Efficacy of Granulocyte Transfusions in the Control of Systemic Candidiasis in the Leukopenic Host

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An experimental canine model was designed to evaluate the effect of granulocyte transfusions on systemic infection with Candida albicans in the granulocytopenic host. Each of a pair of dogs was rendered granulocytopenic with a single intravenous (i.v.) dose of cyclophosphamide (50 mg/kg body weight) and challenged with $10^6$ Candida albicans organisms administered i.v. when granulocyte counts were $\leq 500/\text{mm}^3$. Granulocytes procured by leukofiltration were infused into six experimental dogs 1, 24, 48, and 72 hr after challenge with Candida. An average of $13 \pm 1.3 \times 10^9$ granulocytes were administered per infusion, producing an average 1-hr increment of $588 \pm 146$ granulocytes/\text{mm}^3 over the pretransfusion granulocyte count. Experimental and control dogs were killed 96 hr after challenge and organs examined grossly and by quantitative culture techniques to measure the extent of infection. All animals receiving granulocyte transfusions had significantly less tissue infection than nontransfused controls ($p < 0.05$). It was concluded that granulocyte transfusions are effective in reducing the severity of infection by Candida albicans during periods of leukopenia.

Increasing numbers of reports support the efficacy of granulocyte transfusion therapy for infected leukopenic patients, although some controversy persists regarding the usefulness of this blood component. In part, problems with assessment of the proper employment of granulocyte therapy involve the heterogeneity of clinical circumstances surrounding transfusion attempts, differences in procurement techniques, and difficulties in evaluating granulocyte kinetics following transfusions.

In an attempt to assess critically the effects of granulocyte transfusion for prophylactic or therapeutic intervention during spontaneous or induced infections in leukopenic hosts, a canine model has been developed. Effects of granulocyte transfusions on Escherichia coli and Pseudomonas aeruginosa sepsis in dogs have been documented. In recent years better control of bacterial infection has resulted in an increased incidence of fatal fungal infection associated with granulocytopenia. The major agent involved has been Candida albicans. To date no systematic studies have been performed regarding the role of granulocyte transfusions in systemic candidiasis, although recent evidence has shown that human granulocytes are capable of killing pseudohyphal Candida even though unable to completely engulf the organism. Preliminary studies from this laboratory have indicated that a normal dog can tolerate up to $10^7$ Candida organisms administered intravenously, while...
10^6 organisms invariably results in widespread disseminated candidiasis in leukopenic animals.\textsuperscript{11}

The present study was carried out to determine the effect of multiple granulocyte transfusions on induced systemic candidiasis in leukopenic dogs.

\section*{MATERIALS AND METHODS}

\textbf{Dogs.} Healthy mongrel dogs weighing 15-25 kg were immunized against distemper and hepatitis and dewormed. Pairs of dogs were used for each experiment, with random selection of control animals and those receiving granulocyte transfusions.

\textbf{Production of leukopenia.} Dogs were rendered leukopenic by a single intravenous (i.v.) injection of cyclophosphamide 50 mg/kg body weight. Animals were supported with physiologic saline for anorexia following drug administration. A single dose of cyclophosphamide in these experiments consistently produced leukopenia from day 5 to day 9, when the animal was killed. Carbenicillin (250 mg/kg body weight) and gentamicin (2.5 mg/kg body weight) were administered twice daily to prevent intercurrent bacterial infections.

\textit{Candida albicans} originally isolated from the blood of a burn patient was stored at 4°C on blood agar base plates. A single colony was inoculated into each of three tryptic soy broth tubes 24 hr before candida was to be administered. The following day the \textit{Candida} organisms were washed three times with 0.9% NaCl and adjusted to a concentration of 1.0 × 10^6/ml by hemocytometer counts. Pour plates were also made to verify the hemocytometer counts. Inoculum (yeast phase) (1 ml) was injected i.v.

\textbf{Granulocytes for transfusion} were obtained from healthy donor dogs by continuous-flow filtration leukapheresis as described by Debelak et al.\textsuperscript{11} All granulocytes collected were transfused into the experimental dog.

\textbf{Experimental model.} Six pairs of dogs were studied. Both dogs of a pair received identical treatment except for transfusions. Each dog was injected with a single dose of cyclophosphamide (50 mg/kg) on day 0. Five days later the animals were granulocytopenic (≤500 granulocytes/mm^3 blood). On day 5 each received an i.v. injection of yeast phase \textit{Candida albicans} (10^6 organisms/1 cc saline). One hour after the \textit{Candida} challenge the first transfusion was administered to each of the experimental dogs. A total of four transfusions were administered to each experimental dog, one each on four successive days (days 5-8) at 24-hr intervals.

\textbf{Clinical observations.} Total and differential white blood counts were obtained daily. Quantitative peripheral blood cultures were obtained immediately prior to Candida challenge and 5 and 60 min after injection of the inoculum. Quantitative blood cultures were taken each morning from day 6 until the animal was killed. White cell counts and differentials were performed on each dog each morning and 1 hr after transfusion on the experimental dogs.

\textbf{Autopsies.} On day 9 after peripheral blood samples were taken, both experimental and control dogs were killed. Colonies of \textit{Candida} grossly visible were counted on the various organs and graded as follows: 0, no \textit{Candida} lesions visible on the organ surface or in a random cross-section; +, one to ten colonies; ++, more than ten colonies. Tissue sections from heart, lung, liver, spleen, and kidney were obtained at autopsy for culture. The organ samples were homogenized and diluted in sterile saline for quantification by the agar pour plate method and expressed as colonies/gram tissue weight. Isolated organisms were identified by germ tube test.

\textbf{Statistical analysis.} The Wilcoxon rank sum test for paired experiments (two-tailed test) was employed, and \chi^2 analysis was employed for the analysis of gross pathologic data.

\section*{RESULTS}

\textbf{Granulocyte data.} The average pretransfusion granulocyte count for the four days of transfusion (n = 24) for the experimental dogs was 147 ± 48 (SE) granulocytes/mm^3. There was no significant difference between this number and the mean number of granulocytes the nontransfused dog possessed during the same time intervals, 71 ± 26 granulocytes/mm^3. The mean number of granulocytes transfused into the experimental dog was 13.0 ± 1.3 × 10^6 (range
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The 1-hr posttransfusion blood sample of the experimental dogs showed a mean granulocyte count of $739 \pm 154 \text{ mm}^3$. The mean increment in granulocytes/$\text{mm}^3$ ($588 \pm 146$) from pretransfusion to 1-hr posttransfusion levels was significant ($p < 0.001$).

Figure 1 shows the granulocyte counts of the transfused dogs as compared to the control dogs on each of 4 days granulocyte transfusions were given. There was no significant difference between control and experimental pretransfusion counts. Significant granulocyte increments occurred 1 hr after transfusion in the experimental dogs on each day ($p < 0.05$).

Pathologic data. Table 1 illustrates gross colony counting data of the organs examined at autopsy. In no instance were more lesions visible on the organs of a transfused dog compared to its nontransfused partner. Of 30 pairs of organs examined, the transfused dogs had fewer tissue lesions visible in 24. Only 1 of 30 organs in the experimental group had more than ten lesions visible, while 16 of 30 organs in the control group had more than ten lesions. No gross lesions were present in 20 of 30 organs of the experimental animals, while only 5

| Table 1. Gross Evaluation of Organs Examined at Autopsy From Nontransfused and Transfused Leukopenic Dogs |
|---|---|---|---|---|---|---|---|
| Experiment No. | 1 | 2 | 3 | 4 | 5 | 6 |
| | Organ | NT | T | NT | T | NT | T | NT | T | NT | T |
| Spleen | 0 | 0 | + | 0 | + | 0 | + | 0 | + | 0 | + |
| Kidney | + + | + | + + | + | + | + | + | + | + | + |
| Heart | + + | 0 | + + | 0 | 0 | 0 | 0 | 0 | + | 0 | + |
| Liver | + + | + | + | 0 | + | 0 | + | + | + | + | + |
| Lung | 0 | 0 | + | 0 | 0 | 0 | + | 0 | + | 0 | + |

0, no Candida lesions visibly present; +, one to ten lesions present; + +, more than ten lesions present.

* Nontransfused.

† Transfused (four daily granulocyte transfusions).
organs in the control group had no gross lesions. These differences were significant ($p < 0.01$). Microscopic examination confirmed the typical morphologic feature of *Candida* pseudoabcess formation. Although not quantitated, granulocyte infiltration of lesions appeared more prominent in transfused dogs.

**Bacteriologic data.** All animals had negative blood cultures (both fungal and bacterial) at the time of *Candida* challenge, were positive for *Candida* 5 min after challenge, and reverted to negative at 60 min. All subsequent blood samples continued to be negative, as were all autopsy blood samples.

Despite negative blood cultures, widespread candidiasis was found at autopsy. Figure 2 compares the numbers of organisms cultured from the various tissues sampled in the control and transfused animals. In all tissues of transfused animals a significant reduction in the numbers of candida was found ($p < 0.05$). The greatest reduction occurred in spleen and lung, where there was more than a three-log difference between control and experimental animals. Liver, heart, and kidney showed a one-log reduction in number of organisms.

**DISCUSSION**

The incidence of *Candida albicans* infection in marrow-suppressed patients has increased in recent years. This has resulted in increased morbidity and mortality despite the proper employment of available antibiotics.$^8,13$ The technology of granulocyte transfusions is such that repeated transfusions are feasible, but effectiveness in controlling the spectrum of bacterial and fungal infections has not been rigorously shown in human studies. An animal system where conditions can be controlled is therefore desirable.
The present study clearly shows that therapy with multiple granulocyte transfusions procured by the leukofiltration technique significantly reduced the number of Candida organisms infecting the leukopenic canine host as compared to the nontransfused control animals. Spleen and lung tissue showed a thousandfold reduction in the number of Candida organisms cultured from the transfused animals as compared to the controls (Fig. 2). Liver, heart, and kidney tissue showed a tenfold reduction in numbers of organisms as a result of granulocyte transfusions. It has been suggested that the hyperosmolality of the renal medulla may play a role in diminished ingestion and killing of Candida by granulocytes. This may explain in part the lower reduction in Candida organisms in the kidney.

All animals were killed at the same time after challenge, rather than followed for survival, in order to obtain precise microbiologic data in these paired studies. It is generally accepted that the severity of infection is associated with the number of organisms in the individual. Since the number of organisms in animals receiving granulocyte transfusions was greatly reduced, one would predict longer survival for these animals. The physical appearance of the animals would seem to bear this out; in all cases the nontransfused dog appeared lethargic or moribund, unable to stand, while most of the transfused animals remained active and alert.

Despite the demonstrated beneficial effect of the 4 days of granulocyte transfusions, established lesions persisted in treated animals. Whether or not granulocyte transfusions alone could be curative at lower fungal inocula and the effect of combined therapy with antibiotics and granulocytes remain to be explored. In addition, one cannot extrapolate the present data in a precise manner to the transfusion requirements that may occur in the varied clinical settings associated with disseminated candidiasis. Nevertheless, the present finding supports a role for granulocyte transfusions in leukopenic patients infected with Candida albicans.

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