Basic Research—Significance of Detection and Clinical Impact of *Candida albicans* in Non-Immunosuppressed Patients

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**ABSTRACT**

**Background:** The clinical significance of the detection of *Candida albicans* on mucous membranes of the respiratory or intestinal tract from patients in intensive care units is still not finally clarified. Many patients reveal colonization, although, despite increased risk, there are only a few invasive infections detectable. Therefore, antimycotic therapy in this setting is strongly discouraged. In reality, however, many patients receive antimycotics as a pre-emptive therapy. To elucidate this point, a literature research was performed. **Results:** In the light of new results on the pathogenicity of *C. albicans*, the recommendation not to treat should be discussed anew. Without becoming invasive, *C. albicans* influences the immune system negatively in an anti-inflammatory sense (Th2) by means of at least two distinct mechanisms (action on toll like receptors (TLR), production of farnesol), which will be discussed. **Conclusion:** It is believed that patients in the phase of CARS or MARS can be further endangered by concomitant colonization of mucous membranes by *C. albicans*, i.e., in the sense of an anti-inflammatory immune response. Treatment with azole preparations, like fluconazole, which interacts with ergosterol synthesis in this phase of the disease, may trigger an additional effect on the patient, through increase of farnesol concentration by way of a negative feedback. Results of animal experiments on the immune system and concomitant therapeutic consequences indicate the need for verification through clinical trials.

**Keywords:** *Candida Albicans*, Farnesol, Azole, Echinocandin, TLR, Immune Answer, Th Cell

1. **Introduction**

“The diagnosis of pulmonary moniliasis (*C. albicans*-Infection) is fraught with difficulties … Actually there are no indisputable criteria for establishing the diagnosis…” [1]. Even after 50 years the clinical significance of a detection of *Candida* in the respiratory or intestinal tract is greatly disputed. Based on the results of studies performed on non-neutropenic patients, the detection of *Candida* species in specimen of the deep respiratory tract, even in high concentrations, is regarded as colonization of mucous membranes rather than invasive infection [2]. Therefore, in many cases, administration of anti-fungal drugs is regarded as unwarranted and expensive [3].

On the other hand, colonization of mucous membranes represents a significant risk factor for invasive *Candida*-infections [4-6]. For example, in a previous study, growth of *Candida* in at least one specimen was positive in 215 of 357 patients (60.2%) [7]. *Candida* was mostly detected in secretions of the respiratory tract (49.8%), in rectal smears (48.3%) and in wounds (20.1%). The relative part of *Candida albicans* was 72%, *Candida glabrata* 16%, *Candida tropicalis* 5%, *Candida parapsilosis* 3% and *Candida krusei* 2%. A colonization—particularly in several localizations—together with other risk factors such as loss of skin and mucous membrane barrier function, major surgical procedures (in particular abdominal), burns, total parenteral nutrition, acute renal failure and haemodialysis, high APACHE II scores, antacids and artificial respiration can contribute to an increased risk of invasive *Candida* infections. However, the number of proven invasive infections (detection in blood cultures, Candida endophthalmitis, growth from usually sterile specimen such as pleural or peritoneal fluid) based on the number of patients with colonization, is rather rare. Of a total of 1669 patients, 719 patients had no colonization or
infection; in 883 patients colonization was detected, but only 97 patients (5.8%) had an infection [8]. Consequently the conclusion seems obvious: detection of Candida from the deep respiratory or intestinal tract has no further significance for affected patients, unless they have additional risk factors for an invasive infection e.g. colonization in multiple body sites. Nevertheless, based on personal experience, a large number of ICU patients are treated with antifungals without detection of an invasive infection. The question arises as to whether a pathogenic correlation does exist that justifies the “empirical therapy” (better: prophylaxis or “pre-emptive therapy”).

2. Material and Methods

A literature review was performed using PubMed and the following key words: Candida albicans, immune system, therapy, fluconazole, echinocandin, farnesol, TLR.

3. Results from Basic Research

3.1. An Alternative Way for Pathogenesis

To be clinically relevant, C. albicans would have to possess virulence factors that can negatively influence the homeostasis in a patient, independently from an invasion. A connection between C. albicans and the development of an allergic reaction of the respiratory system has already been postulated over 50 years ago [1]. First publications of systematic studies on this topic appeared almost 20 years ago. In 10 out of 13 children with allergic asthma and C. albicans specific IgE antibodies a reaction with Candida antigen of 46 kDa was detected [9]. In an additional study, sera from 105 patients with C. albicans-specific IgE antibodies reacted with 42 different candida antigens in the immunoblot—42% with the 46 kDa antigen and 28% with a 27 kDa antigen [10]. According to Ito K. et al. [11], the 46 kDa antigen is an enolase; antibodies directed against enolase were detected in 37% of patients, all positive for C. albicans IgE antibodies.

The detection of C. albicans-specific IgE antibodies indicates that the immune system reacts to a C. albicans antigen stimulus with a Th2 (“anti-inflammatory”) response. In animal experiments, the administration of IL-4 and IL-10 (Th2) was the reason for a fatal progression which was linked to the inhibition of IL-12 and a Th2-dominance [12]. In 1999, Talluri G et al. [13] could demonstrate, in patients with candiduria and candidemia, that the production of interleukins of the Th2 cell lineage (IL-4, IL-10; “anti-inflammatory”) was increased and that the IL-2 concentration (Th0, Th1, “inflammatory”) was decreased. The immune system of patients with symptoms of chronic mucocutaneous candidiasis also shows a shift of the T-cells towards the Th2-population [14]. This anti-inflammatory response of the immune system with a prevalence of the Th2-cell lineage results in insufficient or no elimination of pathogens, and thereby in a relative immune weakness.

3.2. Growth Form of C. albicans and Immune Response

There are two different types of C. albicans growth forms: yeast cells (blastoconidia) and hyphae, which can alternate depending on the external conditions (see below). Therefore, a differentiation between these two forms is significant as this controls the interaction between microorganisms and the immune system.

The production of various interleukins as a reaction to C. albicans antigen is controlled by toll-like receptors (TLR) of antigen presenting cells. In animal testing, mice with and without TLR2 were infected with C. albicans. In this case, mice without TLR2 (TLR2-) survived longer than those with TLR2 (TLR2+), the colony counts in the kidneys of TLR2- mice was lower by a factor of 100 ($p < 0.01$). At the same time, the IL-10 production in TLR2- mice was reduced, the IFN-γ concentration increased and the destruction of Candida by macrophages improved [15]. IL-10 appears to be a key to the immune defence of C. albicans infections. In the event of a systemic infection, knockout mice without IL-10 production were able to eliminate significantly more C. albicans cells in the kidneys than controls with IL-10 production ($p > 0.05$). This phenomenon could be caused by a direct influence of IL-10 on the function of neutrophilic granulocytes [16].

The stimulation of dendritic cells with zymosan (derivative of yeast cell walls) leads to an induction of IL-10- and of TGF-β (transforming growth factor beta), with simultaneous suppression of IL-12, IL-6 and TNF-α (pro-inflammatory), whereby both TLR-2 (as heterodimers together with TLR-6) and Dectin-1 control the signal transduction [17,18].

According to the model of Van der Graaf [19], the growth form of C. albicans significantly influences the immune response. If the cell grows as blastoconidium (yeast cell), it interacts with TLR4 of antigen-presenting cells. This trigger leads to a pro-inflammatory (Th1) response with a significant increase of IFN-γ and TNF-α. However, if C. albicans switches to the hyphal form, the anti-inflammatory (Th2) response overbalances with an increase of IL-10 production controlled by TLR2.

3.3. C. albicans and Farnesol Formation—Impact on the Immune System

C. albicans produces a lipophilic substance called farnesol. Farnesol is produced from farnesyl diphosphate, a
molecule that interestingly represents a precursor of cholesterol in humans, ergosterol (cell membrane) in yeasts and staphyloxanthin (yellow pigment, virulence factor) in Staphylococcus aureus. For C. albicans, the E,E-Isomer has the function of a “quorum sensing molecule” (QSM), i.e., it is significant for the communication of yeast cells amongst each other and, for example, controls the transition of the blastoconidia to the hyphal form [20], depending on time of exposure. In addition, the lipophilic farnesol interacts with host cell membranes, i.e., it can possibly make way for an invasive infection. It also interferes with the immune response and protects C. albicans from the impact of oxygen radicals [21,22]. In physiological concentrations, farnesol reduces the effect of H2O2 on the Candida cell, strains with farnesol production are ~20 times more resistant than strains without [23,24].

In an animal model, mice infected with farnesol pre-treated or farnesol-producing C. albicans strains die more quickly [25,26]. In the control group with C. albicans knockout strains without farnesol production, the survival rate was significantly higher (p < 0.0014). Farnesol actually suppresses the production of IL-12 and IFN-γ, both necessary for an adequate defence against C. albicans infections (Th1) and it also increases the IL-5 level (Th2) [22].

In addition, farnesol modulates the expression of genes (TUP1, CRK1, PDE2) which regulate the hyphal formation in terms of an increased formation of hyphae [27].

These experimental data show that C. albicans has a negative impact on the immune system (growth in hyphal form and interaction with TLR2; farnesol production) with a shift of the T-cell response towards Th2 (anti-inflammatory), independent from invasiveness.

### 3.4. Factors That Can Impact the Production of Farnesol through C. albicans

As mentioned previously, farnesol is generated from two molecules of farnesyl diphosphate. Farnesyl diphosphate is an early precursor in the synthesis of ergosterol, a significant part of the cytoplasmic membrane of yeast cells. If C. albicans cells are exposed to azoles, the farnesol production increases significantly. This effect is also used in the various previously described infection models [23,25,28]. As azoles, e.g., fluconazole are inhibiting the ergosterol synthesis through inhibition of the lanosterol 14α-demethylase, a negative feedback must be presumed. Clinical isolates of C. albicans strains produce 2 to 4 μM farnesol with a cell density of 10^8 cells/ml. Under the influence of subinhibitory concentrations of substances which, like azoles, inhibit the sterol biosynthesis, the production increases by the factor of 10 - 45 [27]. Abe et al. (2009) were able to demonstrate that farnesol concentrations of ~56 μM and higher suppress the inhibitory activity of macrophages on hyphae formation of C. albicans and also leads to an apoptosis of the macrophages [29]. Evidently, farnesol also increases the apoptosis of cells of an oral squamous epithelial carcinoma cell line [30].

### 3.5. Mucous Membranes Intersection—C. albicans and the Local Immune System

Anaerobic conditions, as they exist in biofilms on mucous membranes, lead to a growth of C. albicans in hyphal form, the growth form that controls the anti-inflammatory shift of T-cells via TLR2. In experimentally produced biofilms, antifungal drugs such as amphotericin B, clotrimazole, fluconazole, miconazole and ketoconazole do not inhibit the growth of C. albicans [31]. The only effect of the azole would therefore be the increase of farnesol concentration with subsequent suppression of IL12 and IFN-γ. In fact, in the model of a mucocutaneous Candida infection, the suppression of inflammatory leukocytes could be observed [32]. This is also true in recurrent vulvovaginal candidiosis [33].

Candida cells incorporated in biofilms of mucous membranes e.g. of the gut are in close contact to the mucous membrane-associated immune system (MALT). The M-cells of the MALT are situated at the boundary surface both exogenously (gut lumen) and endogenously in close contact with the micro-organisms existing in the gut lumen, like hyphae of C. albicans. Without having to become invasive, C. albicans can control the differentiation of Th0-cells towards Th2-cells via the TLR2 of M-cells [34] in this situation [19]. Animal testing actually demonstrates that the immune system of the intestine is able to influence cytokine levels in lymph nodes and in the blood [35].

In the model of the gastrointestinal Candida infection, the administration of IL-10 and IL-4 lead to an induction of CD4+ cells in the Peyer’s patches with production of high levels of IL-10 and IL-4 [12]. At the same time, this negative effect on the immune system is increased through farnesol (Figure 1).

### 4. Possible Effects for Patients

The colonization of patients with high C. albicans cell counts is in most cases an event of a prolonged hospital stay, often also the result of a previous or existing antibiotic treatment [36,37]. However, this means that the affected persons could be in a CARS or MARS condition [38]. In this phase, multiple organ dysfunctions or organ failure could occur, the condition of the patient deteriorates and the fatalities could increase [39].
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Blastoconidia

\[ \downarrow \]

TLR 4

Hypha

\[ \downarrow \]

TLR 2

Farnesol-diphosphate

Farnesol

\[ \downarrow \]

IL-5 \( \uparrow \)

IL-10 \( \downarrow \)

IL-12

Th1-answer inflammatory

Antigen-presenting cell

Th2-answer anti-inflammatory

Figure 1. Impact of *C. albicans* on the immune system. Blastoconidia cause the stimulation of the inflammatory Th1 response by TLR4 mediated activation of antigen-presenting cells. Hyphae stimulate TLR2 signalling and production of IL-10 which promotes an anti-inflammatory Th2 response. Farnesol leads to up-regulation of IL-5 and down-regulation of IL-12 and IFN\( \gamma \) promoting the Th2 response.

In patients with a colonization of the mucous membranes through *C. albicans*, the immune system could be weakened via the above-mentioned mechanisms during this critical period of illness. If it is now decided—often also because previous antibiotic treatments have not resulted in an improved clinical condition—to eliminate the yeasts by administering antifungal drugs, fluconazole is often selected nowadays.

However, this decision in particular could be associated with serious disadvantages for the patient, in light of the illustrated pathogenic processes. In the case of a sepsis through a Gram-negative pathogen like *Escherichia coli*, the production of IL-12 usually increases via the release of lipopolysaccharides of the cell wall. A Th1-response develops that leads to an inflammatory response and therefore to elimination of the pathogen. However, Navarathna *et al.* [26] demonstrated in their experiment that, under the influence of farnesol, this IL-12 stimulation remains absent: therefore, a significant function of the immune system fails (Table 1).

### 5. Conclusion and Therapeutic Consequences

Basic research of previous years has demonstrated how actively *C. albicans* can impact the host immune system via TLR and farnesol production in terms of an anti-inflammatory (Th2) immune response. Thus, the detection of *C. albicans* on mucous membranes obtains—at least in some patients—a completely new, clinically relevant significance. Precisely in those patients who are in the phase of CARS or MARS with reduced cellular defence, an increase of this process in phases with bacterial translocation could lead to a worsening in the course of the disease.

Due to the results of basic research, a rational basis exists nowadays to treat these patients with antifungal drugs in order to interrupt the pathogenic process of the immune modulation. Azole should subsequently not be prescribed for critically ill patients as this would result in an increase of farnesol formation by a negative feedback through inhibition of the ergosterol synthesis.

Contrary to azoles, echinocandins like caspofungin or anidulafungin do not inhibit the ergosterol metabolism of *C. albicans* and are effective in biofilms [40]: *C. albicans* is eliminated without directly resulting in an increase of the farnesol concentration through negative feedback.

In addition, echinocandins possess a further significant property. They interfere with the cell wall of *C. albicans*, which mainly consists of \( \beta \)-glucans and that have an impact on the immune system in terms of a pro-inflammatory response. However, this characteristic does not usually occur as the \( \beta \)-glucans are not accessible to the immune system through an external mannan layer. Only when *C. albicans* cells are exposed to sub-inhibitory concentrations of caspofungin the level of TNF\( \alpha \) (Th1-response) increases three to four times under experimental conditions, in comparison to untreated *Candida* cells [41].

The model presented here—immune modulating effects of *C. albicans*—could also explain the debated superior clinical outcome of anidulafungin in comparison to fluconazole in *C. albicans* infections [42].

Of specific interest are future clinical studies in which the design is applied such that it can demonstrate which patients with *C. albicans* colonization ideally benefit from an echinocandin therapy.

<table>
<thead>
<tr>
<th>Pre-treatment</th>
<th>Average IL-12 production (pg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>p40</td>
</tr>
<tr>
<td>None</td>
<td>1.5</td>
</tr>
<tr>
<td>Farnesol (100 ( \mu \text{M} ))</td>
<td>0.33</td>
</tr>
<tr>
<td>IFN-( \gamma ) + LPS</td>
<td>3215</td>
</tr>
<tr>
<td>Farnesol + IFN-( \gamma ) + LPS</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Table 1. Changes of the IL-12 production under the influence of LPS, IFN-\( \gamma \) and farnesol [26].
REFERENCES


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**Abbreviations**

- **CARS**: Compensatory anti-inflammatory response syndrome
- **GOLD**: Global Initiative for Chronic Obstructive Lung Disease
- **ICU**: Intensive care unit
- **Ig**: Immunoglobulin
- **IL**: Interleukin
- **CFU**: Colony forming units
- **LPS**: Lipopolysaccharide
- **MARS**: Mixed anti-inflammatory response syndrome
- **TGF**: Tumour growth factor
- **Th**: T helper cells
- **TLR**: Toll like receptor
- **TNF**: Tumour necrosis factor
- **IFN**: Interferon