Exacerbation of Invasive *Candida albicans* Infection by Commensal Bacteria or a Glycolipid Through IFN-γ Produced in Part by iNKT Cells

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**Background.** The commensal yeast *Candida albicans* is a major cause of invasive fungal infections. Despite treatment with antifungal agents, the mortality rate attributed to these types of infection is high. Although numerous cases have been reported regarding a poor outcome for patients with bacterial and *C. albicans* coinfection, the mechanisms by which the coinfecting bacteria exacerbate the *C. albicans* infection remain elusive.

**Methods and Results.** We evaluated how glycolipid-mediated activation of invariant natural killer T (iNKT) cells affects the clearance of *C. albicans*. Surprisingly, *C. albicans*-infected, glycolipid-treated mice exhibited significantly lower survival rates, increased fungal burden, and higher interleukin (IL)-6 production in the kidneys compared with control mice. Glycolipid-induced exacerbation of *C. albicans* infection was not observed in interferon-gamma knockout (IFN-γKO) mice. In the *C. albicans*-infected, glycolipid-treated mice, the number of neutrophils in the blood and bone marrow dramatically decreased in an IFN-γ–dependent manner. Furthermore, mice that were coinfected with *C. albicans* and nonfermentative gram-negative commensal bacteria exhibited increased fungal burden and inflammatory cytokine production in the kidneys that were dependent on IFN-γ and iNKT cells.

**Conclusions.** Our results indicate that coinfecting commensal bacteria exacerbate *C. albicans* infection through IFN-γ produced, in part, by iNKT cells.

**Keywords.** *Candida albicans*; iNKT cell; glycolipid; commensal bacteria; nonfermentative gram-negative bacteria.

*Candida* species are fungi that colonize the skin and mucosal surfaces and are a major cause of hospital-acquired bloodstream infections [1–3]. Despite treatment with antifungal agents, mortality rate attributed to this type of infection is estimated to be 40% [2, 3]. Although not widely recognized, approximately 1 in 4 patients with candidal bloodstream infection are also coinfected with bacteria [4]. It is known that patients with *Candida* and bacterial coinfection have lower survival rates and higher incidence of endocarditis compared with those infected with *Candida* only [5–7]. However, the mechanisms by which the coinfecting bacteria exacerbate *Candida* infection remain elusive.

Neutrophils and macrophages play an important role in the innate immune response against *Candida albicans* [8, 9]. However, studies show that the functions of these phagocytes are affected by invariant natural killer T (iNKT) cells, an innate lymphocyte that expresses an invariant T-cell receptor (TCR)–α chain [10–13]. iNKT cells are involved in bridging innate and adaptive immunity by secreting immunomodulatory...
cytokines in response to CD1d-presented glycolipids, leading to stimulation of other immune cells [10–13].

iNKT cells play an important role in host defense against various microbial infections [11, 14]. Additionally, iNKT cell activation with the prototypical glycolipid antigen, α-galactosylceramide (αGalCer), promotes enhanced clearance of various microbes [15–19]. We and others have shown that iNKT cells respond to various bacteria through the recognition of bacterial or endogenous glycolipids and that this response occurs either with or without inflammatory cytokines [20–25]. It has been reported that a large number of patients with candidal bloodstream infections are also infected with bacteria [4], this iNKT cell activation by coinfecting bacteria might affect fungal clearance during C. albicans infection.

In this study, we investigated the effect of glycolipid antigen–dependent iNKT cell activation on the clearance of C. albicans. We infected mice with C. albicans, and αGalCer was injected subsequently. The survival rate, fungal burden, and cytokine production were analyzed. Because activated iNKT cells primarily secrete interferon-gamma (IFN-γ), we also investigated the role of IFN-γ in the outcome of C. albicans infection. Furthermore, we determined whether coinfection with commensal bacteria affected fungal clearance during C. albicans infection.

MATERIALS AND METHODS

Animals
C57BL/6 mice were purchased from Japan SLC, Inc. (Shizuoka, Japan) and IFN-γ knockout (KO) mice (C57BL/6 background) were purchased The Jackson Laboratory (Bar Harbor, ME). Jtx18 KO mice [26] were provided by Drs Masaru Taniguchi and Hiroshi Watarai (RIKEN Research Center for Allergy and Immunology, Yokohama, Japan). All mice were maintained in a pathogen-free facility at the National Institute of Infectious Diseases in Japan. The Animal Care and Use Committee of the National Institute of Infectious Diseases reviewed and approved all experiments.

Reagents
αGalCer (Funakoshi Co.) was dissolved at 1 mg/mL in a vehicle made of a buffer (pH 7.2) containing sucrose (57 mg/mL), histidine (7.5 mg/mL), and Tween 20 (5 mg/mL). αGalCer and vehicle were diluted with phosphate-buffered saline (PBS) to the desired concentration prior to use.

Infection Models
C. albicans strain SC5314, Novosphingobium capsulatum (ATCC 14666), and Pseudomonas aeruginosa strain PAO1 were grown in yeast extract peptone dextrose (YPD) broth (BD Biosciences), nutrient broth (BD Biosciences), and trypticase soy broth overnight at 30°C, 37°C, and 37°C, respectively. The yeast and bacteria were then washed and resuspended in PBS at the desired concentration. Mice were inoculated with a designated number of colony-forming units of yeast (1.4–6.2 × 10⁴ cfu) via the retro-orbital venous plexus, and 2 µg of αGalCer was injected intraperitoneally 2 hours after the infection. For the coinfection experiments, mice were first intravenously infected with N. capsulatum (1.5 × 10⁶–1.3 × 10⁸ cfu) or P. aeruginosa (7.8 × 10⁶–1.6 × 10⁷ cfu) and then infected intravenously with C. albicans (4.7–9.2 × 10⁴ cfu) 14 hours later.

Determination of Fungal Burden and Cytokine Production
The blood and organs were aseptically collected on days 1 and 2 after infection. The kidneys were homogenized in 2 mL of PBS, and 100 µL of the homogenates was plated on YPD agar plates. The fungal burden was determined after 1 day of incubation at 30°C. The remaining homogenates and blood samples were centrifuged and stored at −30°C until further analysis. Cytokines were measured with enzyme-linked immunosorbent assay kits (interleukin [IL]-6, BD Biosciences; IFN-γ and IL-1β, R&D Systems).

Blood Cell Count
The number of white blood cells in the blood samples was measured with an automatic cell counter (KX-21NV; Sysmex Co.). Classification of white blood cell types was done by visual inspection of May–Grunwald–Giemsa-stained samples at the Japan Pet Life Corporation (Tokyo).

Histology
Mice were euthanized 2 days after infection, and the kidneys and heart were collected followed by fixation in 10% formalin, dehydration with ethanol, and paraffin embedding as described previously [27]. Tissue sections (5 µm) were mounted onto glass slides (Dako Japan) and stained with hematoxylin and eosin or periodic acid-Schiff (PAS). The staining was followed by histological examination.

Cell Isolation and Flow Cytometry
Splenocytes and bone marrow cells collected from 1 femur were counted after lysis of the red blood cells. The cells were then stained with antibodies for Gr-1 (RB6-8C5), CD11b (M1/70), CD19 (6D5), TCR-β (H57-997), IFN-γ (XMG1.2; Biolegend), or PBS-57–loaded mCD1d tetramers (National Institutes of Health tetramer facility). Samples were obtained using a fluorescence-activated cell sorting calibur (BD Biosciences) flow cytometer. Data were analyzed using Flow Jo, version 9.5 (Tree Star, Inc.).

Statistical Analysis
The survival curves were compared using a log-rank test; all other statistical analyses were performed using an unpaired t test. All P values <.05 were considered significant. The results are presented as the mean ± the standard error of the mean.
RESULTS

Glycolipid-Mediated iNKT Cell Activation Exacerbates Invasive C. albicans Infection

To determine the effect of iNKT cell activation on the outcome of C. albicans infection, we infected C57BL/6 mice with C. albicans and injected αGalCer or vehicle. We then examined the survival rate and fungal burden in these mice. Surprisingly, all mice treated with αGalCer died within several days, whereas 50% of the vehicle-treated mice survived for 40 days (Figure 1A). The fungal burden in the kidneys 2 days after C. albicans infection was approximately 100-fold greater in the αGalCer-treated mice compared with the vehicle-treated mice (Figure 1B). Additionally, the amount of IL-6 in the kidneys and plasma was much higher in αGalCer-treated mice than in vehicle-treated mice (Figure 1C and 1D). Two days after C. albicans infection, no significant difference in amounts were seen for other cytokines, including TNF, IFN-γ, IL-10, IL-12, IL-17, and transforming growth factor-β, in the plasma or in other organs, including the kidneys, spleen, and liver (data not shown). Collectively, these data suggest that glycolipid-mediated iNKT cell activation impairs fungal clearance and augments the inflammatory response under invasive C. albicans infection.

IFN-γ Plays a Major Role in Exacerbation of C. albicans Infection in αGalCer-Treated Mice

The activation of iNKT cells by αGalCer induced an increase in the amount of IFN-γ in the plasma and kidneys of C. albicans-infected mice 15 hours after infection (Figure 2A and 2B). Production of IFN-γ by iNKT cells in C. albicans-infected, αGalCer-treated mice was confirmed with intracellular cytokine staining (data not shown). To determine whether IFN-γ played a role in exacerbation of Candida infection in the αGalCer-treated mice, we performed several experiments using IFN-γKO mice. When wild-type (WT) and IFN-γKO mice were infected with C. albicans and treated with αGalCer, the survival rate of the IFN-γKO mice was significantly prolonged compared with the WT mice (Figure 2C). The fungal burden in the kidneys was similar in IFN-γKO mice treated with αGalCer

Figure 1. Exacerbation of invasive Candida albicans infection mediated by glycolipid-activated invariant natural killer T (iNKT) cells. A, The survival curve of C. albicans-infected mice treated with either vehicle (n = 8) or alpha-galactosylceramide (αGalCer) (n = 8) is shown. The fungal burden in the kidneys (B) and interleukin-6 levels in the kidneys (C) and plasma (D) of C. albicans-infected mice treated with vehicle or αGalCer are shown. Data were collected 2 days after infection. All results are expressed as mean ± standard error of the mean and representative of 2 independent experiments (n = 5). Abbreviation: αGC, αGalCer. **P < 0.01.
or vehicle, whereas increased fungal burden was observed in αGalCer-treated WT mice (Figure 2D). Furthermore, αGalCer induced an increase in IL-6 and IL-1β in the kidneys and plasma of WT mice but not of IFN-γ KO mice (Figure 2E-G).

We also performed histological analysis of the mice. In the αGalCer-treated WT mice, many foci of extensive C. albicans growth were observed in the kidneys. However, only a few foci with a smaller number of fungi were observed in αGalCer-treated IFN-γ KO mice and in the 2 control groups (Figure 3A-C). Furthermore, enlarged foci of extensive fungal growth were only observed in the hearts of αGalCer-treated WT mice (Figure 3D). These results suggest that αGalCer-mediated iNKT...
cell activation induces a disseminated Candida infection and that IFN-γ plays a major role in the pathogenesis.

Neutropenia in αGalCer-Treated WT Mice

Notably, we found that the number of neutrophils was markedly decreased in αGalCer-treated mice, although the total numbers of white blood cells, lymphocytes, and monocytes were also decreased in these mice (Supplementary Figure 1A–D). This decrease is characteristic of a severe infection such as sepsis. The quantity of granulocyte-colony stimulating factor (G-CSF) and keratinocyte chemoattractant in the plasma was higher in the C. albicans-infected, αGalCer-treated mice than in C. albicans-infected, vehicle-treated mice (Supplementary Figure 1E and 1F). These results indicate that the decrease in number of neutrophils in the blood of the αGalCer-treated mice is not due to impaired recruitment of these cells to the peripheral blood.

Decreased Number of Neutrophils in Bone Marrow of WT Mice Treated With αGalCer

Next, we examined the number of neutrophils in the bone marrow of αGalCer-treated mice. In previous studies, the differentiation of neutrophils was determined by the expression of Gr-1 and CD11b [28]. Based on these reports [28], we analyzed the frequency of Gr-1$^{\text{high}}$ cells in the bone marrow using flow cytometry by gating on 2 cell populations G1 (Gr-1$^{\text{high}}$, CD11b$^{\text{very high}}$) and G2 (Gr-1$^{\text{high}}$, CD11b$^{\text{high}}$). Notably, the percentages of G1 cells corresponding to segmented neutrophils and of G2 cells corresponding to band neutrophils and metamyelocytes [28] were markedly decreased in C. albicans-infected, αGalCer-treated mice (Figure 4A). The numbers of G1 and G2 cells were also dramatically decreased in the C. albicans-infected, αGalCer-treated mice (Figure 4B and 4C). Notably, G1 and G2 cell numbers were also decreased in uninfected αGalCer-treated mice; however, the decrease was not as prominent as in the C. albicans-infected, αGalCer-treated mice (Figure 4B and 4C). These data indicate that a factor induced by αGalCer-mediated iNKT cell activation, such as IFN-γ, may be responsible for the decrease in the number of neutrophils in the bone marrow.

IFN-γ–Dependent Decrease of Neutrophils in Bone Marrow

We compared the percentage of neutrophils in the bone marrow of the WT and IFN-γKO mice. As expected, a reduction in the percentage of G1 and G2 cells was not seen in C. albicans-infected, αGalCer-treated IFN-γKO mice unlike in C. albicans-infected, αGalCer-treated WT mice (Figure 5A). In addition, similar numbers of G1 and G2 cells were present in αGalCer and vehicle-treated IFN-γKO mice (Figure 5B and 5C). Histological analysis revealed lower cell density in the bone marrow of the C. albicans-infected, αGalCer-treated mice compared with the other 3 groups (Supplementary Figure 2A and 2B). Furthermore, morphological analysis showed that the number of segmented and band neutrophils was significantly decreased only in αGalCer-treated WT mice (Supplementary Figure 2C). We consistently observed that the number of white blood cells and neutrophils in the blood was not significantly different between αGalCer- and vehicle-treated IFN-γKO mice. However, there was a slight trend toward a decrease in the number of neutrophils in the αGalCer-treated IFN-γKO mice (Figure 5D and 5E). These results suggest that IFN-γ plays a major role in the decrease of neutrophils in C. albicans-infected, αGalCer-treated mice.
Coinfection With a Commensal Bacterium Exacerbates C. albicans Infection

Novosphingobium/Sphingomonas species are nonfermentative, gram-negative, commensal bacteria that live in the human intestine [29] and are known to express glycolipid antigens for iNKT cells [21–23]. We evaluated whether infection with Novosphingobium (formerly Sphingomonas) capsulatum affected the clearance of C. albicans. Notably, the fungal burden in the kidney was significantly higher in N. capsulatum- and C. albicans-coinfected mice than in mice infected with C. albicans only (Figure 6A). The quantities of IL-6 and IL-1β in the kidneys were also significantly higher in N. capsulatum- and C. albicans-coinfected mice than mice infected with C. albicans only (Figure 6B and 6C). The majority of CD1d tetramer+ CD19− splenic iNKT cells were activated, and a significant number were positive for intracellular IFN-γ only in N. capsulatum- and C. albicans-coinfected mice (Figures 6D–F). Similar data were obtained from livers of coinfect ed mice (data not shown). The amount of IFN-γ in the plasma was also increased only in coinfected mice (Figure 6G). These data suggest that iNKT cells are a source of IFN-γ in N. capsulatum- and C. albicans-coinfected mice and that coinfection exacerbates C. albicans infection similar to that observed in the C. albicans-infected, αGalCer-treated mice.

IFN-γ and iNKT Cells Play a Major Role in Exacerbation of C. albicans Infection in N. capsulatum Coinfected Mice

When WT and IFN-γKO mice were infected with N. capsulatum and C. albicans, increased fungal burden in the kidneys was observed in coinfected WT mice, whereas this exacerbation in C. albicans infection was absent in IFN-γKO mice (Figure 7A). The coinfected N. capsulatum induced an increase in IL-1β production in the kidneys of WT mice but not in IFN-γKO mice (Figure 7B). A significant decrease in the number of Gr-1high, CD11bvery high G1 cells in the bone marrow was not seen in N. capsulatum- and C. albicans-coinfected IFN-γKO mice compared with those infected with C. albicans alone, whereas a significant decrease was observed in coinfected WT mice (Figure 7C). However, although not significant, a trend toward a decrease in the number of neutrophils was seen in the coinfected IFN-γKO mice, suggesting that an IFN-γ-independent mechanism may also be involved in the decrease in neutrophils.

Figure 4. alpha-galactosylceramide (αGalCer) administration during Candida albicans infection induces neutrophil reduction in the bone marrow. The percentage (A) and number (B, C) of bone marrow G1 (CD11bvery high/Gr-1high) cells and G2 (CD11bhigh/Gr-1high) cells from uninfected or C. albicans-infected mice treated with vehicle or αGalCer are shown. A–C, Data were collected 2 days after infection, and 5 mice/group were analyzed. Results are expressed as mean ± standard error of the mean and representative of 1 of 2 experiments. **P < .01.

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We also determined whether iNKT cells are required for the *N. capsulatum*-mediated exacerbation of candidal infection using iNKT cell–deficient *Jα18 KO* mice for coinfection experiments. Exacerbation was not observed in coinfected *Jα18 KO* mice, demonstrating that the presence of iNKT cells is critical in *N. capsulatum*-mediated exacerbation of candidal infection (Figure 7D). However, a trend toward higher fungal burden in coinfected *Jα18 KO* mice was observed, suggesting that other cells such as NK cells and NKT cells that express diverse TCR may also contribute to the exacerbation of candidal infection.

**Coinfection With *P. aeruginosa* Exacerbates *C. albicans* Infection**

We tested whether infection with *P. aeruginosa*, another nonfermentative gram-negative bacterium, affected the clearance of *C. albicans*. Notably, the fungal burden in the kidney was significantly higher in mice coinfected with *P. aeruginosa* and

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**Figure 5.** alpha-galactosylceramide (αGalCer)-mediated reduction in bone marrow neutrophils during *Candida albicans* infection is dependent on interferon-gamma (IFN-γ). The percentage (A) and number (B, C) of bone marrow G1 (CD11b+very high/Gr-1high) cells and G2 (CD11b(high)/Gr-1(high)) cells from *C. albicans*-infected wild-type (WT) or IFN-γKO (knockout) mice treated with vehicle or αGalCer are shown. Data are representative of 1 of 2 experiments with 5 mice/group analyzed per experiment. The number of white blood cells (D) and neutrophils (E) in the blood of *C. albicans*-infected WT or IFN-γKO mice treated with vehicle or αGalCer are shown. Data are representative of 2 experiments combined (10 mice/group). All data were collected 2 days after infection, and results are reported as mean ± SEM. **P < .01. Abbreviation: ns, not significant.
C. albicans than in mice infected only with C. albicans (Figure 7E). The quantities of IL-6 and IL-1β in the kidneys of the P. aeruginosa- and C. albicans-coinfected mice were also significantly higher than in mice infected only with C. albicans (Figure 7F and 7G). Furthermore, the number of G1 cells in the bone marrow was significantly decreased in the P. aeruginosa- and C. albicans-coinfected mice (Figure 7H). These data show that the exacerbation of C. albicans infection is induced not only by coinfection with N. capsulatum but also by coinfection with P. aeruginosa.
Figure 7. Interferon-gamma (IFN-γ)–dependent exacerbation of *Candida albicans* infection in mice coinfected with commensal bacteria. Fold increase in kidney fungal burden (A) and interleukin (IL)-1β (B) of *C. albicans*/Novosphingobium capsulatum (CA/NC)–coinfected wild-type (WT) (n = 5) and IFN-γKO (knockout) mice (n = 4) are shown compared with mice infected with CA alone. Fold increase was calculated by dividing the fungal burden (cfu/g kidney weight) and IL-1β levels (ng/g kidney weight) for each mice by the mean fungal burden and IL-1β values of the CA group, respectively. C, Comparison of bone marrow G1 (CD11bvery high/Gr-1high) cell numbers in WT (n = 5) and IFN-γKO (n = 4) mice infected with CA only or CA/NC. Mean G1 cell number of the CA group was normalized to 100%, and the G1 cell number for each mouse was expressed as a percentage of the mean G1 cell number of the CA group. D, Fold increase in kidney fungal burden of CA/NC coinfected WT (n = 17) and Jα18KO (n = 15) mice are shown compared with those infected with CA alone (WT, n = 15; Jα18KO, n = 13). Data represent pooled data from 3 experiments. The fold increase in kidney fungal burden (E), IL-6 (F), IL-1β (G), and the number of bone marrow G1 cells (H) in mice infected with CA only or coinfected with CA and *Pseudomonas aeruginosa* (CA/PA) are shown and were analyzed in the same manner as mentioned above (n = 3–6 per group). The data are representative of 2 independent experiments. All data were collected 2 days after the *C. albicans* infection and are expressed as mean ± standard error of the mean. *P < .05, **P < .01. Abbreviation: ns, not significant.
DISCUSSION

Numerous studies have shown that iNKT cell activation by αGalCer treatment ameliorates infections that result from various microbial pathogens [15–19]. However, we found that iNKT cell activation by αGalCer treatment exacerbates C. albicans infection. Few studies have shown that iNKT cell activation by αGalCer treatment is detrimental to the host during microbial infection. During Chlamydia muridarum infection, iNKT cell activation by αGalCer enhances bacterial growth through a decreased Th1 response and an increased Th2 response [30]. Similarly, iNKT cell activation by αGalCer treatment exacerbates visceral leishmaniasis through a decreased Th1 response [31]. To the best of our knowledge, our study is the first to demonstrate that iNKT cell activation by αGalCer treatment exacerbates microbial infection through an IFN-γ–dependent decrease in neutrophils.

IFN-γ has been shown to stimulate macrophages and neutrophils that inhibit the growth of C. albicans [32]. Additionally, IFN-γ KO mice and IFN-γ receptor-KO mice are more susceptible to C. albicans infection [33–35]. These data show that the Th1-mediated response plays an important role in host defense against C. albicans infection. Consistent with these reports, survival rates were lower in vehicle-treated IFN-γ KO mice compared with vehicle-treated WT mice (Figure 2C). However, several studies have demonstrated the detrimental role of IFN-γ to the host during C. albicans infection, which supports our current findings. Mice that received recombinant IFN-γ exhibited a shorter survival time during C. albicans infection [36]. In another study, recombinant IL-12 treatment promoted disease progression during C. albicans infection through increased production of IFN-γ [37]. Collectively, in support of previous findings, our data indicate that increased levels of IFN-γ in the early phase of infection may exacerbate C. albicans infection, although IFN-γ also plays an important role in the acquired immune response against C. albicans.

In this study, we showed that glycolipid-mediated iNKT cell activation exacerbates C. albicans infection through an IFN-γ–dependent decrease in the number of neutrophils (Figure 5). Although the detailed mechanisms by which IFN-γ and C. albicans infection mediate the decrease in neutrophils in bone marrow are not fully understood, previous studies support our findings. For example, IFN-γ has been shown to decrease the number of neutrophils [38–40]. De Bruin et al showed that IFN-γ suppressed the number of neutrophils and increased the number of monocytes during lymphocytic choriomeningitis virus infection [40]. They also showed that IFN-γ impaired the proliferation and differentiation of myeloid progenitors in response to G-CSF. Additionally, when mice were infected with a high dose of Listeria monocytogenes, strong stimulation of the innate immune response via Toll-like receptors by bacterial components induced depletion of bone marrow neutrophils and resulted in lethal outcomes [41]. It was also reported that iNKT cell activation exacerbates lipopolysaccharide-mediated lung inflammation [42]. Therefore, the response to cell wall components of C. albicans, including β-glucan and/or mannan, may also play a role in the decrease in the number of neutrophils in bone marrow. A slight decrease in neutrophils was observed when bone marrow cells were cultured in the presence of heat-killed C. albicans. However, addition of recombinant IFN-γ induced further decrease of neutrophils in the presence of heat-killed C. albicans, although no reduction was seen when cells were cultured with IFN-γ alone (data not shown). Collectively, IFN-γ plays a critical role in inducing neutropenia in some microbial infections. However, it has recently been shown that IFN-γ production induced by microbial infection can directly stimulate the growth of bone marrow progenitor cells during chronic infections [43]. We hypothesize that the timing and quantity of IFN-γ determine the effect on neutrophils and other bone marrow cells because we did not observe an exacerbation of C. albicans infection when the mice were treated with αGalCer at different time points (data not shown). Future studies are needed to clarify the mechanisms of IFN-γ–mediated decrease in neutrophils.

Our data show that coinfection with C. albicans and a nonfermentative gram-negative commensal bacterium N. capsulatum exacerbates fungal infection that is dependent on IFN-γ and iNKT cells (Figures 6 and 7). Importantly, a significant number of iNKT cells produced IFN-γ in mice coinfected with C. albicans and N. capsulatum (Figure 6). These data suggest that IFN-γ–secreting iNKT cells plays a critical role in the exacerbation of C. albicans infection by the coinfected N. capsulatum. Furthermore, the exacerbation of C. albicans infection was also observed by coinfect ed P. aeruginosa, another nonfermentative gram-negative bacterium that is regarded as an important pathogen in hospital-acquired infections (Figure 7). Many nonfermentative gram-negative bacteria, including Pseudomonas, Acinetobacter, and Sphingomonas/Novosphingobium species, are causative agents of bacteremia [44–46], and these bacteria are resistant to several major antibiotics [47–50]. It is possible that these drug-resistant bacteria are responsible for the exacerbation of C. albicans infection seen in some patients who are treated with antifungal agents. Furthermore, iNKT cells may also contribute to C. albicans infection exacerbation by reacting to bacteria other than those studied here because iNKT cells may also respond to bacteria that do not have glycolipid antigens, allowing them to respond to a diverse array of bacteria [20]. Importantly, human iNKT cells respond to microbes in a manner that is similar to that of mouse iNKT cells, so our findings may potentially have direct correlation to humans [21, 22, 24, 25]. Therefore, our findings indicate that iNKT cells may play a role in the exacerbation of C. albicans infection, particularly in patients with bacterial coinfection.

In summary, our data suggest that coinfection with commensal bacteria contributes to the exacerbation of C. albicans
infection through increased IFN-γ production that is mediated, in part, by iNKT cell activation. Our results identify a new mechanism of exacerbation of invasive *C. albicans* infection, and these findings could be useful for developing novel therapies to treat patients with exacerbated *C. albicans* infection.

**Supplementary Data**

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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