# Editorial: **Of Mice and Men—Yet Again**

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RECEIVED APRIL 4, 2013; REVISED MAY 12, 2013; ACCEPTED MAY 13, 2013. DOI: 10.1189/jlb.0413187

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*Candida albicans* infections at skin and mucosal sites are common, but organisms hardly ever invade deeper tissues. *Candida* species, with *albicans* the most common, are typically low-abundance commensals among skin, gastrointestinal, and vaginal flora. Conditions that permit *Candida* overgrowth often provoke surrounding tissues to warmth, redness, itching or pain, and discharge—an inflammatory response. Facilitating factors for these conditions, known as mucocutaneous candidiasis, include persistent moisture; administration of antibacterial antibiotics that reduce competing flora without suppressing candidal growth; high levels of glucose in mucosal secretions, as would be expected in poorly controlled diabetics; or subtle defects in immunity, particularly in IL-17-mediated pathways [1].

Blood and deeper tissue infections with *Candida* species, once rarely encountered, are now more common. Contributing factors include medical practices of (1) prolonged systemic administration of antibacterials that have no direct effect on fungi, (2) long-term use of i.v. catheters, and (3) persistent neutropenia, usually consequent to cancer therapies.

New insights regarding barriers to *Candida* invasiveness emerged with the evaluation, in 1968, of a 49-year-old diabetic who had had two or three deep-seated *Candida* infections in the previous 4 years but with none of the risk factors described above [2]. This individual proved to have a genetic, complete absence of MPO, a phagocyte enzyme that catalyzes the oxidation of chloride by $H_2O_2$ to hypochlorous acid, a potent microbicide. Typically, MPO is packaged in phagocyte (neutrophil and monocyte) cytoplasmic granules that merge with the developing phagocytic compartment. Concurrently, a membrane oxidase is activated to reduce dissolved molecular oxygen, first to superoxide anion and then to $H_2O_2$ (the respiratory burst). Together with chloride from the ambient medium, all components of a potent, intravacuolar, antimicrobial system are assembled and active within the confined space of the phagocytic vacuole. This MPO-mediated microbicidal system overlaps with many other intravacuolar antimicrobial systems, and it is often difficult to establish a hierarchy of importance for each system's contribution (for a recent review, see ref. [3]). For *C. albicans*, however, the MPO-deficient neutrophils from this patient were shown to be impaired extensively in their ability to kill ingested yeasts (**Fig. 1**). Furthermore, they were severely impaired in their capacity to suppress filamentation (**Fig. 2**).

*C. albicans* is a dimorphic fungus, encountered environmentally as a yeast–an oval spheroid–that replicates by budding off daughter yeasts. Under different environmental conditions, notably a temperature shift to 37°C and exposure to host tissue factors, yeasts begin to extend long filaments in a process known as germ-tube formation, germination, or filamentation. Filaments can become the dominant morphology, growing to become complex, intertwined mats. This latter morphology is associated with invasive behavior.

Normal human neutrophils not only kill a large proportion of ingested *C. albicans*, but they also suppress filamentation in the remainder. Optimal suppression requires the oxygen-derived products of the respiratory burst and MPO, with the absence of the former leading to a more severe defect than just the absence of MPO (Fig. 2). When the influx of neutrophils is limited, as in the conduct of a typical in vitro experiment, suppression of filamentation, even by normal neutrophils, is limited in duration. Filaments from surviving fungi may ultimately grow beyond the capacity of the phagocyte to contain them, leading to rupture of the host cell and dissemination of the microbe.

Of note, the proband patient for MPO deficiency did not have a major infection history until age 45, concurrent with the clinical recognition of his diabetes, even though he was, presumably, completely MPO deficient from birth. Also, the proband's sister, with identical MPO deficiency but not diabetes, had no unusual infection history. As additional individuals with complete hereditary MPO deficiency were identified, it became clear that most do not exhibit an unusual susceptibility to bacterial or fungal infections, at least in the setting of modern sanitation practices and health-care interventions. When they do, infections seem to emerge in the setting of diabetes, and *C. albicans* is, by far, the most prominent, unusual pathogen.

Absence of the capacity to generate a phagocyte respiratory burst, which impairs more than just the MPO system, as in CGD, brings overall infection risk to a new, life-threatening level. Still, bacterial infections are more common than fungal, and even within the fungal group, serious aspergillus infections seem to be more prominent than candida, suggesting that even with severely impaired neutrophils, CGD patients retain significant anticandidal defenses overall.

Abbreviations: CGD=chronic granulomatous disease, $H_2O_2$=hydrogen peroxide, MPO=myeloperoxidase

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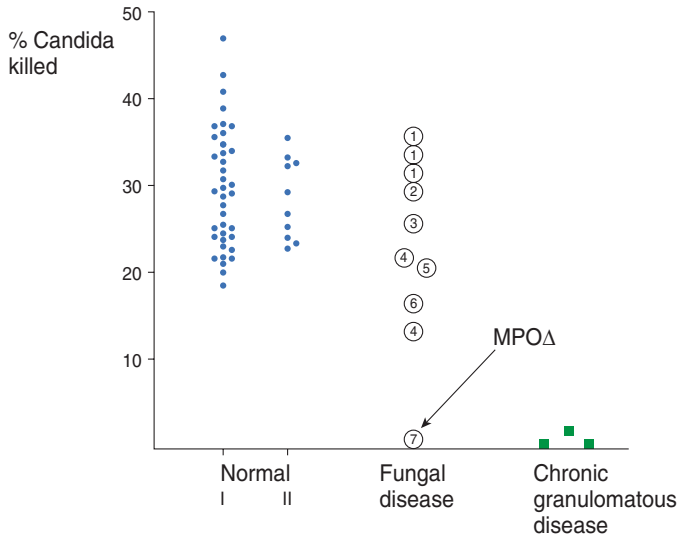



**Figure 1. The ability of human neutrophils from different individuals to kill *C. albicans* cells in 1 h.** Neutrophils from normal individuals ingesting *Candida* yeasts, opsonized with autologous (I) or pooled AB serum (II), are compared with those from individuals with a variety of systemic fungal infections, one of whom presented with systemic candidiasis and complete hereditary MPO deficiency (MPOΔ). Neutrophils from this latter individual and those from patiets with CGD were ineffective at killing *Candida* under these conditions (adapted from Fig. 4 in ref. [4]).

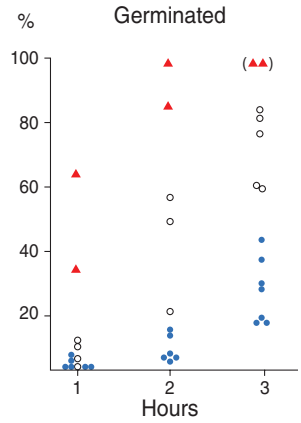



**Figure 2. The ability of human neutrophils to suppress C. albicans germ-tube formation.** Leukocytes were incubated with *C. albicans* yeasts for the indicated times (hours), stained, and assessed for germination (germ-tube formation) by microscopy. Closed circles, Neutrophils from normal individuals; open circles, neutrophils from an indivdual with hereditary complete MPO deficiency; open triangles, individuals with CGD; double-triangle, CGD neutrophils were destroyed by the growing candida after 3 h. Note that given sufficient time, surviving yeasts will germinate, in vitro, even in normal neutrophils (adapted from Fig. 4 in ref. [5]).

The respiratory burst and MPO are not the only elements of phagocyte-mediated immunity to impede candidal invasion. Other neutrophil anticandidal factors are important [6] but appear to be directed more at species of *Candida* other than *albicans*. Among nonoxidative, anticandidal factors, human neutrophils contain small, cysteine-rich granule proteins with anticandidal and antibacterial activity, the defensins that are present in human but not murine neutrophils. Furthermore, as neutrophils lyse during inflammation, they release from their cytoplasm zinc-sequestering calprotectin that has broad, antifungal properties [7, 8]. Calprotectin, active in its anticandidal activity, is a prominent component of neutrophil extracellular traps, a complex mixture of proteins on a DNA scaffold, derived from degenerating neutrophils within inflammatory foci. However, beyond a general observation that additional barriers to invasive *Candida* infections exist and are important, they are not well-defined.

In this issue of the *Journal of Leukocyte Biology*, Ermert and colleagues [9] describe studies comparing human neutrophils with those from C57/BL6 mice, with respect to their capacity to (1) ingest *C. albicans* yeasts, (2) kill most, and (3) suppress filamentation in the survivors. They used yeasts and early germinating organisms tagged with fluorescent antibodies on the expectation that subsequently, synthesized cell envelope structures would be unlabeled. That is, once the label fixes to the *Candida* surface, it will not migrate as the organism buds or filaments. They observe that murine neutrophils kill *Candida* less well than do human neutrophils and that neutrophils from mouse bone marrow are more effective killers than those obtained from mouse peripheral blood. Furthermore, murine neutrophils are more permissive for the germination of surviving yeasts to the point where filamentous growth deforms and destroys the phagocyte. In many respects, normal murine neutrophils resemble human neutrophils that lack MPO or the capacity to mount a respiratory burst.

What then is the primary basis for the diminished anticandidal properties of murine neutrophils? Mouse neutrophils lack defensins [10] and contain just [1/5] (C57 mouse strain; ref. [11]) to [1/2] (C57/BL6; current study) of human amounts of MPO/neutrophil. Estimates of respiratory burst activity in murine cells vary, but the current study found it to be comparable with humans, albeit distributed differently among the secondary oxidants generated. The introduction of defensins into murine neutrophils at levels approximating 10% human improved candidacidal activity but did not much affect the ability to suppress filamentation. Thus, the basis for anticandidal differences among neutrophils (murine vs. human and human normal vs. human MPO-deficient vs. human oxidant-deficient) is only partially explained.

*C. albicans* infections are not endemic in mice, and even among humans with functionally impaired neutrophils, they are not the most prominent of opportunistic pathogens. There are clearly important host factors in *Candida* resistance that remain to be identified.

As acknowledged by Ermert and colleagues [9], mouse models of human diseases yield important insights into pathogenesis and have suggested novel treatment approaches for many human disorders, so much so that the broad paradigm is that similarities of

the two mammalian physiologies vastly outweigh differences. However, in the realm of innate immunity, differences are often substantial. Environmental stresses and the spectrum of microbial threats differ between mice and men. Modifications in gene-expression patterns suggest that innate-immune systems have been quite adaptable and responsive to the differences. Thus, investigators who seek to understand innate immunity must be especially vigilant when extrapolating findings from mouse models to the human condition [12]. The presently described differences in the manner with which neutrophils cope with a *C. albicans* challenge contribute yet another chapter to this established cautionary tale.

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KEY WORDS:
*Candida* · chronic granulomatous disease · hereditary · myeloperoxidase · neutrophils