A Possible Mechanism for Synergy between Antifungal Therapy and Immune Defenses

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(See the major articles by Lamaris et al., on pages 186–92, and Hohl et al., on pages 176–85.)

The host immune response [1, 2] and therapy with antifungal drugs [3] are the major determinants of the outcome of fungal infections. Evidence that these 2 forces often act synergistically has been amassed [4]. The newest available antifungals are the echinocandins, and caspofungin was the initial echinocandin studied and introduced into clinical medicine [5]. These agents act by inhibiting fungal glucan synthase [6], interfering with production of β-1,3-glucan for the fungal cell wall. Although the echinocandins are fungicidal for yeasts (e.g., Candida organisms) [5], and although they are inhibitory for the filamentous Aspergillus molds, they are not fungicidal for Aspergillus species in vitro, and it has been shown that they are active only at the tips and the branch points of the filaments, where β-1,3-glucan synthesis is most active [7]. However, very encouraging results have been obtained regarding the use of echinocandin therapy for aspergillosis in preclinical animal model studies and in clinical studies (with both types of studies performed in highly susceptible hosts) [8–10]. These results present somewhat of a conundrum: how do drugs that are not fungicidal in vitro have such obvious efficacy in vivo (where drug penetration, as well as pharmacologic and toxicologic issues not present in vitro, would likely make demonstration of efficacy much more difficult)? Previous studies examining possible interactions with all 3 of the clinically available echinocandin drugs, as well as with all 3 of the immune effector cell lineages, indicated that a synergistic antifungal interaction could explain the dichotomy [11–13].

Two articles in this issue of the Journal [14, 15] address one possible mechanism for this cooperation. Somewhat counterintuitively, caspofungin, despite its β-1,3-glucan inhibitory effect, has been shown to increase exposure of β-1,3-glucan on the Aspergillus hyphal cell surface as it grows [14, 15]; this finding is the opposite of what occurs in the absence of drug. This apparent contradiction may possibly be explained by caspofungin’s down-regulation of other cell wall constituents (e.g., β-1,6-glucan) and its up-regulation of yet others (e.g., chitin) as a compensatory fungal mechanism, thus remodeling the cell wall [16]. Glucans have long been recognized as immune stimulators [17], although, as a caution, it should be noted that they can also be immunosuppressive [18]. Hohl et al. [14], with macrophages, and Lamaris et al. [15], with neutrophils, showed that caspofungin-treated Aspergillus hyphae stimulate effector cells in vitro to produce such inflammatory mediators as tumor necrosis factor (TNF) and CXCL2; this result, in vivo, could have immunostimulatory and anti-infective effects at the site of infection. Again, it must be cautioned that increasing inflammation locally could also increase tissue destruction and, thus, disease [19].

Hohl et al. [14] showed that echinocandin-treated conidia (the infectious form of the fungus) and germlings (the initial step in the formation of the invasive hyphae) induce diminished secretion of TNF and CXCL2 in murine macrophages, concurrent with decreased β-glucan surface exposure, in contrast to the hyphal effects mentioned above. Consistent with the explanation that β-glucan is responsible for the enhanced cytokine secretion induced by hyphae is the finding that secretion diminishes as the hyphae grow in the absence of drug but that it increases over time if the hyphae are treated with drug. Neither cytokine effect is related to a direct effect of drug on macrophages. Of note, the drug effect was diminished when the amount of drug present was greater than the growth-inhibitory concen-
tation, which may relate to altered wall remodeling [16]. The increase in inflammatory response induced by drug-treated hyphae occurs despite the inhibition of drug-induced fungal growth.

Lamaris et al. [15] also studied other molds, and they suggested that, after caspofungin treatment, β-glucan exposure increases in these molds as well, with the exception of Scedosporium prolificans. However, use of a method with greater specificity for β-glucan might have been preferable [14, 19], and it is not clear that, with non-Aspergillus molds, the effect is associated with clinically relevant drug concentrations [5]. Furthermore, the authors show that the increased exposure occurs at the sites in the hyphal mat where caspofungin acts [20]. Lamaris et al. [15] demonstrate that exposure to caspofungin enhances human neutrophil-associated damage to the molds, as does treatment of the fungi with antibody to β-glucan. Neither exposure damages S. prolificans, thereby providing further support for the proposed mechanism. The studies by Hohl et al. [14] and Lamaris et al. [15] provide evidence that the effect on the phagocytes is mediated either (1) through the mammalian cell receptor for β-glucan, Dectin-1 [21], by neutrophil expression studies demonstrating up-regulation of Dectin-1 mRNA after exposure to drug-treated Aspergillus species (but again, not with S. prolificans) [15], or (2) directly [14], by blocking the effect on cytokines with treatment of macrophages with anti-Dectin-1. Activation of Dectin-1 would presumably lead to increased cytokine production via such triggering transcription factors as nuclear factor κB [22, 23] and activator protein-1 [24]. The studies by Hohl et al. [14] and Lamaris et al. [15] are also complementary, in that, together, they show that the effects demonstrated are not limited [15] to one mammalian cell donor (or even to one species) or to only one [14] fungal strain (or even one species).

It is surprising that antibody to β-glucan is said to increase PMN damage to hyphae [15] and that it has been shown to provide further enhancement of the antifungal effect of PMNs after fungal treatment with caspofungin [15], in that one might have expected that the antibody would block the interaction between β-glucan and Dectin-1 [19], particularly because antibody to Dectin-1 dampens the effector cell effect [14]. An appropriate antibody control for the antibody to β-glucan may have made this more convincing. It is possible that the antibody to β-glucan may, in some way, have increased the presentation to the phagocyte of β-glucan or facilitated the interaction of β-glucan with Dectin-1. Of note, antifungal antibodies have been shown to have a role in treatment of the mycoses [25, 26], and antibody to β-glucan has been proposed to explain the salutary effect in a successful antifungal vaccination [27], with efficacy demonstrated by passive transfer of antibody.

Demonstration of the specificity of the proposed mechanism of echinocandin action on fungi and, thus, on phagocytes would be assisted by fungal treatment with other antifungals that have a different mechanism of action [3] and by demonstration of an absence of the phagocyte effects shown [14, 15]. In addition, it is unclear what the cytokines are doing to result in fungal damage, and it is possible that (1) the drugs alter the fungi in ways that make the fungi more susceptible to toxic oxygen or nitrogen products produced by the phagocytes and (2) the damage to altered fungi could occur even without an up-regulation mediated by the cytokines [4]. Finally, elucidating the effect of treated fungi on other mammalian receptors for fungi, including Dectin-2, would be of interest.

Another concern is that, in both studies [14, 15], the effector cells were studied in fetal calf serum, a commonly used and readily available reagent for murine or human cell cultures. It has been demonstrated, however, that there are factors, such as mannose-binding lectin, that are present in mouse serum but are not found in (or are found in much lesser amounts in) fetal calf serum and that bind to β-glucan and affect phagocyte TNF production in response to fungi [28]. Despite its name, mannose-binding lectin can interact with horizontal 3- and 4-hydroxyl groups of various sugars, including glucose (glucan is a polymer of repeating 1,3-linked glucose molecules) [29], and this collectin is found in human sera as well [30]. In serum homologous with the effector cell, mannose-binding lectin can profoundly block the phagocyte TNF response and dominate over even significant differences in fungal wall composition [28].

If enhanced cytokine production is viewed as an anti-infective asset, then the reduction in cytokines that occurs when conidia and germlings are treated with caspofungin [14] might suggest a deleterious drug effect in the early stages of infection. It might then follow that we would see worse outcomes when caspofungin or other echinocandins are used for prophylaxis or initial empirical use, and this does not appear to be the case [31, 32].

The mechanism described in these 2 studies [14, 15] does not, of course, exclude other possible mechanisms of cooperation. Serum has been reported to increase the anti-Aspergillus activity of caspofungin [33] and other echinocandins, although the effect may be variable [34], and negative effects have also been reported [35, 36]. Abstracts from recent meetings of the Interscience Conference on Antimicrobial Agents and Chemotherapy have presented data indicating that Aspergillus species and caspofungin may act cooperatively to up-regulate expression of Toll-like receptor [37], and that caspofungin and other cytokines, such as granulocyte-macrophage colony-stimulating factor [38], may cooperatively act on effector cell function. Other antifungal–effector cell interactive mechanisms of general interest, for which evidence has been provided [4], include: the effect of antifungals on phagocyte oxidative burst either directly or mediated through cytokines or via effector cell priming, enhancement of drug penetration into phagocytes by cytokine stimulation, reversal by
cytokines of depression of phagocyte function by antifungals, reversal by antifungals of immune depression caused by fungal products, cooperation of antifungals with proinflammatory cytokines to polarize the host response toward a Th1 helper 1 path, alteration of fungal cell membranes by antifungals whose actions affect the membrane [3] (resulting in increased susceptibility to toxic oxygen or nitrogen products), or cooperation of antifungals with innate mammalian constituents, such as chitinase [39] or collectins.

References
