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Effect of Challenge with *Candida albicans* Strains with Different Levels of Virulence on Plasma Proteins in Burned Mice

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Immunoglobulin G, immunoglobulin A, transferrin, and fibrinogen were measured by radial immunodiffusion in plasma samples from burned versus unburned mice challenged with high-virulence *Candida albicans* MY 1044 or its low-virulence mutant, MY 1049. Early decreases in these proteins were found after burn and/or MY 1044 but not MY 1049 challenge. These decreases may contribute to increased susceptibility of mice that were burned and challenged by MY 1044 to lethal candidiasis.

Candida albicans causes morbidity and mortality problems in a variety of compromised patient populations, including burned individuals (9, 16, 20). While understanding of the pathogenesis of *C. albicans* infection is growing, our knowledge is still incomplete. Therefore, we developed a murine model in which challenge with a low dose of a fully virulent, wild-type *C. albicans* (strain MY 1044) was lethal in the burned animal, but not in the unburned control (13). In contrast, challenge with the same dose of a low-virulence mutant (strain MY 1049) of MY 1044 was lethal in neither the burned nor unburned mouse. Other studies utilizing these two strains have examined lethality (11), hyphal formation (11), cell growth (7), proteolytic activity (7, 13), renal pathology (8), and spleen cell chemiluminescence (8). In this study, to understand better the pathogenesis of these infections, we asked whether the mortality might be related to changes in the amounts of immunologically important proteins in the circulation system. Immunoglobulin G (IgG), IgA, and transferrin have each been implicated in host defense against *C. albicans* (3, 4, 15, 19, 21). Therefore, levels of these three proteins were measured in blood samples from normal mice, burned mice, and burned mice challenged with MY 1049 or MY 1044. Levels of a critical, hematologic protein, fibrinogen, were measured as well.

Mice (female, Crl-CFl BR non-Swiss mice; Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were anesthetized with methoxyflurane and given a nonlethal, partial thickness (second degree) flame burn on their dorsa. The burn covered 12 to 15% of the total body surface area of the mouse and was followed by intraperitoneal injection of 0.5 ml of saline as fluid replacement therapy (13). The high-virulence parent *C. albicans* MY 1044 and its temperature-sensitive, serine-auxotrophic low-virulence mutant, MY 1049 (11), were provided by Ann Edison (Merck and Co., Inc., Rahway, N.J.). Challenge with 5×10^5 *C. albicans* per mouse was given intravenously immediately postburn.

For protein levels, blood was collected from anesthetized mice via the retro-orbital sinus, using heparinized, EDTA-coated capillary tubes; plasma protein levels were measured by radial immunodiffusion (10). Total IgG, expressed as milligrams per 100 ml, was quantitated by using a kit (ICN Biomedicals, Inc., Costa Mesa, Calif.). Other proteins were measured by using radial immunodiffusion plates prepared in the laboratory (2) with goat anti-mouse IgA (IgG fraction; Cappel-Worthington Biochemicals, Malvern, Pa.), goat anti-mouse transferrin (IgG fraction), or rabbit anti-mouse fibrinogen (transferrin and fibrinogen from Research Plus, Inc., Bayonne, N.J.). For these proteins, results are presented as percentage of normal (value found in unburned, uninfected mice).

In each experiment, four to five mice were bled at each time point. Each experiment was performed two to four times, and the results were averaged. Statistical differences were determined by analysis of variance followed by Duncan's test, using the University of Cincinnati CLINFO computer services. Groups were considered different at $P < 0.05$.

Results are shown in the four panels of Fig. 1. At 6 h following a burn, whether the mice were challenged or not, there was generally a decrease in the amount of each protein measured (solid symbols). By 6 h, a common pattern had developed for IgG and fibrinogen levels, i.e. burned mice and burned mice challenged with MY 1049 showed protein levels significantly below normal, while burned mice challenged with MY 1044 had levels which were not only significantly below normal but also significantly below levels in burned mice. This pattern persisted for IgG (Fig. 1A) for the entire period of observation (6 to 72 h), but for fibrinogen, this pattern disappeared (Fig. 1D) by 24 h postburn after which fibrinogen levels in all burned mice were equally below normal. A similar pattern appeared for IgA (Fig. 1B) at 24 h in that the burn reduced IgA levels and burn and high-virulence MY 1044 challenge further reduced these levels. Transferrin (Fig. 1C) was the only protein which showed no indication of this pattern. For transferrin, levels in all burned groups were below normal at 6 h, normal at 24 h, and above normal at 72 h.

For Ig, measurements were also made in unburned but *C. albicans*-challenged mice (Fig. 1A and B, open symbols). Mice challenged with high-virulence MY 1044 generally showed lower Ig values than mice challenged with low-virulence MY 1049. When this decrease in Ig due to the MY 1044 challenge (□) was then added to the decrease in Ig due to the burn (▲), a decrease in Ig that was greater than the decrease due to either factor alone resulted (■), suggesting that both the burn and the MY 1044 challenge contributed to the decrease in IgG at 6 to 72 h and in IgA at 24 h.

The finding that all of these proteins which are involved either in immune defense or in normal hemostasis were

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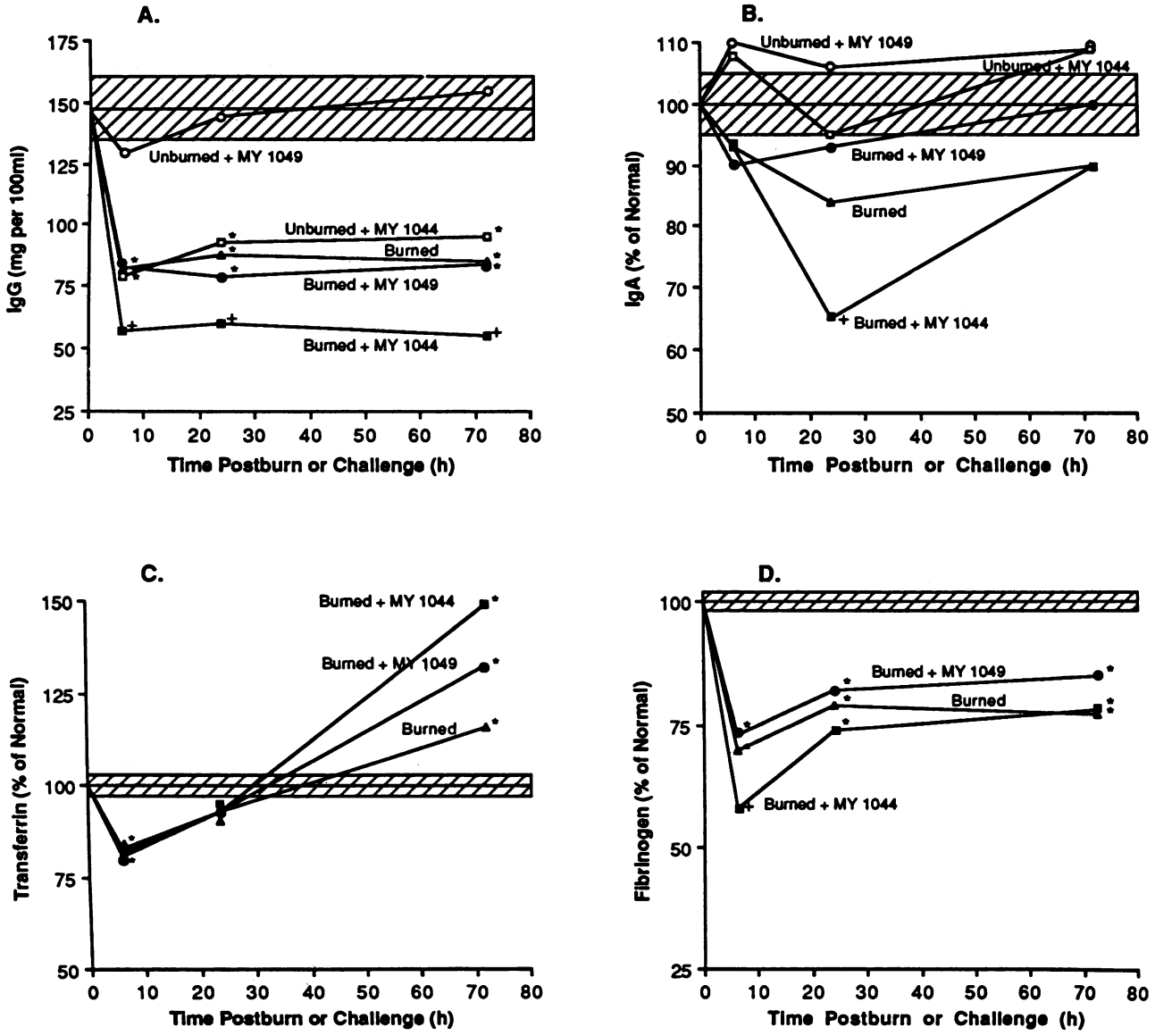

FIG. 1. Levels of IgG (A), IgA (B), transferrin (C), and fibrinogen (D) in normal mice (hatched area, [mean ± standard error of the mean shown]), unburned mice challenged with MY 1049 (○) or MY 1044 (□), burned mice (▲), and burned mice challenged with MY 1049 (●) or MY 1044 (■). Symbols: *, significantly different than normal; +, significantly less than burn only and, when run, also significantly less than MY 1044 challenge to unburned mice.

significantly depressed soon (6 h) after a burn is consistent with findings in burn patients (1, 5, 14, 17, 18) and may explain, in part, why burned hosts are more susceptible to lethal sepsis. The additional findings that challenge with the more-virulent MY 1044 *C. albicans* depressed certain proteins (IgG, IgA, and fibrinogen) more than the decrease in these proteins caused by challenge with the less-virulent MY 1049 mutant suggests that one way in which the wild-type *C. albicans* MY 1044 may be achieving its virulence is by decreasing levels of these proteins in the host's circulation. Previous studies have shown an increased fungal load in burned mice challenged with MY 1044 versus with MY 1049 (12). Since humoral defense mechanisms have been shown to play a role in clearance of *C. albicans* (4, 19), a greater decrease in IgG with MY 1044 challenge than with MY 1049 challenge may be one reason why the fungal load is higher in the MY 1044-challenged animals.

The causes of these changes in protein levels are not known, although it does appear that some specificity was involved, since even though a common pattern was discerned at times for three of the four proteins (IgG, IgA, and fibrinogen), each protein followed this pattern on its own time course, and the fourth protein, transferrin, never demonstrated this pattern. Several possibilities can be suggested as contributing to the observed changes in protein levels. First, fluid resuscitation treatment postburn could decrease the concentration of proteins in plasma by dilution. Variations in the amount of decrease in any particular protein would then depend upon the rate at which that protein was replenished. Second, proteins could be lost through the skin

or sequestered selectively into other compartments outside the circulation. For example, following a burn, pockets of edema fluid, blisters, etc. develop. Various amounts of plasma proteins have been found in these different compartments (6). Third, changes in the proportion of synthesis to degradation of each protein could affect the amount of that protein in the circulation. Relevant to this, total proteolytic activity was measured in serum samples from burned versus unburned mice challenged with MY 1044 or MY 1049, and it was found that this circulating proteolytic activity increases postburn and increases further in burned mice challenged with high-virulence MY 1044 (13). These findings raise the possibility that direct degradation of some of these proteins may have occurred. These possibilities are presently being explored in our laboratory.

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