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Original article

Candida albicans isolates with different genomic backgrounds display a differential response to macrophage infection

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Abstract

Few human pathogens possess the ability exhibited by *Candida albicans* to colonize and cause symptomatic infections at different body sites. The host immune system is the major factor determining whether this opportunistic yeast behaves as a commensal or as a pathogen, since *C. albicans* strains appear capable of expressing similar virulence factors in response to specific body-district cues. This report provides evidence showing that *C. albicans* isolates with diverse genomic backgrounds (b and c karyotypes) differently modulate their pathogenic potential when assayed in cocultures with human monocytic derived macrophages (THP-1 cells). Striking differences were observed in the ability to undergo bud-hypha transition, a relevant *C. albicans* virulence factor, between b and c karyotypes ($P < 0.0001$) upon their internalization by macrophages. All c types were able to develop hyphal forms, resist intracellular killing, replicate, and escape from macrophages. The b type isolates, which were shown to be more efficiently ingested by THP-1 cells than the c type strains ($P = 0.013$), were susceptible to intracellular killing and predominantly found as blastoconidia inside macrophages. Despite their different intracellular disposition, both b and c type isolates were equally able to undergo morphogenesis and to express *NRG1* and *HWP1* genes, markers of the bud-hypha transition program, during in vitro propagation. Since macrophages play a critical role in the host resistance to *C. albicans*, the different response of b and c isolates to macrophage infection suggests that the c type strains are better suited to behave as a more virulent strain cluster.

Keywords: *Candida albicans*; Karyotyping; Macrophage infection; *SAP1–10* gene expression; Morphogenesis

1. Introduction

Among human fungal pathogens, *Candida albicans* plays a dominant role. This opportunistic yeast possesses the remarkable ability to survive and proliferate in a radically changing environment, adapting its growth to physiological extremes of pH, osmolarity, availability of nutrients, and temperature [1]. This versatility may account for the successful behavior of *C. albicans* both as a commensal colonizer of radically different anatomic districts in healthy individuals [2,3], and as a pathogen causing symptomatic infections at such a broad range of body sites [2,4], especially in patients with compromised immune functions [2]. Although the host immune system is the major factor balancing the transition from commensalism to pathogenicity, *C. albicans* expresses several virulence attributes, such as adhesion factors, phenotypic switching, dimorphic behavior, and secretion of hydrolytic enzymes [4–9], which may contribute to the persistence of colonization as well as to development of symptomatic episodes. Nevertheless, the pathogenic mechanisms involved in the onset and progression of *C. albicans* infections are

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not yet completely understood. Various in vivo studies with *C. albicans* mutants lacking specific virulence genes have shown that no single virulence factor can be identified as a dominant determinant in *C. albicans* pathogenesis. Rather, it is generally accepted that *C. albicans* virulence is a multifactorial event governed by the ability of the micro-organism efficiently adapting to environmental changes and finely coordinating the expression of various virulence genes [4,5].

An intriguing aspect of *C. albicans* virulence is given by the genome plasticity of this opportunistic pathogen [10]. This feature addresses the question of whether genetically related strains may be identified as a predominant cluster in disease states. Genome typing of *C. albicans* isolates have produced different although non-controversial results. Highly discriminating DNA-based techniques, such as fragment length polymorphism analysis, Southern blot with species-specific probes, randomly amplified polymorphic DNA (RAPD) [11], and in particular multi-locus sequence typing (MLST) [12], have allowed to reproducibly establish genetic relatedness among isolates, to identify the source of infection, the route of transmission, the selection and/or strain replacement at a given body site, as well as the emergency of strains resistant to specific antimicotic drugs [13–15]. Such techniques, however, have failed in identifying genetically distinct hypervirulent strains. In contrast, poorly discriminatory molecular methods, such as the determination of electrophoretic karyotype, suggest that a link may exists between the genomic background exhibited by *C. albicans* and the expression of its virulence [16,17]. Indeed, since *C. albicans* virulence results from the integration of several cellular functions, each requiring multiple patterns of gene expression, it is likely that different *C. albicans* genotypes may exhibit a similar virulence phenotype, while *C. albicans* strains with a given genomic background may share similar phenotypic features, virulence traits included. However, both highly and poorly discriminative molecular typing methods support the view that all *C. albicans* isolates may potentially behave as commensals or as pathogens, when changes in the host physiology gives such an opportunity.

Given the complexity of *C. albicans* virulence and the critical role played by macrophages in balancing colonization/infection caused by this opportunistic yeast [18], the analysis of *C. albicans* response to macrophage infection may be regarded as a window to dissect the complex relationship existing between the virulence exerted by the yeast and the resistance to *C. albicans* the host may elicit. Indeed, macrophages are essential components of the innate host resistance to *C. albicans*, as they play a significantl role in confining the infectious agent at the site of colonization/infection, in controlling the yeast invasion of deeper tissues, and ultimately in preventing the yeast entering the bloodstream [18]. On the other hand, *C. albicans* may adapt and survive in the hostile environment of the macrophage intracellular milieu, by activating a number of possible cross-talking signal transduction pathways in response to internalization [19–23]. In this context, a special trait of *C. albicans*, which is likely to contribute to the evasion of the host-defence mechanisms, is its ability to switch from unicellular budding yeast to filamentous forms, through the critical stage of the germ-tube formation [2]. Early in vitro studies have shown that *C. albicans* strains capable of forming hyphae escape from macrophages, while *C. albicans* strains unable to undergo this morphological switch remain engulfed inside macrophages [24,25]. In addition, there is much evidence which shows that strains trapped in either the yeast or filamentous state are both significantly less virulent than cells capable of undergoing morphogenesis [26]. Therefore, since both hyphae and blastoconidia are frequently observed in infected tissues, it appears that the developmental program of the bud-hypha transition may represent a mechanism providing the pathogen with the ability to rapidly adapt and survive inside macrophages, thus overcoming the killing activity the macrophages may exert upon yeast internalization.

In this investigation, oral isolates of *C. albicans*, characterized by distinct genome backgrounds, referred to as b and c karyotypes, were assayed in coculture experiments with human monocytic derived macrophages (THP-1 cells). Both b and c type strains were from healthy individuals and from oral candidiasis patients, who were either infected or uninfected with human immunodeficiency virus (HIV). Moreover, in a previous report the two karyotypes were proven to differently express secretory aspartyl proteinase (Sap) encoding genes (*SAP1–10*) [16], which are a relevant virulence factor in *C. albicans* [27]. Thus, we addressed the question of whether differences in *C. albicans* genome backgrounds, which were found to affect the expression of Sap encoding genes, may also modulate the behavioral response of this opportunistic pathogen upon its internalization by macrophages. To this end, *C. albicans* isolates with b and c karyotypes, were analyzed for: (i) the extent of phagocytosis by human monocyte-derived macrophages; (ii) the resistance and/or susceptibility to intracellular killing; (iii) the ability to undergo bud-hypha transition both in vitro and during macrophage infection; and (iv) the expression patterns of *SAP1–10* genes during morphogenesis in vitro and inside macrophages. This report provides the first evidence that oral *C. albicans* isolates with distinct karyotypic profiles exhibit a different virulence phenotype in their interaction with macrophages, since they show a different ability to resist intracellular killing, to undergo transition from yeast to hyphal forms, and to replicate inside macrophages.

2. Materials and methods

2.1. Source of C. albicans isolates

The *C. albicans* isolates used throughout this study were from our strain collection recovered from the oral cavities of healthy carriers (commensal strains) and oral candidiasis patients (infectious strains), who were either infected or uninfected with HIV [16]. The collection was stored at −80 °C in 30% glycerol. The strains included in the study were propagated on Sabouraud agar plates (Difco, Detroit, MI, USA) supplemented with gentamicin and chloramphenicol (Sigma Chemical Co., St. Louis, MO, USA). The karyotype of each strain