In vitro response to Candida albicans in cultures of whole human blood from young and aged donors

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Abstract
Invasive infections with opportunistic fungi, such as Candida albicans, have become an increasing problem in aged adults in recent years. This work investigates the influence of human ageing on C. albicans recognition by toll-like receptors (TLRs), essential components of the innate immune system, using a cohort of 96 young (15–42 years) and aged (>70 years) human volunteers. No significant differences between aged and young donors were observed on (1) cell surface TLR2, TLR6 and TLR4 expression on lymphocytes, monocytes and granulocytes, (2) production of cytokines [IL-8, IL-1β, IL-6, IL-10, tumour necrosis factor (TNF)-α and IL-12p70] and prostaglandin E2 (PGE2) by whole human blood in response to C. albicans and (3) fungicidal activity of whole blood. A statistically significant higher titre of natural anti-C. albicans antibodies was found in plasma of volunteers between 80 and 95 years old when compared with other age groups, probably as a consequence of the increased levels of serum Ig that has been described in elderly subjects. Therefore, the results indicate that the increased susceptibility to C. albicans infections in the elderly is not a consequence of defects in TLRs expression or signalling, nor of an impaired fungicidal activity of blood.

Introduction
Candida albicans is a polymorphic fungus that resides as a commensal in human mucosae and the GI tract. In immunocompromised patients, the delicate balance between the host and this opportunistic pathogen turns into a parasitic relationship, resulting in the development of invasive infections. The incidence of these infections is increasing as a result of the growing population of immunocompromised individuals. Common risk factors for bloodstream candidiasis include immunosuppression, malignancy with leucopenia, major abdominal surgery or trauma, exposure to multiple antibacterial agents, central venous catheterization, prolonged stay in intensive care units, parenteral nutrition, as well as extremes of age (low birth weight infants and the elderly). Systemic candidiasis are associated with high morbidity and mortality, as early diagnosis is difficult and current antifungal therapies are limited by toxicity and resistance (Calderone, 2001; Hajjeh et al., 2004; Pfaller & Diekema, 2007).

Protective immunity to C. albicans involves both innate and adaptive immune responses. Phagocytic cells recognize the pathogen by a variety of pattern recognition receptors (PRRs), including toll-like receptors (TLRs) (Poulaín & Jouault, 2004; Gil & Gozalbo, 2006; Zelante et al., 2007). These cells can ingest and kill the pathogen, releasing several key mediators such as proinflammatory cytokines, and inducing a T-helper type 1 (Th1) immune response that activates fungicidal effector mechanisms, and helps in the generation of a protective antibody response (Romani, 2004).

In old adults, alterations of both innate and adaptive immunity (immunosenescence) lead to increased susceptibility to infections (Effros, 2001; Ginaldi et al., 2001). Alterations in adaptive immunity associated with ageing have been well described (Linton & Dorshkind, 2004; Gorczynski et al., 2007; Zhao et al., 2007). However, the influence of ageing on components of the innate immune system, such as the family of TLRs, remains incompletely understood (Plowden et al., 2004; Gomez et al., 2005; van
Duin et al., 2007). Invasive infections with opportunistic fungi have become an increasing problem in the elderly because they are more likely to be considered for transplantation, receive aggressive regimens of chemotherapy for cancer and take immunosuppressive drugs for nonmalignant diseases (Kauffman, 2001). Moreover, the immunosenescence process in old adults probably enhances the risk and severity of candidaemia (Nucci et al., 1998; Romani, 2005). Recently, it has been shown that aged C57BL/6 mice develop an altered innate and adaptive immune response to C. albicans and are more susceptible to systemic primary candidiasis (Murciano et al., 2006b).

In the present work, using human volunteers, the following has been studied: the influence of ageing on (1) cell surface TLR expression in blood cells, (2) production of cytokines and prostaglandin E₂ (PGE₂) by whole human blood in response to C. albicans, (3) fungicidal activity of whole blood and (4) the presence of C. albicans-specific antibodies in plasma. The present findings represent the first analysis of the influence of human ageing on C. albicans recognition by components of the innate immune system using whole-blood cultures.

Materials and methods

Human volunteers

Whole blood from two groups of healthy male or female volunteers was analysed: young (between 15 and 42 years, n = 49) and old (between 70 and 95 years, n = 47). Volunteers were recruited from an outpatient department (Centro de Atención Primaria Pintor Stolz, Conselleria de Sanitat, Valencia, Spain) and from the University of Valencia. All donors were informed and completed a health questionnaire, and doctor’s consent for their inclusion in the study was obtained. Only healthy donors were considered; volunteers with antiinflammatory or immunosuppressive medication, diabetes mellitus, chronic inflammatory disease or symptoms of recent infection were excluded.

Analysis of TLR expression by flow cytometry

Blood was collected by venipuncture using heparinized syringes (lithium heparin; Sarstedt, Barcelona, Spain). The levels of TLR2, TLR4 and TLR6 in blood cells were measured by flow cytometry: 0.1 mL of whole blood was incubated with 5 μL of fluorescein isothiocyanate (FITC)-labelled monoclonal antibodies (TLR2, clone TL2.1; TLR4, clone HTA 125; or TLR6, clone 86B1153.2) or with the respective FITC-labelled isotype control (Immunok, AMS Biotechnology Ltd, UK) for 20 min at 4°C. Whole blood without a label was used as a control. After incubation, cells were treated with ImmunoPrep Reagent System in an Epics Immunology Workstation (Beckman Coulter) for erythrocyte lysis and cell fixation. Samples were analysed by flow cytometry using an EPICS XL-MCL flow cytometer (Beckman Coulter). The fluorescence mean intensity was measured in lymphocytes, monocytes and granulocytes, gated by its properties of side and forward scatter. Flow-Set Fluorospheres (Beckman Coulter) were used for the standardization of light scatter and fluorescence intensity to avoid day-to-day variations.

Ex vivo stimulation of whole blood

Whole blood was diluted 1:5 into RPMI 1640 culture medium, supplemented with 1% penicillin/streptomycin (Gibco, Barcelona, Spain) and 2.5 U mL⁻¹ heparin sodium salt (Sigma, Madrid, Spain) at a final volume of 0.2 mL. Samples were challenged with the indicated stimuli in a 96-well tissue culture plate for 24 h at 37°C in a 5% CO₂ atmosphere. The stimuli used were lipopolysaccharide from Escherichia coli O111:B4 (5 μg mL⁻¹; Sigma, Madrid, Spain), the yeast cell wall particle zymosan (30 × 10⁶ particles mL⁻¹; Molecular Probes, Invitrogen) and paraformaldehyde-fixed C. albicans American type culture collection (ATCC) 26555 yeasts or hyphae [500 μg (dry weight) cells mL⁻¹], obtained as reported elsewhere (Gil-Navarro et al., 1997; Gozalbo et al., 1998). Briefly, starved yeast cells were inoculated [200 μg (dry weight) cells mL⁻¹] in a minimal synthetic medium, and incubated for 3 h at 28°C to obtain yeasts, or at 37°C to obtain hyphae; > 90% of the cells exhibited well-defined germ tubes (true hyphae) at 37°C, whereas only yeasts were observed at 28°C. In order to kill the cells, yeasts and hyphae were treated for 1 h with 4% paraformaldehyde (fixation buffer; eBioscience, San Diego, CA) (5 × 10⁶ cells mL⁻¹). After inactivation, fungal cells were extensively washed in phosphate-buffered saline (PBS) and brought to the desired cell density in cell culture medium. Blood samples without stimuli were used as a control.

All the assays were performed under conditions designed to minimize endotoxin contamination. Endotoxin-free water and PBS were used; fungal culture media were passed through a detoxi-gel endotoxin-removing gel (Pierce, Rockford, IL) and tested for the absence of endotoxin by the E-toxate assay (Sigma, Madrid, Spain).

Cytokine and PGE₂ measurement

After stimulation, cell-free supernatants of blood cultures were harvested and the levels of cytokines and PGE₂ were measured. Six different cytokines [IL-8, IL-1β, IL-6, IL-10, tumour necrosis factor (TNF)-α and IL-12p70] were determined by flow cytometry using the ‘Human Inflammation Kit’, a Cytometric Bead Array (CBA) (BD Biosciences, San Diego, CA), according to the manufacturer’s instructions. The intensity of the fluorescence signal was measured on an
EPICS XL-MCL flow cytometer (Coulter Beckman), and analysed using the manufacturer’s indications. The levels of PGE₂ were measured using the Enzyme Immunoassay Kit (Assay Designs, Michigan), a commercial competitive immunoassay for the quantitative determination of PGE₂.

**Candida albicans survival in blood**

Yeasts of *C. albicans* strain ATCC 26555 were grown in YPD medium (1% yeast extract, 2% peptone, 2% glucose) at 28 °C up to the late exponential growth phase (A₆₀₀nm 0.6–1), and then collected and washed with water. Cells were resuspended in water at the same cell density (A₆₀₀nm 0.6–1) and maintained for 3 h at 28 °C with shaking and afterwards at 4 °C for 24 or 48 h (starved yeast cells), as described previously (Gil-Navarro et al., 1997; Gozalbo et al., 1998). The suspension was finally washed, diluted in RPMI 1640 culture medium (Gibco, Barcelona, Spain) and brought to the desired cell density. 5 × 10⁵ *C. albicans* yeast cells (5 μL) were inoculated in 0.1 mL of whole blood or plasma from each donor, and incubated for 1 h at 37 °C. Samples of plasma were obtained by centrifugation (5 min at 3000g) of heparinized blood. Viable fungal cells (CFU) were determined as follows: aliquots of the samples were withdrawn at t = 0 and 1 h, diluted in water, vigorously vortexed, plated on Sabouraud/glucose agar and incubated overnight at 37 °C. The percentages of survival were calculated as follows: % CFU blood = [CFU blood (1 h)/CFU blood (0 h)] × 100; % CFU plasma = [CFU plasma (1 h)/CFU plasma (0 h)] × 100; % survival = (% CFU blood/ % CFU plasma) × 100; % killing = 100 – % survival.

**Semi-quantitative determination of *C. albicans*-specific antibodies**

Human plasma samples were assayed by enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescence (IIF) to determine *C. albicans*-specific antibodies, as described previously (Villamón et al., 2004b,c). Microtitre plates were coated with 50 μL of 6 × 10⁻² M sodium carbonate (pH 9.6) containing soluble cell extracts (5 μg of protein per well), obtained from yeasts or hyphae by boiling the cells in PBS supplemented with 1% sodium dodecyl sulphate and 1% DTT. After overnight incubation at 4 °C, the wells were washed with 0.05% (v/v) Tween 20 in PBS (washing buffer) and blocked with 0.1 mL of PBS supplemented with 1% bovine serum albumin (BSA), for 1 h at room temperature. Three additional washes were followed by the addition of 50 μL per well of serial dilutions (1 : 2) of plasma in PBS with 0.01% of Tween 20 and 0.1% BSA and further incubation for 2 h at 37 °C. After washing, 50 μL of rabbit anti-human IgG (whole molecule) peroxidase-conjugated (Sigma, Madrid, Spain) 1 : 10 000 diluted (in PBS with 0.01% of Tween 20 and 0.1% BSA) was added to the wells, incubated for 1 h at room temperature, and after extensive washing, the absorbance was determined at 450 nm following the addition of substrate mixture.

For IIF fixed *C. albicans* ATCC yeasts, obtained as mentioned above, were incubated with 1 : 100 dilution of human plasma in PBS (1 × 10⁶ yeasts in 0.1 mL), for 1 h at 37 °C. After three washes with PBS, 0.1 mL of a 1 : 100 dilution of rabbit anti-human IgG (whole molecule) FITC-conjugated (Sigma, Madrid, Spain) was added and incubated for 1 h at 37 °C. After incubation, the samples were washed three times with PBS and the fluorescence mean intensity and the percentage of FITC-labelled yeasts were analysed by flow cytometry in an EPICS XL-MCL flow cytometer (Beckman Coulter). Control experiments were performed by omitting incubation of cells with the plasma samples.

**Statistical analysis**

For statistical analysis, the human samples were grouped into four age categories: two groups of young donors (from 15 to 25 years and from 26 to 42 years, respectively) and two groups of aged donors (from 70 to 79 years and from 80 to 95 years, respectively). Student’s two-tailed t-test was used to compare TLR expression, cytokine and PGE₂ production, fungicidal activity of blood and levels of anti-*C. albicans* antibodies in human plasma. Data are expressed as mean ± SE. Significance was accepted at the P < 0.05.

**Results**

**TLR surface expression in blood cells**

To determine the influence of ageing on *C. albicans* recognition by components of the innate immune system, 96 healthy volunteers belonging to two age groups were enrolled: 49 donors between 15 and 42 years and 47 donors >70 years old. The release of several key mediators such as proinflammatory cytokines is important for protecting the host against disseminated candidiasis (Romani, 2004). This host-secretory immune response is triggered through recognition of fungal ligands by PRRs such as TLRs (Gil & Gozalbo, 2006; Zelante et al., 2007). The authors’ group has described that TLR2 is essential for murine resistance to primary invasive candidiasis, triggers the production of proinflammatory cytokines and is involved in the induction of a Th1 response, whereas TLR4 appears not to have a relevant role in these events (Villamón et al., 2004a,c; Gil & Gozalbo, 2006; Murciano et al., 2006a). Other authors have described a role for TLR2 and TLR4 in resistance to primary infection as well as their involvement in the *in vitro* cytokine production in response to *C. albicans* cells (Bellocchio et al., 2004; Neta et al., 2006). Therefore, to define the effect of ageing on the cell surface expression of the TLRs involved in host defence against candidiasis, baseline TLR2 and TLR4
surface expression was assessed on blood granulocytes, monocytes and lymphocytes of young and old donors. The results showed no significant differences in TLR2 and TLR4 expression in all three blood cell populations from the individuals of the study clustered into four age groups (Fig. 1). As TLR2 generally functions as a heterodimer with either TLR1 or TLR6, and zymosan induces signalling through a TLR2/TLR6 heterodimer (West et al., 2006), TLR6 expression was also measured in blood cells. Although TLR6 levels tend to decrease in granulocytes and monocytes from aged volunteers (80–95 years), the results showed no statistical significant differences in TLR6 expression in all three blood cell populations from the individuals of the four age groups (Fig. 1). This observation does not exclude possible defects in signal transduction pathways triggered by TLRs associated with ageing.

**Cytokine and PGE2 production in whole blood in response to C. albicans**

Whole blood was used to measure ex vivo cytokine and PGE2 production in response to yeasts and hyphae as these conditions most closely recreate the in vivo situation (Fig. 2). In this system, cell populations that are important for the defence against C. albicans (granulocytes, monocytes and lymphocytes), along with complement, antibodies and other serum factors can interact with each other and with the fungus. Unstimulated cultures served as negative controls, and known TLR4 (lipopolysaccharide) and TLR2/TLR6 (zymosan) agonists were included as controls. As expected, no cytokine could be detected in unstimulated samples in contrast to all other stimulated cultures. However, no significant differences between old and young volunteers were observed for IL-8, IL-1β, IL-6, IL-10, TNF-α, IL-12p70 and PGE2 production upon stimulation with lipopolysaccharide, zymosan or C. albicans yeasts or hyphae, even when samples were clustered into four age categories for statistical analysis (Fig. 2).

**Fungicidal activity of whole blood**

In order to investigate the effect of ageing on the fungicidal activity of whole human blood, the survival of C. albicans yeast cells was determined following incubation with blood samples from young and old donors. Blood samples were inoculated with C. albicans cells at a density of $5 \times 10^5$ fungal cells per 0.1 mL, which roughly corresponds to 1 : 1 yeast/leucocyte ratio. As a control, fungal cells were also incubated in the plasma from each donor, under the same conditions. The actual fungal growth was determined after 1 h of incubation (Table 1). The results indicated that the viability of the fungus is not reduced by plasma components alone, and even that fungal cells were capable of budding in the presence of plasma. However, the survival in fresh whole blood was reduced to 39–54%, which represents a blood-killing activity of 45–60%. No significant differences in the fungicidal activity between blood from old and young donors were observed even when samples were clustered into four age categories for statistical analysis (Table 1).

**Candida albicans-specific antibodies in plasma**

Although the levels of anti-C. albicans circulating antibodies may not correlate with protection, there is evidence indicating the protective role of some specific antibodies during infection (Calderone, 2001; Romani, 2005). In addition, low levels of circulating antibodies against some abundant C. albicans enzymes are often present in healthy subjects (Pitarch et al., 2004). Therefore, C. albicans-specific antibodies were measured in plasma from aged and young donors. IgG levels were measured by ELISA using microtitre
Fig. 2. Cytokine and PGE2 production in response to Candida albicans. Whole-blood cultures from young and old volunteers were challenged for 24 h with Escherichia coli lipopolysaccharide (5 μg mL$^{-1}$), zymosan (30 × 10$^6$ particles mL$^{-1}$) and paraformaldehyde-fixed C. albicans ATCC 26555 yeasts or hyphae [500 μg (dry weight) cells mL$^{-1}$]. The concentrations of cytokines and PGE2 were measured in the cell-free culture supernatants by flow cytometry using a Cytometric Bead Array (CBA), and by a competitive immunoassay, respectively. Graphs show the mean production ± SE of IL-8, IL-1β, IL-6, IL-10, TNF-α, IL-12p70 and PGE2 in the four age groups.
plates coated with soluble cell extracts obtained from yeasts or hyphae (Fig. 3). A slight but statistically significant higher titre was found in plasma from old volunteers (between 80 and 95 years) when compared with the three other age groups \( (P < 0.05, \text{for both yeasts and hyphae extracts})\).

However, the diversity of antigens recognized by plasma of old adults, assessed by Western blot analysis using the same cell extracts from yeasts or hyphae, was quite similar to that from young volunteers (data not shown). The \textit{C. albicans}-specific antibodies were also measured against cell wall surface antigens by IIF and flow cytometry (Fig. 4). Once again, no significant differences were found in fluorescence mean channel or in the percentage of labelled yeast even when samples were clustered into four age categories for statistical analysis.

### Discussion

In the elderly, deterioration of immune function and increased incidence and lethality of infectious diseases is well documented (Effros, 2001; Ginaldi \textit{et al.}, 2001). It has been described that aged mice develop an altered innate and adaptive immune response to \textit{C. albicans} and are more susceptible to systemic primary candidiasis (Murciano \textit{et al.}, 2006b). In this report, the influence of ageing on \textit{C. albicans} recognition by components of the human innate immune system such as TLRs has been examined.

Murine-aged macrophages secrete significantly lower levels of proinflammatory cytokines than young macrophages in response to different ligands of TLRs (Renshaw \textit{et al.}, 2002; Boehmer \textit{et al.}, 2004, 2005), although different explanations have been offered for this observation.

### Table 1. \textit{Candida albicans} survival in plasma and blood from young and old volunteers clustered in four age groups

<table>
<thead>
<tr>
<th>Age groups</th>
<th>% CFU blood</th>
<th>% CFU plasma</th>
<th>% survival</th>
<th>% killing</th>
</tr>
</thead>
<tbody>
<tr>
<td>15–25 y ((n=22))</td>
<td>47.41 ± 4.72</td>
<td>113.20 ± 6.10</td>
<td>44.77 ± 4.96</td>
<td>55.23 ± 4.96</td>
</tr>
<tr>
<td>26–42 y ((n=25))</td>
<td>43.14 ± 5.22</td>
<td>114.42 ± 6.11</td>
<td>39.45 ± 6.51</td>
<td>60.55 ± 5.61</td>
</tr>
<tr>
<td>70–79 y ((n=31))</td>
<td>54.92 ± 5.01</td>
<td>116.78 ± 4.99</td>
<td>46.16 ± 3.58</td>
<td>53.84 ± 3.58</td>
</tr>
<tr>
<td>80–95 y ((n=13))</td>
<td>55.23 ± 7.85</td>
<td>109.60 ± 7.56</td>
<td>54.15 ± 8.21</td>
<td>45.85 ± 8.21</td>
</tr>
</tbody>
</table>

\(y\), years old; \% CFU blood = [CFU blood \((t=1)/\text{CFU blood \((t=0)\}\) \times 100; \% CFU plasma = [CFU plasma \((t=1)/\text{CFU plasma \((t=0)\}\) \times 100; \% survival = (\% CFU blood/\% CFU plasma) \times 100; \% killing = 100 – % survival.

Data represents mean ± SE.

\(A_{450 \text{ nm}}\), absorbance; \(A_{405 \text{ nm}}\), absorbance; % labelled yeasts, percentage of FITC-labelled yeasts.

![Fig. 3. \textit{Candida albicans}-specific antibodies in human plasma determined by ELISA.](https://academic.oup.com/femspd/article-abstract/51/2/327/889280/0)

![Fig. 4. \textit{Candida albicans}-specific antibodies in human plasma determined by IIF.](https://academic.oup.com/femspd/article-abstract/51/2/327/889280/1)
Renshaw et al. (2002) found that macrophages from aged mice express lower levels of TLRs, whereas Boehmer et al. (2004, 2005) concluded that decreased expression of mitogen-activated protein kinases (MAPKs) could be the mechanism responsible for age-related deterioration of TLR-mediated signalling. A reduction of in vitro TNF-α production by resident peritoneal macrophages from aged mice has been described previously in response to both yeasts and hyphae of C. albicans (Murciano et al., 2006b).

In contrast to the findings in aged mice, a defect in TLR2, TLR6 or TLR4 function and expression in the aged human individuals was not observed. Granulocytes, monocytes and lymphocytes of old adults express baseline levels of TLR2, TLR6 and TLR4 similar to cells from young donors. In whole blood, the effect of ageing on cellular pathways signalling for cytokine and PGE₂ production upon stimulation by the fungal stimuli (zymosan and C. albicans yeasts and hyphae) has also been determined. The authors’ group has described that TLR2 triggers the production of proinflammatory cytokines and PGE₂ in response to C. albicans cells whereas TLR4 appears not to have a relevant role in these events (Villamón et al., 2004a, c, 2005; Gil & Gozalbo, 2006; Murciano et al., 2006a). Other authors have described a role for TLR2 and TLR4 in the cytokine production in response to fungal stimuli (Bellocchio et al., 2004; Netea et al., 2006). The present results showed that cytokine and PGE₂ production in response to all fungal stimuli, as well as to lipopolysaccharide, was similar in young and old donors. This is in accordance with the results by van Duin et al. (2007), who found that TLR1/TLR2-induced TNF-α production is diminished in old adults, but TLR4- and TLR2/TLR6-induced cytokine production appears to be largely intact in monocytes. These authors have also found that TLR1 surface expression was 36% lower on purified monocytes from old adults than on those from young adults, TLR2 surface expression was not significantly altered between the age groups and TLR4 expression is slightly diminished on monocytes from old volunteers, although no accompanying alteration in lipopolysaccharide-mediated cytokine production was observed (van Duin et al., 2007). Similarly, Fulop et al. (2004) showed that there are no ageing-associated changes in the expression of TLR2 and TLR4 receptors on human neutrophils. Whether the function of any other TLR is altered in the context of human ageing remains to be determined. The older group of volunteers who were analysed was free of comorbid conditions, and therefore, it is possible to speculate that age-associated TLR2 and TLR4 functional defects may arise in populations with increased levels of disability and/or diseases, leading, at least in part, to the increased incidence and lethality of infectious diseases in the elderly.

In order to determine whether there are defects in the ability of aged innate cells to kill C. albicans, survival assays were performed in blood samples. Neutrophils govern the immediate defence against C. albicans in whole blood, by dominating the viability and growth of the fungus, as well as their morphology and transcript profile (Fradin et al., 2005). The results concerning changes in neutrophil functions with ageing are controversial. Over the past few years, it has been demonstrated that neutrophil-specific receptor-driven effector functions are indeed altered with ageing, although some functions have consistently been found not to change with ageing (Fulop et al., 2004). The present experimental approach has shown no significant differences between the fungicidal activity of blood from old and young donors, suggesting that neutrophils from healthy aged individuals can normally kill C. albicans cells in blood.

Finally, the levels of C. albicans-specific antibodies in plasma from young and aged donors were also determined. Pitarch et al. (2004) have demonstrated that low levels of circulating antibodies against some abundant C. albicans proteins were often present in healthy subjects. The presence of these natural anti-C. albicans antibodies may be attributable to the continuous exposure of these antigens during harmless colonization of C. albicans. Alternatively, considering the ubiquitous nature, the great abundance and the high degree of sequence homology of these proteins across species, they could, therefore, cross-react with antibodies elicited by other human commensal or infectious agents. A statistically significant higher titre was found in the plasma of volunteers between 80 and 95 years when compared with the three other age groups. Probably, this result is a consequence of the increased levels of serum Ig that has been detected in elderly subjects (Ginaldi et al., 2001). However, when the C. albicans-specific antibodies against cell wall surface antigens were measured by IIF and flow cytometry, no significant differences were found between the four age groups. Moreover, the pattern of C. albicans antigens recognized by Western blot analysis, in both yeast and hyphal extracts, was similar for young and old plasma samples.

In summary, no significant differences between old and young donors were observed concerning (1) cell surface TLR expression in blood cells, (2) production of cytokines and PGE₂ by whole human blood in response to C. albicans and (3) fungicidal activity of whole blood. Therefore, the present results suggest that the increased susceptibility to C. albicans infections in the elderly is not a consequence of defects in TLRs expression or signalling, or a consequence of impaired fungicidal activity of blood.

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