Effect of Thermal Injury on the Adherence of *Candida albicans* to Murine Splenic Tissue

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In a mouse model of thermal injury, an increase in burn size produced a decrease in the ratio of *Candida albicans* cells adherent to the marginal zone to those adherent to the white pulp of the spleen, an increase in the number of *Candida* cells in the circulation and in the kidneys, and an increase in mortality.

Adherence of *Candida albicans* to host tissues can have two consequences—one favors the host, the other favors the microorganism. On the one hand, *C. albicans* attachment is a critical initial step in the subsequent phagocytosis and killing of yeast (11). This process, which depends upon polymorphonuclear neutrophils and macrophages fixed in the liver, lungs, spleen, bone marrow, and adrenals, is the primary means of clearance of systemic *Candida* (17). On the other hand, attachment to nonphagocytizing cells is a crucial first step in the establishment of a *Candida* infection (12). Hence, any factor which could influence whether *Candida* adheres to a phagocytic cell versus a nonphagocytic one could significantly impact the outcome of *Candida* exposure.

Using an ex vivo adherence assay which has been shown to accurately reflect the adherence pattern of *Candida* in vivo following candidemia (8), studies have indicated that in normal mice *C. albicans* adheres quite specifically and in large numbers to the macrophage-rich marginal zone of the spleen (8, 13). In contrast, in mice that are immunosuppressed either genetically, by irradiation, or by drugs, either *Candida* adherence in the marginal zone decreases or the relative distribution of *Candida* cells in the spleen shifts so that the ratio of *Candida* cells in the marginal zone to those in the white pulp is decreased (5, 6, 14). This decrease in *C. albicans* binding in the marginal zone is related to a decrease in the ability of the host to clear the *Candida* organisms and therefore to an increase in susceptibility of the mice to fatal candidiasis (6, 18).

Burns cause immunosuppression in patients (1, 4, 9, 21) and increase their susceptibility to candidiasis (2, 10). Using a burned-mouse model, we have determined that a 12% total body surface area (TBSA) burn increases the susceptibility of mice to fatal candidiasis (15). Here, we ask if progressively larger burns (0 to 12 to 24% TBSA) will progressively increase fatalities caused by *Candida* and if these fatalities will be associated with a progressive change in *C. albicans* adherence in the spleens of the burned mice.

All animal procedures were approved by the University of Cincinnati Institutional Animal Care and Use Committee and utilized female Hsd:NSA (CF-1) mice (Harlan Sprague Dawley, Indianapolis, Ind.). Burn size was calculated with Meeh’s formula (19) with the constant 8.95 for female mice (3) and an average mouse weight of 27 g. Hair was clipped from the backs and sides of the mice, and the animals were anesthetized with methoxyflurane (Metofane; Pitman-Moore, Inc., Mundelein, Ill.). For the 12% TBSA burn, a Teflon template with a 1-by 1.5-in. window was placed in the center of the back, the window was filled with 0.5 ml of ethanol, and the ethanol was ignited and allowed to burn for 10 s. For the 24% TBSA burn, the template was first applied from the midline of the dorsum down the right side of the mouse and then the procedure was repeated for the left side. Immediately postburn, all mice received 1.0 ml of saline intraperitoneally as fluid replacement therapy.

To determine whether burn size would affect the susceptibility of burned mice to fatal candidiasis, 45 mice were divided into three equal groups, which received no burn (0% TBSA), 12% TBSA burns, or 24% TBSA burns. All groups were challenged intraperitoneally immediately postburn with 1.3 × 10⁷ CFU of *C. albicans* CA-1 (courtesy of Jim Cutler, Montana State University, Bozeman, Mont.). As burn size increased from 0 to 12 to 24% TBSA, survival decreased (Fig. 1). Comparing lengths of survival for the three groups by the Kruskal-Wallis test indicated overall significant differences (P < 0.0001) among the groups, with Wilcoxon tests showing significant differences for all possible comparisons: 0 and 12% TBSA (P < 0.004), 0 and 24% TBSA (P < 0.0002), and 12 and 24% TBSA (P < 0.03).

To determine if the deaths could be attributed to *C. albicans* dissemination and fatal sepsis, quantitative cultures (16) of the blood and of a target organ, the kidneys, were performed for dissemination and fatal sepsis, quantitative cultures (16) of the blood and of a target organ, the kidneys, were performed for six mice each in the 0% and the 24% TBSA groups. Samples were taken at 36 h postchallenge, a time when survival in the 24% TBSA group was steadily decreasing (Fig. 1). While there were very few yeast cells in the circulation of the unburned animals (1.0 ± 1.0 CFU/ml), there were a measurable number in the blood of the burned mice (112.5 ± 48.7 CFU/ml). In addition, while there was only 2.0 log₁₀ units of *Candida* in the kidneys of the unburned mice, there was significantly (P < 0.002) more (5.5 log₁₀ units) in these target organs of the burned animals.

To establish the ex vivo adherence assay in our laboratory, normal (0% TBSA) mice were injected with luconyl blue dye (no. 7080; courtesy of Greg Perrone, BASF Corp., Rensselaer, N.Y.), sacrificed, and processed in an ex vivo assay (8, 20). As reported by others (5, 6, 14, 20), the blue dye localized in the vascular areas, readily allowing distinction between marginal zones and white pulp, and the *C. albicans* cells heavily concentrated in the marginal zones of the spleen sections (Fig. 2A).

To determine whether a burn affected the adherence of *Candida* to splenic tissue, mice were given a 0, 12, or 24% TBSA burn and spleens were harvested 3 days later. For quan-
In the white pulp, marginal zone, or red pulp, and the total number of Candida cells per section was calculated as the sum of the yeast cells in all three areas. If the counts by the two technicians were ±30% of the average of the two counts, then the counts were considered to agree and were accepted. Occasionally, the divergence of the counts was >30%, in which case the section was recoded and given back to both technicians for recounting. If the difference was still >30%, then that section was eliminated from the study. Less than 10% of the sections were eliminated. Examination of the eliminated sections indicated that these sections were of poor technical quality, i.e., exhibited shredding, poor staining, etc., thereby contributing to the inconsistencies of the counting. The ratio of Candida cells in all marginal zones to those in all white pulp areas was calculated per section, and these ratios were averaged for each treatment. Statistical comparisons were made by analysis of variance followed by Tukey’s test.

As the coded slides were counted, each technician made notes as to her impression of the sections. No consistent differences in general histology were noted among the three groups. Quantitatively, however, despite considerable variability in the total number of Candida cells adherent per cross-section, an increase in burn size tended to decrease the number of adherent yeast cells per section (Table 1 and Fig. 2B). To help compensate for the difference in general Candida adherence from one section to another and to compare the number of Candida cells adherent to the phagocytic marginal zones with the number adherent to the white pulp areas, the ratio of

FIG. 1. Effect of burn size on the survival of mice challenged with equal numbers of C. albicans cells. *, significantly less survival than in the 0% TBSA group; †, significantly less survival than in the 12% TBSA group.

FIG. 2. (A) Section of spleen from a normal mouse injected with lucyl blue dye (magnification, ×62; bar, 160 μm). (B) Section of spleen from a mouse with a 24% TBSA burn (magnification, ×62; bar, 160 μm). MZ, marginal zone; W, white pulp. Arrows, C. albicans cells.
as burn size increased, the ratio of white pulp areas was calculated for each splenic cross-section. As burn size increased, the ratio of *C. albicans* cells adherent to the phagocytic marginal zones to those adherent to the nonphagocytic white pulp areas decreased (Table 1). These data are consistent with current information available about burns and candidiasis. First, burn patients are more susceptible to lethal *Candida* infection than are unburned individuals (2, 10), and fatalities from burns continue to be related to burn size (7, 9, 22), as found in this animal model (Fig. 1). The findings that the deaths occurred when *Candida* counts in the circulation and in the kidneys were high suggest that the deaths were indeed due to *Candida* sepsis.

Secondly, the pattern of *Candida* adherence in the normal (0% TBSA) mice (Fig. 2) was similar to what has been reported by other laboratories, i.e., *C. albicans* bound primarily to the marginal zones (6, 8, 14). Also, others have commented about the variation in *Candida* binding from spleen to spleen and zone to zone (20). We found this same variation, as indicated by the wide range of total *C. albicans* counts from section to section (Table 1). Two methods were used to counter this variation. (i) Numerous sections (22 to 28) were evaluated, and the entire cross-section, rather than just a designated part of it, was counted. Hence, the sample size was large, thereby at least partially offsetting the individual variation from sample to sample. (ii) As suggested by Lopez-Ribot et al. (14), to counter the difference in adherence from spleen to spleen, the ratio of *Candida* cells bound to the marginal zones to those bound to the white pulp areas was calculated. Using this ratio reduced the variation enough to show a statistically significant difference in the pattern of binding in the 0% compared to the 24% TBSA mice (Table 1).

The significance of the decreased *Candida* binding in the splenic marginal zones of the burned mice is not known. However, similar decreases in *Candida* adherence in mice immunocompromised genetically, by radiation, or by drugs (5, 6, 14, 18) indicate that the change in adherence is related to the ability of the host to clear the *Candida* challenge. For example, when Qian et al. (18) eliminated the macrophages from splenic marginal zones, *Candida* adherence in the marginal zones decreased, clearance of yeast from the blood was slower, *Candida* counts in the kidney were higher, and more of the mice died. While at this point we do not know if the *Candida* cells in the macrophage-rich marginal zones in the present study were bound specifically to macrophages, we do know that when the burns produced a decrease in *Candida* binding in the marginal zone areas, *Candida* counts in the circulation and in the kidneys increased and survival decreased. It is perhaps not surprising that the patterns in burned hosts should resemble those in animals immunosuppressed by a number of different means, since serious burns do immunocompromise both humans and animals (1, 4). However, to date this is the first suggestion that part of that immunosuppression might include a relative decrease in the ability of the spleen to bind *Candida* in its phagocytic marginal zones.

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**REFERENCES**


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**TABLE 1.** Effect of burn size on the adherence of *C. albicans* to splenic tissue

<table>
<thead>
<tr>
<th>TBPA burned (%)</th>
<th>No. of sections</th>
<th><em>C. albicans</em>/section (median [range])</th>
<th>Ratio ± SE*</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>22</td>
<td>200 (24–641)</td>
<td>41.1 ± 8.6</td>
</tr>
<tr>
<td>12</td>
<td>26</td>
<td>127 (14–537)</td>
<td>30.2 ± 5.2</td>
</tr>
<tr>
<td>24</td>
<td>28</td>
<td>92 (3–583)</td>
<td>18.1 ± 3.5*</td>
</tr>
</tbody>
</table>

* C. albicans adherent to marginal zone/C. albicans adherent to white pulp.
* P < 0.05 compared to 0% burn.