Pathophysiology of *Candida albicans* Meningitis in Normal, Neutropenic, and Granulocyte Transfused Dogs

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

*Candida albicans* meningitis was induced in normal dogs and in dogs rendered neutropenic (<500/cu mm) in order to follow cerebrospinal fluid (CSF) granulocyte migration patterns, quantitative fungal cultures, and clinical events. Dogs received an intrathecal challenge with 10⁷ *C. albicans*, and CSF samples were monitored. Neutropenia was produced by administration of cyclophosphamide 5 days before fungal challenge. Granulocytes for transfusion were derived from normal donors by semicontinuous centrifugation. In normal dogs, CSF neutrophilic pleocytosis began 3 hr after fungal challenge and reached a maximum within 24 hr. These dogs cleared their infection and remained clinically well. Nontransfused neutropenic dogs failed to show granulocyte responses in CSF and died within 84 hr of meningitis. In a controlled study of 8 pairs of neutropenic dogs, one partner received a single granulocyte transfusion 24 hr following fungal challenge. Transfusions were followed by significant increments (p < 0.025) in CSF pleocytosis compared to controls that correlated with concurrent peripheral blood increments (r = 0.8). Six of eight transfused dogs survived longer than their nontransfused partners. Four dogs that had received prior immunization with donor tissue failed to show CSF granulocyte increments following transfusion. It was concluded that: (A) the model provides for assay of in vivo kinetics of transfused granulocytes during a local infection, (B) in normal dogs the initial CSF granulocyte response is associated with resolution of the infection; (C) transfused granulocytes migrate into the CSF in proportion to peripheral blood increments; and (D) infusion of granulocytes into sensitized recipients demonstrates no biologic effect.

Infection with opportunistic organisms remains a major source of morbidity and mortality in the leukopenic host. Improved antibiotic therapy and the introduction of granulocyte transfusions as prophylactic or therapeutic support have contributed to recovery of most patients from initial episodes of intercurrent bacterial infections. However, the participation of granulocyte transfusions in controlling clinically defined local and systemic infections with specific organisms remains uncertain.

Canine models for the study of such specific infections with gram-negative bacteria have documented a role for granulocyte transfusion therapy. Exploration of mycotic infections, where diagnosis is often difficult and available antibiotics are of limited value, may also be useful. Increasingly, fungal infections, particularly with *C. albicans*, have emerged as a significant clinical problem in patients with prolonged periods of leukopenia due to chemotherapy. Information regarding a possible role for granulocyte transfusions in invasive candidiasis have been derived from the in vitro demonstration that granulocytes can kill pseudohyphal forms of the organism and recent in vivo canine studies indicating reduced severity of systemic candidiasis in transfused leukopenic dogs.

The present studies were performed in order to assess, in a more precise fashion, the granulocyte kinetics involved in the pathophysiology of a locally induced *C. albicans* infection in normal, leukopenic, and granulocyte transfused dogs. The central nervous system was selected as a site that permitted quantitative evaluation of cellular, biochemical, and microbial events not readily available from other tissues. Movement of granulocytes into the CSF following intrathecal challenge could be monitored in normal dogs and in neutropenic animals following transfusion from normal donors. In addition, the results of granulocyte transfusion into sensitized recipients could be assessed.

**MATERIALS AND METHODS**

**Dogs**

Mongrel dogs weighing between 10 and 25 kg were dewormed and immunized against hepatitis and distemper. All dogs were observed for 3–4 wk prior to use and appeared in good health.

**Production of Leukopenia**

Dogs were rendered leukopenic by a single intravenous injection of cyclophosphamide (50 mg/kg body weight). Animals were supported with physiologic saline for anorexia or vomiting following drug administration. A single dose of cyclophosphamide in these experiments consistently produced neutropenia (<500/cu mm) by day 5 following administration. Tricarcillin (15 mg/kg) was administered twice daily when white counts were below 1000/cu mm.

**Candida albicans Inoculum**

*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 candida organisms were washed 3 times with 0.9% NaCl and adjusted to a concentration of 10⁷/ml by hemocytometer count. Counts were verified quantitatively by the pour plate technique using mycobiotic agar and blood agar base. Under pentobarbital anesthesia, cisterna
magna punctures were performed with a 20-gauge 1.5 in needle. After an initial CSF specimen was obtained, 10⁵ C. albicans diluted in a volume of 1 ml of saline were injected intrathecally.

**Granulocyte Procurement and Transfusion**

Healthy dogs were used as granulocyte donors. Granulocytes were procured by semicontinuous flow centrifugation (Haemonetics Corp., Natick, Mass.) using trisodium citrate as the anticoagulant and hydroxyethyl starch as the sedimenting agent. Buffy coat cells were collected at a rate of 20 ml/min for 4 min. After 3 periods of collection, an additional separation was performed that included the buffy coats originally obtained at 25 ml/min for 6 min in order to concentrate the total fluid volume. Transfusions were administered over a period of 10 min.

**Experimental Design**

Three studies were performed. Study 1 included 6 normal dogs challenged intrathecally with an inoculum of 10⁵ C. albicans. Cerebrospinal fluid was serially examined for total leukocytes and differential count, protein, sugar, and quantitative C. albicans culture. The clinical course was monitored in 5 dogs, the sixth being sacrificed for histologic study 72 hr postchallenge. In study 2, dogs were rendered neutropenic with 50 mg/kg body weight of cyclophosphamide. Preliminary serial monitoring of the CSF was performed in 5 dogs. Eight pairs of neutropenic dogs were then randomly allocated so that one member of the pair received a granulocyte transfusion 24 hr after C. albicans challenge and the other member served as a nontransfused control. Increments in 3-hr posttransfusion CSF and peripheral blood granulocyte counts were determined and compared to controls. In study 3, 4 dogs were immunized by 4 weekly 50-ml whole blood transfusions followed by a skin graft from the corresponding donors 1 mo prior to the experiment. The recipients were given a fifth injection of 50 ml whole blood from the same donor 7 days before the administration of cyclophosphamide and fungal challenge. Leukoagglutination by undiluted recipient serum was detected in all 4 instances when tested against donor granulocytes.

**Autopsies**

Autopsies were performed on all dogs, and tissues were examined grossly and histologically. Brains were removed in their entirety and sections prepared from the basilar meninges and ventricular walls. Slides were stained with hematoxylin-eosin and methenamine silver.

**Statistical Analysis**

The comparison of CSF granulocyte increments in transfused and nontransfused dogs was determined by Student’s t test. Linear regression analysis was employed to determine correlations of CSF with peripheral blood increments following transfusion and associations between survival and CSF increments in transfused dogs. Paired survival data were analyzed by the Wilcoxon signed rank test.

**RESULTS**

**Cerebral Spinal Fluid Findings in Normal and Neutropenic Dogs Challenged With C. albicans**

The CSF of 16 neutropenic and 6 normal dogs contained no granulocytes prior to fungal challenge and were comparable for levels of protein and glucose. In Fig. 1, the kinetics of granulocyte migration in normal animals following C. albicans challenge are illustrated. Concurrent quantitative fungal cultures from CSF are also shown, and the median values are plotted. In these normal animals, pleocytosis was evident by 3 hr (median 28; mean ± SE, 144 ± 114) consisting predominantly of polymorphonuclear leukocytes that peaked within the first 24 hr (median 6525; mean 6119 ± 790) and subsequently gradually declined. Following an initial exponential clearance of organisms, a plateau was reached with subsequent complete clearance of C. albicans by 72 hr. Table 1 compares the migration of granulocytes over a period of 72 hr in leukopenic versus normal dogs. An absence of granulocyte response was seen in the neutropenic animals. The initial decline of CSF candida counts in leukopenic dogs paralleled that seen in normals (Fig. 2). In contrast to normal dogs that showed eventual fungal clearance and recovery, leukopenic animals demonstrated persistently positive CSF cultures, clinical deterioration with signs of meningitis, and died within 4 days of challenge. Figure 3 illustrates the invasive meningeal infection noted at autopsy in the neutropenic dogs. CSF protein elevation and glucose depression paralleled the course of the disease in the two groups studied, as shown in Fig. 4. Neutropenia
clearly failed to influence the typical biochemical diagnostic findings associated with CNS infections.

Granulocyte Transfusions in Neutropenic Dogs

Eight pairs of dogs were rendered granulocytopenic with 50 mg/kg of cyclophosphamide. All dogs had less than 500 granulocytes/cu mm 5 days later and were challenged intrathecally with $10^7$ C. albicans. Twenty-four hours after challenge, one member of the pair received a single granulocyte transfusion while the other member served as a nontransfused control. Transfusions consisted of between 2.4 and $9.0 \times 10^8$ granulocytes/kg body weight. Table 2 summarizes the pretransfusion and 3-hr posttransfusion peripheral blood and the CSF increments compared to control dogs examined at the same time intervals together with survival of individual animals. All transfused dogs had significant blood and CSF granulocyte increments compared to their nontransfused partner ($p < 0.025$). In addition to the appearance of granulocytes in the CSF, a good correlation between peripheral blood and CSF increments existed ($r = 0.8$, $p < 0.05$). In 6 of 8 pairs, transfused dogs lived longer than nontransfused controls ($p = 0.054$), suggesting possi-

Table 1. Median CSF Granulocyte Counts / cu mm in Normal and Neutropenic Dogs Post C. albicans Challenge

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Normal Median</th>
<th>Range</th>
<th>n</th>
<th>Neutropenic Median</th>
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<td>4</td>
<td>2</td>
<td>0-426*</td>
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</table>

*Two dogs showed early marrow recovery at day 8 following cyclophosphamide injection.

Fig. 2. Median Candida albicans counts in CSF of normal and neutropenic dogs following intrathecal challenge.

Fig. 3. Methenamine silver stain of leptomeninges and underlying cortex in a neutropenic dog dying 72 hr after Candida albicans challenge. The invasive characteristics of the infection are illustrated. Original magnification, $\times 1000$.

Fig. 4. CSF glucose and protein levels in normal and neutropenic dogs following challenge with Candida albicans.
Survival benefit from the single granulocyte transfusion. When survival of transfused dogs was analyzed separately, a correlation of the duration of survival with CSF increments was present ($r = 0.76, p < 0.05$).

**Granulocyte Transfusion in Sensitized Recipients**

Four sensitized dogs with positive leukoagglutinin crossmatches against donor granulocytes were rendered neutropenic and received a single granulocyte transfusion from the corresponding donor 24 hr following challenge. Figure 5 compares increments of granulocytes seen in transfused nonsensitized, sensitized, and nontransfused dogs. Mean granulocyte counts at 3 hr in blood and CSF averaged $588 \pm 181$ and $440 \pm 172/cu \ mm$, respectively, in nonsensitized animals as compared to sensitized dogs in which blood and CSF granulocytes averaged 0 and $3 \pm 3/cu \ mm$.

**DISCUSSION**

Candidiasis is an uncommon infection of the CNS, often due to hematogenous spread from a primary focus. Difficulties in establishing experimental bacterial or fungal meningitis in animals has been a barrier in studying the pathogenesis of the disease. Direct inoculation of the subarachnoid space with pathogenic organisms remains the method of choice for consistently producing inflammation of the meninges. In the present study, intrathecal challenge with *C. albicans* was selected in order to produce a central nervous system infection. Events occurring during infection, particularly the migration of granulocytes across the blood–brain barrier in normal animals and in neutropenic dogs in which granulocyte transusions were given, could be quantitatively monitored. Following challenge with *C. albicans*, it was apparent that a system for clearing these organisms did exist. An initial exponential clearance rate analogous to that seen following the systemic challenge with *C. albicans* in both normal and neutropenic dogs was observed. Moxon et al. showed that *Hemophilus influenzae* penetrating the dura after intranasal inoculation was cleared from the subarachnoid space. Evidence in

<table>
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<tr>
<th>Dog No.</th>
<th>Granulocytes Transfused x 10^6/kg</th>
<th>Blood Granulocytes (per cu mm)</th>
<th>CSF Granulocytes (per cu mm)</th>
<th>Postchallenge Survival* (hr)</th>
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<td></td>
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<td>3 hr Posttransfusion</td>
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</table>

*Limits of time when death may have occurred.

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**Table 2. Pre- and Posttransfusion Blood and CSF Granulocyte Counts 24 hr Post *C. albicans* Challenge**

![Fig. 5. Blood and CSF 3 hr posttransfusion, granulocyte increments (mean ± SE) in transfused, nontransfused, and sensitized recipients.](image-url)
lizards and rats show that arachnoid-associated macrophages may be responsible for the initial bacterial or fungal clearance.20,21 Similar to the events following systemic challenge with C. albicans in neutropenic dogs, initial microbial clearance occurred in the CSF. In the absence of granulocytes, however, eradication of the infection failed, and progressive meningitis was noted in all animals. Spontaneous resolution was associated with the migration of granulocytes into the CSF of normal animals beginning at 3 hr postchallenge. Smith et al. have shown the crucial importance of early granulocyte migration in limiting the extent of tissue infection.22 The appearance of CSF neutrophil pleocytosis provides a model for testing the efficacy of transfusion with granulocytes collected by different methods. In the present studies, following granulocyte transfusion, significant migration of the cells across the blood–brain barrier into the inflammatory site was evident. Granulocytes appearing in the CSF posttransfusion correlated best with peripheral blood granulocyte increments achieved in individual dogs. Contrary to reports that the demonstration of granulocyte increments are unimportant for clinical effectiveness, the present studies suggest that leukocyte increments following transfusion are a significant factor for assessing what may be accomplished at the tissue level.23,24 This may be analogous to platelet transfusion therapy in which the achievement of platelet increments in the peripheral blood is a necessary prerequisite to effective patient management.25,26

Neutrophils have the capability to phagocytize and kill the yeast phase of C. albicans.27 Recent in vitro studies have also indicated that granulocytes are active in the killing of the mycelial phase of the C. albicans.28 These findings were confirmed by in vivo observations of significant reductions in the level of tissue infection following granulocyte transfusion in neutropenic dogs challenged systemically with C. albicans.29 Based on clinical data, the study of multiple transfusion schedules in the candida model will be important to clearly demonstrate therapeutic effectiveness.30 In the present study, survival of transfused dogs suggested that a single transfusion was of some value. A more intensive granulocyte transfusion schedule will be necessary to precisely define the benefits of such therapy in this model. However, a correlation could be demonstrated between the duration of survival and posttransfusion peripheral blood or CSF granulocyte increment.

The adverse effects of circulating antileukocyte antibody on the appearance of transfused granulocytes in sites of infection were demonstrated. An intensive immunization schedule was used in these dogs to assure high levels of antileukocyte antibody. Sensitized dogs, in addition to showing only minimal increments after granulocyte transfusion, had an absence of granulocytes in the CSF. This confirms previous work in canines suggesting the importance of presensitization and the necessity for developing adequate cross-matching procedures in multitransfused individuals to detect leukocyte antibodies.28,29

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