Elevated Candida Antigen Titers Are Associated with Neutrophil Dysfunction after Injury

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This study was undertaken to determine if impaired neutrophil (polymorphonuclear leukocytes [PMNL]) function is associated with an elevated Candida antigen titer after injury. PMNL from eight severely injured adults with Candida antigen titers of $\geq 1:4$ (titer positive) were evaluated for the ability to inhibit growth of Candida albicans in vitro by using a $[^3]$Hglucose incorporation assay. PMNL from eight severely injured adults with titers of $< 1:4$ (titer negative) and from eight healthy volunteers were studied for comparison. PMNL from the titer-positive patients had suppressed ability to inhibit C. albicans growth compared with PMNL from titer-negative patients and healthy volunteers. In vitro, PMNL function against C. albicans could be augmented significantly by cytokines. Granulocyte macrophage–colony-stimulating factor was most potent at augmenting function, followed by interleukin-8 and gamma interferon. Injured patients with elevated candida antigen titers have impaired PMNL function against C. albicans, and this function can be restored by cytokines.

Candida albicans is a saprophyte which forms part of the normal human flora shortly after birth (1). Despite constant exposure to this organism, humans rarely develop systemic infection. The transformation from candida colonization to systemic infection is a result of complex interactions involving candida virulence factors and host defenses. It is only in the setting of host immune suppression that the balance between host defenses and candida is altered, allowing progressive candida invasion (2, 6, 16). In an attempt to detect occult Candida invasion in severely injured adults, we have begun utilizing the Candida antigen titer. This involves a latex agglutination method for the detection of an unspecified Candida cell wall antigen which is felt to have been modified by host interaction. Although the Candida antigen titer has had a controversial sensitivity and specificity for the diagnosis of Candida invasion, we have found a correlation between elevated Candida antigen titers and mortality in this patient population (17). The basis of this association is unknown, but it may be secondary to host immune dysfunction. In undertaking this study, we hypothesized that Candida antigen titers are associated with host immune dysfunction. Because the neutrophil is the primary effector cell responsible for control of Candida growth (7), this study was undertaken to determine if an elevated Candida antigen titer is associated with neutrophil dysfunction in adults following major injury.

Patients with an injury severity score (ISS) (3) of 18 or more were included in this study. The ISS is a means of numerically describing the severity of a patient's injuries, with an ISS of 18 or more representing injuries to at least three separate organ systems. Through protocol, and with Institutional Review Board approval, injured adults with an ISS of 18 or more had Candida antigen titers determined weekly (Cand-Tec latex agglutination test; Ramco Laboratories, Houston, Tex.). Positive and negative controls were tested simultaneously. Positive Candida antigen titers were defined as titers of 1:4 or greater.

With the development of a positive Candida antigen titer, prior to initiation of antifungal therapy, 20 ml of whole blood was drawn into heparinized Vacutainer tubes. Similarly, whole blood was obtained from healthy volunteers and injured adults (ISS $\geq 18$) with negative Candida antigen titers (i.e., titers $< 1:4$). The C. albicans strain used for in vitro assessment of the function of polymorphonuclear leukocytes (PMNL) was an isolate from a patient suffering chronic mucocutaneous candidiasis. It was identified according to the taxonomic criteria of Lodder (15). The C. albicans was propagated by biweekly transfer onto fresh Sabouraud's dextrose agar slants and incubated at 28°C. At the time of use, C. albicans was obtained from the agar slant with a pipet and washed once with Hanks' balanced salt solution by centrifugation at 200 $x$ g for 10 min.

Whole blood was collected in heparinized tubes from patients, diluted 1:2 in balanced salt solution, layered on 10 ml of Ficoll-Hypaque solution (Pharmacia Fine Chemicals, Piscataway, N.J.), and then centrifuged at 400 $x$ g for 30 min. For recovery of PMNL, the leukocyte layer obtained by centrifugation was collected and lysed free of erythrocytes by hypotonic shock with sterile distilled water for 30 s. The PMNL were then washed twice in balanced salt solution before being readjusted to the desired concentration. Previously, such preparation has been documented to produce a cell population of more than 99% PMNL.

The conditions for assay of PMNL function against C. albicans have been previously described (10). Human PMNL were diluted to $1 \times 10^5, 3 \times 10^5,$ and $1 \times 10^5$ in the growth medium (RPMI 1640 medium containing 2% fetal bovine serum, 2 mM l-glutamine, 100 U of penicillin per ml, 100 $\mu$g of streptomycin per ml, and 5 mM HEPES [N-2-hydroxethylpiperazine-N'-2-ethanesulfonic acid]). Fifty microliters of each PMNL-containing medium was added to triplicate wells of a 96-well flat-bottomed microplate. Fifty microliters of medium containing C. albicans organisms at $10^5$ was added to all wells with PMNL-containing medium, yielding effector cell/target cell ratios of 100:1, 30:1, and 10:1, and to empty wells to serve as controls. After the cell mixtures were incubated at 37°C for 18 h, the medium in the wells was discarded.

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and 50 μl of [3H]glucose (New England Nuclear, Boston, Mass.) diluted to 10 μCi/ml in sterile water was added. Sterile water lysed the PMNL, leaving behind the mycelial colonies that had grown overnight. After an additional 3 h of incubation, the proliferating fungus that had incorporated the radiolabel was harvested onto glass filter paper after 50 μl of 5.25% sodium hypochlorite had been added to all wells. The mean number of counts per minute due to the incorporated [3H] in the triplicate cultures was determined. Standard error of the mean was seldom more than 5% of the mean. The percent growth inhibition of Candida was calculated at each of the effector cell/target cell ratios: % growth inhibition = [(Candida cpm alone - PMNL-Candida cpm)/Candida cpm alone] × 100. Percent growth inhibition was plotted against effector cell/target cell ratios to form a line through linear regression analysis. The slope of the line corresponding to 20% growth inhibition was defined as 1 growth-inhibitory unit. Results are reported in growth-inhibitory units per 10^7 PMNL.

PMNL were preincubated for 30 min at 37°C with 1,000 U each of granulocyte macrophage–colony-stimulating factor (GM-CSF; Immunex Corp., Seattle, Wash.) or gamma interferon (IFN-γ; Hoffmann-La Roche, Inc., Nutley, N.J.) per ml or with 10 ng of interleukin-8 (IL-8; a generous gift of Kouji Matsushima, Laboratory of Immunoregulation, National Cancer Institute, Frederick, Md.) per ml. These are previously determined optimal doses which activate PMNL function against C. albicans (4, 8, 9). Fifty microliters of medium containing C. albicans organisms at 10^6/ml was then added to each well, and the cell mixtures were processed as previously described.

The statistical software package KWIKSTAT 2.0 was utilized for data analysis. Differences between groups of patients were compared with a nonpaired Student's t test, while differences within groups were compared with a paired Student's t test. Significance was accepted when P was < 0.05. All data are reported as means ± standard errors of the mean.

Eight patients with elevated Candida antigen titers and eight patients with negative Candida antigen titers were studied. There was no significant difference between the two groups in terms of sex, mechanism and severity of injury, amount of blood transfused, or number of documented bacterial infections (Table 1). Although patients with negative Candida antigen titers tended to be older and received antibiotic therapy for a shorter period than patients with elevated titers, the differences between the two groups were not statistically significant. Only two of the eight patients with elevated Candida antigen titers had positive Candida cultures during their hospital course; each had a single positive culture (sputum culture in one case and urine culture in the other). None of the titer-negative patients had a culture which grew Candida organisms.

The antifungal function of PMNL isolated from the eight titer-positive patients, eight titer-negative patients, and eight healthy volunteers was determined by using a [3H]glucose incorporation assay (Fig. 1). PMNL from all persons tested were able to inhibit the growth of C. albicans in vitro. However, compared with the antifungal function of PMNL from healthy volunteers and from injured adults with negative Candida antigen titers, the antifungal function of PMNL isolated from patients with elevated titers was significantly impaired (Fig. 1). One patient in the titer-negative group was excluded from the statistical analysis, because the antifungal function of PMNL from this patient was more than 8 standard deviations greater than the mean antifungal function of PMNL from the remaining seven titer-negative patients. PMNL from the titer-positive patients, titer-negative patients, and healthy volunteers demonstrated significantly enhanced antifungal function following exposure to cytokines (Fig. 2). GM-CSF was the most potent stimulator of PMNL function against C. albicans, followed by IL-8 and IFN-γ. Although the absolute inhibition of C. albicans growth by the cytokine-stimulated PMNL from titer-positive injured adults appeared to be less than that demonstrated by similarly stimulated PMNL from Candida antigen titer-negative patients and healthy volunteers, the differences between groups were not statistically significant. More importantly, the percent increase in antifungal function above the baseline, manifested by cytokine-stimulated PMNL from the injured adults with elevated Candida antigen titers, was comparable to that

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FIG. 2. Cytokine stimulation improves the anticandidal function of PMNL from healthy volunteers (Vol), injured patients with negative Candida antigen titers (Titer−), and injured patients with elevated Candida antigen titers (Titer+). ■, medium alone; □, GM-CSF; ◆, IL-8; ⧲, IFN-γ. Error bars represent standard deviation. *P < 0.05, compared to mean. †, greater than the anticandidal function of respective PMNL incubated in medium alone (P < 0.05, paired Student’s t test).

of the cytokine-stimulated PMNL from injured patients with negative Candida antigen titers and from healthy volunteers.

After injury, host immune response to Candida infection is complex and multilayered. Candida invasion generates humoral and cell-mediated immune responses; the relative importance of each has been debated (1). However, it is generally believed that phagocytic cells, particularly PMNL, play a primary role in the control of Candida growth (7). Using a [3H]glucose incorporation assay developed in our laboratory (10), we evaluated the anticandidal function of PMNL isolated from injured adults with elevated Candida antigen titers. This radiolabelled assay is a rapid and reproducible method for the objective evaluation of effector cell function against Candida proliferation in vitro. This study shows that PMNL from injured patients with negative Candida antigen titers and from healthy volunteers have similar abilities to inhibit C. albicans growth in vitro. In addition, the results clearly demonstrate that PMNL from seriously injured adults with elevated Candida antigen titers have a depressed ability to inhibit the growth of C. albicans in vitro. We have previously demonstrated that GM-CSF, IFN-γ, and IL-8 are potent activators of PMNL anticandidal function (4, 8, 9). We were therefore interested in whether these cytokines could overcome the defect in anticandidal function found in PMNL from injured patients with elevated Candida antigen titers. Our results demonstrate that PMNL from the titer-positive patients manifested significantly enhanced function against C. albicans following stimulation with GM-CSF, IFN-γ, or IL-8. Furthermore, compared with baseline PMNL function against C. albicans, the augmentation in anticandidal function was on a par with that demonstrated by PMNL from healthy volunteers and from titer-negative injured adults. Therefore, although PMNL from injured adults with elevated Candida antigen titers have impaired anticandidal function, these results suggest that the mechanisms required for priming and activation of PMNL functions following cytokine stimulation remain intact.

There is little disagreement that invasive candidal infections are associated with a poor outcome in critically ill patients. However, considerable difficulty surrounds the differentiation between Candida colonization and Candida invasion. In an attempt to resolve this issue, several serologic markers for occult invasive Candida infection have been investigated (12, 13, 18). We have focused our attention on the Candida antigen titer. This involves a latex agglutination method for the detection of an unspecified Candida cell wall antigen which has probably been modified by host interaction. The Candida antigen assay has had a controversial sensitivity and specificity in the detection of Candida infection in several studies (5, 11, 14). Of the patients studied herein, only two of eight patients with elevated titers had microbiologic evidence of Candida invasion. This is not unusual, as injured patients that develop an elevated Candida antigen titer in our institution are immediately started on antifungal therapy and generally do not develop microbiologic evidence of invasive candidiasis. However, when the utility of Candida antigen titers in severely injured adults is being discussed, it is important to focus on the implications of an elevated titer rather than on the sensitivity or specificity of the assay. Previous studies from our laboratory have shown that following injury, elevated Candida antigen titers are associated with an increased mortality rate due to sepsis and multisystem organ failure (17). The present study demonstrates that injured patients with elevated Candida antigen titers have impaired PMNL function against C. albicans, which in part explains the poor clinical outcome in these patients. Further investigation of immune function in injured adults with elevated Candida antigen titers is currently under way. Identification of specific immune defects may lead to directed immunotherapies and potential reconstitution of deficient immune responses in this patient population.

REFERENCES