithmetic mean of organisms recovered from the liver, kidney, and spleen. Figure 3 relates the total number of granulocytes transfused per kilogram to the number of colonies per gram tissue for each level of tissue burden. The relationships between C. albicans and transfusion therapy are shown in Figs. 3 and 4. Each point represents the Candida albicans colony count per gram tissue expressed as the arithmetic mean of organisms recovered from the liver, kidney, and spleen. Figure 3 relates the total number of granulocytes transfused per kilogram to the number of colonies per gram tissue for each level of tissue burden.

Table 1. Granulocyte Increments Following Transfusion in Neutropenic Control and C. albicans Infected Dogs

<table>
<thead>
<tr>
<th>Group</th>
<th>Numbers of Transfusion</th>
<th>Granulocytes Transfused ($\times 10^8$/kg)</th>
<th>1-hr Increment/cu mm</th>
<th>Corrected† Increment/cu mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>16</td>
<td>5.0 ± 0.7</td>
<td>888 ± 116</td>
<td>204 ± 26</td>
</tr>
<tr>
<td>II</td>
<td>12</td>
<td>3.0 ± 0.6</td>
<td>682 ± 85</td>
<td>202 ± 27</td>
</tr>
<tr>
<td>III</td>
<td>14</td>
<td>4.6 ± 0.6</td>
<td>218 ± 46</td>
<td>50 ± 11</td>
</tr>
</tbody>
</table>

*Data expressed as mean ± SE.
†Corrected increments/cu mm = (1 hr granulocyte increments/cu mm)/(granulocytes transfused $\times 10^8$/kg).
infection studied. Tissue infection was observed at both levels of *C. albicans* challenge and at all doses of granulocytes administered. Approximately a tenfold difference in tissue levels of organisms occurred between groups II and III dogs. A clear association of antimicrobial effect (\( p < 0.01 \)) was demonstrated between the number of granulocytes administered per kilogram body weight and residual tissue infection. This was true for groups II and III animals. A significant negative correlation (\( p < 0.05 \)) was also demonstrated (Fig. 4) between the 1-hr granulocyte increments/cu mm and the number of colonies per gram tissue. When each tissue was considered separately, significant negative correlations were observed for the kidney and spleen. Such relationships could not be demonstrated in the liver due to a low recovery of *C. albicans*. Even in the heavily infected dogs of group III, which demonstrated a reduction in increments

![Graph showing survival of circulating granulocytes expressed as percent of 1-hr posttransfusion increments.](image)

**Fig. 2.** Survival of circulating granulocytes expressed as percent of 1-hr posttransfusion increments (mean ± SE).

![Graph showing regression analysis for *C. albicans* cultured from tissue and total number of transfused granulocytes administered in two consecutive transfusions.](image)

**Fig. 3.** Regression analysis showing inverse relations of *C. albicans* cultured from tissue and total number of transfused granulocytes administered in two consecutive transfusions. Six points shown for \( 10^4 \) inoculum.
following transfusion, the increase in granulocyte counts reflected tissue antimicrobial potential.

**DISCUSSION**

The present study addresses several biologic questions relevant to the effect of granulocyte transfusions. Under the standard conditions of this experimental model it could be demonstrated that although systemic tissue infection exists (group II), the kinetics of transfused granulocytes, as measured by increments achieved and the half-time of survival in the circulation, are not significantly altered as compared to the uninfected host. These findings are in accord with transfusion data in animals and suggestive evidence in man that the presence of tissue infection does not necessarily preclude achieving satisfactory granulocyte increments or circulating survival times.14,15 On the other hand, severe systemic infection, as present in group III, was shown to result in significantly reduced increments and half-life of transfused granulocytes. Accelerated utilization of granulocytes sufficient to eliminate increments in granulocytes following transfusion has been inferred from experimental data as well as clinical findings in severely infected nonleukopenic and leukopenic hosts.3,5,16 In the present study, some increments following transfusions were detected in group III dogs with severe systemic infection.

The quantitative relationship of granulocyte transfusion dose and tissue burden of infection is well established in the present studies. This relationship holds within the limits of the tenfold differences of microbial challenge. A linear inverse correlation exists between the number of granulocytes transfused per kilogram body weight or granulocyte increments and the colonies of organisms obtained per gram of tissue.

In man, studies of leukocyte transfusions using large quantities of granulocytes obtained from patients with chronic granulocytic leukemia (CGL) demonstrated a correlation between the number of cells transfused, granulocyte increments, and the clinical improvement of infected neutropenic patients.17,18 Responses were observed following transfusions of $10^9 \times 10^{10}$ CGL granulocytes. With a dose of $5 \times 10^{10}$, increments of 1000/cu mm were demonstrated, while significant increments failed to occur when less than $10^8$ cells were transfused.17 With available leukapheresis techniques and normal donors, $1-2 \times 10^{10}$ granulocytes are usually collected.12,19,20 These numbers of granulocytes may be of only marginal value. In canine studies, transfusion of $10^{10}$ granulocytes into 20-kg recipients results in increments of about 1000/cu mm.10 Presumably, size accounts for much of the difference between human and canine increments. Findings in pediatric patients of a relation between granulocyte dose and increments emphasize the importance of the size of the recipient.21 Pfleger and coworkers have established a positive correlation between the blood granulocyte increments and accumulation of transfused granulocytes in the oral cavity of a child with mucous membrane ulcer following 14
separate transfusions. Hence, the failure to demonstrate the effectiveness of granulocyte transfusions in adults may well be a technologic problem in procurement of adequate numbers of cells from normal donors.

Since the antimicrobial activities of transfused granulocytes cannot readily be assessed in human studies, extrapolations of the data obtained from group II dogs were used to speculate on potential quantitative effects of granulocyte transfusions in patients. Calculations are based on the following assumptions: (1) the clinical effectiveness of the administration of \(10^6\) canine granulocytes in a 20-kg neutropenic dog may be comparable to the transfusion of \(5 \times 10^6\) granulocytes to a 70-kg man; (2) the intravascular kinetics of transfused granulocytes may be comparable to the noninfected and infected neutropenic dog. Table 2 summarizes the analysis of the quantitative data correlating the dose of transfusion, the posttransfusion blood increments, and the effects on the tissue infection. In order to clear 90% of the candida in the tissues during a 2-day course of transfusions, a dose of \(4 \times 10^6\)/kg/day of granulocytes would have to be administered to maintain daily posttransfusion increments of 800/cu mm in dogs with moderate candidiasis. On the other hand, doses of \(1.35 \times 10^8\) granulocytes/kg/day could produce blood increments of 200/cu mm and clear about 50% of the candida in the tissues. The transfusion of two doses of 1–2\( \times 10^6\) granulocytes into a 70-kg man could, according to the canine data, have the potential of eliminating 50% of the fungi in the tissues. This dose of granulocytes would produce granulocyte increments following transfusion of less than 500/cu mm, which is consistent with the findings in clinical transfusion experience.7,8

As illustrated in the current studies, the number of granulocytes transfused is a crucial element in the therapeutic response to granulocyte transfusions. Current controversies regarding optimal employment of granulocytes are not likely to be resolved until technologic progress can provide more cells for infusion.

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