Immunoglobulins G could prevent adherence of *Candida albicans* to polystyrene and extracellular matrix components

Marie-Helene Rodier, Christine Imbert, Catherine Kauffmann-Lacroix, Gyslaine Daniault and Jean-Louis Jacquemin

Unité de recherche en biologie parasitaire et fongique, Laboratoire de parasitologie et mycologie médicales, CHU La Milétrie, 86021 Poitiers Cedex, France

INTRODUCTION

*Candida albicans*, a saprophyte of the human digestive tract, is frequently responsible for systemic infections in immunocompromised patients, even if other species of *Candida* are reported with increased frequency (Vincent et al., 1998). According to a wide-ranging American study, the rate of invasive fungal infections among hospital patients approximately doubled between 1980 and 1990, and the incidence of nosocomial candidaemia alone increased fivefold (Beck-Sague & Jarvis, 1993).

*C. albicans* possesses virulence factors that are required for the establishment of candidiasis, involved in processes such as adhesion, phenotypic switching and morphogenesis (Calderone & Fonzi, 2001). Adhesion of the organism to mucosal epithelium is a prerequisite for colonization and is therefore regarded as the initial step in the process leading to infection. Furthermore, adhesion to endothelium and extracellular matrix (ECM) components are required for dissemination of *C. albicans* (Klotz, 1992). A number of ECM proteins bind to the yeast, including fibronectin (Klotz et al., 1994), laminin (Sakata et al., 1999), vitronectin and type I and IV collagens (Klotz et al., 1993). Moreover, *C. albicans* is also able to adhere to the surface of intravascular catheters, usually colonized by intra- or extraluminal migration of *Candida* spp. from the skin surface (Flanagan & Barnes, 1998). Candidaemia occurs commonly in the presence of a colonized intravascular catheter (Rex, 1996).

Nevertheless, the ability of this yeast to cause human infectious disease relates more to the immunological status of the host than to obvious virulence factors produced by the fungus. *C. albicans* is an opportunistic yeast that becomes a pathogen in hosts whose local or systemic immune functions are impaired, for whatever reason (Matthews & Burnie, 1996). Neutropenia represents a crucial risk factor, because neutrophils are indispensable for antifungal immunity (van Sprael et al., 2001). Derangements in antibody immunity often accompany defective cellular immunity. The role of antibody immunity in fungal infections remains a controversial subject. Many *in vivo* or *in vitro* studies have provided evidence for or against the importance of antibody immunity to *C. albicans* (Casadevall, 1995).

Some authors have reported that antibody immunity against *C. albicans* may participate in host defence by preventing attachment (Han & Cutler, 1995). Most *in vitro* studies have...
METHODS

Polyclonal antiserum. C. albicans strain 2091, obtained from the Pasteur Institute (Paris, France), was grown for 48 h at 37 °C on Sabouraud’s dextrose agar slants (Sanofi Diagnostics Pasteur). The cells were harvested in Tris-buffered saline (TBS: 140 mM NaCl, 10 mM Tris.HCl, pH 7.2), washed three times with PBS (pH 7.2), and resuspended in 100 μg/ml lysostaphin in 4 mM EDTA (both from Sigma) and 0.2% Triton X-100 (v/v) before use.

Immunization and to determine the titre of the specific antibodies. Antiserum directed against the cell wall components of C. albicans, compared with its adherence after contact of the yeast with non-specific IgG.

Verification of the immunization of rabbits. Pooled sera from rabbits before and after immunization were obtained after six subcutaneous injections, at 15 day intervals, of this strain. The titre of serum obtained after immunization was 1:1280. Whereas the titre of serum obtained after immunization was negative, whereas the titre of serum obtained after immunization was 1:1280.

Adherence of C. albicans to ECM proteins. C. albicans blastospores were added, after pre-incubation with or without IgG at various concentrations, in culture plates as inocula of 10^7 cells ml^-1 in 150 μl PBS alone for control or containing the IgG at the same concentrations. They were then allowed to adhere to polystyrene for 2 h at 37 °C; half of the wells were then washed twice with PBS to remove non-adherent yeasts. Thereafter, 300 μl XT TETRAMOZIUM (pH 6.8) was added to each well. Plates were incubated for 3 h at 37 °C without shaking and then gently agitated and XT TETRAMOZIUM was measured by monitoring the absorbance in unwashed wells divided by the absorbance in washed wells. Background absorbance was determined with plates that contained PBS only or PBS, XT TETRAMOZIUM and menadione; these values did not exceed 0.085 and were therefore not significant. All experiments were performed at least twice in six samples.

Statistical analyses. Analysis of variance and Scheffe’s test were conducted to determine differences among the test groups (P < 0.05).

RESULTS

Verification of the immunization of rabbits. Pooled sera from rabbits before and after immunization were tested by an immunofluorescence assay using C. albicans blastoconidia. The pooled serum obtained before immunization was negative, whereas the titre of serum obtained after immunization was 1:1280.

Influence of IgG on adherence of C. albicans to different surfaces. Incubation of blastospores with IgG directed against cytoplasmic extract of C. albicans at different concentrations...
Table 1. Effect of IgG on the capacity of four strains of *C. albicans* to adhere to polystyrene, fibronectin and ECM proteins

Adherence was determined as described in Methods. Non-specific (NS) and specific (S) IgG were used at the concentrations indicated. Control incubations contained PBS alone.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Control</th>
<th>800 µg ml⁻¹</th>
<th>300 µg ml⁻¹</th>
<th>100 µg ml⁻¹</th>
<th>50 µg ml⁻¹</th>
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<tbody>
<tr>
<td></td>
<td>NS</td>
<td>S</td>
<td>NS</td>
<td>S</td>
<td>NS</td>
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<tr>
<td>Polystyrene</td>
<td></td>
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<tr>
<td>1066</td>
<td>48.7 ± 5.1</td>
<td>25.2 ± 4.3**</td>
<td>24.0 ± 0.7a</td>
<td>25.3 ± 0.4a</td>
<td>24.2 ± 1.1a</td>
</tr>
<tr>
<td>2091</td>
<td>47.2 ± 1.3</td>
<td>17.3 ± 3.0a</td>
<td>20.3 ± 3.0a</td>
<td>18.4 ± 0.9a</td>
<td>19.6 ± 1.0a</td>
</tr>
<tr>
<td>NIH 311</td>
<td>71.4 ± 2.5</td>
<td>29.3 ± 6.1a</td>
<td>27.2 ± 2.5a</td>
<td>31.6 ± 0.5a</td>
<td>28.2 ± 3.2a</td>
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<tr>
<td>165-CA</td>
<td>377 ± 1.2</td>
<td>30.2 ± 3.6a</td>
<td>23.3 ± 2.5a</td>
<td>21.2 ± 1.4a</td>
<td>25.3 ± 4.0a</td>
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<td>Fibronectin</td>
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<td>67.2 ± 9.2</td>
<td>25.7 ± 4.2a</td>
<td>23.9 ± 2.1a</td>
<td>27.2 ± 3.5a</td>
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<tr>
<td>2091</td>
<td>25.3 ± 2.7</td>
<td>16.4 ± 2.9a</td>
<td>17.3 ± 2.7a</td>
<td>18.2 ± 1.2a</td>
<td>17.7 ± 2.7a</td>
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<tr>
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<td>33.9 ± 3.8a</td>
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<td>37.2 ± 2.5a</td>
<td>35.3 ± 1.7a</td>
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<td>165-CA</td>
<td>557 ± 7.8</td>
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<td>ECM proteins</td>
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<td>20.4 ± 3.2a</td>
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<tr>
<td>2091</td>
<td>25.2 ± 1.3</td>
<td>20.0 ± 2.7a</td>
<td>18.2 ± 3.2a</td>
<td>21.5 ± 0.2a</td>
<td>21.2 ± 0.3a</td>
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<tr>
<td>NIH 311</td>
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<tr>
<td>165-CA</td>
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<td>226 ± 4.8</td>
<td>22.1 ± 1.5</td>
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</table>

*Significant differences (P < 0.001) are indicated in comparison with: a, control; b, non-specific IgG.
induced a significant decrease in the adherence of the four tested strains to polystyrene, fibronectin and ECM proteins (P < 0·001) when compared with the adherence capacity of these strains incubated with PBS only. Only low concentrations of IgG were unable to decrease adherence, in the case of strains 2091 and 165-CA to fibronectin and of strain NIH 311 to fibronectin and ECM gel. Moreover, for strain 165-CA, there was no effect of the presence of IgG (specific or not) on adherence to ECM protein. We found the same results after incubation of the yeasts with non-specific IgG from rabbits before immunization, in comparison with the same controls (Table 1).

**Influence of specific IgG on the capacity of C. albicans to adhere to different surfaces**

Regardless of the surface studied and the conditions used, we found only two significant cases where the specificity of the IgG for *C. albicans* antigens influenced (at the highest concentration) the capacity of adherence of the yeast: adherence of strain 165-CA to polystyrene and of strain NIH 311 to fibronectin (Table 1). In all the other cases, there was no significant difference between the adherence capacities of the strains incubated with specific or non-specific IgG, regardless of concentration.

**DISCUSSION**

In high-risk patients suffering from disseminated candidiasis, predisposition to infection has many components, including both immunodeficiency (humoral and cellular), related to the pathology itself, and the results of immunosuppression, related to treatment (Tsiodras et al., 2000). Delayed immune reconstitution represents an increased risk of infectious complications (LaRocco & Burgert, 1997). The incidence of invasive fungal infections is particularly high in bone-marrow transplant recipients. The conditional regimen used to prepare the host is a major determinant of host tissue injury and may lead to mucositis diarrhoea, facilitating transmucosal arrival of blood-stream infections (van Burik & Weisdorf, 1999). Invasive monitoring with intravascular catheters predisposes to colonization and infection with *Candida* spp. (Flanagan & Barnes, 1998). In intensive-care units, patients are also immunosuppressed following major surgery, trauma, burns or corticosteroid administration. In these patients, this is often associated with a decrease in intestinal mucosal barrier function (Flanagan & Barnes, 1998).

Adhesion events to endothelium and ECM components are required for dissemination of *C. albicans*. This process could begin by yeasts gaining access to the blood stream through gastrointestinal peroration, by seeding from the biofilm of a medical device or by inoculation consecutive to a trauma (Glee et al., 2001). For these reasons, adherence to implanted devices and to ECM are of great importance for development of the disease. The adhesins of *C. albicans* are diverse, reflecting the ability of the organism to colonize and invade a variety of host cells and tissues (Bailey et al., 1995).

It has been reported in some in vitro studies that antibody immunity may contribute to host defence by direct candidal activity, by providing opsonins for more efficient phagocytosis, by binding to immunomodulating polysaccharides, by neutralizing extracellular proteases and by inhibiting the yeast-to-mycelium transition, but also by preventing attachment (Casadevall, 1995).

Administration of immune serum has been protective in some animal studies, but not in others. Polyclonal preparations are complex mixtures of antibodies, differing in isotype and specificity. This may explain why antibody protection has been observed only in some studies (Casadevall, 1995). Specific antibodies to mannanproteins and hisp90 have been shown to be protective against murine candidiasis (Matthews & Burnie, 1996; Cassone et al., 1995). Specific antibody induction has been investigated as an immunotherapeutic preventative measure (Han & Cutler, 1995). Recently, oral administration of bovine anti-*Candida* antibodies has been used for passive immunization in allogeneic bone-marrow transplant recipients (Tollefson et al., 1999).

The ability of a protein to block adherence may have potential in ameliorating or eliminating disease associated with this organism (Klotz & Smith, 1995), even if blocking of invasion of tissue by *C. albicans* in order to reduce infection has had modest success in several animal models dealing with different forms of candidiasis (Pendrak & Klotz, 1995).

In this study, we have investigated the effect of specific and non-specific IgG at different concentrations on the capacity of *C. albicans* to adhere to polystyrene and ECM components. IgG does adsorb spontaneously to polymer surfaces both in *vitro* and in *vivo* (Tang et al., 1993; Inoue et al., 1997). It has also been demonstrated that a biochemical interaction occurs between fibronectin and the Fc portion of IgG (Rostagno et al., 2002). Antibodies used in this study were directed against a cytoplasmic extract of *C. albicans*. Nevertheless, these specific IgG were able to recognize proteins localized in the fungus cell wall, as proved by the immunofluorescence assay. We have shown that the presence of IgG, specific or not, at concentrations close to the *in vivo* situation, reduced the capacity of *C. albicans* to adhere to polystyrene and ECM components. More interestingly, this study highlights the fact that the hypogammaglobulinaemia found in immunocompromised patients (Hammarström et al., 1994, 2000) could play a role in the dissemination of *Candida* infections, not only because of the decrease in specific antibodies, but also because total IgG could present a barrier to the interaction between pathogens and host components or medical device surfaces. This could have clinical implications: survey of the total level of immunoglobulins and maintenance of a sufficient amount of these immune proteins until the recovery of the immune system could have potential in the prevention of systemic candidiasis.
REFERENCES


