Morphology-Independent Virulence of *Candida* Species during Polymicrobial Intra-abdominal Infections with *Staphylococcus aureus*

Evelyn E. Nash,^a Brian M. Peters,^{b*} Paul L. Fidel,^{a,b} Mairi C. Noverr^{a,b,c}

Department of Microbiology, Immunology, and Parasitology, School of Medicine, Louisiana State University Health Sciences Center, New Orleans, Louisiana, USA^a; Department of Oral and Craniofacial Biology, Dental School, Louisiana State University Health Sciences Center, New Orleans, Louisiana, USA^b; Department of Prosthodontics, Dental School, Louisiana State University Health Sciences Center, New Orleans, Louisiana, USA^c

Intra-abdominal polymicrobial infections cause significant morbidity and mortality. An experimental mouse model of *Candida albicans*-*Staphylococcus aureus* intra-abdominal infection (IAI) results in 100% mortality by 48 to 72 h postinoculation, while monomicrobial infections are avirulent. Mortality is associated with robust local and systemic inflammation without a requirement for *C. albicans* morphogenesis. However, the contribution of virulence factors coregulated during the yeast-to-hypha transition is unknown. This also raised the question of whether other *Candida* species that are unable to form hyphae are as virulent as *C. albicans* during polymicrobial IAI. Therefore, the purpose of this study was to evaluate the ability of non-*albicans* *Candida* (NAC) species with various morphologies and *C. albicans* transcription factor mutants (*efg1/efg1* and *cph1/cph1*) to induce synergistic mortality and the accompanying inflammation. Results showed that *S. aureus* coinoculated with *C. krusei* or *C. tropicalis* was highly lethal, similar to *C. albicans*, while *S. aureus*-*C. dubliniensis*, *S. aureus*-*C. parapsilosis*, and *S. aureus*-*C. glabrata* coinoculations resulted in little to no mortality. Local and systemic interleukin-6 (IL-6) and prostaglandin E₂ (PGE₂) levels were significantly elevated during symptomatic and/or lethal coinfections, and hypothermia strongly correlated with mortality. Coinoculation with *C. albicans* strains deficient in the transcription factor Efg1 but not Cph1 reversed the lethal outcome. These results support previous findings and demonstrate that select *Candida* species, without reference to any morphological requirement, induce synergistic mortality, with IL-6 and PGE₂ acting as key inflammatory factors. Mechanistically, signaling pathways controlled by Efg1 are critical for the ability of *C. albicans* to induce mortality from an intra-abdominal polymicrobial infection.

Intra-abdominal infection (IAI) is a broad term used to describe numerous infections caused by the invasion of microorganisms into the abdominal cavity. These infections can range from appendicitis to complicated peritonitis and are initiated by microbes entering the abdominal cavity through a variety of routes, including bowel perforation, laparotomy, intestinal hernias, and insertion of medical devices such as peritoneal catheters (reviewed in reference 1). These infections often are polymicrobial in nature, which complicates diagnosis and treatment. Accordingly, infections with multiple microbes can result in amplified pathogenicity, also known as “infectious synergism” (2–4), which may accelerate disease progression or protect microbes from host responses (5), resulting in higher morbidity and mortality (6, 7).

Polymicrobial IAIs involving fungi result in mortality rates between 50 and 75%, compared to 10 to 30% for polymicrobial bacterial infections (8–11). The majority of polymicrobial fungal IAIs are caused by *Candida albicans* (12, 13). However, IAIs caused by non-*albicans* *Candida* (NAC) species are becoming more common (14). *C. albicans* most often is detected together with enteric bacteria (15), but the ubiquitous bacterial pathogen *Staphylococcus aureus* can be coisolated in up to 50% of polymicrobial fungal IAIs (16–18). *C. albicans* and *S. aureus* both are nosocomial pathogens that cause opportunistic infections in similar host niches. They are the most common causative agents of late-onset sepsis in very-low-birth-weight infants (19) and are rated among the top four pathogens causing bloodstream infections (20). Additionally, they can form polymicrobial biofilms on a variety of surfaces, including peritoneal catheters, which serve as a source of contamination leading to development of IAIs (21, 22).

Using an experimental murine model of fungal-bacterial IAI,

our laboratory has demonstrated that coinfection with *C. albicans* and *S. aureus* resulted in dissemination, significant local and systemic inflammation, and extremely high mortality (upwards of 100% by 48 h) (23, 24). In contrast, monomicrobial infections resulted in no morbidity or mortality, even when fungal inocula were increased to the levels used in coinfections. Surprisingly, mortality in coinfections correlated with increased inflammatory mediators but not increased dissemination or microbial burden in target organs (24).

The intra-abdominal route of infection also represents one route of systemic candidiasis, with the other major route being the intravenous one derived from contaminated catheters. The intravenous (i.v.) model of systemic candidiasis has been used extensively to analyze fungal virulence and immunity. However, these two routes of infection lead to distinct syndromes with different

Received 25 August 2015 Returned for modification 15 September 2015

Accepted 10 October 2015

Accepted manuscript posted online 19 October 2015

Citation Nash EE, Peters BM, Fidel PL, Noverr MC. 2016. Morphology-independent virulence of *Candida* species during polymicrobial intra-abdominal infections with *Staphylococcus aureus*. Infect Immun 84:90–98. doi:10.1128/IAI.01059-15.

Editor: G. S. Deepe, Jr., University of Cincinnati

Address correspondence to Mairi C. Noverr, mnover@lsuhsc.edu.

* Present address: Brian M. Peters, College of Pharmacy, University of Tennessee Health Sciences Center, Memphis, Tennessee, USA.

Supplemental material for this article may be found at <http://dx.doi.org/10.1128/IAI.01059-15>.

Copyright © 2015, American Society for Microbiology. All Rights Reserved.

target organs and niche-specific differences in innate defenses (reviewed in reference 25). Therefore, virulence traits or genes identified using the i.v. model may not necessarily be required for the IAI model. The morphological transition from yeast to hyphae is considered to be a major virulence trait of *C. albicans*, which is accompanied by an increased ability to penetrate tissues, evade immune defenses, and cause cell damage, and it leads to the up-regulation of virulence factors not directly involved in morphogenesis (reviewed in references 26–28). Surprisingly, we have demonstrated that *C. albicans* morphogenesis is not required to induce synergistic lethality during IAIs with *S. aureus* (23). Recognizing that NAC species vary in morphology and ability to undergo morphogenesis (29, 30), reside within the gastrointestinal tract (31, 32), and have been shown to cause IAIs (8, 33), it is important to extend the pathogenesis studies to NAC species and further investigate the role of pathways that regulate morphogenesis and virulence during IAIs.

MATERIALS AND METHODS

Strains and growth conditions. The methicillin-resistant *S. aureus* strain NR-46069 (previously referred to as NRS383) was provided by the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) for distribution by BEI Resources and used in all experiments. NR-46069 is positive for toxic shock syndrome toxin (*tst*) and delta-toxin genes. A single colony of *S. aureus* was transferred to 10 ml of Trypticase soy broth (TSB) (Becton, Dickinson, Sparks, MD) and incubated with shaking at 37°C overnight, diluted 1:100 in fresh TSB, and incubated similarly for 3 h (log phase). The wild-type (WT) *C. albicans* strain, DAY185, is a prototrophic control strain derived from a triple auxotrophic strain (BWP17; parent, SC5314) and is commonly used to produce knockout and complemented strains of *C. albicans* (34). Strains HLC52 (*efg1/efg1*), HLC74 (*efg1/efg1 EFG1*), J9 (*cph1/cph1*), and HLC54 (*efg1/efg1 cph1/cph1*) were kindly provided by G. R. Fink (Whitehead Institute, Cambridge, MA). *C. krusei* strain 14130.1 is an oral isolate from an HIV⁺ individual (Fidel specimen bank). *C. dubliniensis* strain 962926 and *C. glabrata* strain LF574.92 (also referred to as BG2) are vaginal isolates from a recurrent vulvovaginal candidiasis (VVC) patient and were provided by J. Sobel at Wayne State University School of Medicine. *C. tropicalis* and *C. parapsilosis* were obtained from the American Type Culture Collection (ATCC) (*C. tropicalis*, ATCC 44508; *C. parapsilosis*, ATCC 90018). *Saccharomyces cerevisiae* FY4 was provided by Fred Winston (Harvard University). Frozen stocks of fungal strains were kept at –80°C and streaked onto Sabouraud dextrose agar (SAB) (BD) or CHROMagar *Candida* (CHROMagar, Paris, France). A single colony was transferred to 20 ml of yeast extract-peptone-dextrose (YPD) broth and incubated with shaking at 30°C for 18 h (stationary phase). For inoculation, all isolates were washed 3 times by centrifugation in 0.1-μm sterile filtered phosphate-buffered saline (PBS) (HyClone), counted on a hemocytometer, and diluted in sterile PBS to the desired inocula. For UV radiation-killed *C. albicans*, cells were grown and washed as described above and then exposed in a thin liquid suspension to 4 doses of radiation (100 mJ/cm²) in a UV Stratalinker. One hundred percent killing was confirmed by plating 100-μl aliquots of UV-treated *C. albicans* onto SAB plates and incubating at 30°C for 24 h.

Mouse model of IAI. All animals were housed and handled according to institutionally recommended guidelines. All animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the LSU Health Sciences Center New Orleans. Peritoneal infection was conducted as previously described (23, 24), with some modifications. Briefly, 6-week-old female outbred NIH Swiss mice, purchased from Charles Rivers, Frederick, MD, were injected intraperitoneally (i.p.) with 3.5×10^7 CFU of various *Candida* species or *S. cerevisiae* and 8×10^7 CFU of *S. aureus* in 0.2 ml of PBS. Mice injected i.p. with 0.2 ml of saline served as a negative infection control and are referred to as naive. After

inoculation, mice were observed over 10 days for morbidity and mortality. Morbidity scores for each coinfection were designated by tallying the number of moribund characteristics observed during each infection out of a total of four characteristics (hunched posture, lethargy, ruffled fur, and porphyrin ocular discharge) over the 10-day period. In addition, separate groups of mice were sacrificed at 4 and 24 h postinoculation, and specimens were immediately collected. Whole blood was collected by retro-orbital bleeding, and serum was separated by centrifugation in Microtainer serum separator tubes (BD) at $10,000 \times g$ for 2 min. Peritoneal cavities were lavaged by injection of 2 ml of sterile PBS containing protease inhibitor (complete, EDTA-free; Roche, Basel, Switzerland) followed by gentle massaging of the peritoneal cavity. Peritoneal lavage fluid then was removed using a pipette inserted into a small incision in the abdominal cavity. Spleen and brains were removed, weighed, and mechanically homogenized prior to CFU analysis.

Microbial burden. Microbial burdens in the peritoneal lavage fluid, spleen, and brain were enumerated after serial dilutions and plating onto SAB agar plates containing 40 μg/ml gentamicin (Invitrogen, Carlsbad, CA) and 2 μg/ml vancomycin (Sigma Chemicals Co., St. Louis, MO) or CHROMagar plates for fungal quantification. Trypticase soy agar (TSA) plates containing 2.5 μg/ml amphotericin B (Sigma) and 40 μg/ml gentamicin were used for *S. aureus* quantification. SAB and CHROMagar plates were incubated at 35°C for 48 h, and TSA plates were incubated at 37°C for 24 h. Spleen and brain CFU were normalized to tissue weights (in grams).

Prostaglandin analysis. Recovered peritoneal lavage fluid was centrifuged at $500 \times g$ for 5 min and the supernatant transferred to clean microcentrifuge tubes. Prostaglandin E₂ (PGE₂) rapidly converts to the 13,14-dihydro-15-keto metabolite *in vivo*, so the prostaglandin E metabolite competitive enzyme immunoassay (EIA) was used to measure PGE₂ as a function of its breakdown product according to the manufacturer's directions (Cayman Chemical, Ann Arbor MI).

Cytokine analysis. Concentrations of interleukin-6 (IL-6), IL-1β, and tumor necrosis factor alpha (TNF-α) in serum and peritoneal lavage supernatants were determined by single-plex enzyme-linked immunosorbent assays (ELISAs) (eBioscience, San Diego, CA). Total protein levels also were measured in peritoneal lavage supernatants using a bicinchoninic acid (BCA) protein assay kit (Thermo Scientific, Rockford, IL), and cytokine data from lavage fluid were expressed as picograms of cytokine per milligram of total protein.

Body temperature. Rectal temperature was measured using a three-quarter-inch rectal probe (ThermoWorks, American Fork, UT). At designated times postinoculation, mice were anesthetized with 3% isoflurane followed by insertion of the probe into the rectum. Body temperature was measured in degrees Celsius.

Statistics. All experiments used groups of 5 mice and were independently repeated, except where noted. For all assays, samples were tested in duplicate, and the results were averaged. Survival curves were compared using the log rank (Mantel-Cox) test. Fungal and bacterial burdens were analyzed in several ways using nonparametric tests. Kruskal-Wallis was used to compare fungal or bacterial CFU between all organisms as well as those representing lethal and nonlethal outcomes. Additionally, fungal and bacterial burdens were analyzed between lethal coinfections (*C. albicans*, *C. krusei*, and *C. tropicalis*) grouped together and compared to the burden from each nonlethal coinfection (*C. dubliniensis*, *C. parapsilosis*, *C. glabrata*, and *S. cerevisiae*) as well as the reverse (nonlethal coinfections grouped together and compared to each lethal coinfection) using the Mann-Whitney U test. In addition, microbial burden was compared between each individual infection using the Mann-Whitney U test. Cytokine and PGE₂ concentrations from pathogenic coinfections were individually compared to concentrations from each nonpathogenic coinfection using the unpaired Student's *t* test. Significant differences were defined at *P* < 0.05. All statistical analyses were performed using Prism software (Graph Pad, San Diego, CA).

FIG 1 Survival following coinoculation with NAC species and *S. aureus*. Mice ($n = 5$ mice/group) were injected intraperitoneally with 8×10^7 CFU of *S. aureus* and 3.5×10^7 CFU of *C. albicans*, *C. krusei*, *C. tropicalis*, *C. dubliniensis*, *C. parapsilosis*, or *C. glabrata*. Mice inoculated with *S. aureus* and 3.5×10^7 CFU of *S. cerevisiae* or UV-killed *C. albicans* served as negative controls. Mice were monitored for 10 days postinoculation (p.i.). Data are cumulative from two repeat experiments. Mortality compared to that of *C. albicans* coinfection was measured by log-rank (Mantel-Cox) test. *, $P < 0.001$; **, $P < 0.0001$.

RESULTS

NAC species vary in ability to induce morbidity and mortality during polymicrobial IAI. Several non-*albicans* *Candida* species have been isolated during IAIs, but most have never been experimentally tested as causative agents (8, 33). Using the polymicrobial IAI model, five NAC species that vary in ability to undergo morphogenesis were tested for virulence. All *Candida* species were inoculated as yeast, and *in vivo* morphologies were examined in peritoneal lavage fluid (see Fig. S1 in the supplemental material). Mice coinoculated with *S. aureus* and *C. krusei* (yeast, pseudohyphae) or *C. tropicalis* (yeast, true hyphae) exhibited high mortality rates (80 and 90%, respectively) by day 10 postinoculation (p.i.), similar to the 100% mortality rate observed for *C. albicans* (yeast, true hyphae)-*S. aureus* coinfections (positive control) (Fig. 1). Importantly, monomicrobial infections with either *C. krusei* or *C. tropicalis* were avirulent (no morbidity or mortality) (data not shown), in agreement with our previously published observations with *C. albicans* and *S. aureus* monomicrobial infections (24). This indicates that coinfection with several *Candida* spp. and *S. aureus* leads to synergistic effects on mortality during IAI. In contrast, *S. aureus*-*C. dubliniensis* (yeast, true hyphae) coinoculation resulted in 20% mortality by day 10 p.i., while coinoculation with *C. parapsilosis* (yeast, pseudohyphae) or *C. glabrata* (yeast only) were nonlethal, similar to negative controls that included mice inoculated with *S. aureus*-*Saccharomyces cerevisiae* and *S. aureus*-UV-killed *C. albicans* (yeast form) (Fig. 1).

The morbidity of coinoculated mice also was monitored during the survival studies. Unsurprisingly, mice that were coinoculated with *C. albicans*, *C. krusei*, and *C. tropicalis* (lethal outcome) all exhibited significant moribund characteristics, including ruffled fur, lack of mobility, hunched posture, and porphyrin ocular discharge (morbidity scores of 3 to 4) (Table 1). Remarkably, mice coinoculated with *C. dubliniensis* (nonlethal) also exhibited similar signs of morbidity (morbidity score of 3). Mice coinoculated with *C. parapsilosis* and *C. glabrata* showed little to no sign of morbidity, similar to the negative-control coinoculations (*S. cerevisiae* and UV-killed *C. albicans*) (morbidity score of 0). Furthermore, the onset of morbidity correlated with mortality as early as day 1 p.i. for lethal coinfections and was delayed for *C. dubliniensis* (nonlethal) coinfection (day 3) (Table 2).

TABLE 1 Morbidity scores following NAC species-*S. aureus* coinoculations

Species coinoculated with <i>S. aureus</i>	Ruffled fur	Lack of mobility	Hunched posture	Ocular discharge	Final score ^a
<i>C. albicans</i>	+	+	+	+	4
<i>C. krusei</i>	+	+	+	+	4
<i>C. tropicalis</i>	+	+	+	—	3
<i>C. dubliniensis</i>	+	+	+	—	3
<i>C. parapsilosis</i>	+	—	—	—	1
<i>C. glabrata</i>	—	—	—	—	0
<i>S. cerevisiae</i>	—	—	—	—	0
UV-killed <i>C. albicans</i>	—	—	—	—	0

^a Scores reflect the additive value of the various moribund characteristics, with a maximum score of 4.

Inflammation and hypothermia, but not microbial burden, are closely associated with morbidity and mortality during polymicrobial infection. During polymicrobial IAI with *C. albicans*, we previously demonstrated that heightened inflammation, as opposed to microbial burden, was more closely associated with mortality (23). To confirm that differences in mortality between NAC species coinfections were not due to variations in fungal or bacterial burden, mice coinoculated with *S. aureus* and NAC species were sacrificed and the microbial burden was assessed in the peritoneal lavage fluid. While we observed intraspecies variability in fungal and bacterial burden that resulted in significant differences via multiple comparisons as groups (lethal/nonlethal) or independently, no patterns or trends were observed within or between lethal (*C. albicans*, *C. krusei*, and *C. tropicalis*) and nonlethal (*C. dubliniensis*, *C. parapsilosis*, and *C. glabrata*) coinfections to support a particular outcome (lethal/nonlethal) (Fig. 2; also see Tables S1 and S2 in the supplemental material). This result was further confirmed in spleen and brain homogenates (data not shown).

Proinflammatory cytokine levels (IL-6, IL-1 β , and TNF- α) positively correlate with mortality during *C. albicans*-*S. aureus* coinfection (23), and the immunomodulatory eicosanoid PGE₂ is required for lethality (24). At 24 h p.i., PGE₂ (Fig. 3A) and IL-6 (Fig. 3B) levels were significantly elevated during symptomatic coinfections with *C. albicans*, *C. krusei*, *C. tropicalis*, and *C. dubliniensis* compared to asymptomatic coinfections (*C. parapsilosis*, *C. glabrata*, *S. cerevisiae*, and UV-killed *C. albicans*). The level of IL-1 β also was elevated during symptomatic coinfection (Fig. 3C), while TNF- α levels did not correlate with morbidity or mortality (Fig. 3D). Because PGE₂ and IL-6 strongly correlated with mortality at 24 h p.i., we assessed these mediators at earlier time points as possible markers of future disease severity. However, elevated concentrations of PGE₂ and IL-6 were detected only in *C. albicans* and *C. krusei* coinfections (Fig. 3E and F).

Similar to local inflammatory responses, NAC species coinfection

TABLE 2 Onset of morbidity during NAC species-*S. aureus* coinfections

Species coinoculated with <i>S. aureus</i>	Morbidity score on day:		
	1	2	3
<i>C. albicans</i>	4	4	4
<i>C. krusei</i>	4	4	4
<i>C. tropicalis</i>	3	3	3
<i>C. dubliniensis</i>	0	0	3

FIG 2 Microbial burden in peritoneal lavage fluid during NAC species-*S. aureus* coinfections. Mice were infected with *S. aureus* and NAC species as described in the legend to Fig. 1. Mice ($n = 5$ mice/group) were sacrificed at 24 h p.i. to quantify fungal and bacterial burden in peritoneal lavage fluid. Results are expressed as the median CFU. Shown are cumulative data from two repeat experiments. Fungal and bacterial burden from *C. albicans* (CA), *C. krusei* (CK), and *C. tropicalis* (CT) coinfections were grouped and individually compared to *C. dubliniensis* (CD), *C. parapsilosis* (CP), *C. glabrata* (CG), and *S. cerevisiae* (SC) coinfections. Data were analyzed using the Mann-Whitney U test.

tions that cause morbidity also had significantly higher serum IL-6 (Fig. 4A) and IL-1 β (Fig. 4B) levels than asymptomatic coinfections. Again, TNF- α levels did not correlate with morbidity or mortality (Fig. 4C). Interestingly, serum IL-6 levels were significantly increased in all lethal coinfections compared to the levels in nonlethal coinfections, suggesting that IL-6 is closely associated with mortality and is a predictor of lethal sepsis.

Hypothermia also is closely associated with mortality during sepsis and represents a potentially viable and less invasive marker of disease severity in the clinical setting (35, 36). Core body temperatures of coinfecting mice were monitored daily throughout the course of infection. Coinfecting mice that eventually succumb to infection (including mice infected with *C. albicans*, *C. krusei*, *C. tropicalis*, and *C. dubliniensis*) have significantly decreased core temperature at 4 h p.i. compared to those of mice that survive coinfection (including mice infected with *C. parapsilosis*, *C. glabrata*, and *C. dubliniensis*) (Fig. 5A). This hypothermic state is even more pronounced at 24 h p.i. (Fig. 5B).

Differential ability of *C. albicans* nonhyphal mutants to induce mortality during polymicrobial IAI. We previously demonstrated that *C. albicans* strains that are genetically engineered to remain locked in yeast form (t-NRG1; overexpresses a negative regulator of morphogenesis) or locked in hyphal form (t-UME6; overexpresses a positive regulator of morphogenesis) both were capable of causing 100% mortality, similar to wild-type *C. albicans* infections (23), indicating that *C. albicans* morphogenesis is not required for virulence during IAI. Morphogenesis is regulated through complex signaling pathways controlled by a group of transcription factors with overlapping function. The activation of these signaling pathways results in the transcription of hypha-coregulated genes such as adhesins, secreted aspartyl proteases (SAPs), and factors involved in biofilm formation (reviewed in reference 27). Because the yeast-locked *C. albicans* mutant has all morphogenetic signaling pathways intact, there is the possibility that genes not directly involved in morphogenesis are induced in the yeast-locked strain *in vivo* and play a role in virulence during IAI. Therefore, we tested *C. albicans* mutants deficient in various

transcription factors that regulate morphogenesis along with other coregulated virulence traits, such as biofilm formation and white-opaque switching in the polymicrobial IAI model. These included a homozygous mutant in *CPH1* (*cph1/cph1*), which is regulated through the mitogen-activated protein kinase (MAPK) pathway and fails to make hyphae under certain laboratory stress conditions (37); a homozygous mutant in *EFG1* (*efg1/efg1*), which signals downstream of the cyclic AMP (cAMP)/protein kinase A (PKA) pathways and has severe defects in morphogenesis under a variety of conditions (38, 39); and a homozygous double mutant strain (*efg1/efg1 cph1/cph1*) that fails to produce hyphae *in vivo* and in response to nearly all *in vitro* culture conditions (38, 40, 41). Interestingly, coinfections with either *efg1/efg1* or the double-knockout strain, but not *cph1/cph1*, significantly reduced mortality (<10%) compared to the 100% mortality for the WT (Fig. 6). Furthermore, reconstitution of the *efg1/efg1* mutant with a wild-type copy of *EFG1* restored mortality (80%), confirming a role for Efg1-regulated pathways in *C. albicans* virulence during polymicrobial IAI.

DISCUSSION

The differential results following coinoculation with *S. aureus* and several NAC species (*C. krusei* and *C. tropicalis* have high mortality and heightened inflammation, similar to *C. albicans* coinfections [23], while *C. parapsilosis* and *C. glabrata* show no mortality or heightened inflammation) was not dependent on morphogenesis or any particular morphological form of NAC species. Having confirmed the *in vivo* morphologies, including yeast, pseudohyphae, and true hyphae, we conclude that hyphal formation is not required or sufficient for inducing synergistic lethality with *S. aureus*. Interestingly, coinfection with *C. dubliniensis* resulted in an intermediate phenotype with low mortality, despite significant morbidity and hyphal formation *in vivo*. In addition, a 2-fold increase in the *C. dubliniensis* inocula also failed to result in mortality (data not shown). Together, these results further emphasize the lack of a requirement for morphogenesis in lethality. *C. dubliniensis* also exhibits reduced mortality in the i.v. model of systemic candidiasis, and it was postulated that this is due to reduced hyphal formation compared to that of *C. albicans*. However, a hyperfiliamentous *C. dubliniensis* mutant strain (deficient in NRG1) also was less virulent than *C. albicans* despite being more resistant to macrophages *in vitro* (42). This suggests that *C. dubliniensis* lacks *C. albicans* virulence mechanisms independent of morphogenesis, which also may be required for virulence in the polymicrobial IAI model.

Despite the fact that all of the NAC species disseminated to retroperitoneal organs during polymicrobial IAI, only three species caused lethal sepsis. This is consistent with results using the i.v. model of systemic candidiasis, with high mortality observed with *C. albicans* and *C. tropicalis* and low mortality with *C. glabrata*, *C. dubliniensis*, and *C. parapsilosis* (43, 44). The exception is *C. krusei*, which causes variable mortality in disseminated i.v. models of systemic candidiasis (43, 44). Note that all of these species cause mortality clinically, mostly in immunocompromised or neonatal patients, and the incidence of candidemia due to NAC has increased in the past several years (45). This indicates a need to consider the role of host immunity and/or age in experimental virulence testing for NAC species. This is particularly important for polymicrobial infections involving *Candida* and

FIG 3 Local immune mediators during peritoneal coinfections with NAC species and *S. aureus*. Mice ($n = 5$ mice/group) were infected with *S. aureus* and NAC species as described in the legend to Fig. 1. At 4 or 24 h p.i., peritoneal lavage fluid was collected and analyzed for PGE₂ (A and E), IL-6 (B and F), IL-1β (C), and TNF-α (D). Results are expressed as the mean cytokine or eicosanoid concentration $\pm$ standard errors of the means (SEM). Shown are cumulative data from two repeat experiments. Cytokine or eicosanoid concentrations from *C. albicans* (CA), *C. krusei* (CK), and *C. tropicalis* (CT) coinoculations were individually compared to those of *C. dubliniensis* (CD), *C. parapsilosis* (CP), *C. glabrata* (CG), *S. cerevisiae* (SC), and UV-killed *C. albicans* (UV-killed CA) coinoculations or those of naive mice. Data were analyzed using the unpaired Student's *t* test. n.s., not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Graphs in which multiple groups are significantly different are labeled with an asterisk, representing the least significant comparison.

Staphylococcus spp., which often are observed in neonatal ICU patients (46–48).

There are several intra-abdominal infection and sepsis models that have been used to examine mortality with *Candida* species (49–52). In contrast to our results, another group reported 100% mortality following i.p. inoculation with *C. glabrata* and sterilized

feces, using the same strain as that used in our studies (53). The conflicting results likely are due to differences in the inoculum size (Cheng et al. inoculated with 5×10^8 CFU, while we used 3.5×10^7 CFU in our study) and the fact that our studies included coinoculation with a single species of viable bacteria. Interestingly, inocula of 1×10^8 and 1×10^7 CFU were sublethal in the Cheng

FIG 4 Systemic immune mediators during peritoneal coinfections with NAC species and *S. aureus*. Mice ($n = 5$ mice/group) were inoculated with *S. aureus* and NAC species as described in the legend to Fig. 1. At 24 h p.i., serum was collected and analyzed for IL-6 (A), IL-1β (B), and TNF-α (C). Results are expressed as the mean cytokine concentrations $\pm$ SEM. Shown are cumulative data from two repeat experiments. Cytokine concentrations from *C. albicans* (CA), *C. krusei* (CK), and *C. tropicalis* (CT) coinoculations were individually compared to those of *C. dubliniensis* (CD), *C. parapsilosis* (CP), *C. glabrata* (CG), *S. cerevisiae* (SC), and UV-killed *C. albicans* (UV-killed CA) coinoculations. Data were analyzed using the unpaired Student's *t* test. n.s., not significant; *, $P < 0.05$; **, $P < 0.01$. Graphs in which multiple groups are significantly different are labeled with an asterisk, representing the least significant comparison.

et al. study. Moreover, in our studies, we used lower inoculum levels that are not lethal during monomicrobial infections to be able to observe synergistic lethality during coinfection. Thus, it is possible that higher inocula of *C. glabrata* or *C. parapsilosis* with *S. aureus* would be lethal. Of note, though, inoculation with 2-fold higher concentrations of *C. dubliniensis* (7×10^7) during polymicrobial IAI did not result in any higher mortality (data not shown).

NAC species coinfections that cause morbidity (*C. krusei*, *C. tropicalis*, and *C. dubliniensis*) are characterized by elevated levels

of inflammatory mediators, namely, PGE₂, IL-6, and IL-1β (Fig. 3), at concentrations similar to what was observed during *C. albicans*-*S. aureus* infections (23). Furthermore, this study has identified elevated PGE₂ and IL-6 in peritoneal lavage fluid as hallmarks of highly pathogenic polymicrobial IAI, and systemic IL-6 is closely associated with lethality. Future studies using knockout mice or immunological or pharmacological means to block/neutralize mediators are needed to determine their full contribution to morbidity and mortality. Nevertheless, these data suggest that PGE₂ and IL-6 are putative prognostic indicators of disease severity and possibly mortality. Our group and others have reported that mice are protected from *C. albicans*-*S. aureus* IAI after indomethacin treatment (24, 54), and exogenous administration of

FIG 5 Murine core temperature during NAC species-*S. aureus* coinfections. Mice ($n = 5$ to 10 mice/group) were coinoculated with *S. aureus* and *C. albicans*, *C. krusei*, *C. tropicalis*, *C. dubliniensis*, or *C. parapsilosis*. Survival and rectal body temperature were monitored daily for 10 days p.i. Core temperature at 4 (A) and 24 (B) h p.i. was compared between mice that succumbed to infection and mice that survived infection to day 10 p.i. Shown are representative results from two experiments. Body temperatures were analyzed using the Mann-Whitney U test. *, $P < 0.05$; ***, $P < 0.001$.

FIG 6 Morphogenetic signaling in *C. albicans* is crucial for synergistic mortality during polymicrobial infection with *S. aureus*. Mice ($n = 5$ mice/group) were inoculated with 8×10^7 CFU of *S. aureus* and 3.5×10^7 CFU of wild-type (WT) *C. albicans* or the *efg1/efg1 cph1/cph1*, *cph1/cph1*, *efg1/efg1*, or *efg1/efg1 EFG1* mutant. Survival was monitored for 5 days p.i. Results are cumulative from two repeat experiments, except for those for the *efg1/efg1 EFG1* plus *S. aureus* experiment, which was performed once.

PGE₂ in a less robust IAI model (lower inocula with less mortality) restores mortality to control levels (24). The contribution of IL-6 to mortality during IAI is more controversial. Studies using IL-6 knockout mice during *Escherichia coli*-induced peritonitis or cecal ligation and puncture (CLP) have observed no differences or even enhanced mortality in knockout mice compared to controls (55, 56). In contrast, other studies reported that IL-6 antibody depletion protects mice from IAI (57). It is possible that some IL-6 is required to control infection (incomplete neutralization via antibody treatment versus complete elimination via gene knockout).

Reduced body temperature is associated with higher mortality rates in sepsis patients (35), with mortality rates doubling in hypothermic patients compared to those in febrile sepsis patients (58, 59). Animals also exhibit hypothermia during experimental abdominal sepsis (60–62), and therefore hypothermia has been proposed as an effective indicator of mortality (63). Indeed, our model of polymicrobial IAI shows strong association between hypothermia and mortality at 24 h postinfection. The cause of this hypothermia is not well understood. One theory suggests it is a marker of immune dysfunction (64). Indeed, mild hypothermia profoundly reduces phagocytosis and oxidative killing by neutrophils (65). Neutrophils are clearly observed in infiltrates of the peritoneal cavity during fungal IAI (23, 24, 66). Alternatively, prostaglandins released from macrophages have been proposed as key regulators in temperature change during abdominal sepsis (67). This explanation is supported by our current data, and future studies will focus on the role of peritoneal macrophages in inflammation during polymicrobial IAI.

The morphogenesis of *C. albicans* results in the formation of hyphae as well as the upregulation of other virulence factors not directly involved in filamentation, such as adhesins and degradative enzymes, which also contribute to pathogenesis. The genes encoding these virulence factors are activated through the same signaling pathways and transcription factors that induce hyphal formation. Therefore, it is difficult to separate the roles of the morphogenesis and coregulated virulence factors. This was exemplified in experiments using a *C. albicans* tetracycline-regulatable mutant, in which morphology is controlled by varying the expression of the negative regulator of hyphal formation (Nrg1). In the absence of inducer, *NRG1* is overexpressed and *C. albicans* remains locked in the yeast form and is avirulent in the i.v. model of systemic candidiasis (68). Conversely, a similar yeast-locked *C. albicans* mutant (t-NRG1) caused high mortality, dissemination, and inflammation in the polymicrobial IAI model (23). However, testing *C. albicans* mutants deficient in transcription factors that control morphogenesis as well as other virulence factors coregulated during morphogenesis demonstrated that Efg1, but not Cph1, is critical for both morphogenesis and virulence during IAI. The differential virulence of the two nonhyphal mutants (t-NRG1 and *efg1/efg1*) in the IAI model provides an opportunity to identify virulence factors and regulatory machinery that are required for virulence but not morphogenesis.

Several NAC species (those in the CTG clade along with *C. albicans*) are known to have orthologs of *EFG1* (69, 70), although gene targets can be different and some conserved targets have different functions (71, 72). Nevertheless, we hypothesize that a similar role for Efg1-regulated pathways independent of morphogenesis is critical to the lethal versus nonlethal phenotypes observed in the IAI model. In particular, the *EFG1* ortholog was only recently discovered in *C. tropicalis*, in a region that contains a gap

in the current genome (70). We speculate that Efg1-regulated pathways also will be required for virulence of *C. tropicalis* in the IAI model, which is a member of the CTG clade and forms true hyphae, similar to *C. albicans*. The outlier is *C. krusei*, which is a member of the *Saccharomyces* clade and would not be expected to have an *EFG1* ortholog; however, the genome has not yet been annotated (70). Overall, this study advances the concept that fungal morphogenesis is a complex process that is required for adaptation to the host environment but that physical morphology *per se* does not determine virulence.

In conclusion, we have demonstrated that NAC species can induce sepsis and mortality similar to those of *C. albicans* during polymicrobial IAIs. This model is highly significant in light of the fact that intra-abdominal candidiasis is associated with high mortality rates. In addition, infections caused by NAC species are becoming more common due to inherent resistance to antifungals, and infection rates are increasing in at-risk populations (73). We also have demonstrated that hypothermia and inflammation are closely associated with mortality, suggesting that reduced core body temperature is an early prognostic indicator for highly pathological IAIs, and pharmacologic agents aimed at reducing PGE₂ and IL-6 concentrations may be therapeutic options. Finally, we have identified a critical role for the transcription factor Efg1 in the lethal phenotype associated with *C. albicans*. Additional investigation into the morphological signaling pathways involved in *Candida* pathogenesis will be important to identify new therapeutic targets to treat these highly lethal polymicrobial IAIs.

ACKNOWLEDGMENTS

We kindly thank Aaron Mitchell (Carnegie Mellon University) for providing *C. albicans* strain DAY185, G. R. Fink (Whitehead Institute) for providing the transcription factor-deficient *C. albicans* mutants, Jack Sobel (Wayne State University) for providing the NAC species, and Fred Winston (Harvard University) for providing the *S. cerevisiae* FY4 strain.

FUNDING INFORMATION

HHS | NIH | National Institute of Allergy and Infectious Diseases (NIAID) provided funding to Mairi C Noverr under grant number R01-A172406.

The funding agency (NIH NIAID) did not participate in study design, data collection, interpretation, or the decision to submit the work for publication.

REFERENCES

1. Heemken R, Gandawidjaja L, Hau T. 1997. Peritonitis: pathophysiology and local defense mechanisms. *Hepatogastroenterology* 44:927–936.
2. Dowd SE, Wolcott RD, Sun Y, McKeehan T, Smith E, Rhoads D. 2008. Polymicrobial nature of chronic diabetic foot ulcer biofilm infections determined using bacterial tag encoded FLX amplicon pyrosequencing (bTEFAP). *PLoS One* 3:e3326. <http://dx.doi.org/10.1371/journal.pone.0003326>.
3. Sibley CD, Duan K, Fischer C, Parkins MD, Storey DG, Rabin HR, Surette MG. 2008. Discerning the complexity of community interactions using a *Drosophila* model of polymicrobial infections. *PLoS Pathog* 4:e1000184. <http://dx.doi.org/10.1371/journal.ppat.1000184>.
4. Kuriyama T, Nakagawa K, Kawashiri S, Yamamoto E, Nakamura S, Karasawa T. 2000. The virulence of mixed infection with *Streptococcus constellatus* and *Fusobacterium nucleatum* in a murine orofacial infection model. *Microbes Infect* 2:1425–1430. [http://dx.doi.org/10.1016/S1286-4579\(00\)01296-X](http://dx.doi.org/10.1016/S1286-4579(00)01296-X).
5. Goldstein EJ. 2002. Intra-abdominal anaerobic infections: bacteriology and therapeutic potential of newer antimicrobial carbapenem, fluoroquinolone, and desfluoroquinolone therapeutic agents. *Clin Infect Dis* 35:S106–S111. <http://dx.doi.org/10.1086/341930>.
6. Edey M, Hawley CM, McDonald SP, Brown FG, Rosman JB, Wiggins

- KJ, Bannister KM, Johnson DW. 2010. Enterococcal peritonitis in Australian peritoneal dialysis patients: predictors, treatment and outcomes in 116 cases. *Nephrol Dial Transplant* 25:1272–1278. <http://dx.doi.org/10.1093/ndt/gfp641>.
7. Barraclough K, Hawley CM, McDonald SP, Brown FG, Rosman JB, Wiggins KJ, Bannister KM, Johnson DW. 2010. Polymicrobial peritonitis in peritoneal dialysis patients in Australia: predictors, treatment, and outcomes. *Am J Kidney Dis* 55:121–131. <http://dx.doi.org/10.1053/j.ajkd.2009.08.020>.
8. Dupont H, Paugam-Burtz C, Muller-Serieys C, Fierobe L, Chosidow D, Marmuse JP, Mantz J, Desmots JM. 2002. Predictive factors of mortality due to polymicrobial peritonitis with *Candida* isolation in peritoneal fluid in critically ill patients. *Arch Surg* 137:1341–1347. <http://dx.doi.org/10.1001/archsurg.137.12.1341>.
9. Blot SI, Vandewoude KH, De Waele JJ. 2007. *Candida* peritonitis. *Curr Opin Crit Care* 13:195–199. <http://dx.doi.org/10.1097/MCC.0b013e328028fd92>.
10. Calandra T, Bille J, Schneider R, Mosimann F, Francioli P. 1989. Clinical significance of *Candida* isolated from peritoneum in surgical patients. *Lancet* ii:1437–1440.
11. Montravers P, Gauzit R, Muller C, Marmuse JP, Fichelle A, Desmots JM. 1996. Emergence of antibiotic-resistant bacteria in cases of peritonitis after intra-abdominal surgery affects the efficacy of empirical antimicrobial therapy. *Clin Infect Dis* 23:486–494. <http://dx.doi.org/10.1093/clinids/23.3.486>.
12. Hasibeder W, Halabi M. 2014. *Candida* peritonitis. *Minerva Anestesiol* 80:470–481.
13. de Ruiter J, Weel J, Manusama E, Kingma WP, van der Voort PH. 2009. The epidemiology of intra-abdominal flora in critically ill patients with secondary and tertiary abdominal sepsis. *Infection* 37:522–527. <http://dx.doi.org/10.1007/s15010-009-8249-6>.
14. Carneiro HA, Mavrakis A, Mylonakis E. 2011. *Candida* peritonitis: an update on the latest research and treatments. *World J Surg* 35:2650–2659. <http://dx.doi.org/10.1007/s00268-011-1305-2>.
15. Singh N, Limaye AP. 2015. Infections in solid-organ transplant recipients, p 3440–3452. In Bennett JE, Dolin R, Blaser MJ (ed), *Mandell, Douglas, and Bennett's principles and practice of infectious diseases*, 8th ed. Elsevier/Saunders, Philadelphia, PA.
16. Miles R, Hawley CM, McDonald SP, Brown FG, Rosman JB, Wiggins KJ, Bannister KM, Johnson DW. 2009. Predictors and outcomes of fungal peritonitis in peritoneal dialysis patients. *Kidney Int* 76:622–628. <http://dx.doi.org/10.1038/ki.2009.202>.
17. Govindarajulu S, Hawley CM, McDonald SP, Brown FG, Rosman JB, Wiggins KJ, Bannister KM, Johnson DW. 2010. *Staphylococcus aureus* peritonitis in Australian peritoneal dialysis patients: predictors, treatment, and outcomes in 503 cases. *Perit Dial Int* 30:311–319. <http://dx.doi.org/10.3747/pdi.2008.00258>.
18. Benedetti E, Gruessner AC, Troppmann C, Papalois BE, Sutherland DE, Dunn DL, Gruessner RW. 1996. Intra-abdominal fungal infections after pancreatic transplantation: incidence, treatment, and outcome. *J Am Coll Surg* 183:307–316.
19. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, Lemons JA, Donovan EF, Stark AR, Tyson JE, Oh W, Bauer CR, Korones SB, Shankaran S, Laptook AR, Stevenson DK, Papile LA, Poole WK. 2002. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. *Pediatrics* 110:285–291. <http://dx.doi.org/10.1542/peds.110.2.285>.
20. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. 2004. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin Infect Dis* 39:309–317. <http://dx.doi.org/10.1086/421946>.
21. Shirliff ME, Peters BM, Jabra-Rizk MA. 2009. Cross-kingdom interactions: *Candida albicans* and bacteria. *FEMS Microbiol Lett* 299:1–8. <http://dx.doi.org/10.1111/j.1574-6968.2009.01668.x>.
22. Bender FH, Bernardini J, Piraino B. 2006. Prevention of infectious complications in peritoneal dialysis: best demonstrated practices. *Kidney Int Suppl* 70:S44–S54. <http://dx.doi.org/10.1038/sj.ki.5001915>.
23. Nash EE, Peters BM, Palmer GE, Fidel PL, Noverr MC. 2014. Morphogenesis is not required for *Candida albicans-Staphylococcus aureus* intra-abdominal infection-mediated dissemination and lethal sepsis. *Infect Immun* 82:3426–3435. <http://dx.doi.org/10.1128/IAI.01746-14>.
24. Peters BM, Noverr MC. 2013. *Candida albicans-Staphylococcus aureus* polymicrobial peritonitis modulates host innate immunity. *Infect Immun* 81:2178–2189. <http://dx.doi.org/10.1128/IAI.00265-13>.
25. Lionakis MS. 2014. New insights into innate immune control of systemic candidiasis. *Med Mycol* 52:555–564. <http://dx.doi.org/10.1093/mmy/myu029>.
26. Mayer FL, Wilson D, Hube B. 2013. *Candida albicans* pathogenicity mechanisms. *Virulence* 4:119–128. <http://dx.doi.org/10.4161/viru.22913>.
27. Kumamoto CA, Vines MD. 2005. Contributions of hyphae and hypha-core-regulated genes to *Candida albicans* virulence. *Cell Microbiol* 7:1546–1554. <http://dx.doi.org/10.1111/j.1462-5822.2005.00616.x>.
28. Jacobsen ID, Wilson D, Wachtler B, Brunke S, Naglik JR, Hube B. 2012. *Candida albicans* dimorphism as a therapeutic target. *Expert Rev Anti Infect Ther* 10:85–93. <http://dx.doi.org/10.1586/eri.11.152>.
29. Thompson DS, Carlisle PL, Kadosh D. 2011. Coevolution of morphology and virulence in *Candida* species. *Eukaryot Cell* 10:1173–1182. <http://dx.doi.org/10.1128/EC.05085-11>.
30. Furlaneto MC, Tardeli de Jesus Andrade CG, Furlaneto-Maia C, Galvao de Franca EJ, Pereira Moralez AT. 2012. Pathogenic attributes of non-*Candida albicans* species revealed by SEM. In Kazmiruk V (ed), *Scanning electron microscopy*. InTech, Rijeka, Croatia.
31. Dharwad S, Dominic S. 2011. Species identification of *Candida* isolates in various clinical specimens with their antifungal susceptibility patterns. *J Clin Diagn Res* 5:1177–1181.
32. Schulze J, Sonnenborn U. 2009. Yeasts in the gut: from commensals to infectious agents. *Dtsch Arztebl Int* 106:837–842.
33. Sartelli M, Catena F, Ansaloni L, Leppaniemi A, Taviloglu K, van Goor H, Viale P, Lazzareschi DV, de Werra C, Marrelli D, Colizza S, Scibe R, Alis H, Torer N, Navarro S, Catani M, Kauhanen S, Augustin G, Sakakushev B, Massalou D, Pletinckx P, Kenig J, Di Saverio S, Guercioni G, Rausei S, Laine S, Major P, Skrovina M, Angst E, Pittet O, Gerych I, Tepp J, Weiss G, Vasquez G, Vladov N, Trana C, Vettoretto N, Delibegovic S, Dziki A, Giraudo G, Pereira J, Poiasina E, Tzerbinis H, Hutan M, Vereczkei A, Krasniqi A, Seretis C, Diaz-Nieto R, Mesina C, Rems M, et al. 2012. Complicated intra-abdominal infections in Europe: preliminary data from the first three months of the CIAO Study. *World J Emerg Surg* 7:15. <http://dx.doi.org/10.1186/1749-7922-7-15>.
34. Davis D, Edwards JE, Jr, Mitchell AP, Ibrahim AS. 2000. *Candida albicans* RIM101 pH response pathway is required for host-pathogen interactions. *Infect Immun* 68:5953–5959. <http://dx.doi.org/10.1128/IAI.68.10.5953-5959.2000>.
35. Drewry AM, Fuller BM, Skrupky LP, Hotchkiss RS. 2015. The presence of hypothermia within 24 hours of sepsis diagnosis predicts persistent lymphopenia. *Crit Care Med* 43:1165–1169. <http://dx.doi.org/10.1097/CCM.0000000000000940>.
36. Kushimoto S, Gando S, Saitoh D, Mayumi T, Ogura H, Fujishima S, Araki T, Ikeda H, Kotani J, Miki Y, Shiraishi S, Suzuki K, Suzuki Y, Takeyama N, Takuma K, Tsuruta R, Yamaguchi Y, Yamashita N, Aikawa N, JAAM Sepsis Registry Study Group. 2013. The impact of body temperature abnormalities on the disease severity and outcome in patients with severe sepsis: an analysis from a multicenter, prospective survey of severe sepsis. *Crit Care* 17:R271. <http://dx.doi.org/10.1186/cc13106>.
37. Liu H, Kohler J, Fink GR. 1994. Suppression of hyphal formation in *Candida albicans* by mutation of a STE12 homolog. *Science* 266:1723–1726. <http://dx.doi.org/10.1126/science.7992058>.
38. Lo HJ, Kohler JR, DiDomenico B, Loebeberg D, Cacciapuoti A, Fink GR. 1997. Nonfilamentous *C. albicans* mutants are avirulent. *Cell* 90:939–949. [http://dx.doi.org/10.1016/S0092-8674\(00\)80358-X](http://dx.doi.org/10.1016/S0092-8674(00)80358-X).
39. Sonneborn A, Bockmuhl DP, Gerads M, Kurpanek K, Sanglard D, Ernst JF. 2000. Protein kinase A encoded by TPK2 regulates dimorphism of *Candida albicans*. *Mol Microbiol* 35:386–396. <http://dx.doi.org/10.1046/j.1365-2958.2000.01705.x>.
40. Staib P, Kretschmar M, Nichterlein T, Hof H, Morschhauser J. 2002. Transcriptional regulators Cph1p and Efg1p mediate activation of the *Candida albicans* virulence gene SAP5 during infection. *Infect Immun* 70:921–927. <http://dx.doi.org/10.1128/IAI.70.2.921-927.2002>.
41. Peters BM, Palmer GE, Nash AK, Lilly EA, Fidel PL, Jr, Noverr MC. 2014. Fungal morphogenetic pathways are required for the hallmark inflammatory response during *Candida albicans* vaginitis. *Infect Immun* 82:532–543. <http://dx.doi.org/10.1128/IAI.01417-13>.
42. Moran GP, MacCallum DM, Spiering MJ, Coleman DC, Sullivan DJ. 2007. Differential regulation of the transcriptional repressor NRG1 accounts for altered host-cell interactions in *Candida albicans* and *Candida*

- dubliniensis*. Mol Microbiol 66:915–929. <http://dx.doi.org/10.1111/j.1365-2958.2007.05965.x>.
43. Arendrup M, Horn T, Frimodt-Moller N. 2002. *In vivo* pathogenicity of eight medically relevant *Candida* species in an animal model. Infection 30:286–291. <http://dx.doi.org/10.1007/s15010-002-2131-0>.
 44. Koga-Ito CY, Komiya EY, Martins CA, Vasconcellos TC, Jorge AO, Carvalho YR, do Prado RF, Balducci I. 2011. Experimental systemic virulence of oral *Candida dubliniensis* isolates in comparison with *Candida albicans*, *Candida tropicalis* and *Candida krusei*. Mycoses 54:e278–e285. <http://dx.doi.org/10.1111/j.1439-0507.2010.01899.x>.
 45. Pfaller M, Neofytos D, Diekema D, Azie N, Meier-Kriesche HU, Quan SP, Horn D. 2012. Epidemiology and outcomes of candidemia in 3648 patients: data from the Prospective Antifungal Therapy (PATH Alliance) registry, 2004–2008. Diagn Microbiol Infect Dis 74:323–331. <http://dx.doi.org/10.1016/j.diagmicrobio.2012.10.003>.
 46. Carey AJ, Saiman L, Polin RA. 2008. Hospital-acquired infections in the NICU: epidemiology for the new millennium. Clin Perinatol 35:223–249. <http://dx.doi.org/10.1016/j.clp.2007.11.014>.
 47. Kaufman DA, Brown AT, Eisenhuth KK, Yue J, Grossman LB, Hazen KC. 2014. More serious infectious morbidity and mortality associated with simultaneous candidemia and coagulase-negative staphylococcal bacteremia in neonates and in vitro adherence studies between *Candida albicans* and *Staphylococcus epidermidis*. Early Hum Dev 90(Suppl 1):S66–S70.
 48. Pammi M, Zhong D, Johnson Y, Revell P, Versalovic J. 2014. Polymicrobial bloodstream infections in the neonatal intensive care unit are associated with increased mortality: a case-control study. BMC Infect Dis 14:390. <http://dx.doi.org/10.1186/1471-2334-14-390>.
 49. de Repentigny L, Phaneuf M, Mathieu LG. 1992. Gastrointestinal colonization and systemic dissemination by *Candida albicans* and *Candida tropicalis* in intact and immunocompromised mice. Infect Immun 60:4907–4914.
 50. Kennedy MJ, Volz PA. 1983. Dissemination of yeasts after gastrointestinal inoculation in antibiotic-treated mice. Sabouraudia 21:27–33. <http://dx.doi.org/10.1080/00362178385380051>.
 51. Blyth W. 1964. Experimental candidiasis due to *Candida tropicalis*: the effect of oxytetracycline, hydrocortisone and metabolic imbalance. Sabouraudia 3:261–272. <http://dx.doi.org/10.1080/00362176485190471>.
 52. Brieland J, Essig D, Jackson C, Frank D, Loebenberg D, Menzel F, Arnold B, DiDomenico B, Hare R. 2001. Comparison of pathogenesis and host immune responses to *Candida glabrata* and *Candida albicans* in systemically infected immunocompetent mice. Infect Immun 69:5046–5055. <http://dx.doi.org/10.1128/IAI.69.8.5046-5055.2001>.
 53. Cheng S, Clancy CJ, Hartman DJ, Hao B, Nguyen MH. 2014. *Candida glabrata* intra-abdominal candidiasis is characterized by persistence within the peritoneal cavity and abscesses. Infect Immun 82:3015–3022. <http://dx.doi.org/10.1128/IAI.00062-14>.
 54. Carlson EC. 1988. Synergism of *Candida albicans* and delta toxin producing *Staphylococcus aureus* on mouse mortality and morbidity: protection by indomethacin. Zentralbl Bakteriol Mikrobiol Hyg A 269:377–386.
 55. Dalrymple SA, Slattery R, Aud DM, Krishna M, Lucian LA, Murray R. 1996. Interleukin-6 is required for a protective immune response to systemic *Escherichia coli* infection. Infect Immun 64:3231–3235.
 56. Remick DG, Bolgos G, Copeland S, Siddiqui J. 2005. Role of interleukin-6 in mortality from and physiologic response to sepsis. Infect Immun 73:2751–2757. <http://dx.doi.org/10.1128/IAI.73.5.2751-2757.2005>.
 57. Starnes HF, Jr, Pearce MK, Tewari A, Yim JH, Zou JC, Abrams JS. 1990. Anti-IL-6 monoclonal antibodies protect against lethal *Escherichia coli* infection and lethal tumor necrosis factor- α challenge in mice. J Immunol 145:4185–4191.
 58. Clemmer TP, Fisher CJ, Jr, Bone RC, Slotman GJ, Metz CA, Thomas FO. 1992. Hypothermia in the sepsis syndrome and clinical outcome. The Methylprednisolone Severe Sepsis Study Group. Crit Care Med 20:1395–1401.
 59. Arons MM, Wheeler AP, Bernard GR, Christman BW, Russell JA, Schein R, Sumner WR, Steinberg KP, Fulkerson W, Wright P, Dupont WD, Swindell BB. 1999. Effects of ibuprofen on the physiology and survival of hypothermic sepsis. Ibuprofen in Sepsis Study Group. Crit Care Med 27:699–707.
 60. Miao P, Kong Y, Ma Y, Zeng H, Yu Z. 2013. Hypothermia predicts the prognosis in colon ascendens stent peritonitis mice. J Surg Res 181:129–135. <http://dx.doi.org/10.1016/j.jss.2012.05.078>.
 61. Eiseman B, Wotkins RS, Hirose H. 1964. Hypothermia and infection: three mechanisms of host protection in type 3 pneumococcal peritonitis. Ann Surg 160:994–1006. <http://dx.doi.org/10.1097/0000658-196412000-00009>.
 62. Cauvi DM, Song D, Vazquez DE, Hawisher D, Bermudez JA, Williams MR, Bickler S, Coimbra R, De Maio A. 2012. Period of irreversible therapeutic intervention during sepsis correlates with phase of innate immune dysfunction. J Biol Chem 287:19804–19815. <http://dx.doi.org/10.1074/jbc.M112.359562>.
 63. Nemzek JA, Xiao HY, Minard AE, Bolgos GL, Remick DG. 2004. Humane endpoints in shock research. Shock 21:17–25.
 64. Young PJ, Bellomo R. 2014. Fever in sepsis: is it cool to be hot? Crit Care 18:109. <http://dx.doi.org/10.1186/cc13726>.
 65. Wenisch C, Narzt E, Sessler DI, Parschall B, Lenhardt R, Kurz A, Graninger W. 1996. Mild intraoperative hypothermia reduces production of reactive oxygen intermediates by polymorphonuclear leukocytes. Anesth Analg 82:810–816.
 66. Cheng S, Clancy CJ, Xu W, Schneider F, Hao B, Mitchell AP, Nguyen MH. 2013. Profiling of *Candida albicans* gene expression during intra-abdominal candidiasis identifies biologic processes involved in pathogenesis. J Infect Dis 208:1529–1537. <http://dx.doi.org/10.1093/infdis/jit335>.
 67. Derijk RH, Van Kampen M, Van Rooijen N, Berkenbosch F. 1994. Hypothermia to endotoxin involves reduced thermogenesis, macrophage-dependent mechanisms, and prostaglandins. Am J Physiol 266:R1–R8.
 68. Saville SP, Lazzell AL, Monteagudo C, Lopez-Ribot JL. 2003. Engineered control of cell morphology *in vivo* reveals distinct roles for yeast and filamentous forms of *Candida albicans* during infection. Eukaryot Cell 2:1053–1060. <http://dx.doi.org/10.1128/EC.2.5.1053-1060.2003>.
 69. Connolly LA, Riccombeni A, Grozer Z, Holland LM, Lynch DB, Andes DR, Gacser A, Butler G. 2013. The APSES transcription factor Efg1 is a global regulator that controls morphogenesis and biofilm formation in *Candida parapsilosis*. Mol Microbiol 90:36–53.
 70. Mancera E, Porman AM, Cuomo CA, Bennett RJ, Johnson AD. 2015. Finding a missing gene: EFG1 regulates morphogenesis in *Candida tropicalis*. G3 5:849–856. <http://dx.doi.org/10.1534/g3.115.017566>.
 71. Ding C, Vidanes GM, Maguire SL, Guida A, Synnott JM, Andes DR, Butler G. 2011. Conserved and divergent roles of Bcr1 and CFEM proteins in *Candida parapsilosis* and *Candida albicans*. PLoS One 6:e28151. <http://dx.doi.org/10.1371/journal.pone.0028151>.
 72. Holland LM, Schroder MS, Turner SA, Taff H, Andes D, Grozer Z, Gacser A, Ames L, Haynes K, Higgins DG, Butler G. 2014. Comparative phenotypic analysis of the major fungal pathogens *Candida parapsilosis* and *Candida albicans*. PLoS Pathog 10:e1004365. <http://dx.doi.org/10.1371/journal.ppat.1004365>.
 73. Deorukhkar SC, Saini S, Mathew S. 2014. Nonalbicans *Candida* infection: an emerging threat. Interdiscip Perspect Infect Dis 2014:615958.