Published in final edited form as:

Early Hum Dev. 2009 August ; 85(8): 531–535. doi:10.1016/j.earlhumdev.2009.05.011.

Oxidative Burst and Phagocytosis of Neonatal Neutrophils Confronting *Candida albicans* and *Candida parapsilosis*

Kisha G. Destin¹, Jennifer R. Linden², Sonia S. Laforce-Nesbitt³, and Joseph M. Bliss^{2,3,*}

¹Shady Grove Adventist Hospital, Rockville, MD

²Graduate Program in Pathobiology, Brown University, Providence, RI

³Department of Pediatrics, Women & Infants Hospital of Rhode Island, Warren Alpert Medical School of Brown University, Providence, RI

Abstract

Background—*Candida albicans* and *Candida parapsilosis* are important causes of sepsis among premature neonates. The neutrophil is a key element in the control of *Candida* infections, yet specific neutrophil mechanisms that may contribute to the susceptibility of the premature neonate to candidiasis are not well understood.

Aims—The hypothesis for this study is that neonatal neutrophils have a developmental deficiency in their capacity to generate an oxidative burst in response to *Candida* species.

Study Design—Neutrophils were isolated from cord blood of term and preterm infants and from peripheral blood of adult volunteers. Neutrophils were exposed to *Candida* species, and assays of oxidative burst and phagocytosis were conducted.

Results—Oxidative burst of neutrophils from term and preterm (22-29 weeks) neonates exposed to *C. albicans* hyphae was similar to adult neutrophils. No detectable burst was induced in either group by exposure to *C. parapsilosis* yeast, and was attenuated by exposure to *C. albicans* yeast. Because no deficiency in oxidative burst was seen, phagocytosis was also studied. Phagocytosis of unopsonized *C. albicans* yeast was low in both adult and neonatal neutrophils (10-12%), but was more efficient with *C. parapsilosis* as target (76-88%). Neutrophils from both term and preterm infants were capable of phagocytosis equivalent to adults.

Conclusion—A deficiency in generation of an oxidative burst or phagocytosis may not contribute to the increased susceptibility of preterm neonates to infections with *Candida*.

Introduction

Candida species have become increasingly important pathogens in premature neonates, particularly among very low birth weight (VLBW) infants. Invasive *Candida* infections are the third most common cause of late-onset sepsis in this immunocompromised population, with an incidence ranging from 7% to 12% [1,2]. *Candida albicans* is the third most frequent organism isolated from blood cultures after coagulase-negative *Staphylococcus* and *S. aureus*.

* Corresponding Author: Dept. of Pediatrics, Women & Infants Hospital of RI, 101 Dudley St., Providence, RI 02905, Phone: 401-274-1100; Fax: 401-453-7571, Email: jbliss@wihri.org.

Conflict of Interest Statement: None declared.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Candida parapsilosis is also associated with invasive disease in this population with rates that approach that of *C. albicans* [1,2]. These infections continue to cause significant morbidity and mortality, even among infants receiving appropriate antifungal therapy [1,3].

The neutrophil has long been recognized as a key effector cell in fungal infection. As the first line of defense, these cells phagocytose and kill microorganisms through a combination of mechanisms [4] including production of reactive oxygen species [5]. They are well adapted to confront *C. albicans* by inhibiting hyphal formation and promoting efficient killing [6]. Deficiencies in either neutrophil number or function at any age are closely linked to the susceptibility to systemic disease [7,8]. The general features of neutrophil function from neonates, in comparison to adults, have been extensively studied in term infants and to a lesser extent in preterms [9]. Functional differences between adult and neonatal neutrophils have been observed, including reduced neutrophil supply in response to stress and diminished chemotaxis in the latter [9]. Preterm neutrophils have also been noted to have reduced ability to generate an oxidative burst in response to bacterial stimuli [10]. Capacity for phagocytosis has also received considerable attention with mixed results, and efficiency varies primarily with the degree of opsonization of the target [9]. Opsonization status is particularly relevant for *C. albicans* yeast, in that phagocytosis is quite low in the absence of opsonins, and improves dramatically when they are provided [11,12]. Very little work has focused on the function of neonatal neutrophils in fungal infections. The response of neutrophils to *Candida* may be quite different than the response to bacterial pathogens. Fungi have a unique cell wall and are significantly larger than bacteria. Further, in a hyphal form, fungal cells are considerably larger than the neutrophil and thus not amenable to phagocytosis.

In this study, we focused on the function of neonatal neutrophils when presented with a relevant fungal target, *C. albicans* or *C. parapsilosis*. Because *C. albicans* (but not *C. parapsilosis*) exists in both a yeast and hyphal form, and because both forms are important for virulence, we included both growth morphologies in these analyses. Opsonins were intentionally omitted from this study so that innate neutrophil function could be evaluated at its most basic level. This design avoids the additional complexities introduced by the various types and sources of opsonins that have confounded previous work. We hypothesized that relative to adult neutrophils, neonatal neutrophils have reduced capacity to generate an oxidative burst in response to *Candida* species, and that this reduction will be more pronounced in preterm than in term infants. We reasoned that this reduction may account for the increased susceptibility of preterm infants to invasive candidiasis.

Methods

Subjects

Adult neutrophils used in this study were obtained from peripheral blood of healthy volunteers. Neonatal neutrophils were obtained from cord blood collected immediately following delivery of the placenta. Clinical characteristics of the neonates and their mothers are summarized in Table 1. The protocols involving human subjects were reviewed and approved by the Institutional Review Board at Women & Infants Hospital of Rhode Island.

Candida strains

Candida albicans strain 3153A [13,14] and *Candida parapsilosis* strain RO75-R1 [15] were used in this study. Starter cultures of *C. albicans* or *C. parapsilosis* for burst or phagocytosis assays were grown 16 h at 37°C with vigorous agitation in YEPD medium (1% yeast extract, 2% peptone, 2% dextrose). Cultures were predominantly (>99%) yeast forms following this incubation. Yeast were washed in Hanks Balanced Salt Solution (HBSS) and enumerated on a hemacytometer prior to use in assays.

Oxidative burst assay

Adult and neonatal leukocytes were isolated from peripheral or cord blood, respectively, by density gradient centrifugation on Histopaque-1077 (Sigma) following the manufacturer's instructions. Neutrophils were further purified from red blood cells by dextran sedimentation and subsequent hypotonic lysis. Cells were adjusted to 5×10^6 cells/ml in HBSS + Ca/Mg, and were >95% pure as determined by Wright-Giemsa stain. To detect oxidative burst, neutrophils were loaded with 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H₂DCFDA, Molecular Probes) according to manufacturer's instructions (Linden *et al.*, submitted). This cell-permeant indicator becomes fluorescent upon intracellular oxidation, allowing quantification of oxidative burst. Loaded neutrophils were washed and resuspended in HBSS + Ca/Mg and seeded in 50 μ l aliquots into a 96-well dish. Hyphal growth of *C. albicans* was induced the day prior to the experiment by seeding yeast forms in the 96-well dish at 7000 cells per well, followed by growth in Medium 199 for 16-24 h at 37°C. *C. albicans* and *C. parapsilosis* yeast were added at a ratio of 10 yeast to 1 neutrophil. Phorbol 12-Myristate 13-Acetate (PMA) was used as a positive control at a final concentration of 20 nM. The plate was incubated at 37°C for 60 min. Oxidative burst activity was quantified using a fluorescence plate reader at excitation/emission wavelength settings 485/530 nm, respectively. Total fluorescence was read at 0 and 60 min. After correcting for baseline fluorescence at the start of the incubation period, fluorescence was normalized to PMN incubated in the absence of stimulus. Individual experiments were conducted in replicates of 4 to 8.

Phagocytosis assay

The phagocytosis assay was conducted essentially as described [11]. Neonatal neutrophils, adult neutrophils, and *Candida* yeast were prepared as described in the oxidative burst assay. *Candida* yeast were adjusted to 6×10^7 cells per ml. For ease of interpretation, yeast were heat-killed at 60-65°C for 90 min. Extensive studies using live yeast in this assay have demonstrated very similar rates of phagocytosis using live and heat killed yeast (Linden *et al.*, submitted). Yeast were labeled with FITC at 10 μ g/ml in the dark with rotation for 30 min at 37°C followed by extensive washes with HBSS. Neonatal and adult neutrophils were combined with the labeled *Candida* species at a ratio of 10 yeast to 1 neutrophil. Cells were pelleted at $400 \times g$ for 2 min, incubated on ice for 30 min to give a pool of cells with surface-bound *Candida*, then at 37°C for 30 min to allow phagocytosis. Cells were pelleted at $400 \times g$ for 2 min and resuspended in 20 μ L HBSS. 2.5 μ L of ethidium bromide (100 μ g/ml) was mixed with 2.5 μ L of the sample on the surface of a microscope slide and examined by fluorescence microscopy. Intracellular *Candida* were differentiated from extracellular by retention of green fluorescence (Fig. 3). A minimum of 100 PMN were counted, and the percent that had undergone phagocytosis of yeast was calculated.

Statistical analysis

Comparisons of oxidative burst and phagocytosis were made by one-way analysis of variance (ANOVA). Between groups comparisons were made by Newman Keuls test with p values < 0.05 being considered significant.

Results

Oxidative burst induced by *Candida* in adult neutrophils

To determine normative adult patterns of oxidative burst induced by *Candida* in our assay system, a total of 17 independent experiments were performed using 7 individual healthy adult donors (Fig. 1). Data were normalized to background activity of the same neutrophil preparation incubated in the absence of *Candida* or stimulus. Adult neutrophils exposed to *C.*

albicans hyphae exhibited an approximately 70% increase in burst activity relative to control neutrophils, which was equivalent to the burst induced by the positive control, PMA. No measurable burst was detected after incubation with *C. parapsilosis* yeast. *C. albicans* yeast significantly attenuated oxidative burst activity of adult neutrophils when compared to the absence of stimulus ($p < 0.01$). Overall, the response of adult neutrophils to *Candida* varied sharply depending on the growth form and species of the organism.

Oxidative burst induced by *Candida* in term and preterm infant neutrophils

Neutrophils were isolated from cord blood of 8 term neonates and 10 preterm neonates (22-29 wk gestation) immediately following delivery of the placenta (Table 1). Oxidative burst induced by *Candida* was measured using the conditions described above for adult neutrophils, and an adult control was included in each experiment. No significant differences in magnitude of oxidative burst compared to adult neutrophils were detected using neutrophils isolated from either term or preterm neutrophils (Fig. 2). Although the variance of the data was higher for preterm neutrophils than for term neutrophils, the variance of the adult controls was also higher in the preterm assays, suggesting that the difference was due to assay conditions. These data support the notion that neutrophils collected at birth from neonates are not deficient in their capacity to mount an oxidative burst to *Candida*, regardless of the gestational age of the infant.

Phagocytosis of *Candida* yeast by adult and neonatal neutrophils

Results of burst assays suggested that a deficiency in oxidative burst may not account for the increased susceptibility of preterm neonates to candidiasis. However, no burst was detectable against *C. parapsilosis*, and *C. albicans* yeast appeared to inhibit the burst activity. Previous work in our laboratory has demonstrated a marked difference in phagocytosis efficiency of these two species in adult neutrophils (Linden *et al.*, submitted). To determine whether differences in phagocytosis may exist between adult and neonatal neutrophils for these species that may account for susceptibility in preterm neonates, phagocytosis assays were conducted with neutrophils from 4 term and 3 preterm infants (22-27 wk gestation). Because the opsonic milieu is quite varied among adults, term, and preterm neonates, opsonins were omitted from these studies. Assays were conducted on yeast forms only, as *C. albicans* hyphae are too large to undergo phagocytosis.

A differential staining design was used for phagocytosis assays. *Candida* yeast were labeled with FITC, coincubated with neutrophils to allow phagocytosis to occur, and examined by fluorescence microscopy. Slides were counterstained with ethidium bromide to differentiate intracellular from extracellular *Candida*. Because ethidium bromide is excluded from live neutrophils, intracellular FITC-labeled *Candida* remained green in appearance on fluorescence microscopy while extracellular *Candida* appeared orange (Fig. 3). Phagocytosis was quantified by scoring the number of neutrophils containing intracellular *Candida*. Similar to previous work, phagocytosis of *C. albicans* yeast was low in adult neutrophils (11%), and markedly higher for *C. parapsilosis* (83%) (Fig. 4). No differences in phagocytosis efficiency of *Candida* yeast were observed using neutrophils from term or preterm neonates.

Discussion

Candida infections are a common cause of late-onset sepsis in premature neonates and are associated with significant mortality and neurodevelopmental impairment, with *C. albicans* and *C. parapsilosis* as the predominant implicated species [1,3]. Invasive *Candida* infections are uncommon outside the setting of immune compromise. Many deficiencies of host defense have been described in premature infants that contribute to their susceptibility to infections. Because of its important role in defense against *Candida*, we chose to focus on the neutrophil in this study, and in particular on the production of an oxidative burst in response to this

organism. Recent studies have examined various aspects of the oxidative burst response generated by phagocytes in response to *C. albicans*. In agreement with our findings in adult cells, human neutrophils have been demonstrated by others to generate a burst in response to unopsonized hyphae [16], and the cell wall component, β -glucan appears to play a role in this response through the neutrophil receptor, Dectin-1 [12]. Further, *C. albicans* has been shown to be capable of inhibiting the oxidative burst in a variety of murine and human phagocytes [17]. The authors suggest that such inhibition may represent an immune evasion mechanism. We saw a similar inhibition in our studies, despite the use of a different assay technique, and the effect was similar in neonatal neutrophils.

In contrast to studies of phagocyte function derived from animal models, cultured cell lines, or adult human cells, very few studies have focused on the response of neonatal neutrophils to a fungal pathogen. Using a whole blood assay, premature infants were found to have reduced non-directed neutrophil migration and phagocytosis of *Candida*, relative to adult controls and term infants [18]. The effect persisted whether autologous or control plasma was used in the experiment. Oxidative burst was not measured in this analysis. The whole blood approach differs significantly from the current study in which purified neutrophils were used in the absence of any added opsonins. This design was chosen to limit the influence of confounding factors inherent in the use of additional blood components or opsonins from varied sources. In contrast, phagocytic activity against *C. albicans* was found to be very similar in term (≥ 37 weeks) and preterm (< 37 weeks) neutrophils isolated from Chinese infants [19]. These cells were provided with human group AB serum “to provide needed opsonins.” Oxidative burst in response to *E. coli* lipopolysaccharide, as measured by nitroblue tetrazolium reduction, was also found to be similar between term and preterm infants in this cohort. *Candida* was not investigated as a stimulus. Another study found a reduction in phagocytosis of *C. albicans* in 13 preterm infants (28-36 weeks) relative to term infants and adults, while phagocytosis of *S. aureus* was identical among the groups [20]. Pooled serum was also included in these phagocytosis assays. Again, similar to the present study, no differences in superoxide release were detected among the groups in response to PMA, but response to *Candida* was not included in this report. Finally, a study of preterm leukocytes in autologous serum using *C. albicans* as target showed no difference in phagocytosis among preterm, term, and adult samples [21]. Oxidative burst was not studied. Taken together, these studies with conflicting results underscore the relevance of the target of phagocytosis that is studied and the varied observations that can be made when using different methods and different sources of opsonins. To our knowledge, no prior studies have investigated oxidative burst in response to *Candida* in neonates. In addition, the functions of neonatal neutrophils against *C. parapsilosis* have not been studied, despite the increasing prevalence of this organism in neonatal infections.

C. parapsilosis is of particular relevance in premature infants, where it is responsible for up to 43% of blood cultures positive for a *Candida* species and carries a mortality of 20% [1]. Recent studies of this organism have documented its ability to damage oral epithelial and epidermal tissues in reconstituted human tissue models [22] and have demonstrated the role of secreted lipases in virulence [23]. Significant differences were observed between these species in our assays. *C. albicans* yeast attenuated oxidative burst while *C. parapsilosis* did not, and phagocytosis of the latter was far more efficient. Although we found no differences in the activity of preterm neutrophils relative to adults, the marked differences between the species underscore the complexity of these interactions. In animal models, *C. parapsilosis* has been shown to be less virulent than *C. albicans* as it lacks the ability to undergo hyphal morphogenesis and is less adherent and penetrable to endothelium [24]. A recent surveillance study in preterm infants < 1500 g birth weight showed a lower rate of deep-seated infections among infants infected with *C. parapsilosis* relative to *C. albicans* with a similar mortality [25]. However, others have seen a lower mortality than *C. albicans* in preterm neonates [1,

26]. The differences in neutrophil response observed in this study may partially account for the apparent lower virulence of *C. parapsilosis* in these infants.

Our experiments were conducted in the absence of opsonins. There were several reasons for this design. Previous work in preterm infants has demonstrated that their neutrophils function in a milieu that is relatively deplete of opsonins, including profound hypogammaglobulinemia and deficiencies in complement activity [9], suggesting that omission of opsonins in these assays may be more relevant to the preterm infant *in vivo*. Multiple studies showing diminished phagocytosis activity in preterm neutrophils against a variety of targets have found marked improvement when opsonins are provided [9]. Given the variability among different sources of opsonins in terms of specific antibody components and/or complement activity, opsonization in this study would introduce additional complexity that could confound the observations. Finally, previous work in our laboratory has demonstrated a very efficient, opsonin-independent pathway for phagocytosis of *C. parapsilosis* (Linden *et al.*, submitted). We wished to evaluate whether that pathway was equally efficient in neonates, particularly given their propensity for infection with this species.

Although some insights into the interaction between neonatal neutrophils and *Candida* species can be gained from this study, there are additional variables that have not been addressed. The volume of blood required for these assays makes collection from preterm infants impractical, and has limited our observations to cord blood. The function of these neutrophils may change significantly after birth, and is likely to be affected by the clinical course of the infant. Likewise, a high proportion of the preterm infants were exposed to pathological conditions and medications prior to birth including chorioamnionitis, preeclampsia, betamethasone and magnesium (Table 1), which may have affected neutrophil function in cord blood samples. All of our term infant subjects were delivered by Cesarean section. An increased generation of superoxide anion has been observed in cord blood neutrophils collected after vaginal delivery compared to Cesarean section, suggesting that labor impacts burst activity [27]. Since we found no deficiency in burst activity in these infants, and similar results in preterm infants who were delivered by both routes, the high rate of Cesarean delivery is unlikely to have significantly biased our results. Because the quantity of blood that could be collected from a given placenta was sometimes limited, particularly in preterm infants, the number of patients included in the phagocytosis assays was lower than the number studied in burst assays. This smaller sample size limits the conclusions that can be drawn from the phagocytosis experiment; however, there was relatively low variance in these observations. Finally, although difference in oxidative burst and phagocytosis were not observed among groups, we have not excluded a deficiency in the ability of neonatal neutrophils to kill the fungal cells. Phagocytosis and oxidative burst are important elements in the ultimate killing of invading microbes, but other important mechanisms that may be deficient in neonatal cells may exist. Killing of *Candida* by these cells is a current area of active investigation in our laboratory. Nonetheless, the results of this study suggest that a deficiency of oxidative burst or phagocytosis may not significantly contribute to the increased susceptibility of preterm neonates to infections with *Candida*.

Acknowledgments

We are grateful for the assistance of Melanie Wellington and Kristy Dolan with the phagocytosis assay, Matthew Maccani in data collection and Sunil Shaw for useful discussions.

This work was supported by a March of Dimes Basil O'Connor Award (FY05-1211), a National Institute of Health grant (K08 AI064919), and a NIH COBRE grant (P20 RR018728).

References

1. Benjamin DK Jr, Stoll BJ, Fanaroff AA, McDonald SA, Oh W, Higgins RD, et al. Neonatal candidiasis among extremely low birth weight infants: risk factors, mortality rates, and neurodevelopmental outcomes at 18 to 22 months. *Pediatrics* 2006;117(1):84–92. [PubMed: 16396864]
2. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. *Pediatrics* 2002;110(2 Pt 1):285–91. [PubMed: 12165580]
3. Stoll BJ, Hansen NI, Adams-Chapman I, Fanaroff AA, Hintz SR, Vohr B, et al. Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection. *JAMA* 2004;292(19):2357–65. [PubMed: 15547163]
4. Segal AW. How neutrophils kill microbes. *Annu Rev Immunol* 2005;23:197–223. [PubMed: 15771570]
5. Fang FC. Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. *Nat Rev Microbiol* 2004;2(10):820–32. [PubMed: 15378046]
6. Fradin C, De Groot P, MacCallum D, Schaller M, Klis F, Odds FC, et al. Granulocytes govern the transcriptional response, morphology and proliferation of *Candida albicans* in human blood. *Mol Microbiol* 2005;56(2):397–415. [PubMed: 15813733]
7. Walsh TJ, Hiemenz JW, Anaissie E. Recent progress and current problems in treatment of invasive fungal infections in neutropenic patients. *Infect Dis Clin North Am* 1996;10(2):365–400. [PubMed: 8803625]
8. Liese J, Kloos S, Jendrossek V, Petropoulou T, Wintergerst U, Notheis G, et al. Long-term follow-up and outcome of 39 patients with chronic granulomatous disease. *J Pediatr* 2000;137(5):687–93. [PubMed: 11060536]
9. Carr R. Neutrophil production and function in newborn infants. *Br J Haematol* 2000;110(1):18–28. [PubMed: 10930976]
10. Kallman J, Schollin J, Schalen C, Erlandsson A, Kihlstrom E. Impaired phagocytosis and opsonisation towards group B streptococci in preterm neonates. *Arch Dis Child Fetal Neonatal Ed* 1998;78(1):F46–50. [PubMed: 9536841]
11. Wellington M, Bliss JM, Haidaris CG. Enhanced phagocytosis of *Candida* species mediated by opsonization with a recombinant human antibody single-chain variable fragment. *Infect Immun* 2003;71(12):7228–31. [PubMed: 14638823]
12. Kennedy AD, Willment JA, Dorward DW, Williams DL, Brown GD, DeLeo FR. Dectin-1 promotes fungicidal activity of human neutrophils. *Eur J Immunol* 2007;37(2):467–78. [PubMed: 17230442]
13. Morrow B, Ramsey H, Soll DR. Regulation of phase-specific genes in the more general switching system of *Candida albicans* strain 3153A. *J Med Vet Mycol* 1994;32(4):287–94. [PubMed: 7983573]
14. Vargas K, Wertz PW, Drake D, Morrow B, Soll DR. Differences in adhesion of *Candida albicans* 3153A cells exhibiting switch phenotypes to buccal epithelium and stratum corneum. *Infect Immun* 1994;62(4):1328–35. [PubMed: 8132340]
15. Bliss JM, Basavegowda KP, Watson WJ, Sheikh AU, Ryan RM. Vertical and horizontal transmission of *Candida albicans* in very low birth weight infants using DNA fingerprinting techniques. *Pediatr Infect Dis J* 2008;27(3):231–5. [PubMed: 18277930]
16. Lavigne LM, Albina JE, Reichner JS. Beta-glucan is a fungal determinant for adhesion-dependent human neutrophil functions. *J Immunol* 2006;177(12):8667–75. [PubMed: 17142767]
17. Wellington M, Dolan K, Krysan DJ. Live *Candida albicans* suppresses production of reactive oxygen species in phagocytes. *Infect Immun* 2009;77(1):405–13. [PubMed: 18981256]
18. Al-Hadithy H, Addison IE, Goldstone AH, Cawley JC, Shaw JC. Defective neutrophil function in low-birth-weight, premature infants. *J Clin Pathol* 1981;34(4):366–70. [PubMed: 7240423]
19. Wan T, Yeung C, Tam A. Neutrophil functions of Chinese neonates. *J Paediatr Child Health* 2000;36(2):153–8. [PubMed: 10760014]
20. Bektas S, Goetze B, Speer CP. Decreased adherence, chemotaxis and phagocytic activities of neutrophils from preterm neonates. *Acta Paediatr Scand* 1990;79(11):1031–8. [PubMed: 2267919]

21. Xanthou M, Valassi-Adam E, Kintsonidou E, Matsaniotis N. Phagocytosis and killing ability of *Candida albicans* by blood leucocytes of healthy term and preterm babies. *Arch Dis Child* 1975;50(1):72–75. [PubMed: 1092273]
22. Gacser A, Schafer W, Nosanchuk JS, Salomon S, Nosanchuk JD. Virulence of *Candida parapsilosis*, *Candida orthopsilosis*, and *Candida metapsilosis* in reconstituted human tissue models. *Fungal Genet Biol* 2007;44(12):1336–41. [PubMed: 17391997]
23. Gacser A, Trofa D, Schafer W, Nosanchuk JD. Targeted gene deletion in *Candida parapsilosis* demonstrates the role of secreted lipase in virulence. *J Clin Invest* 2007;117(10):3049–58. [PubMed: 17853941]
24. Weems JJ Jr. *Candida parapsilosis*: epidemiology, pathogenicity, clinical manifestations, and antimicrobial susceptibility. *Clin Infect Dis* 1992;14(3):756–66. [PubMed: 1345554]
25. Clerihew L, Lamagni TL, Brocklehurst P, McGuire W. *Candida parapsilosis* infection in very low birthweight infants. *Arch Dis Child Fetal Neonatal Ed* 2007;92(2):F127–9. [PubMed: 17337658]
26. Faix RG. Invasive neonatal candidiasis: comparison of *albicans* and *parapsilosis* infection. *Pediatr Infect Dis J* 1992;11(2):88–93. [PubMed: 1741204]
27. Ambruso DR, Stork LC, Gibson BE, Thurman GW. Increased activity of the respiratory burst in cord blood neutrophils: kinetics of the NADPH oxidase enzyme system in subcellular fractions. *Pediatr Res* 1987;21(2):205–10. [PubMed: 3029658]

Figure 1.

Oxidative burst of adult neutrophils induced by *Candida* species. Neutrophils (PMN) isolated from peripheral blood of healthy adult volunteers ($n = 7$; 17 total independent experiments) were combined with PMA, *C. parapsilosis* (*C. para*) yeast, or *C. albicans* (*C. alb*) yeast or hyphae. Fluorescence, proportional to oxidative burst activity, was measured after 60 min and normalized to control neutrophils incubated in the absence of stimulus (PMN only). Error bars represent standard deviation. PMA and *C. albicans* hyphae stimulate a significant burst over control (* - $p < 0.001$), while *C. albicans* yeast inhibit burst (** - $p < 0.01$).

Figure 2.

Comparison of oxidative burst of adult and infant neutrophils induced by *Candida* species. Neutrophils (PMN) isolated from cord blood of term infants (Panel A, n = 8) or preterm infants (Panel B, n = 10) were combined with PMA, *C. parapsilosis* (*C. para*) yeast, or *C. albicans* (*C. alb*) yeast or hyphae. Fluorescence, proportional to oxidative burst activity, was measured after 60 min and normalized to control neutrophils incubated in the absence of stimulus (PMN only) (white bars). Adult neutrophils were run in parallel in each experiment (black bars). Error bars represent standard deviation. No significant differences were observed in burst activity between term and adult neutrophils or preterm and adult neutrophils under any condition. Significant differences relative to control were found in comparing burst activity to the various

stimuli within an age category, similar to that seen in Figure 1 (* - $p \leq 0.02$, ** - $p < 0.001$ for adult, $p = 0.06$ for infant). Because of the increased variance in Panel B, statistical significance was not achieved for *C. albicans* yeast or hyphae relative to control for either adult or preterm neutrophils.

Figure 3.

Phagocytosis assay by fluorescence microscopy method. *Candida* yeast were labeled with FITC and combined with adult or infant neutrophils. After allowing phagocytosis to occur, cells were spotted on microscope slides and stained with ethidium bromide. Since ethidium bromide stains yeast orange but is excluded from live neutrophils, yeast that have undergone phagocytosis retain green fluorescence. Rates of phagocytosis were calculated by counting a minimum of 100 neutrophils and calculating the percentage that have intracellular (green) yeast. This photomicrograph is a portion of a representative field from a phagocytosis assay with *C. parapsilosis* and illustrates the distinct color difference between extracellular (orange) and intracellular (green) yeast.

Figure 4.

Comparison of phagocytosis rates by adult and infant neutrophils against *Candida* species. Phagocytosis assays were conducted with *C. albicans* and *C. parapsilosis* yeast using neutrophils isolated from adults (black bars; n = 6), term infants (white bars; n = 4), and preterm infants (gray bars; n = 3). Phagocytosis rates were calculated by enumerating the percentage of neutrophils containing intracellular (green) yeast. Error bars represent standard deviation. Although the rates of phagocytosis of *C. parapsilosis* yeast were significantly higher than rates of *C. albicans* yeast ($p < 0.001$), there were no significant differences among neutrophils from adults, term, or preterm infants.

Table 1**Clinical characteristics of subjects**

	Term N = 8	Preterm N = 10
Maternal Age – mean (SD)	30.9 (4.6)	26.6 (5.5)
Primiparous – n (%)	1 (12.5)	5 (50)
Cesarean section – n (%)	8 (100)	5 (50)
Male gender – n (%)	3 (37.5)	4 (40)
Gestational age – median (range)	39 (38-39)	27 (22-29)
Birth weight, g – mean (SD)	3691 (548)	1153 (326)
Maternal Conditions		
Preeclampsia – n (%)	1 (12.5)	1 (10)
Chorioamnionitis – n (%)	0 (0)	6 (60)
Systemic infection – n (%)	0 (0)	2 (20)
Maternal Medications		
Betamethasone – n (%)	0 (0)	9 (90)
Magnesium – n (%)	0 (0)	4 (40)
Other tocolytics – n (%)	0 (0)	5 (50)
Antibiotics – n (%)	0 (0)	7 (70)