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Effects of Neutrophils and In Vitro Oxidants on Survival and Phenotypic Switching of *Candida albicans* WO-1

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The relationship to pathogenesis of the spontaneous phenotypic switching of *Candida albicans* is uncertain. Since neutrophils are critical in containment of disseminated candidiasis, we used these cells and some of their potentially microbicidal oxidative products to define effects on a *C. albicans* strain (WO-1) that exhibits characteristic, easily recognized switching between the white and opaque phenotypes. Blastoconidia of the opaque phenotypes were more susceptible than those of the white to killing by either intact neutrophils or cell-free oxidants, including reagent hydrogen peroxide or the myeloperoxidase-H_2O_2-Cl^- system. Paralleling these findings, opaque blastoconidia were 2.8- to 3.6-fold more potent stimuli of neutrophil superoxide generation than were the white cells. In addition, both neutrophils and oxidants (reagent H_2O_2 or hypochlorous acid as well as the myeloperoxidase-H_2O_2-Cl^- system) induced unidirectional increases in spontaneous rates of switching from white to opaque phenotypes. Differences in expression of *C. albicans* phenotypes therefore may determine relative susceptibility to neutrophil fungicidal mechanisms, and neutrophils themselves appear to be capable of selectively augmenting the switching process.

Unlike most other causes of systemic opportunistic mycoses, species of *Candida* commonly colonize mucocutaneous or gastrointestinal epithelial surfaces. Local infections may then develop and disseminate via the bloodstream, causing progressive, potentially life-threatening systemic lesions in susceptible hosts (6, 7). *Candida albicans*, the leading cause of opportunistic mycoses, recently was shown to undergo reversible, high-frequency phenotypic switching (22). Phenotypic switching was noted to be a genetically programmed phenomenon (21, 26) that occurred in both laboratory and clinical strains, in vivo as well as in vitro (25, 29, 30). It has been suggested that this ability might provide an adaptive mechanism to facilitate survival of organisms at local and systemic sites of infection (20–23, 25–30). Strains obtained from patients with vaginitis (25) and systemic infections (30) suggested that a high-frequency switching mode is sustained once it is established. Phenotypic differences in colonial morphology appeared to reflect differences in surface properties of blastoconidia and pseudohyphae (1, 2). These corresponded to disparate propensities for coadherence and attachment to epithelial cells, suggesting that phenotypic switching might influence pathogenicity (11).

Other investigators noted that growth on selective media could induce phenotypic alterations in surface properties that influenced interactions of *C. albicans* with phagocytic defense mechanisms (10). In addition, sublethal doses of UV light can increase phenotypic switching rates (20, 22). Since UV light and leukocytes were noted to share abilities for oxygen radical generation (4, 5, 12, 14, 32), we reasoned that they might also share an analogous potential to induce increased spontaneous phenotypic switching rates (20, 22). Neutrophils (PMN) appear to be primarily responsible for prevention and containment of disseminated candidiasis (5–7). To determine whether intact human PMN and some of their putative oxidative products might affect switching, we used the well-characterized *C. albicans* WO-1 strain. We chose this particular strain because it originated from a patient with disseminated candidiasis and manifested easily recognizable, spontaneous, bidirectional switching between the white and opaque phenotypes at rates on the order of 10^{-2} to 10^{-3} (2, 23). Typical round, smooth, shiny, white phenotype colonies of strain WO-1 can be distinguished easily from the wider, flatter, duller colonial morphology of opaque phenotypes, and the latter selectively stain red when grown on medium incorporating the dye phloxine B (20, 23). Blastoconidia of the opaque phenotype proved to be more sensitive to the candidacidal activity of intact PMN as well as cell-free oxidants and were more potent stimuli of PMN superoxide anion generation than were organisms from white colonies. Moreover, PMN and oxidants induced an increased unidirectional switching rate from the white to the opaque phenotype.

MATERIALS AND METHODS

Growth, maintenance, and use of stock cultures. *C. albicans* isolate WO-1 was kindly supplied by David Soll, University of Iowa, Iowa City, Iowa. The culture was maintained as described by Slutsky et al. (23), with isolation and separation of white and opaque phenotypes on the medium of Lee et al. (13) supplemented with arginine, zinc, and phloxine B. For use in experiments, cultures of both phenotypes were shaken for 24 h at 25°C, diluted into fresh medium, grown to mid-log phase, centrifuged, washed once in buffer, and used immediately. When required, blastoconidia were opsonized by tumbling for 10 min at 37°C with pooled normal human serum, washed twice with Ca^{2+}- and Mg^{2+}-free modified Hanks balanced salt solution (mHBSS; GIBCO, Grand Island, N.Y.), suspended in Hanks balanced salt solution (HBSS), and used immediately.

Neutrophil isolation. All reagents were obtained from Sigma Chemical Co. (St. Louis, Mo.) unless otherwise specified. PMN were obtained from heparinized peripheral venous blood of normal volunteers by sedimentation of erythrocytes with 3% dextran (Pharmacia Fine Chemicals, Piscataway, N.J.) in 0.9% NaCl and centrifugation on His-

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topaque 1083 (Metrizoate-Ficoll). After hypotonic lysis of remaining erythrocytes, PMN were suspended in mHBSS and kept on ice until used.

Superoxide anion generation. Release of O_2^- by PMN was determined by quantitative superoxide dismutase (SOD)-inhibitable reduction of cytochrome *c* (3). Prior to incubations, PMN and stimuli were warmed separately for 5 min in a 37°C water bath. In 240 μM horse cytochrome *c* with and without 100 μg of SOD and 50 μg of catalase (33), 2×10^6 PMN were stimulated by shaking for 10 min with the following ratios of one or the other phenotype of opsonized *C. albicans* blastoconidia to PMN: 2:1, 4:1, 10:1, 20:1, and 50:1. As a positive control to verify that each cell preparation functioned normally, PMN were also stimulated with 10^{-6} M formyl-methionyl-leucyl-phenylalanine (fMLP) in the presence of 5 μg of cytochalasin B. To test whether the presence of blastoconidia inhibited the production of O_2^- by PMN, blastoconidia were combined with 10^{-6} M fMLP. Assay conditions were as described above. After the incubation, tubes were plunged into an ice slurry and centrifuged. Supernatants were diluted with 1 ml of buffer and then read at 550 nm (Perkin-Elmer spectrophotometer model 576ST). Addition of catalase to incubation tubes did not significantly change cytochrome *c* reduction under the conditions used with these stimuli. Duplicate samples containing 100 μg of SOD were analyzed in parallel. Results are expressed as nanomoles of SOD-inhibitable ferricytochrome *c* reduced per 2×10^6 stimulated PMN per 10 min, using the previously described extinction coefficient of 21.1 $M^{-1}\,cm^{-1}$ (19), followed by subtraction of calculated values for corresponding SOD and unstimulated control PMN measured simultaneously (<1 nmol/2×10^6 PMN per 10 min). Controls containing stimuli without PMN did not release detectable O_2^-.

Neutrophil candidacidal assays. In sterile 1.5-ml microfuge test tubes (Eppendorf, Fremont, Calif.), blastoconidia of both phenotypes, in 10% human serum, were incubated with PMN (at ratios of 2:1, 5:1, and 10:1, blastoconidia to PMN) by tumbling for 1 h at 37°C. After PMN lysis in sterile distilled water with gentle sonication, blastoconidia were diluted and plated on *Candida* medium with phloxine B as described by Morrow et al. (20). Colonies were counted after incubation of plates for 6 days at 25°C, and percent survival was determined by the formula [(CFU at time zero − CFU surviving after 60 min)/CFU at time zero] × 100. White and opaque colony phenotypes were quantitated separately. At all times, organisms in suspension were maintained at concentrations of $\leq 10^6$/ml. For all assay conditions there was microscopic examination for clumping of the blastoconidia in every experiment. No significant clumping of either phenotype was noted under the experimental conditions used.

Effects of cell-free oxidants. Both phenotypes were exposed to ranges of concentrations of HOCl and H_2O_2 (between 10^{-1} and 10^{-8} M in HBSS) or a reconstituted myeloperoxidase (MPO)-H_2O_2-Cl^- system. MPO, originally purified from human pus as in our prior studies (5), was generously supplied by Robert A. Clark (University of Iowa, Iowa City). The system consisted of 400 mU of MPO plus 100, 10, or 1 mM NaCl and 10^{-3} to 10^{-5} M H_2O_2. H_2O_2 concentrations were determined spectophotometrically before each experiment as in our prior studies (5) by using the extinction coefficient of 81 $M^{-1}\,cm^{-1}$. Mixtures of blastoconidia and oxidants were tumbled for 1 h at 37°C. Survival and colony phenotype were determined as in PMN candidacidal assays.

Phenotypic switching. Switching was determined by direct counts of surviving CFU of each phenotype. Within cultures of each phenotype were low numbers of colonies expressing the other phenotype (i.e., plated cultures of predominantly white phenotype colonies contained a few opaque CFU, and vice versa). As noted above, the fungicidal effects of PMN or oxidants were determined in each experiment by incubation with inocula composed predominantly of blastoconidia expressing one of the two phenotypes (e.g., almost entirely opaque blastoconidia). For the purposes of analyses of switching, we assumed that equivalent killing of that same phenotype would occur during incubations in which it represented the nonpredominant *C. albicans* phenotype present (e.g., inocula for incubations composed almost entirely of white phenotype cells but also containing low numbers of opaque-type blastoconidia). The expected numbers of CFU of that phenotype following exposure to PMN or oxidants was then calculated from the formula: expected CFU of the nonpredominant phenotype surviving incubations with PMN or oxidants = observed CFU of that same, nonpredominant phenotype in unincubated controls × [(observed CFU after incubation of PMN or oxidants with inocula with that same predominant phenotype)/(observed CFU in unincubated controls with that same predominant phenotype)]. Deviations in observed from expected CFU were compared as noted below.

Statistics. According to standard procedures for analysis with parametric methods, data expressed as percentages were first normalized by transformation by using the arcsin of the square root of values. Candidacidal assays and superoxide anion generation were then analyzed by the appropriate Student *t* test for paired or unpaired observations. Frequencies of phenotype switching were compared by either a Fisher 2 × 2 exact test or chi-square analysis, depending on sample size (24).

RESULTS

Candidacidal effects. We first examined the relative sensitivities of white and opaque phenotypes of *C. albicans* WO-1 to the candidacidal activity of intact PMN. We noted no significant clumping of organisms microscopically under any of the experimental conditions employed. In addition, there was equivalent phagocytosis of white and opaque phenotypes. Nevertheless, differences in the susceptibility of white and opaque phenotypes became apparent when more optimal conditions for fungicidal activity were created by reducing blastoconidia-PMN ratios in incubations to below 10:1 (Fig. 1). The adherence of the two phenotypes to plastic incubation tubes differed minimally. After 1 h of incubation in microfuge tubes, the white phenotype inoculum was totally recovered, while there was only an 11.6% loss of the opaque phenotype. We incorporated this reduction in control inocula in calculating killing of the opaque phenotype cells by PMN. For example, at a 2:1 ratio of blastoconidia to PMN, 50.8% of the white and 35.3% of the opaque phenotype organisms survived, compared with 90.4% survival by white versus 74.4% by opaque phenotype cells at a 5:1 ratio ($P < 0.02$ for both of these ratios by the paired-sample Student *t* test).

Since PMN candidacidal effects appear to require an active respiratory burst response to stimulation (5, 7, 12, 14), we next compared the relative abilities of PMN to generate O_2^- during phagocytosis of white versus opaque blastoconidia (Fig. 2). Blastoconidia of the white phenotype, which were more resistant to PMN fungicidal effects, were also significantly less potent initiators of O_2^- release than were

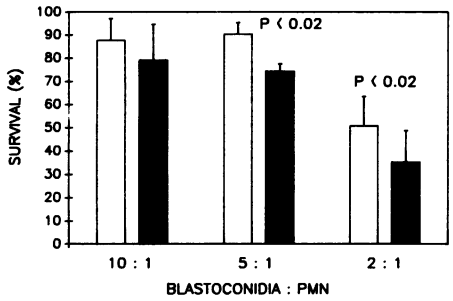

FIG. 1. Survival of strain WO-1 phenotypes following incubation of opsonized blastoconidia with neutrophils (PMN). Means ± standard deviations of four separate experiments are shown for white (open bars) and opaque (solid bars) phenotypes, representing the percentages of CFU surviving after incubations divided by CFU in unincubated controls, with *P* values determined by the Student *t* test for paired samples.

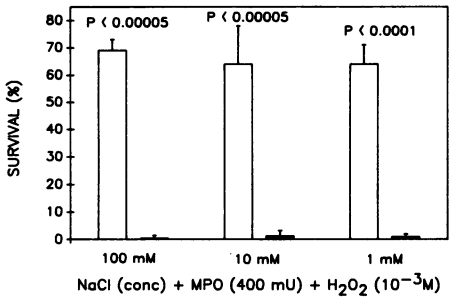

FIG. 3. Survival of strain WO-1 phenotypes following incubation of blastoconidia with a reconstituted MPO system, comprising MPO, H_2O_2, and various concentrations of Cl^- ions. Means ± standard deviations of three separate experiments are shown for white (open bars) and opaque (solid bars) phenotypes, representing the percentages of CFU surviving after incubations divided by CFU in unincubated controls, with *P* values determined by the Student *t* test for paired samples.

opaque cells. Blastoconidia by themselves did not release detectable levels of O_2^-. In addition, neither white nor opaque blastoconidia interfered with PMN respiratory burst responses elicited by fMLP (data not shown). Therefore, observed differences in stimulation of O_2^- generation seem attributable neither to disparate oxygen scavenging nor to selective suppression of PMN responses by white versus opaque cells.

Oxidants released by the PMN respiratory burst are presumed to participate in the killing of *C. albicans* (5, 7, 12, 14). Therefore, we compared the effects of cell-free oxidants on survival of white and opaque blastoconidia. The narrow concentration ranges between totally fungicidal and subfungicidal concentrations of these reagents made it difficult to discern phenotypically determined differences in susceptibility. For example, concentrations of $\geq 10^{-5}$ M HOCl or 10^{-2} M H_2O_2 were completely lethal for cells of both phenotypes, but survival of blastoconidia was totally unaffected by $\leq 10^{-7}$ M HOCl or 10^{-4} M H_2O_2. The opaque phenotype (73.87%) proved to be more susceptible than the white (95.6%, $P < 0.02$) to 10^{-3} M H_2O_2, though no differences in susceptibility to 10^{-6} M HOCl were detectable.

To better mimic the presumed mechanisms of PMN candidacidal activity (7, 12, 14), we then used purified reagents to reconstitute an MPO-H_2O_2-Cl^- system (5, 14). First, we determined the concentrations of constituents sufficient to kill but not totally eliminate strain WO-1 blastoconidia. With these experimental conditions, there were striking differences in the relative susceptibilities of the two phenotypes, as approximately 70% of white but <5% of opaque blastoconidia survived (Fig. 3). Various concentrations of MPO from 4 to 400 mU did not affect the results.

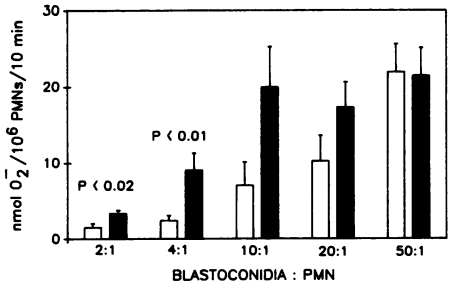

FIG. 2. Stimulation of PMN superoxide generation by incubation of various ratios of blastoconidia to PMN. Open bars (white phenotype) and solid bars (opaque phenotype) represent means ± standard deviations of four separate experiments, with *P* values determined by the Student *t* test for paired samples.

Effects on frequencies of white-opaque phenotypic switching. We next determined the effects of PMN and oxidants on rates of phenotypic switching. In each experiment, we counted total CFU of the predominant and nonpredominant phenotypes after incubation with or without PMN or oxidants. To eliminate effects of CFU reduction by concomitant killing and permit analyses of switching, we made the conservative assumption that each phenotype would survive at an equivalent rate whether it represented the predominant or nonpredominant type in any given set of experimental conditions. For example, with *Candida* predominantly of the opaque phenotype at a 5:1 ratio of blastoconidia to PMN, opaque colonies were reduced from 6,581 to 3,959 CFU. We assumed that incubations containing equivalent 5:1 ratios of PMN to predominantly white-phenotype blastoconidia would cause corresponding reductions in CFU of the nonpredominant opaque phenotype. Even if reduced absolute numbers of nonpredominant opaque blastoconidia had influenced the results disproportionately, such an effect would have led to underestimation of phenotypic switching frequency, if anything. Therefore, it seemed unlikely that our assumption would cause overestimation of switching rates. As noted above, PMN candidacidal activity increased as the ratio of blastoconidia to PMN decreased. Accordingly, we counted 2 CFU of the nonpredominant opaque phenotype in controls unexposed to PMN and expected to find this reduced to ≤1 opaque CFU by virtue of PMN fungicidal effects. Instead, we noted 34 opaque colonies (Fig. 4), suggesting that PMN significantly increased the frequency of switching from the white to the opaque phenotype ($P < 0.00005$). Likewise, both 10^{-6} M HOCl and 10^{-3} M H_2O_2 induced significant opaque-to-white phenotypic switching (Fig. 5). Corresponding unidirectional effects on switching frequency of the MPO-H_2O_2-Cl^- system were even more striking (Fig. 6). In contrast, neither PMN nor oxidants altered the rates of switching from the opaque to white phenotype (data not shown).

DISCUSSION

Since the original description of high-frequency phenotypic switching by *C. albicans* (22), evidence has supported

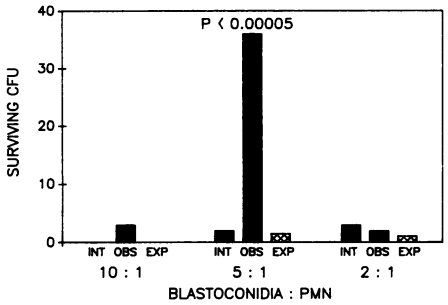

FIG. 4. Effects of incubation with PMN on the frequency of phenotypic switching. After incubation of PMN with inocula predominantly comprising white blastoconidia, increases above expected numbers of surviving opaque-phenotype CFU were determined. Bars represent total CFU in three separate experiments, depicting the low numbers of opaque-type CFU present in initial inocula (INT) and comparisons of observed CFU surviving after incubations (OBS) with expected CFU (EXP). Expected CFU were calculated as if CFU had been reduced by fungicidal effects without any phenotypic switching (*P* values determined by the Fisher 2 × 2 exact test).

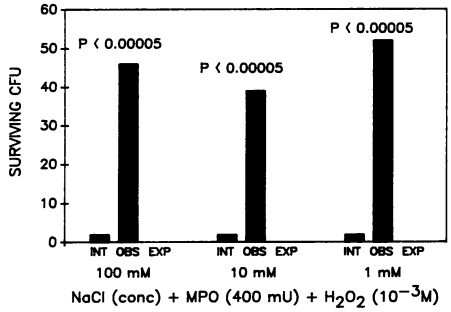

FIG. 6. Effects of incubation with a reconstituted MPO-H_2O_2-Cl^- system on the frequency of phenotypic switching. After incubation of reagents with inocula comprising predominantly white blastoconidia, increases above expected numbers of surviving opaque-phenotype CFU were determined. Bars represent the total CFU in three separate experiments, depicting the low numbers of opaque-type CFU present in initial inocula (INT) and comparisons of observed CFU surviving after incubations (OBS) with expected CFU (EXP), calculated as described in the legend to Fig. 4 (*P* values determined by the Fisher 2 × 2 exact test).

its occurrence in local and systemic infections in vivo (25, 28, 30) and a role in virulence has been hypothesized for the phenomenon (2, 20, 22, 25, 28, 30). Our new findings reported here verify that switches in phenotypic expression by *C. albicans* strains can alter interactions with PMN, the host cells primarily responsible for prevention of disseminated candidiasis (6, 7). We noted that the potency of PMN fungicidal effects varied according to the phenotype expressed by *C. albicans* WO-1. This strain, originally obtained from a patient with disseminated candidiasis, characteristically undergoes bidirectional, high-frequency switching between the white and opaque colony phenotypes (23). That differences in colonial morphology of these two phenotypes reflect disparate surface properties is verified by corresponding distinctions in individual cell sizes as well as budding and germination patterns (1, 2, 20, 23). Our data suggest that these structural properties may determine the relatively increased susceptibility of blastoconidia from opaque-phenotype colonies to critical host defense mechanisms.

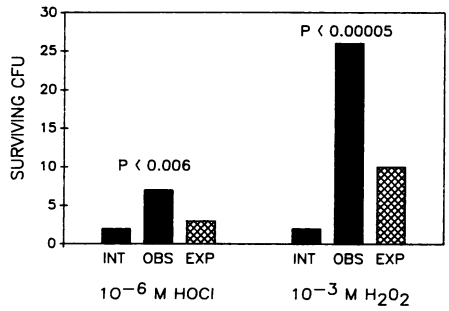

FIG. 5. Effects of incubation with HOCl and H_2O_2 on the frequency of phenotypic switching. After incubation of reagents with inocula predominantly comprising white blastoconidia, increases above expected numbers of surviving opaque-phenotype CFU were determined. Bars represent total CFU in three separate experiments, depicting the low numbers of opaque-type CFU present in initial inocula (INT) and comparisons of observed CFU surviving after incubations (OBS) with expected CFU (EXP), calculated as described in the legend to Fig. 4 (*P* values determined by the Fisher 2 × 2 exact test).

The ability of PMN to combat disseminated candidiasis appears to depend in large part on their release of microbicidal oxidants during stimulation by contact with organisms (6, 7, 9). Significantly, the relatively increased killing of opaque versus white blastoconidia by PMN paralleled the relative sensitivities of these phenotypes to the fungicidal effects of cell-free oxidants in our experiments. In addition, differences in susceptibility to PMN were not related to differential phagocytosis or clumping of the two phenotypes. H_2O_2 is derived from dismutation of O_2^- that is released during respiratory burst activation of intact PMN (3, 12). HOCl, the primary product of interactions between released H_2O_2 and MPO with Cl^- ions (8, 12, 14, 31), did not differentially affect the survival of opaque versus white blastoconidia. However, the small differences between ineffective and totally lethal concentrations of reagent HOCl made this an inherently insensitive experimental system. To further examine whether HOCl and/or other potent oxidative products might contribute to the observed PMN effects, we used purified reagents to reconstitute a simulated MPO-H_2O_2-Cl^- system (12). It seemed unlikely that H_2O_2 directly mediated the candidacidal effects of intact PMN, since Lehrer noted previously that killing of blastoconidia requires high concentrations of this reagent (14, 15). Our data with the WO-1 strain confirm these observations. With MPO release from PMN granules, chlorinating agents such as HOCl and chloramines are produced (8, 12, 14, 31). These account for 28% or more of the oxygen consumed after PMN stimulation (8) and are presumed to be generated within phagocytic vacuoles in high concentrations (12). In our studies, reagent HOCl proved to be a highly potent fungicidal agent for strain WO-1 blastoconidia, as $\geq 10^{-5}$ M HOCl was totally lethal, making it difficult to discern any differences in its effects on the survival of white and opaque phenotypes. However, when we reconstituted a suboptimally fungicidal MPO system from purified reagents, disparities in the relative sensitivities of the opaque and white phenotypes became readily apparent. Opaque blastoconidia also proved to be more potent stimuli of PMN respiratory burst responses than equivalent numbers of white-phenotype cells. This may be related in large part to the greater sizes and surface areas of opaque organisms (2), which would be expected to provoke increased PMN oxidant

release (9). However, biochemical differences in surface cell wall components also might have facilitated increased PMN responses to opaque blastoconidia. Our data make it unlikely that the lesser respiratory burst responses elicited by white-phenotype cells were related to selective inhibition of PMN activation mechanisms, since fMLP-induced PMN O_2^- generation was unaffected by the addition of these blastoconidia. While these studies were not intended to define specific mediators responsible for PMN fungicidal activity, they did establish a remarkable consistency in the relative patterns of susceptibility of strain WO-1 opaque- and white-phenotype blastoconidia to both oxidant- and PMN-mediated damage. Our studies did not address oxidant-independent mechanisms that may also contribute significantly to the candidacidal activity of intact PMN (15, 16). However, our data indicated that genetically programmed phenotypic changes in *C. albicans* affected the efficacy of host defense mechanisms and demonstrated that at least one of several potential mechanisms, PMN oxidant production, might have contributed to this phenomenon.

In addition, we also found that both PMN and oxidants could increase rates of phenotypic switching, but apparently only unidirectionally, from the white to the opaque phenotype. Given the suggested mechanisms for the UV irradiation effect on high-frequency switching (20, 21, 26), it was surprising that bidirectional effects were not noted in our experiments. At this point, explanations for these observations are speculative. As yet undefined differences within populations of blastoconidia comprising each phenotype might be one factor contributing to the unidirectionality of effects. For example, among white blastoconidia, the same cells might exhibit both increased susceptibility to lethal effects and promotion of switching by PMN or oxidants. Conversely, it is possible that opaque blastoconidia newly derived from switched white-phenotype cells might be more sensitive to fungicidal agents. Alternatively, there may be phenotypic differences in accessibility to the various multiple targets potentially subject to oxidant and PMN effects, so that sites affecting switching frequency might be influenced selectively.

The virulence of an opportunistic infectious agent like *C. albicans* is a complex phenomenon, since pathogenicity involves a multiplicity of factors. Some properties of *C. albicans* have been correlated with increased virulence, although the basis for this is as yet undefined. For example, acid protease production by *C. albicans* has been suggested as a putative pathogenetic mechanism (17, 18). Only opaque-phenotype blastoconidia and not the white produce an acid protease (C. D. Payne, T. L. Ray, and D. R. Soll, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, F-45, p. 465). However, multiple stages are involved in the genesis of disseminated candidiasis, including adherence and survival on mucocutaneous surfaces, tissue penetration, and evasion of phagocytic host defenses in the bloodstream and systemic tissues. Expression of a particular phenotype might then provide factors that foster more favorable conditions for accomplishing one or more of these steps. Host resistance factors responsible for any single specific stage in the development of disseminated candidiasis may be compromised selectively or independently. Pathogenicity of one phenotype within any given infectious focus might then be facilitated if the host defect were to eliminate specific obstacles to growth and survival of that distinctive form of the organism.

Thus, the effects of phenotypic switching on pathogenicity would depend on whether resultant infections were local or systemic, as well as the natures of both concomitant local or systemic host defense defects and infecting strains. For instance, *C. albicans* WO-1 was originally isolated from lesions of disseminated candidiasis. Our results suggest that the fungicidal activity of PMN would favor the survival of white-phenotype blastoconidia of this strain, but there would also be an increased frequency of switching of surviving organisms to expression of opaque-phenotype characteristics. However, most cases of disseminated candidiasis develop because PMN are absent or deficient, so that relatively increased PMN susceptibility might not invariably preclude pathogenicity (6, 7). Other properties of white and opaque organisms, such as differences in surface adherence and requirements for germination (1, 2), might then assume greater significance in the establishment and progression of local and disseminated infections (7, 9).

Therefore, delineation of virulence factors will require further definition of the specific biochemical differences responsible for apparent phenotypic alterations, as well as complete characterization of the interactive host defense mechanisms that ultimately determine how such changes in organisms affect pathogenicity. Our observations were made with a single, well-characterized clinical isolate. The biological relevance to the pathogenicity of mucocutaneous and disseminated candidiasis ultimately will depend on whether or not effects on host cell interactions prove to be general phenomena observed with isolates undergoing high-frequency phenotype switching. Thus, it will be important to screen and identify additional clinical strains that are amenable to study by virtue of sufficiently differing colonial morphologies of their high-frequency-switching phenotypes. In any case, our data establish that phenotypic switching in *C. albicans* has the potential for significant effects on interactions with host defense mechanisms.

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