Candida albicans impairs macrophage function and facilitates *Pseudomonas aeruginosa* pneumonia in rat*

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Objective: **To determine whether *Candida albicans* airway colonization influences *Pseudomonas aeruginosa* pneumonia prevalence in rats and by which mechanism.**

Design: **Prospective, randomized, controlled animal study.**

Setting: **Research laboratory of a university.**

Subjects: **Male adult Wistar rats weighing 275–300 g.**

Interventions: In vivo: P. aeruginosa **pneumonia was induced by bronchial instillation of *P. aeruginosa* in rats previously instilled or not with live or ethanol-killed *C. albicans*. *In vitro*: Alveolar macrophages were incubated with or without live or ethanol-killed *C. albicans.***

Measurements and Main Results: **Quantitative cultures of lung were done. Lung tumor necrosis factor alpha, interferon gamma, and interleukin-6 levels were measured along with reactive oxygen species (ROS) production by alveolar macrophages. *P. aeruginosa* pneumonia prevalence was higher in rats given live but not ethanol-killed *C. albicans.* Instilling live *C. albicans* alone increased lung tumor necrosis factor-alpha and interferon-gamma but not interleukin-6, and was not associated with clinical or histologic signs of infection. These three cytokines were more abundant in lungs instilled with live *C. albicans* and *P. aeruginosa* than in those instilled with *P. aeruginosa* alone or with ethanol-killed *C. albicans* and *P. aeruginosa*. Alveolar macrophages incubated with live *C. albicans* had decreased ROS production.**

Conclusions: C. albicans **impedes alveolar macrophage ROS production and is correlated with an increase of *P. aeruginosa* pneumonia prevalence in rats. These results highlight the previously overlooked impact of airway fungal colonization on lung bacterial infection, and indicate the need for studies on the potential for antifungal therapy to prevent the onset of ventilator-associated pneumonia caused by *P. aeruginosa*. (Crit Care Med 2009; 37:1062–1067)**

KEY WORDS: nosocomial infections; ventilation; mechanical; bacterial infections and mycoses; experimental animal models; cytokines

Despite considerable efforts to implement guidelines for prevention of ventilator-associated pneumonia (VAP) (1, 2), this complication remains common among critically ill patients (3–5). VAP increases duration of ventilation (6), intensive care unit stay and, consequently, costs (4), and contributes to increased mortality (4).

Although most if not all VAP are due to bacteria, the role if any of fungal pathogens is unclear in immunocompetent hosts. Indeed, many studies have reported the presence of *Candida* species in the lungs of immunocompetent, ventilated patients (2, 7, 8), but it is usually accepted that lung infection is quite rare in these patients (9), and, thus, there is no specific recommendation for the management of nonimmunocompromised ventilated patients with *Candida*-positive airway specimens (10).

Despite these uncertainties, some clinical findings suggest a possible interaction between bacterial pathogens, mainly *Pseudomas aeruginosa* and *Candida* species. Indeed, *Candida* species and *P. aeruginosa* were among the most common microorganisms retrieved from endotracheal tube biofilm and tracheal secretions in patients with VAP (11). Even more intriguingly, a recent observational study found a statistical link between airway colonization with *Candida* species and *P. aeruginosa* VAP (12). Obviously, this does not imply causality between the two events, but these findings highlight the importance of clarifying whether *Candida* airway colonization is a risk factor for *P. aeruginosa* pneumonia and not a bias reflecting a concealed immunocompromised pulmonary status. The demonstration of a causal link between previous candidal colonization and subsequent infection with *P. aeruginosa* would open to a new approach in the prevention of VAP. This approach—that would consider eradication of such colonization (an option that has never been tested)—would obviously only be part of the already validated measures of VAP prevention. If proved valuable, such an approach would be worth trying because death rates associated with *P. aeruginosa* pneumonia are particularly high, ranging from 70% to >80% (13–15). But enthusiasm must be tempered pending the results of well-conducted experimental studies that demonstrate or rule out an actual sequence of events linking fungal colonization with bacterial infection. To our knowledge, such an experimental demonstration has never been performed.

***See also p. 1164.**

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We, therefore, studied the relationship between *C. albicans* colonization and the occurrence of *P. aeruginosa* pneumonia in immunocompetent rats.

MATERIALS AND METHODS

Experiments were performed in pathogen-free male Wistar rats weighing 275–300 g in compliance with the recommendations of the French Ministry of Agriculture and approved by the French Veterinary Services.

Microorganisms

A nonmucoid *P. aeruginosa* wild-type strain was recovered from the peripheral blood of a patient with *P. aeruginosa* VAP. Fluconazole-susceptible *C. albicans* was recovered from the bronchoalveolar lavage of a nonimmunocompromised patient also ventilated in our intensive care unit. The strains were stored at −80°C in Luria-Bertani (LB) media with 10% glycerol.

P. aeruginosa was grown on LB agar for 24 hours, then one colony was picked up and grown overnight in LB broth at 37°C. After two washes with saline, it was diluted to obtain the chosen concentration. *C. albicans* was plated on Sabouraud agar with chloramphenicol for 48 hours at 37°C. One colony was diluted in saline at the chosen concentration. Ethanol-killed *C. albicans* were obtained by 30 minutes exposure to 100% ethanol. Ethanol was removed by four successive washes in saline. *P. aeruginosa* and live *C. albicans* concentrations were controlled for each experiment.

Microorganisms were inoculated by transglottic instillation (16, 17) under light anesthesia with pentobarbital (Sigma, France) and sevoflurane (Abbott, France). Live or ethanol-killed *C. albicans* (2×10^6 colony-forming units [CFU]) were instilled intratracheally. *P. aeruginosa* (10^4 CFU) was instilled in a main bronchus. Remaining instillate was cultured to make sure the desired concentration of the appropriate microorganism had been instilled.

Histologic Analysis

The macroscopic aspect of each lung was rated for the presence or absence of an inflammation by two independent observers, unaware of rat group allocation. A macroscopic pulmonary inflammation was defined by the presence of a red or a gray congestion (18) (macroscopic scoring grid above 2 [19]). Fifteen additional rats were instilled in the trachea with 10 mL 10% formaldehyde. The trachea was tied and the lungs immersed in 10% formaldehyde. Paraffin-embedded sections were stained with hematoxylin-eosin and periodic acid Schiff.

In Vivo Experiments

Part 1: Instillations of Live or Ethanol-Killed C. albicans *Without* P. aeruginosa. Rats (n = 18) were instilled on days 1, 2, and 3 with either live (n = 6), ethanol-killed (n = 6) *C. albicans*, or saline (n = 6). Two additional rats per group enabled histologic analysis.

Part 2: Instillations of Live or Ethanol-Killed C. albicans *With* P. aeruginosa. Rats (n = 70) were instilled on day 1, day 2, and day 3 with either live (n = 30), ethanol-killed (n = 10) *C. albicans* or saline (n = 30). *P. aeruginosa* instillation was performed on day 2. Three additional rats per group enabled histologic analysis.

Rats were weighed each day and killed on day 4.

Organ Cultures. The right and left lungs were aseptically removed, homogenized, and plated accordingly for culture. Quantitative bacterial counts were obtained by plating serial dilutions on Drigalski agar, LB agar with fluconazole, and Sabouraud agar with chloramphenicol (Biomérieux, France) to confirm that no other microorganism than those instilled grew.

Pneumonia Definition. P. aeruginosa pneumonia was defined as the conjunction of a macroscopic pulmonary inflammation and a lung bacterial count $>10^4$ CFU per lung (a normal rat lung weighs <1 g) (20).

Cytokines. Lung homogenates were stored at −80°C before cytokines assays. Tumor necrosis factor-alpha (TNF-α), interleukin-6, and interferon-gamma (IFN-γ) were assayed in lung homogenates using specific rat enzyme-linked immunosorbent assay kits (R&D Systems, France and Biosource Europe, Belgium). Detection threshold were respectively 5, 30, and 13 pg/mL.

In Vitro Experiments

Reactive Oxygen Species Production by Alveolar Macrophages. Macrophage stimulation induces the activation of the nicotinamide ad-

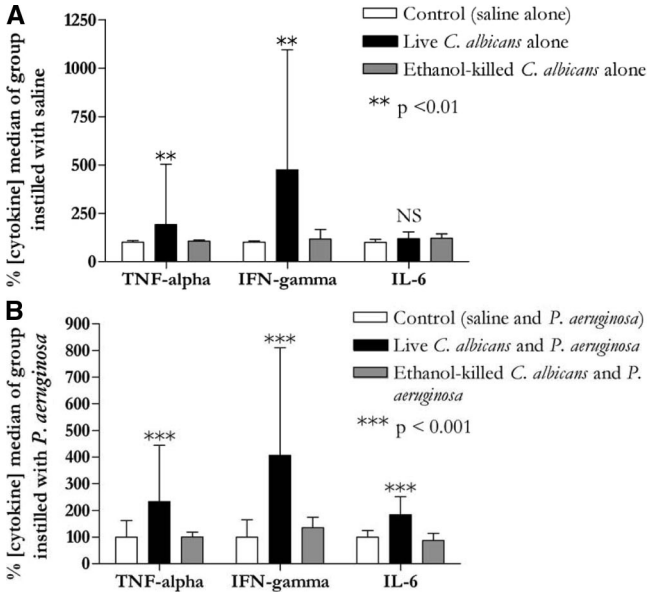

Figure 1. *A*, Lung homogenate cytokine concentrations in the absence of *P. aeruginosa* instillation (ordinate: % of cytokine concentrations in rats instilled with saline alone). In the absence of *P. aeruginosa* instillation: cytokine concentrations in lung homogenates of rats instilled with ethanol-killed or live *C. albicans* compared with rats instilled with saline. Tumor necrosis factor (*TNF*)-α concentrations were significantly higher in rats instilled with live *C. albicans* ($p < 0.01$, Kruskal-Wallis test, $p < 0.05$ compared with rats instilled with saline only or to rats instilled with ethanol-killed *C. albicans*, not significant [NS] comparing rats instilled with saline or ethanol-killed *C. albicans*, Dunn's *post hoc* test). Interferon (*IFN*)-γ concentrations were significantly higher in rats instilled with live *C. albicans* ($p < 0.01$, Kruskal-Wallis test, $p < 0.01$ compared with rats instilled with saline only and $p < 0.05$ compared with rats instilled with ethanol-killed *C. albicans*, NS comparing rats instilled with saline only or with ethanol-killed *C. albicans*, Dunn's *post hoc* test). IL-6 concentrations were not different (NS, Kruskal-Wallis test). *B*, Lung homogenate cytokine concentrations after an instillation of 10^4 colony-forming units (*CFU*) of *P. aeruginosa* (ordinate: % of cytokine concentrations in rats instilled with *P. aeruginosa* alone). With *P. aeruginosa* instillation: cytokine concentrations in lung homogenates of rats instilled with ethanol-killed or live *C. albicans* before *P. aeruginosa* instillation compared with rats instilled with saline before *P. aeruginosa* instillation. TNF-α, IFN-γ, and interleukin (IL)-6 concentrations were significantly higher in rats instilled with live *C. albicans* and *P. aeruginosa* ($p < 0.0001$, Kruskal-Wallis test, all $p < 0.05$ comparing rats instilled with live *C. albicans* and *P. aeruginosa* either with rats instilled with saline and *P. aeruginosa* or with rats instilled with ethanol-killed *C. albicans* and *P. aeruginosa*, NS comparing the two last groups, Dunn's *post hoc* test).

enine dinucleotide phosphate oxidase, which produces superoxide anions and generates other reactive oxygen species (ROS), such as hydrogen peroxide. This macrophage function can be evaluated by luminol-amplified chemiluminescence technique, as previously described (21). Rat alveolar macrophages obtained by bronchoalveolar lavage were incubated in the presence of either live, ethanol-killed *C. albicans* or control (Hank's buffered salt solution) for 30 minutes. Cells were further stimulated with 0.5 mg/mL opsonized zymosan (Sigma). ROS production was assessed by changes in chemoluminescence (21–25) over a 180-minute period. Experiments were repeated four times.

Statistical Analysis. Results are presented as mean ± SEM in case of gaussian distribution and as median in other cases. Statistical analyses were performed with GraphPad Prism (La Jolla, CA). Categorical data were compared by a Fisher's exact test. Rat weight changes were compared by two-way analysis of variance (ANOVA). Cytokine concentrations and changes in chemoluminescence were compared either by the Mann-Whitney *U* test or by the Kruskal-Wallis test with Dunn's *post hoc* test. $p < 0.05$ was considered significant.

RESULTS

Part 1: Effect of C. albicans *in the Absence of* P. aeruginosa *Instillation.* Mean rat weight increased in the control group and the groups given live or ethanol-killed *C. albicans* (5.6 ± 1.4%, 2.3 ± 0.8%, and 2.3 ± 0.2, respectively, not significant [NS], two-way ANOVA). Lung cultures showed $3.0 \pm 0.2 \times 10^6$ CFU of *C. albicans* when live *C. albicans* was instilled and no growth in the two other groups. TNF-α and IFN-γ were significantly higher in lung homogenates of rats instilled with live *C. albicans* compared with those from the two other groups ($p < 0.01$, Kruskal-Wallis test) (Fig. 1*A*). Interleukin-6 was similar in rats instilled with saline, live or ethanol-killed *C. albicans* (NS, Kruskal-Wallis test) (Fig. 1*A*).

Part 2: Effect of C. albicans *With* P. aeruginosa *Instillation.* Two rats died during *P. aeruginosa* instillation (one in the live *C. albicans* group, the other in the saline control group). Weight variation over time differed significantly ($p < 0.0001$, two-way ANOVA): mean weight of rats instilled with *P. aeruginosa* decreased by 2.2 ± 0.9% on day 3 in the group given live *C. albicans* but increased by 1.9 ± 0.3% and 1.0 ± 0.4% in the group instilled with saline and the one instilled with ethanol-killed *C. albicans*, respectively.

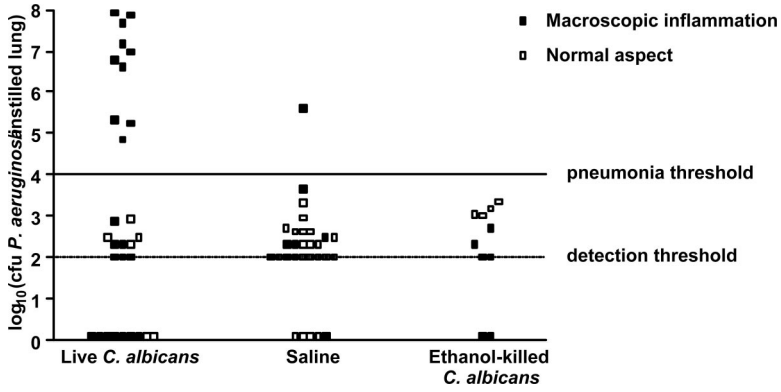

Figure 2. Quantitative lung cultures of *P. aeruginosa* in the three groups 48 hours after an instillation of 10^4 colony-forming units (*CFU*) of the bacteria. Pneumonia was defined as a macroscopic aspect of pulmonary inflammation and a lung bacterial count $>10^4$ CFU per instilled lung (*solid line*). *Open circles* indicate lungs with normal macroscopic aspect and *black circles* indicate lungs with macroscopic inflammation. *P. aeruginosa* detection threshold is shown by the dotted line (10^2 CFU/lung). Rats instilled with live *C. albicans* before *P. aeruginosa* presented more often with a macroscopic aspect of pulmonary inflammation than rats instilled with saline or ethanol-killed *C. albicans* before *P. aeruginosa* ($p = 0.001$, χ^2 test). Microbiological pneumonia was more frequent in rats instilled with live *C. albicans* before *P. aeruginosa* compared with rats instilled with saline before *P. aeruginosa* ($p < 0.01$, Fisher's test) or ethanol-killed *C. albicans* before *P. aeruginosa*. ($p < 0.05$, Fisher's test). *P. aeruginosa* counts in lung homogenates.

Macroscopic pulmonary inflammation, whatever *P. aeruginosa* count on lung culture, was significantly more frequent in rats given live *C. albicans* (23 of 29) than in the rats given saline or ethanol-killed *C. albicans* (9 of 29 and 6 of 10, respectively, $p = 0.001$, chi-square test) and was always noticed when bacterial count was $>10^4$ CFU per lung. *P. aeruginosa* pneumonia was significantly more frequent in rats given *C. albicans* (10 of 29) than saline (1 of 29) ($p < 0.01$, Fisher's test) or ethanol-killed *C. albicans* (0 of 10) ($p < 0.05$, Fisher's test). Figure 2 shows *P. aeruginosa* counts in rats given saline, live or ethanol-killed *C. albicans*. Quantitative lung cultures for *C. albicans* were similar whether or not rats developed *P. aeruginosa* pneumonia ($1.4 \pm 0.2 \times 10^6$ and $1.5 \pm 0.2 \times 10^6$ CFU per lung, respectively; NS, Mann-Whitney test) in rats that received live *C. albicans* instillation. No candidal growth was observed in the two other groups.

Rats given live *C. albicans* and instilled with *P. aeruginosa* had higher TNF-α, IFN-γ, and interleukin-6 pulmonary concentrations than those instilled with saline or ethanol-killed *C. albicans* and *P. aeruginosa* (all $p < 0.0001$, Kruskal-Wallis test) (Fig. 1*B*).

Pathologic Analysis. In the absence of *P. aeruginosa* (part 1), none of the lungs instilled with saline, live or ethanol-killed *C. albicans* displayed macroscopic or microscopic aspect of pneumonia. Live but not ethanol-killed *C. albicans* was responsible for rare spots of peribronchial inflammation (Fig. 3*A*). Yeast forms were largely prevalent, but a few pseudohyphae could be disclosed after extensive search (Fig. 3*B*).

Figure 4*A* shows a typical aspect of macroscopic pneumonia seen after *P. aeruginosa* instillation (part 2). A typical histologic aspect of *P. aeruginosa* pneumonia (widespread alveolitis with infiltration of polymorphonuclear cells [PMN]) is shown in Figure 4*B*. *C. albicans* (be it yeast or pseudohyphae forms) were never observed in these zones of *P. aeruginosa* pneumonia.

Production of ROS by Alveolar Macrophages. Live *C. albicans* inhibited luminol-amplified chemiluminescence of alveolar macrophages stimulated with opsonized zymozan ($p < 0.001$, Kruskal-Wallis test and $p < 0.001$, Dunn's *post hoc* test). In contrast, ethanol-killed *C. albicans* was without effect (NS, Dunn's *post hoc* test) (Fig. 5).

DISCUSSION

To our knowledge, our prospective randomized animal study constitutes the first demonstration of a phenomenon of facilitation between airway colonization with *Candida* species and *P. aeruginosa* pneumonia. These findings might have important clinical relevance. Indeed, given the high frequency of *Candida* species colonization of the airway of mechanically ventilated patients (2, 7, 8) and

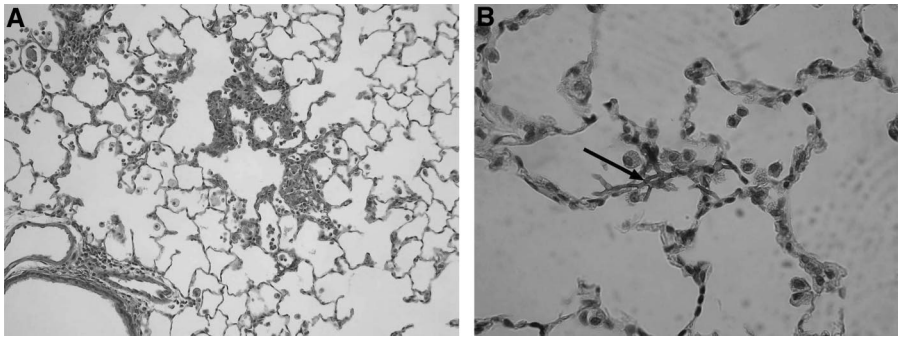

Figure 3. *A*, Lung microscopic aspect of a rat colonized with *C. albicans* that was not given *P. aeruginosa* (hematoxylin-eosin staining). Colonization with *C. albicans* produced a small number of zones of moderate inflammation. *B*, *C. albicans* pseudohyphae (*arrow*) were rarely observed in colonized lungs (periodic acid-Schiff staining). Few leukocytes accompanied these fungi.

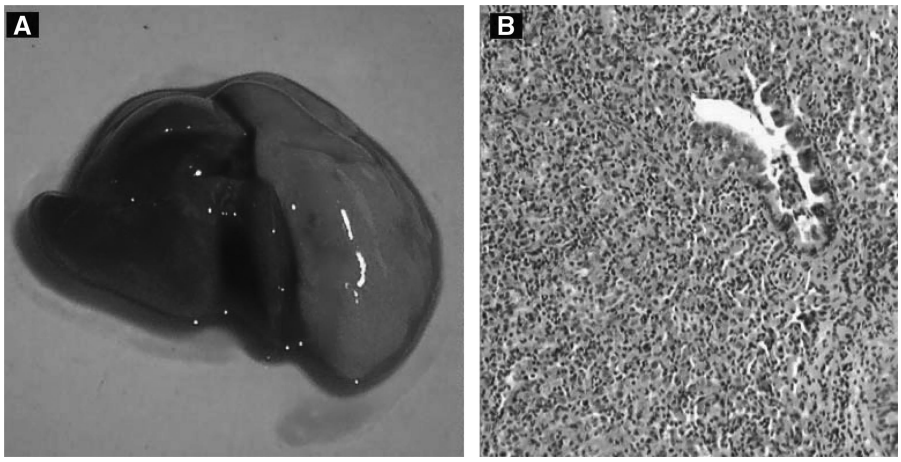

Figure 4. *A*, Example of a macroscopic pulmonary inflammation with $<10^2$ *P. aeruginosa* colony-forming units (CFU) 48 hours after instillation of 10^4 CFU of bacteria in a rat colonized with *C. albicans*. *B*, Histologic aspect (periodic acid-Schiff) of the lung of a colonized rat that developed a macroscopic inflammation after a *P. aeruginosa* instillation. No yeast or pseudohyphae forms of *C. albicans* were seen in the zone of pneumonia.

the important number of patients who develop *P. aeruginosa* pneumonia (13–15), these experimental results provide a strong rationale for performing clinical studies aimed at implementing the arsenal of VAP prevention through treatment of fungal colonization. If proved valuable, this totally new concept might help reduce the severe toll paid by patients to *P. aeruginosa* VAP in terms of morbidity and mortality. The most striking observation of this study was that a *P. aeruginosa* inoculum that does not lead to bacterial pneumonia does so in the presence of *C. albicans* in the airways. This effect was not observed with ethanol-killed *C. albicans*. In addition, we found that ROS production by alveolar macrophages was significantly depressed by live but not ethanol-killed *C. albicans*. Taken together, these results suggest that live *C. albicans* impairs alveolar macrophage function, which may in turn favor *P. aeruginosa* pneumonia development.

We aimed to reproduce *C. albicans* airway colonization by repeated instillations of the fungus. Because rats maintained spontaneous breathing and efficient cough after *C. albicans* or saline instillation, we repeated instillations to maintain the same level of colonization during the experiments. Only minimal peribronchial inflammation was seen on histologic analysis. Cytokine measurements confirmed the inflammatory process, with an increase in TNF-α and IFN-γ in lung homogenates, in agreement with TNF-α production by cell lines exposed to *C. albicans* yeasts (26). Because rats instilled with *C. albicans* alone gained weight in the same way as rats not given the fungus, and because lung *C. albicans* counts were always below initial inoculum, we believe that this presence of *C. albicans* was closer to colonization than infection. Indeed, the presence of proinflammatory cytokines and mild peribronchial inflammation is compatible with colonization, that must be seen, as recalled by Casadevall and Pirofski (27), "as a state of infection that results in a continuum of damage from none to great." Finally, additional rats given *C. albicans* (data not shown) were studied up to 14 days and evolved uneventfully.

Preliminary experiments found that 10^4 CFU *P. aeruginosa* was the highest bacterial inoculum not associated with pneumonia development when given alone. In the present study, only 3% (1 of 29) of rats not given *C. albicans* and instilled with this bacterial inoculum displayed *P. aeruginosa* pneumonia, thus confirming our preliminary finding. Conversely, when rats were given *C. albicans*, this small *P. aeruginosa* inoculum produced pneumonia in 34% of the rats. This *P. aeruginosa* pneumonia development was accompanied by a significant weight loss, whereas animals without pneumonia gained weight. Consistently, lung concentration of proinflammatory cytokines after *P. aeruginosa* instillation was higher in the group given the fungus. A certain degree of alveolar-capillary barrier injury was also observed in this group with an increased dry lung weight over body weight ratio (data not shown). There was no evidence of systemic dissemination, bacterial culture of the spleen being negative (data not shown). To ensure the robustness of our findings, experiments were repeated with different strains of *C. albicans* (ATCC 10231) and *P. aeruginosa* (ATCC 15692), and identical results were obtained (i.e., a significantly higher prevalence of *P. aeruginosa* in rats colonized with *C. albicans*) (data not shown).

Two possibilities were explored to explain these observations. First, an effect of *C. albicans* on *P. aeruginosa* responsible for increased pathogenicity of the latter; second, a decreased immunity of the host induced by *C. albicans* that would favor *P. aeruginosa* growth.

We could reasonably rule out the first hypothesis. Presence of live *C. albicans* was found to increase local production of proinflammatory cytokines in our study. These cytokines have, in turn, been shown in some (28) but not all studies (28–30) to modulate growth and virulence of some bacteria. We studied *P. aeruginosa* growth *in vitro* in different media (data not shown) and found—in

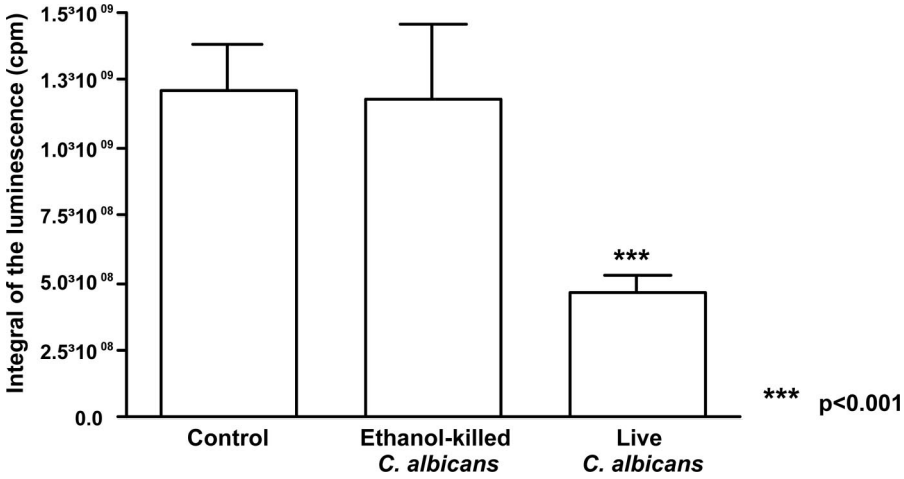

Figure 5. Reactive oxygen species (ROS) production by alveolar macrophages. Effect of ethanol-killed or live *C. albicans* on ROS production by opsonized zymosan-activated alveolar macrophages. Alveolar macrophages were incubated with or without either ethanol-killed or live *C. albicans* for 30 minutes, and then stimulated by opsonized zymosan. Luminol-amplified chemiluminescence was measured in chemiluminometer. Presence of live *C. albicans* decreased significantly ROS production ($p < 0.001$, Kruskal-Wallis test, $p < 0.001$ compared with saline and $p < 0.05$ compared with ethanol-killed *C. albicans*, Dunn's *post hoc* test). No difference was observed between saline and ethanol-killed *C. albicans* (not significant, Dunn's *post hoc* test).

agreement with these latter studies—no effect of cytokines. *C. albicans* may have favored *P. aeruginosa* growth by itself: *P. aeruginosa* can attach to *C. albicans* filaments and kill the fungus after dense biofilm formation (31). We rarely observed *C. albicans* in a filamentous form (pseudohyphae or hyphae) in rat lungs (Fig. 4*B*), making it unlikely that *P. aeruginosa* growth *in vivo* was due to effects like those observed by Hogan and Kolter (31) *in vitro*. We did not measure any increase in *P. aeruginosa in vitro* growth with live or ethanol-killed *C. albicans* (data not shown).

Second, we explored the possibility that *P. aeruginosa* pneumonia was facilitated by a decrease in host immunity. The immune cells implicated in the first defense against *C. albicans* are macrophages (32). *C. albicans* could have modulated innate immune response by impairing lung clearance of *P. aeruginosa* by alveolar macrophage. Because ROS production is a major component of macrophage's anti-pseudomonas activity (32, 33), we tested this hypothesis by studying macrophage production of ROS *in vitro*. We found that alveolar macrophages incubated with live *C. albicans* had a significantly reduced ROS production compared with controls. This finding is in agreement with *C. albicans* impairment of PMN production of superoxide anion reported by Hilger and Danley (34). To further ensure that this effect was specific to *C. albicans* and not a purely mechanical effect, we repeated experiments using ethanol-killed *C. albicans*. Results showed that macrophage incubated with ethanol-killed *C. albicans* produced the same level of ROS as controls, thus indicating that viable *C. albicans* were necessary to decrease macrophage activity. We acknowledge, however, the fact that the role of alveolar macrophages in innate immunity against *P. aeruginosa* lung infection is quite controversial. Numerous studies support their importance (35, 36), whereas others refute it (37). Nonetheless, we believe that alveolar macrophages are important to recruit PMNs (depletion of alveolar macrophages has been shown to decrease neutrophil chemotaxis to *Pseudomonas* pulmonary infection) and to ensure some phagocytic activity. We deliberately focused on alveolar macrophages because they are the first line of cell-mediated defenses against *C. albicans* (32). PMNs obviously also play an important role both against *C. albicans* and even more so against *P. aeruginosa*. Further studies are required to investigate the respective role of PMNs and alveolar macrophages during *P. aeruginosa* infection in the context of *C. albicans* colonization.

Thus, *in vitro* results were consistent with *in vivo* findings. We then postulated that airway colonization with *C. albicans* impairs alveolar macrophage function, resulting in diminished clearance of *P. aeruginosa*. This sequence of events allows development of this latter microorganism that otherwise would have been cleared from the lungs, as observed in animals not given *C. albicans*. This hypothesis parallels a similar but less acute previously described phenomenon: the alteration of macrophage antibacterial activity after viral infection (38).

Our study may provide the rationale for clinical studies aimed at evaluating the effect of decreasing *C. albicans* airway colonization on the rate of VAP caused by *P. aeruginosa*. Indeed, in a recent study (12), airway colonization with *Candida* species was found in 26.6% of patients under mechanical ventilation, *C. albicans* being the main species. *P. aeruginosa* VAP was found associated with *Candida* colonization (odds ratio 2.22, $p = 0.049$). Further evidence for the potential role of *C. albicans* colonization on *P. aeruginosa* VAP is given by the retrospective finding that antifungal therapy was associated with reduced risk for *P. aeruginosa* VAP in patients with *Candida* species colonization (39).

CONCLUSION

Our results clearly indicate that presence of *C. albicans* significantly increases the prevalence of *P. aeruginosa* pneumonia. Because *P. aeruginosa* VAP may be fatal in up to 71% cases (13) and associated with the highest risk of VAP recurrence (40), our results provide a rational for investigating the potential for antifungal treatment to decrease *P. aeruginosa* VAP prevalence. Interestingly, this approach has recently proved its efficacy in an experimental model of neonatal infection caused by coagulase-negative staphylococci and *C. albicans* (41).

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