MYCOLOGY

Interactions between *Candida* species and platelets

M. D. P. WILLCOX*†, B. C. WEBB†, A. THAKUR* and D. W. S. HARTY†

*Cornea and Contact Lens Research Unit, School of Optometry and Cooperative Research Centre for Eye Research and Technology, University of New South Wales, NSW 2052 and †Institute of Dental Research, Sydney, NSW 2010, Australia

Candida spp. are able to cause disseminated disease in immunocompromised patients. This study examined the interactions of *Candida* spp. with platelets, complement and polymorphonuclear leucocytes (PMNLs). With the exception of *C. albicans*, all other *Candida* spp., including a *C. albicans* strain previously classified as *C. stellatoidia*, aggregated human platelets at a ratio of yeast cells: platelets of 1:80. Usually, those species and strains that aggregated platelets were either killed or prevented from growing in platelet-rich plasma indicating that the aggregation released microbicidal or microbistatic substances that were active against *Candida* spp. All *Candida* spp. were resistant to attack by complement in 50% serum. However, all species activated complement as determined by the presence of C3 fragments on their surface, in particular a 195-kDa fragment corresponding to C3c, two fragments at 67 and 40 kDa corresponding to iC3b, and a 33-kDa fragment corresponding to C3d. When strains were tested for their ability to stimulate the release of pro-inflammatory substances from platelets and PMNLs, it was found that most strains stimulated PMNLs to release interleukin(IL)-8 but not IL-1 β or leucotriene B₄. The ability of *C. albicans* to evade complement-mediated killing and not to aggregate platelets may contribute to the survival of this species in the blood during vascular infections.

Introduction

Candida spp. are important opportunist pathogens that infect medically compromised individuals. The most common oral disease caused by *Candida* spp. is denture stomatitis. Although all species of *Candida* can produce denture stomatitis, the most commonly isolated species is *C. albicans* [1]. *C. albicans* is the commonest of the *Candida* spp. found within the oral cavity; other species that have been isolated include *C. glabrata*, *C. tropicalis*, *C. guilliermondii* and *C. krusei* [2]. However, if *Candida* spp. enter the blood stream, they can cause severe life-threatening diseases such as infective endocarditis, vascular catheter sepsis and infections of vascular prostheses [3, 4]. Several species of *Candida* have been isolated from patients with endocarditis, with *C. albicans* being the most common, followed by *C. parapsilosis*, *C. tropicalis*, *C. stellatoidia* (currently classified in the species *C. albicans*) and *C. krusei* [1, 5, 6].

The mechanisms utilised by *Candida* spp. to initiate vascular infections are believed to be complex, involving interactions of fungal cells with plasma proteins, e.g., fibronectin [7], endothelial cells [8] and platelets [9], and avoidance of plasma host defences such as complement and phagocytosis. It is well known that *Candida* cells can activate the alternative complement pathway, a major effector pathway of inflammation, and this will release the inflammatory mediators C3a which is vasodilatory, and C5a which activates and recruits white blood cells to sites of infection. Recently, it has been demonstrated that activation of complement on the surface of *Streptococcus sanguis* may be involved in platelet aggregation [10]. Activation of white blood cells, e.g., polymorphonuclear leucocytes (PMNLs), macrophages, mast cells and platelets also releases inflammatory mediators, e.g., histamine and arachidonic acid metabolites and antimicrobial peptides such as human neutrophil peptides [11, 12] and platelet microbicidal proteins [13]. It has been clearly demonstrated that PMNLs are of vital importance in preventing the development of candidosis [14]. The aim of this investigation was to examine different species of *Candida* for their ability to interact with these elements of the host defence system.

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Corresponding author: Dr M. D. P. Willcox.

Materials and methods

Candida strains and growth conditions

All strains were obtained from the culture collection of the Institute of Dental Research, Sydney, Australia. *C. albicans* NCYC 1473 (GRI 682) was originally isolated from a cervical smear, *C. albicans* NCYC 1467 (GDH 2346) was isolated from the mouth of a patient with denture stomatitis, *C. albicans* Y5499 and *C. albicans* (formerly *stellatoidia*) Y523, *C. tropicalis* Y9, *C. parapsilosis* Y316, *C. krusei* Y301, *C. kefyr* Y83 and *C. guilliermondii* Y324 were all isolated from the saliva of patients attending the United Dental Hospital of Sydney. All strains were grown for 24 h in Sabouraud's broth (containing Neopeptone 10 g/L, glucose 40 g/L; pH 5.6), cells were harvested by centrifugation (10 min, 3000 g), washed three times in phosphate-buffered saline (PBS; NaCl 8.0 g/L, KCl 0.2 g/L, Na₂HPO₄ 1.15 g/L, KH₂PO₄ 0.2 g/L, pH 7.4) and finally resuspended in PBS to an OD₆₀₀ of 1.0 (1.5×10^6 cfu/ml), unless otherwise stated.

Interactions of *Candida* cells with platelets

Two methods were used to assess the interaction of *Candida* cells with platelets. Firstly, the ability of *Candida* spp. to aggregate platelets was measured and secondly the ability of platelet-rich plasma (PRP) to kill *Candida* spp. was determined. PRP was obtained from whole citrated blood by centrifugation for 10 min at 250 g and removal of the supernate. Platelet-poor plasma (PPP) was obtained by recentrifugation of the pellet from the PRP for 10 min at 3000 g and removal of the supernate.

For examination of the ability of *Candida* spp. to aggregate platelets, *Candida* cells were mixed with platelets (2.5×10^8 platelets/ml) in differing ratios of *Candida* cells to platelets, ranging from 1:1000 to 1:10, by concentration of the *Candida* cells by centrifugation, or dilution of the cells in PBS. In either a single-channel recording platelet aggregometer (Model 430; Chrono-log Corporation, Havertown, PA, USA) or a dual-channel aggregometer (Model 540), *Candida* cells and platelets were mixed and allowed to interact at 37°C for 25 min. Aggregation was recorded as a drop in the OD. Adenosine diphosphate (ADP), a natural agonist, was added after incubation for 22 min to measure platelet function and also the degree of aggregation caused by *Candida* spp.

Potential inhibitors (Sigma Aldrich, Sydney, Australia) were dissolved in PBS and added to plasma to the following final concentrations: 40 mM disodium ethylene-diamine-tetra-acetic acid (EDTA), apyrase (grade V) 10 mg/mL, 1 mM quinacrine or 2 mM imipramine. The inhibitors (25 µl) were added to both PPP and PRP, incubated at ambient temperature for 10 min and then incubated at 37°C for 5 min before the addition of *Candida* cells. Platelet aggregation was then

measured. The control consisted of 25 µl of PBS. An examination of the ability of platelets obtained from different blood group subjects was also performed, with subjects who were A positive, A negative, B positive, O negative, O positive and AB negative.

For examination of the ability of platelets to kill *Candida* spp., PRP and *Candida* cells were mixed in the optimal ratio for platelet aggregation and incubated at 37°C for 4 h with end-over-end rotation. At time 0 and after 30 min, 1 h and 4 h, samples of the mixture were removed and a Miles and Misra plate assay [15] was performed to determine the number of viable *Candida* cfu. In addition, the numbers of yeast cells in clumps of yeast plus platelets was examined microscopically and this number was taken into account when the numbers of viable cfu were calculated in the Miles and Misra assay.

Interaction of *Candida* spp. with complement

Cells were allowed to interact with complement in two ways. Firstly, the ability of complement in serum to kill *Candida* cells was measured and secondly the ability of *Candida* cells to bind to complement component C3 was examined.

For determination of complement susceptibility, *Candida* cells were resuspended to an OD₆₀₀ of 1.0 in Dulbecco's phosphate-buffered saline (CSL, Sydney, Australia) and then mixed with pooled normal human serum (obtained from four healthy donors) to give a final concentration of 50% serum. The mixtures were allowed to interact together at 35°C for 4 h with end-over-end rotation; samples were removed at times 0, 30 min, 1 h and 4 h after incubation. A Miles and Misra plate assay was then performed to determine the number of viable *Candida* cfu.

For examination of the binding and activation of complement C3 by *Candida* strains, the method of Gordon *et al.* [16] was used. Briefly, *Candida* cells were opsonised in serum 50% for 1 h at 35°C, then washed three times in PBS containing sodium dodecylsulphate (SDS) 1% w/v. Pellets were then resuspended in a solution of 1 M hydroxylamine/SDS 1% and incubated for 1 h at 37°C. The *Candida* cells were then removed by centrifugation and the supernate containing C3 fragments was collected and stored at -70°C. Before separation of C3 fragments by SDS-PAGE, C3 fragments were reduced by boiling with 10 mM dithiothreitol for 10 min. SDS-PAGE was then performed with pre-poured 4–20% gradient gels (BioRad, Sydney, Australia). The separated proteins were then transferred to nitrocellulose membranes by Western blotting in Towbin's buffer. The membrane was blocked for 2 h in bovine serum albumin (Sigma Aldrich) 1% w/v in PBS containing Tween 20 0.05% v/v. Subsequently, a 1 in 100 dilution of sheep

antiserum to human C3c (The Binding Site; Birmingham) in PBS containing Tween 20 0.05% (PBST) was added to the membrane and incubated at ambient temperature for 4 h. After washing three times in PBST, peroxidase-conjugated sheep antiserum to rabbit IgG (Sigma Aldrich; 1 in 500 dilution) was added and incubated for 1 h at ambient temperature. The nitrocellulose was then washed three times in PBS and developed with chloronaphthol (Sigma Aldrich) 12 mg in methanol 25% v/v and H₂O₂ 0.001% v/v in PBS.

Survival of Candida spp. in the presence of PMNLs

Whole blood was collected in sodium heparin-treated Vacuettes (Greiner GesmbH, Kremsmunster, Austria) and separated with mono-poly resolving medium according to the manufacturer's instructions (ICN Biomedicals, Sydney, Australia). Briefly, 7 ml of blood was layered on 6 ml of mono-poly resolving medium and centrifuged at 400 *g* for 45 min. Red blood cells were centrifuged through the mono-poly resolving medium, leaving monocytes and PMNLs in two distinct bands; PMNLs were the pipetted off with a sterile disposable Pasteur pipette. Purified PMNLs were washed three times by centrifugation at 250 *g* in PBS with heat inactivated fetal calf serum (CSL, Sydney, Australia) 10% v/v. The PMNLs were then resuspended in DPBS plus glucose 0.1% w/v (DPBSG), counted on an automated cell counter (Sysmex NE-8000, Sysmex, Kobe, Japan) and adjusted to 2×10^6 PMNLs/ml. The purity of the preparation was checked and used when >90% of white blood cells were PMNLs. After PMNLs had been equilibrated for 1 h at 4°C, they were mixed with the *Candida* suspension at a ratio of 1 *Candida* cell to every 10 PMNL. The mixture was incubated for 4 h at 37°C with end-over-end rotation and samples were removed at time 0, and after incubation for 1 h and 4 h. A Miles and Misra plate assay was then performed to determine the number of viable *Candida* cfu. In addition, the numbers of yeast cells in clumps of yeast plus PMNLs was examined microscopically and this number was taken into account when the number of cfu, were calculated in the Miles and Misra assay.

Release of inflammatory mediators

The supernate from the interactions of *Candida* cells with either PMNLs or platelets were assayed for interleukin-1 β (IL-1 β), IL-8 and leucotriene β_4 (LTB₄) with commercially available enzyme-linked immunosorbent assay (ELISA) kits obtained from R&D systems, MN, USA (IL-1 β , IL-8) and Perspective diagnostics, MA, USA (LTB₄). Samples were tested for IL-1 β and IL-8 by an antibody sandwich principle. LTB₄ was measured by competition inhibition ELISA. The methods for sample preparation and analysis followed protocols supplied by the manufacturers. Accurate sample concentration was calculated from the linear correlation made with standard antigen concentration *versus* absorbency. All strains were incubated for 30 min and 4 h with PMNLs at a ratio of 10 PMNLs to 1 *Candida* cell or platelets at a ratio of 1 *Candida* cell to 80 platelets and examined for the release of IL-8 and LTB₄. The release of IL-1 β was determined only after incubation of *C. albicans* NCYC 1467, *C. albicans* (stellatoidia) Y523, *C. kefyr* Y83 and *C. guilliermondii* Y324 with PMNLs for 4 h. Controls were PMNLs or platelets incubated without the addition of the yeast cells.

Statistical analysis

Correlations between data sets were analysed by Pearson's correlation coefficient.

Results

Interactions of Candida spp. with platelets

All strains, with the exception of *C. albicans* NCYC 1473, *C. albicans* NCYC 1467 and *C. albicans* Y5499, were able to aggregate platelets (Table 1); *C. tropicalis* Y9 aggregated platelets within the shortest incubation time. A *Candida* cell: platelet ratio of < 1:80 showed either no aggregation, a lag phase of > 25 min, or < 100% aggregation when compared to ADP. At a *Candida* cell: platelet ratio of 1:80 strains showed minimal lag phase and final percentage aggregation approaching 100%. For the higher ratios, the final percentage aggregations were > 100% indicating that both aggregation and agglutination of platelets was

Table 1. Aggregation of human platelets by *Candida* spp.

Species and strain no.	Mean (SD) lag phase (min)*	Mean (SD) time to maximum aggregation (min)	Mean (SD) aggregation† (%)
<i>C. albicans</i> (stellatoidia) Y523	8.0 (3.0)	21.5 (5.8)	83 (15)
<i>C. tropicalis</i> Y9	6.5 (1.0)	19.7 (2.2)	100 (0)
<i>C. parapsilosis</i> Y316	10.4 (1.8)	16.1 (3.8)	97 (5)
<i>C. krusei</i> Y301	15.7 (2.6)	21.8 (4.1)	100 (0)
<i>C. kefyr</i> Y83	7.7 (7.0)	19.4 (4.9)	91 (15)
<i>C. guilliermondii</i> Y324	14.0 (6.8)	18.2 (7.6)	100 (0)

*Lag phase was the time for the trace to vary from baseline after addition of the *Candida* cells.

†Percentage aggregation was determined by using the aggregation given by ADP as 100%.

occurring. Thus the optimum *Candida* cell: platelet ratio for those strains showing aggregation was 1:80. Fig. 1 demonstrates the aggregation profile of platelets in the presence of *C. tropicalis* Y9.

To test for mechanisms of platelet aggregation by *Candida* spp., several different inhibitors were used with *C. tropicalis* (Table 2). EDTA and imipramine were particularly potent inhibitors of aggregation, and quinacrine and apyrase were moderately antagonistic, reducing the final percentage aggregation to 60% and 35%, respectively. The use of blood from different blood group donors did not affect the ability of *Candida* spp. to aggregate platelets, with *Candida* cells aggregating platelets from all blood groups.

Two strains, *C. albicans* (*stellatoida*) Y523 and *C. tropicalis* Y9 were killed in the presence of platelets, losing between 40 and 55% viability (Table 3, Fig. 2). *C. parapsilosis* Y316, *C. krusei* Y301 and *C. kefyr* Y83 were not killed in the PRP, but could not grow in

Table 2. The effect of platelet aggregation inhibitors on aggregation of human platelets by *C. tropicalis* Y9

Inhibitor	Lag phase (min)*	Aggregation (%)†
Control	9.7	100
EDTA	> 25	0
Imipramine	> 25	0
Quinacrine	13.0	60
Apyrase	10.0	35

*Lag phase was the time for the trace to vary from baseline after addition of the *Candida*.

†Percentage aggregation was determined by using the aggregation given by *C. tropicalis* control (with no inhibitor present) as 100%.

Fig. 1. The aggregation of platelets in the presence of *C. tropicalis* Y9.

this medium. All *C. albicans* strains produced hyphae in PRP. Fig. 2 demonstrates the effect of PRP, complement and PMNLs on two strains, *C. albicans* NCYC 1473 and *C. tropicalis* Y9. There was a negative correlation between survival in serum (complement) and survival in PRP ($p = 0.047$).

Interactions of *Candida* spp. with complement

All *Candida* spp. were resistant to the action of complement in serum, and most strains were able to grow after incubation for 4 h (Table 3). However, *C. albicans* NCYC 1473, NCYC 1467 and Y5499 and *C. kefyr* Y83 did not grow in the serum, nor were these strains killed in serum, indicating that these strains were resistant to complement attack but unable to grow in serum. *C. albicans* NCYC 1473, NCYC 1467 and Y5499 produced hyphae when incubated in serum.

Fig. 3 demonstrates that all *Candida* strains bound and activated complement component C3. Several C3 activation fragments were released from the surface of the strains. Fragments of 195 kDa corresponded to the C3c fragment of C3; fragments of 105 kDa corresponded to C3b; fragments 75 kDa corresponded to the common β chain of iC3b and C3b; fragments of 67 kDa and 40 kDa corresponded to iC3b; fragments of 33 kDa corresponded to C3d.

Survival of *Candida* spp. in the presence of PMNLs

Three strains were killed in the presence of PMNLs (Table 3), while all other strains were able to survive.

Table 3. The ability of *Candida* spp. to survive in the presence of complement, platelets and whole blood

Species and strain no.	Mean (SD) survival (%) in*		
	serum	PRP	PMNLs
<i>C. albicans</i> NCYC 1473	96 ^H (4)	99 ^H (0)	105 (3)
<i>C. albicans</i> NCYC 1467	95 ^H (3)	100 ^H (2)	93 (5)
<i>C. albicans</i> Y5499	98 ^H (2)	100 ^H (3)	93 (2)
<i>C. albicans</i> (<i>stellatoidia</i>) Y535	180 (13)	40 (10)	25 (5)
<i>C. tropicalis</i> Y9	160 (17)	54 (8)	37 (10)
<i>C. parapsilosis</i> Y316	140 (26)	100 (1)	130 (5)
<i>C. guilliermondii</i> Y324	135 (3)	120 (7)	105 (12)
<i>C. krusei</i> Y301	160 (8)	100 (0)	104 (5)
<i>C. kefyr</i> Y83	104 (13)	103 (3)	22 (9)

PRP, platelet rich plasma; PMNLs, polymorphonuclear leucocytes; H, hyphal production.

*Survival: 100% = the same number of cfu/ml at T = 0 h and T = 4 h; decreasing numbers indicates cell death, increasing numbers indicates cell growth. Survival in the presence of serum is a measure of the ability of strains to evade the complement pathway.

Fig. 2. The interaction of *C. albicans* NCYC 1473 and *C. tropicalis* Y9 with platelet-rich plasma (PRP), complement and polymorphonuclear leucocytes (PMNLs). The viability of the cells was determined after incubation for up to 4 h by Miles and Misra plate assays. NCYC 1473 + complement, - · -; NCYC 1473 + PRP, - - -; NCYC 1473 + PMNL, - · - ·; Y9 + complement, —; Y9 + PRP, — — —; Y9 + PMNL, ———.

Generally, the ability of strains to survive in the presence of PMNLs mimicked their survival in platelet-rich plasma, and the correlation coefficient was statistically significant ($p = 0.048$).

Release of inflammatory mediators

Table 4 shows the production of inflammatory mediators from PMNLs or platelets by the different *Candida* strains. No strain induced the release of leukotriene B₄. All strains, with the exception of *C. parapsilosis* Y316, induced the release of IL-8 from PMNLs, usually showing an increase in the amount of

IL-8 after incubation for 4 h. Certain strains were able to induce IL-8 release from platelets. Of the strains tested, only *C. albicans* NCYC 1467 and *C. albicans* (*stellatoidia*) Y523 were able to induce the release of IL-1 β from PMNLs.

Discussion

This investigation has clearly demonstrated that *Candida* spp. are able to aggregate human platelets. While previous studies have shown that *C. albicans* can interact with platelets and platelet-fibrin clots [9,

Fig. 3. Complement peptides on the surface of *Candida* strains after incubation with serum. Lane 1, *C. albicans* NCYC 1473; 2, *C. albicans* NCYC 1467; 3, *C. parapsilosis* Y316; 4, *C. tropicalis* Y9; 5, *C. albicans* Y5499; 6, *C. kefyr* Y83.

Table 4. The induction of inflammatory mediators from PMNLs or platelets during incubation with *Candida* strains

Species and strain no.	Mean (SD) mediator concentration (pg/ml)				
	PMNLs incubated for 30 min	PMNLs incubated for 4 h		Platelets incubated for 30 min	Platelets incubated for 4 h
	IL-8	IL-8	IL-1 β	IL-8	IL-8
<i>C. albicans</i> NCYC 1473	59 (12)	0	ND	57 (31)	0
<i>C. albicans</i> NCYC 1467	129 (32)	287 (34)	60 (6)	44 (15)	0
<i>C. albicans</i> Y5499	0	517 (102)	ND	0	0
<i>C. albicans</i> (stellatoidia) Y523	124 (17)	85 (3)	55 (18)	0	0
<i>C. tropicalis</i> Y9	0	565 (194)	ND	0	76 (43)
<i>C. parapsilosis</i> Y316	0	0	ND	0	0
<i>C. krusei</i> Y301	269 (48)	291 (45)	ND	0	59 (3)
<i>C. kefyr</i> Y83	85 (2)	847 (201)	0	0	0
<i>C. guilliermondii</i> Y324	108 (51)	351 (49)	0	0	36 (32)

No strain stimulated the release of LTB₄ from PMNLs. ND, not determined.

17], it has generally been accepted that whole yeast cells are unable to aggregate platelets whereas cell fragments can [18, 19]. The present study confirms these findings, as *C. albicans* was the only species unable to aggregate platelets. This study is the first to demonstrate that other species of *Candida* are able to aggregate platelets. True platelet aggregation was occurring, as the specific inhibitors of platelet aggregation, EDTA that chelates cations, apyrase that reduces the availability of ADP, quinacrine that inhibits the cyclo-oxygenase pathway and imipramine that affects platelet membrane stability, inhibited the aggregation process. Of interest was the fact that platelets derived from different blood group donors

were equally able to be aggregated by the yeasts, indicating that blood group sugars are probably not involved in the platelet-*Candida* interactions, although they are involved in adhesion of *Candida* spp. to epithelial surfaces [19].

A recent publication focused attention on the role of complement in the aggregation of platelets by streptococci [10], with the assembly of the membrane attack complex (C5b-9) on the surface of the bacteria being hypothesised as a key to allowing platelet aggregation. The current study examined *Candida* cells for their ability to activate complement by measuring their survival in serum and the molecular

nature of the C3 deposited on the surface of the fungi. No *Candida* spp. were killed in serum, indicating that either the membrane attack complex of complement is not able to damage *Candida* cells or that *Candida* cells do not allow complement activation to proceed to this terminal stage. Complement C3 was deposited on the surface of all strains of *Candida* and similar fragments were generated. The current study did not show evidence that complement, or C3 alone, is responsible for the aggregation of platelets by *Candida* spp., but – as with the streptococci [10] – it is possible that complement together with another unidentified plasma component may mediate platelet aggregation by *Candida* spp. Such plasma components may be fibrinogen, fibronectin or vitronectin, all of which have been shown to bind to platelets [20–22] and can attach to *Candida* cells [9, 23].

There was an apparent discrepancy between the finding that *C. albicans* strains did not aggregate platelets and yet this species is the most commonly isolated *Candida* spp. from cases of endocarditis [1, 5, 6]. It has been stated that the ability of micro-organisms, in particular *S. sanguis*, to aggregate platelets is associated with a more virulent clinical disease [24], even though it is known that activation of platelets can release antimicrobial proteins [13]. These microbicidal proteins, PMPs, have been shown to be active against *C. albicans* [13]. Thus, the present study determined whether the activation of platelets by *Candida* species could induce killing of the fungal cells. The incubation of certain strains with platelets did reduce the numbers of viable cells and other strains were not able to grow in PRP, but could grow in serum, indicating the microbistatic nature of PRP. These findings were to some extent dependent on the ability of the fungi to aggregate platelets, as *C. albicans* did not aggregate platelets and was not killed but prevented from growing in platelet-rich plasma. Thus, we conclude that, for *Candida* species, platelet aggregation by *Candida* cells may allow the host to clear the fungi more readily.

Incubation of *Candida* spp. with PMNLs led to a similar pattern of survival or killing as incubation in platelet-rich plasma, indicating that similar mechanisms may be involved. The microbicidal proteins of platelets have been shown to be cationic peptides [13, 25] and the defensins of human neutrophils are also cationic proteins [12].

In the blood stream, or as part of a thrombotic vegetation, *Candida* cells will come into contact with PMNLs. It has been noted that neutropenic patients (with reduced numbers of PMNLs) are highly susceptible to the development of systemic candidosis [14] indicating the importance of PMNLs in defending the body against *Candida* spp. In the present study *C. tropicalis*, *C. albicans* (*stellatoidia*) and *C. kefyr* were killed in the presence of PMNLs and the *C. albicans*

strains were unable to grow in their presence. The activation of complement by *Candida* spp. and the production of C3 fragments on cell surfaces indicates that the chemotactic fragment C5a is probably produced by *Candida* cells in blood. This will recruit PMNLs to sites on infection. The present study also demonstrated that *Candida* cells can stimulate PMNLs to produce the inflammatory cytokines IL-8 and, to a lesser extent, IL-1 β . IL-8 is a member of the α chemokine family, a specialised protein that attracts PMNLs. This attraction leads to the accumulation of PMNLs at the site of infection and promotes the development of inflammation. IL-8 also activates PMNLs, increasing their release of tissue destructive substances such as proteases [26]. This activation of tissue destruction may contribute to the destruction of heart that occurs during endocarditis. The current study also demonstrated that interaction of *Candida* with platelets can also release IL-8. Thus, IL-8 can be produced from platelets, PMNLs and endothelial cells [8] upon stimulation with yeast cells.

In conclusion, this study has shown for the first time that non-*albicans* *Candida* spp. can aggregate platelets. Certain strains were killed in the presence of platelets, presumably by the release of platelet microbicidal proteins. Furthermore, strains were able to activate complement and activate PMNLs to produce the chemokine IL-8, increasing the recruitment of PMNLs to the site of infection and potentially contributing to tissue destruction by the activation of the PMNLs and release of proteases.

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