Platelet immunology in fungal infections

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Summary
Up to date, perception of platelets has changed from key players in coagulation to multitaskers within the immune network, connecting its most diverse elements and crucially shaping their interplay with invading pathogens such as fungi. In addition, antimicrobial effector molecules and mechanisms in platelets enable a direct inhibitory effect on fungi, thus completing their immune capacity. To precisely assess the impact of platelets on the course of invasive fungal infections is complicated by some critical parameters. First, there is a fragile balance between protective antimicrobial effects and detrimental reactions that aggravate the fungal pathogenesis. Second, some platelet effects are exerted indirectly by other immune mediators and are thus difficult to quantify. Third, drugs such as antifungotics, antibiotics, or cytostatics, are commonly administered to the patients and might modulate the interplay between platelets and fungi. Our article highlights selected aspects of the complex interactions between platelets and fungi and the relevance of these processes for the pathogenesis of fungal infections.

Keywords
Platelets, fungi, invasive infection, innate immunity

Introduction
Platelets are small anucleate cell fragments derived from megakaryocytes in the bone marrow. With a concentration of 150–400 x 10^9 per litre, they make up the most abundant solid component of human blood. This abundance implies that each pathogen is confronted with a superior number of platelets on entering the bloodstream. The key role of platelets in haemostasis and wound healing often distracts attention from their participation in a number of other processes, like tumour growth, atherosclerosis, inflammatory reactions, pathogen attack and modulation of innate and adaptive immunity (1).

Upon stimulation, platelets release a variety of biologically active molecules from their storage granules. More than 300 different proteins can be secreted after activation (2), including soluble and membrane-bound components (more detailed in [1, 3, 4]). The α-granules contain a multitude of effector molecules like growth and mitogenic factors, proteases, clotting and fibrinolytic factors, cytokines and chemokines, antimicrobial peptides, whereas serotonin, ADP and calcium are the main components in dense bodies (3).

Invading pathogens entering the blood stream are faced with the immunocompetence of platelets, which comprises direct antimicrobial effects as well as interactions with cells of the innate and adaptive immune system. Platelets sense microorganisms by various receptors, including toll-like receptors (TLRs) (5–8); the subsequent direct antimicrobial attack of activated platelets can occur by secretion of platelet microbicidal peptides (PMPs, thrombocidins and kinocidins), or generation of reactive oxygen species (ROS) (1, 4, 9, 10). Furthermore, phagocytosis of particles and pathogens is discussed for platelets ([11]; further references in [4]).

The immune functions of stimulated platelets are completed by an intense crosstalk with other immune cells by secreted (e.g. cytokines, chemokines) and membrane-bound molecules (e.g. CD154 that binds to CD40 on endothelial cells, neutrophils, monocytes, DCs, B cells and T cells) (1, 4, 12–17). This crosstalk includes attraction and activation of neutrophils and monocytes, stimulation of monocytes differentiation to macrophages and dendritic cells (DCs), induction of DC maturation and triggering the secretion of proinflammatory cytokines from macrophages. Platelets also build bridges to adaptive immunity by direct and indirect interplay with B cells (inducing proliferation, differentiation, memory cell generation) and T cells (differentiation, activation, cytokine production). More detailed facts about platelets in immune and inflammatory processes are given in (1, 3, 4, 12–18–22). In the following, we describe how platelets apply these antimicrobial functions to the situation of invasive fungal infections. The different beneficial and detrimental consequences of platelet-associated reactions are highlighted as well as the influence of some additional players, such as the complement system and drugs (antifungotics, antimycotics, cytostatics).

Invasive fungal infections
Invasive fungal infections (IFIs) represent a major threat, particularly to immunocompromised patients. Haematological and transplant patients are primarily affected, but also individuals admitted...
to intensive care units (ICU) are at risk, due to complex surgical procedures, invasive medical devices, and prescription of long-term, broad-spectrum antibiotics. Both yeasts and moulds significantly contribute to morbidity and mortality of affected patients and provoke longer hospital stays and increased health care costs. Aspergillus, Candida, and Cryptococcus are the most prevalent causative species with varying distribution regarding geography, hospital units and underlying patient condition. The mucormycetes (formerly called zygomycetes) represent a further mould group with ascending incidence (23, 24).

The poor clinical outcome despite all recent advances in diagnosis and antifungal therapy draws the attention to the elucidation of IFI immunology. In the following, the knowledge about the relevance of platelets as immune cells is summarized for different fungal pathogens.

Interplay between platelets and fungi: at different sites and with different fungal molecules

Platelets are believed to interact with invading fungi at different stages of the infection and also at different sites of the body.

In most invasive mould infections, the pathogens enter the body as spores via the respiratory tract. In the lung, the fungi may come for the first time in contact with platelets, since bleeding and haemoptysis are rather common symptoms of IFI (25–27). In further course of infection, the moulds can penetrate the endothelial lining of the blood vessels of the lung; hyphal fragments might break away and circulate in the bloodstream (28), giving rise to further dissemination or even fungal sepsis.

Transmission of fungal pathogens can also start independently from the lung. Candida, that causes the majority of IFI and fungal sepsis, mostly starts the infection endogenously from colonisation of gastrointestinal, oral or vaginal mucosa; otherwise disturbed physical integrity of the skin barrier (e.g. by intravascular access devices, wounds, surgery) allows infection and dissemination of the pathogen into the systemic circulation (29, 30).

Regardless of the precise way of infection, the bloodstream represents the main site of contact and mutual affectation between fungi and platelets. Sequestration of IFI in various organs affords another opportunity of interaction between fungi and platelets, when bleeding into the affected organs occurs (31).

The overall image of platelet-fungus interaction not only includes different body sites where the contact can occur, but also different fungal elements and products. Platelets can interact with the fungal cell surface, with circulating cell wall fragments, as well as with metabolites and other secreted fungal compounds (32–34).

Platelets recognise the surface of yeast or hyphal cells, and (e.g. in the lung) of spores. Examples for this direct interaction are given in Figure 1, showing the binding of platelets to fungal hyphae (panel A) and to conidia (panel B). Furthermore, fungal cell wall components such as 1,3-β-glucan or galactomannan are released into the environment during growth or tissue invasion (35, 36). Circulating in the blood stream, they might bind to as yet unknown receptors on the platelets and thus affect their activation status; putative receptor candidates might be TLRs (37) or protease-activated receptors (PARs). In addition, each fungal species secretes, dependent on the growth conditions, various compounds (32–34) that might interact with platelets (4). Proteases are of special interest within this fungal secretome, since platelets express protease-activated receptors (PAR), which react on proteolytic cleavage with induction of a signalling cascade. Mycotoxins, secondary fungal metabolites, are also known to change the biological activity of platelets (4, 38).

Antifungal platelet activities and modulating factors

The contact with fungal molecules, either surface-bound or secreted, can stimulate the platelets to exert a variety of antifungal

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Figure 1: Thrombocytes interact with fungal hyphae and conidia. Platelet-rich plasma was incubated with hyphae (A) or conidia (B) of A. fumigatus; interaction was visualised with a field-emission scanning electron microscope (photos by K. Pfaller).
Infections and the role of plasma proteins and platelets activities (Figure 2). A direct fungistatic or fungicidal effect might be mediated by the release of PMPs, but other molecules in the granules such as serotonin (39) may have a similar impact. Platelets also act synergistically with antimycotic drugs, making the fungi more vulnerable for their action. Other mediators contributing to the antifungal activity of platelets include the complement system and phagocytes. The complement cascade is triggered by activated platelets and can result in opsonisation of the pathogens, thus facilitating their phagocytic clearance (see below). Platelets further support the elimination of invading pathogens by chemotactically attracting, directly or via complement products, the phagocytes and by activating them. A more detailed discussion is given below for the different fungi.

The putative consequences of the platelet–fungus interaction for the affected patient are multifaceted (Figure 3). The beneficial outcome might be a decrease of fungal load due to the antimicrobial activity of the platelets as well as the support of adaptive and innate immunity. This effect might significantly help to reduce morbidity and mortality in infected patients. However, side effects might accompany this big advantage of platelet activity. Tissue damage might be induced if the triggered inflammatory reaction is exaggerated or when the stimulated platelets aggregate and form thrombi in the course of the fungal infection. Since activated platelets are cleared from the bloodstream, platelet loss and thrombocytopenia might be further harmful consequences of their antimicrobial activity. Analogous to the situation in viral infections, it can even be hypothesised that platelets might endocytose spores or small hyphal fragments and transport them throughout the body. This protection of the pathogen from immune attack might contribute to dissemination ([40, 41]; reviewed in [4]).

Not only the divergence of positive outcome and negative side-effects makes it difficult to weigh the putative relevance of platelet activity for the patient, but also the spectrum of factors that modulate the interplay between platelets and fungi (Figure 4). Activated platelets themselves are also opsonised by complement and thus a target for phagocytic elimination. This feedback mechanism protects the body, but also limits the time span for the antifungal activity of platelets. Fungi have also evolved mechanisms to restrict platelets attack: metabolites inhibit the activation process of platelets, and fungal surface molecules can help to evade this cell type. In addition, drugs that are frequently prescribed to patients with risk for IFIs further modify the extent of platelet activity. Some antibiotics contribute to platelet elimination by triggering drug-induced immune thrombocytopenia (DIT), and cytostatic drugs generally impair production and maturation of platelets in the bone marrow. These effects are discussed below in detail.

Platelets and Aspergillus

Aspergillus is a ubiquitous mould in the environment; after inhalation the small conidia can germinate in the lungs of immunosuppressed individuals. A. fumigatus is the predominant human pathogenic species to induce invasive aspergillosis (IA), an infection which presents as a disease with high lethality and elevated treatment costs. The rate of IA has steadily risen within the last decades due to medical progress and thus higher numbers of immunocompromised patients (42, 43).

Platelets attach to Aspergillus fumigatus conidia and hyphae, and this interaction is associated with a strong activation of the
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Platelets, as seen by increased exposure of CD62P (marker for α-granule release) and CD63 (marker for dense granule release) and by secretion of pro-inflammatory molecules (44, 45). Laser microscopy visualised this activation, showing that the platelets aggregated around the *Aspergillus* conidia and covered their surface (46) (see also ▶Figure 1). Platelet activation in IA might also be triggered by soluble fungal compounds. During growth, *Aspergillus fumigatus* secretes factors, e.g. proteases and mycotoxins, which induce granule release, aggregation, and membrane conversion (47).

Aspergillus-driven platelet activation results in fungal damage, as visible by loss of wall integrity and decreased viability (44, 46). The relevance of this effect for infected patients can be proven statistically: a low baseline platelet count is a highly significant predictor for bad IA outcome and allows the stratification of patients into risk categories (48).

However, as mentioned above, fungus-induced platelet activation might also harbour negative effects with excessive inflammation and thrombosis. A strong hint comes from a comparative study between two *A. terreus* isolates; the isolate that triggered platelet activation more efficiently, induced faster death of infected animals (49).

**Platelets and Candida**

The yeast *Candida* (C.) *spp* is a frequent part of the oral, vaginal, and gastrointestinal flora and most *Candida* infections are endogenous. Perturbation of skin or mucosa integrity (intravascular access devices, wounds), disturbed host defence and antibiotic treatment are main risk factors for infection and dissemination. *C. albicans* accounts for about half of invasive candidiasis cases, but the non-albicans species (*C. glabrata, parapsilosis, krusei and tropicalis*) increase in frequency (23, 50).

Former studies proved attachment of *C. albicans* yeast cells and germ tubes to aggregated platelets, whereas other species (*C. tropicalis, parapsilosis, krusei*) adhered to a much lesser extent (51, 52). The adherence was confirmed *in vivo*; blood samples from *Candida*-infected mice revealed that virtually all yeast cells were bound to platelets (53). Interestingly, no difference between *albicans* and the non- *albicans* species was detected in these animal experiments.

The consequences of *Candida* – platelet interaction are reported somewhat contradictory. Willcox et al. demonstrated that platelets react on the contact with *Candida* cells with activation and aggregation (54). Confirmation comes from studies in patients with *C. albicans* sepsis, where increased levels of microparticles derived from activated platelets were detected in the blood (55). In contrast, Carvalho-Neiva et al. showed that *C. albicans* cells were unable to aggregate platelets; instead, preincubation of platelets with the yeast even inhibited subsequent stimulation by collagen or ADP (56). Similarly, Bertling et al. found that *C. albicans* cells inhibit platelet aggregation and fibrinogen binding (38).

Secreted compounds might have additional effects on the platelets, although these results are contradictory, too. While Bertling et al. demonstrated that gliotoxin, a metabolite secreted by *C. albicans*, inhibits fibrinogen binding of platelets and aggregation (38), Carvalho reported that culture supernatants from *C. albicans* had no inhibitory effect on platelet aggregation (56). Own experiments even indicated an activation of platelets by gliotoxin (47).

The *Candida*-induced modification of platelets retroactively affects the viability of the yeast cells. Platelet-rich plasma has antimicrobial effectiveness against *Candida* and inhibits its growth (54, 57). Detailed analyses allowed the identification of three antimicrobial peptides secreted by platelets and harbouring antifungal activity: the chemokine RANTES, platelet factor-4 (PF-4) and thrombocidin-1 (TC-1) (58, 59).
Platelets and other fungi

Mucormycetes

Mucormycosis is caused by the ubiquitous filamentous mucormycetes, formerly called zygomycetes; it has emerged as a common mycosis in patients with immunosuppression, iron overload, or diabetes mellitus (60). The high tendency of angioinvasion implies that contact with platelets occurs particularly frequent.

Platelets adhere to hyphae and spores of different mucormycetes species, such as *Rhizomucor*, *Mucor*, *Lichtheimia* and *Rhizopus*. This adherence runs parallel to exposure of the activation marker CD62P on the platelet surface (61). Platelet adhesion and activation result in hyphal damage and suppression of spore germination, indicating a critical role of this cell type also in mucormycosis (61). However, the frequently reported thromboses (62) indicate the detrimental side effects for the beneficial platelet-derived antimicrobial capacity.

Cryptococcus

The encapsulated yeast *Cryptococcus* induces severe infections mainly in HIV-infected individuals. The lung is most commonly affected, but meningitis is also a frequent manifestation. Dissemination from lung to brain implies contact with platelets and a putative role of these cell fragments in the outcome of infection.

Similar to the results with *Candida*, Carvalho-Neiva et al. found *Cryptococcus* to inhibit platelet activation (56). More detailed analyses revealed differences in the platelet interaction between capsular and non-encapsulated cryptococcal strains. Platelets exclusively attached to non-encapsulated isolates, which were also the only ones to be susceptible to antifungal activity of the platelet microbicidal peptide tPMP (63). Further platelet-derived proteins that act fungicidal against *Cryptococcus* are thrombocidins, RANTES, PF-4 and the fibrinopeptins (9, 59).

Single reports also imply that platelets might play a role in other fungal infections, e.g. by *Fusarium*, *Pneumocystis* or *Scedosporium*, however, analyses for these pathogens are yet too fragmentary.

Additional players in the interplay platelets – fungi

A variety of additional factors influence and modify the interplay between platelets and fungi, including cellular (e.g. neutrophils, monocytes/macrophages) and non-cellular (e.g. complement, antimycotics, antibiotics, cytostatics) ones. These factors can have dual effects: on one hand they can enhance the antimicrobial activity of platelets or contribute to their antifungal effect; on the other side they might interfere with the platelet-associated immune functions (see ▶ Figure 3 and ▶ Figure 4). Due to limited space only some selected players are discussed here.

Complement

The complement system is a potent element of the soluble innate immunity. Being activated by three different pathways, the complement cascade can fulfil various functions (reviewed in [64–66]): (i) complement proteins can form pores in the membrane of pathogens or cells with subsequent lysis; (ii) complement factors opsonise pathogens or cells to target them for phagocytic clearance; (iii) complement-derived anaphylatoxins (C3a, C5a) attract phagocytes to the site of infection; (iv) complement activates lymphocytes, thus guaranteeing effective adaptive immunity.

![Figure 4: Factors interfering with platelet-associated antifungal functionality. AB: antibiotics, CS: cytostatics, DIT: drug-induced immune thrombocytopenia.](image-url)
In the course of IFI, complement can be hypothesized to modulate the platelet-fungus interaction in several respects, which are described in the following. Platelets that have been activated by fungi might initiate the complement cascade and thus become a target for complement-induced lysis or phagocytic clearance. Indications for that come from reports that platelets can trigger the complement cascade by different mechanisms. CD62P on the surface of activated platelets can start the alternative complement pathway (67), and the classical pathway might be induced by binding of its starter molecule C1q to receptors on platelets (68). Furthermore, chondroitin sulphate that is released from platelet granules can subsequently bind to the surface of activated platelets and interact with complement proteins (69, 70). Complement activation can trigger lysis, but also opsonisation of the platelet surface. As a consequence, phagocytes bearing corresponding receptors (e.g. CR3) can bind and eliminate the oposonised platelets. A complement-dependent loss of activated platelets by lysis or phagocytic clearance was shown for diseases such as immune thrombocytopenic purpura (ITP), where complement deposition on platelets correlates with the degree of thrombocytopenia (71).

Alternatively, fungus-induced platelet activation and subsequent opsonisation might support formation of thrombosis, a hallmark of IFI pathogenesis. A correlation of complement deposition on platelets with appearance of arterial thrombosis was demonstrated for patients with systemic lupus erythematoses (72, 73). Some putative mechanisms how complement participates in thrombus formation are described in literature. First, endothelial cells might interact directly with oposonised platelets, since they express the complement receptors CR1 (CD35) and CD4 (CD11c/CD18) (74). Second, C5a up-regulates the expression of ICAM-1 on endothelial cells, thus enhancing their stickiness for platelets and phagocytes that express the counter-receptor complement receptor 3 (CR3) (75). C5a also upregulates tissue factor expression on endothelial cells, a membrane protein that serves as cofactor for blood coagulation and thrombus formation (76, 77). Third, complement activation on platelets results in formation of C5b-9, which stimulates procoagulant activity (78). Fourth, platelets expressing complement receptors (4) can bind to oposonised platelets, thus further increasing the formation of thrombi (79). It is intriguing to speculate that these mechanisms also contribute to thrombosis in IFI patients.

Antimycotic drugs

Antimycotic drugs in therapy and prophylaxis can also influence the interplay between platelets and fungi.

Fluconazole treatment significantly diminishes Candida adherence to platelets; the presence of PMPs further enhances this effect (80). Consequently, dissemination of the yeast and its binding to the endothelium via aggregated platelets might be reduced. On the other hand, this process might interfere with platelets’ anti-candidal function (trapping of Candida cells, presenting them to phagocytes, release of antimicrobial peptides). A rabbit model of Candida-induced endocarditis evaluated these two aspects and revealed that fluconazole and PMPs collaborate to support fungal clearance in the infected animals (81). In vitro studies confirmed this positive effect, showing increased fluconazole efficacy when Candida isolates are susceptible to PMPs (81).

Besides fluconazole, other antimycotic drugs have also been shown to interact with platelets, supporting their action and being supported by them in the antifungal effect. The combination of platelets and anidulafungin significantly reduced the germination rate of A. fumigatus conidia and hyphal elongation (82). Furthermore, platelets and amphoterin B or posaconazole had a more prominent effect on A. fumigatus germination than the antimycotic or the platelets alone (83). A similar effect could be demonstrated for some Aspergillus species other than A. fumigatus (84). Platelet-derived serotonin was also able to enhance the amphoterin activity against A. fumigatus (85).

Own unpublished results highlight another aspect of the interplay between antimycotic drugs and platelets. “Drugs can not only increase the susceptibility of the fungus for the antimicrobial action of the platelets and vice versa, but also induce platelet activation and thus support their antifungal activity, as shown for the echinocandins caspofungin and anidulafungin (Speth et al., manuscript in preparation).

Other drugs (cytostatics, antibiotics)

Most immunosuppressed patients at risk of fungal infections also receive other drugs, particularly antibiotics and cytostatics; at least some of them may modify the relation between platelets and fungi. For that reason, some selected aspects are discussed in the following.

Various antibiotics and cytostatics can induce platelet loss with consequent risk of bleeding, but also a loss of platelet-associated antifungal activity. A DIT can result either from decreased platelet production by inhibition of haematopoiesis in the bone marrow or from increased platelet destruction by an immune-mediated mechanism. Linezolid, an oxazolidinone antibiotic that inhibits the bacterial protein synthesis, suppresses the bone marrow with subsequent thrombocytopenia (86). The same myelosuppressive effect is induced by chemotherapeutic drugs used for patients with malignancies (87). In contrast, the beta-lactam antibiotic penicillin induces DIT by increasing platelet destruction: although not immunogenic itself, penicillin can form a linkage with platelet proteins and thus induce the formation of antibodies that recognize the platelet protein and trigger platelet removal in the spleen and the liver by cells of the mononuclear phagocyte system (88).

Not only the number, but also the functions of platelets may be affected by drugs. For example, beta-lactam antibiotics were shown to exert inhibitory effects on platelet activation (89, 90).

Summary and outlook

Understanding the interaction of platelets with disseminating fungal pathogens might provide important insights in the pathogenesis of IFIs. Pivotal aspects, like attraction of immune cells, complement activation, and release of microbial proteins, have al-
ready been clarified and will surely be complemented in the near future. A central principle is the bivalence of the outcome of platelet-associated reactions: beneficial antifungal effects with decrease of disease burden are faced with harmful consequences such as excessive inflammation, thrombosis and thrombocytopenia. Additional valuable insights in the role of platelets in IFIs might, in the far future, provide the prerequisites for the development of novel platelet-based adjunctive therapies.

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Conflicts of interest
None declared.

References


