Experimental Aspiration Pneumonia Caused by *Candida albicans* in Mice

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**Abstract**: Aspiration pneumonia is a type of pneumonia to which compromised hosts, such as elderly people, are particularly susceptible. A wide range of indigenous microorganisms found in the oral cavity may be involved in the emergence of aspiration pneumonia. Based on the high concentrations of *C. albicans* found in the oral cavity of elderly people, the present study investigated the pathogenicity of *C. albicans* in cases of aspiration pneumonia using a mouse model. *C. albicans* was inoculated into ICR mice administered with prednisolone. At one day after inoculation, the lungs were removed from the mice to prepare tissue sections. These sections showed marked neutrophil infiltration and congestion in the alveoli, as well as bronchiolar epithelial cell detachment. These inflammatory changes persisted even five days after inoculation. Periodic acid-Schiff staining specific to *C. albicans* confirmed numerous *C. albicans* hyphae in the alveoli. Measurements of tumor necrosis factor-α production in infected murine pulmonary tissue showed marked production, peaking three days after infection. These findings suggest that indigenous *C. albicans* in the oral cavity may lead to aspiration pneumonia in immuno-compromised hosts, including the elderly.

**Key words**: experimental aspiration pneumonia, *Candida albicans*, elder, tumor necrosis factor-α, compromised host

**Introduction**

Aspiration pneumonia is a type of pneumonia that commonly occurs in compromised hosts, such as elderly people¹, ². A wide range of indigenous microorganisms found in the oral cavity may be involved³⁻⁵. Therefore, attempts to reduce the frequency of aspiration pneumonia have focused on appropriate oral hygiene⁶. In recent years, periopathogenic bacteria have been studied as etiologic agents and their pathogenicity in pneumonia has also been investigated⁷⁻⁹. The prevalence of *Candida albicans* (*C. albicans*) in the oral cavity is known to increase with age⁸, ¹⁰. *C. albicans* readily adheres to dentures and is the primary etiologic agent for denture stomatitis in the elderly with low immunity¹¹. If microorganisms in the oral cavity can cause aspiration pneumonia, *C. albicans* may also play a role. Given the high incidence of aspiration pneumonia among the elderly, *C. albicans* may play a more significant role than periopathogenic bacteria in the onset of the illness. However, to date, the correlation between aspiration pneumonia and *C. albicans* has not been determined. To better clarify this relationship, we conducted a transmission experiment by investigating the pathogenicity of *C. albicans*, which was removed from the oral cavity of mice and brought in contact with the lungs.

**Materials and Methods**

1. **Organisms**

*C. albicans* OH-1 isolated from the oral cavity of an elderly individual was used⁸. The *Candida* cells
were cultured aerobically at 37 degrees Celsius for 20 hours in Candida GE plates (Nissui Pharmaceutical, Tokyo, Japan). The cells were harvested by microspatula and suspended in physiological saline. This suspension was adjusted to a final concentration of $2.5 \times 10^8$ viable cells.

2. Experimental pneumonia model

Two milligrams of prednisolone (Mitaka Pharmaceutical, Tokyo, Japan) were subcutaneously injected into 5-week old female ICR mice (Clea Japan Inc., Tokyo, Japan). The following day, a 0.1 ml normal saline solution containing $5 \times 10^7$ units of C. albicans was administered via nasal passage. In the control group, an equal amount of normal saline solution was similarly administered.

The mice were sacrificed under anesthesia and the lungs were then removed at different points in time. Each experimental group consisted of four mice. The mice were bred in an isolated breeding room to avoid contact with other infected animals, with a constant temperature of maintained 25°C. Periodic tests were conducted to ensure that the room was not contaminated by pathogenic microorganisms. The mice were supplied with laboratory animal feedstuff and tap water. Animal experiments were conducted in accordance with Ohu University's guidelines regarding the treatment of experimental animals.

3. C. albicans count, tumor necrosis factor-α (TNF-α) measurement

Each excised lung was broken up into small pieces, using tweezers, in 3 milliliters of physiological saline. The levels of tumor necrosis factor-α (TNF-α) in the pulmonary tissue solution were measured with an ELISA system (Bender MedSystems, Vienna, Austria).

4. Pulmonary tissue section preparation

Pulmonary tissue sections were stained using hematoxylin-eosin and periodic acid-Schiff (PAS), following formalin fixation.

Results

1. Pulmonary infection by C. albicans

Pulmonary tissues were analyzed on the day following inoculation of C. albicans into the nasal cavity of mice (Figure 1). Marked infiltration of neutrophils and hemorrhage in the pulmonary alveoli, as well as detachment of bronchiolar epithelial cells were observed. PAS staining specific to C. albicans showed numerous Candida hyphae in the alveoli (Figure 2).
2. Changes in murine pulmonary tissue with respect to different doses of *C. albicans*

*C. albicans* was inoculated at the following three doses: $5 \times 10^7$, $5 \times 10^6$, and $5 \times 10^5$. Pulmonary tissues were examined a day after inoculation (Figure 3). As previously described, congestion and inflammatory cell infiltration in alveoli were most marked at a dose of $5 \times 10^7$ cells. Slightly less pronounced but still marked inflammatory changes were observed at a dose of $5 \times 10^6$ cells.

3. Chronological changes in the lung after *C. albicans* inoculation

We analyzed pulmonary tissue every day for five days after *C. albicans* inoculation. The results showed that neutrophil infiltration and congestion were most pronounced at one day following inoculation (Figure 4). Compared to the control group, inflammatory changes remained visible even at five days after inoculation.

4. TNF-α production in pulmonary tissue following *C. albicans* inoculation

Marked TNF-α production in pulmonary tissue is one factor causing *C. albicans* to elicit strong inflammatory reactions in the lung (Figure 5). Measurements of TNF-α production levels in the pulmonary tissue of infected mice showed that while levels of TNF-α production for the control remained below the detection limit, significant TNF-α production was seen in the lung one and three days after inoculation.

Discussion

Some reports have suggested that periopathogenic bacteria may be etiological agents for aspiration pneumonia, but no conclusive findings pertaining to *C. albicans* have been published. Although the present study was conducted on mice, the findings showed that *C. albicans* causes powerful inflammatory symptoms in the lungs. Given that the elderly have significant amounts of *C. albicans* in their oral cavities, we posit that *C. albicans* may be one cause of aspiration pneumonia in this population.

In the present study, *C. albicans*, isolated from the oral cavity of an elderly person, was inoculated...
Figure 4 Chronological changes in pulmonary tissue following *C. albicans* infection
(a) day 1, (b) day 2, (c) day 3, (d) day 4, (e) day 5, (f) control. (original magnification: ×40)

Figure 5 Chronological changes in TNF-α production following *C. albicans* infection

into mice. The results showed that regardless of whether *C. albicans* was inoculated into the nasal cavity or oral cavity, it was followed by marked inflammatory cell infiltration, mainly consisting of neutrophils, in the alveoli. These findings suggest that pneumonia may occur if a compromised host ingests the *C. albicans* occurring in the oral cavity. Marked inflammatory changes occurred with the administration of > 5 × 10^6 cells. Unless dentures are cleaned, the large quantities of *C. albicans* adhering to the denture plates can increase the risk of aspiration pneumonia. The findings obtained with mice in the present study may also hold true
Numerous studies have assessed the pathogenicity of *C. albicans* in pulmonary diseases\(^{10-12}\). The cytokines produced by *C. albicans* infection appear to play significant roles in the progression of inflammatory reactions\(^{13-15}\). In particular, TNF-\(\alpha\), an inflammatory cytokine, has a significant impact on leukocyte function in candidiasis\(^{16,17}\). TNF-\(\alpha\) was previously believed to enhance the anticandidal activity of leukocytes\(^{18,19}\). However, Futenma et al. reported that TNF-\(\alpha\) increases the pathogenicity of *C. albicans* in pulmonary candidiasis in granulocytopenic mice\(^{10}\). Their report indicates that TNF-\(\alpha\) production was reduced and mortality was lowered in mice, suggesting that TNF-\(\alpha\) is a septic shock mediator. In the present study, TNF-\(\alpha\) levels in pulmonary tissue increased soon after inoculation and decreased as the severity of neutrophil infiltration and congestion improved. TNF-\(\alpha\) appears to increase inflammation and pathogenicity in *C. albicans*-induced pneumonia.

TNF-\(\alpha\) produced in the lung induces apoptosis and causes neutrophil apoptosis. Trautmann et al. reported that TNF-\(\alpha\) induces apoptosis of bronchial epithelial cells in asthma cases\(^{39}\). Histopathological investigations of the lung confirmed bronchial epithelial cell detachment, induced by large quantities of TNF-\(\alpha\) produced in the lung by *C. albicans* infection.

In an earlier study, we used a mouse model to show that *C. albicans* in the oral cavity can cause oral candidiasis in immuno-compromised hosts\(^{31}\). Based on this model, the present study discovered marked pneumonia-related symptoms, furthering support for appropriate oral care in compromised hosts. One study, which investigated the relationship between the onset of pneumonia and implementation of oral care at a residential home for the elderly requiring daily nursing care, showed that daily oral care suppresses the onset of pneumonia\(^{19}\). However, the actual microorganisms involved in the onset of pneumonia remain unidentified. *C. albicans* is commonly found in high concentrations in the oral cavity of elderly people\(^{6,7}\). Therefore, appropriate oral care, including reducing *C. albicans* populations in the oral cavity, may lower the incidence of pneumonia.

**References**


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Candida albicans によるマウスの実験的嚥下性肺炎

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抄録：嚥下性肺炎は高齢者の易感染性宿主でおこる多い肺炎である。原因菌としては口腔内に常在するさまざまな微生物が考えられている。高齢者の口腔内に常在する頻度の高いCandida albicans (C. albicans)の嚥下性肺炎における病原性についてマウスモデルを使用して調べた。プレドニゾロンを投与したICMマウスの鼻腔からC. albicansを感染させた。感染1日後に肺を摘出して組織標本を作製した。肺胞内への好中球の著しい浸潤と充血が観察された。細気管支の上皮細胞の脱落も認められた。これらの炎症反応は感染5日後でも認められた。さらにC. albicansを特異的に染色するPAS染色をおこなうと肺胞内に多くのC. albicansの菌糸が認められた。感染させたマウスの肺組織におけるTNF-αの産生量を調べると感染3日後までをピークとして著しい産生が認められた。これらの結果から高齢者を含む易感染性宿主では感染防御機能の低下に伴って口腔内に常在するC. albicansが嚥下性肺炎をおこす可能性が示唆された。

キーワード：実験的嚥下性肺炎, Candida albicans, 高齢者, tumor necrosis factor-α, 易感染性宿主

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