The role of platelets in defence against pathogens

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Summary
Many more platelets are present in healthy mammals than are necessary for routine haemostasis. Thus, they could have other functions. Platelets have many of the attributes of innate immune function including Toll-like receptors. They also contain a wide range of anti-microbial peptides in storage granules. Platelets play an important role in bacterial infections, both in disease progress and in defence mechanisms depending on circumstances. Similar mechanisms are used in defence against fungi. Platelets are also involved in viral diseases, either in protecting from the immune system or in killing viruses that activate platelets. Finally, platelets have a role in defence against parasitic diseases, in particular malaria, that should not be ignored, and may aggravate some of the worst aspects. Platelets also have receptors for IgE and are implicated via parasitic disorders in development and problems of allergy.

During their evolution from haemocytes to platelets these functions mainly became separated to leukocytes and erythrocytes, which specialize in immunology and oxygen transport, respectively. There is more and more evidence that platelets still carry additional residual functions from this common haemocyte origin. Platelets are well known to have a critical role in

- haemostasis,
- prevention of bleeding after injury,
- maintenance of the vascular system.

In addition, they have a major role in pathological conditions such as thrombosis, including cardiac infarction and stroke. Although the normal blood platelet count lies between 150000 and 350000 platelets/μl, only a small part of these (<10000 platelets/μl) are actually needed to prevent bleeding under normal conditions, although severe trauma or major surgery may lead to more consumption.

This suggests that the large numbers of platelets in the circulation have other, additional functions.

It remains unclear what the evolutionary advantages of small, anuclear, discoid platelets are, versus larger nucleated thrombocytes but one possibility is that this parallel change was required to allow placental development in order to handle the increase in haemostatic challenges during birth and menstruation.

Detailed analysis of platelet receptors has revealed a wide range of newly discovered molecules including several that are important in immunology, particularly innate immunity.

In addition, both in patients and animal models, there is considerable evidence that thrombocytopenia (platelets levels <150000 but often much lower) con-

In evolution, platelets first appear along with monotremes, marsupials and mammals. In vertebrates, such as fish, reptiles, amphibians and birds, and probably even dinosaurs, the nucleated thrombocyte carries out platelet functions, whereas in invertebrates the haemocyte incorporates these functions as well as oxygen transport and immunological defence. The haemocyte

- resembles the macrophage,
- expresses toll-like receptors (TLR),
- secretes anti-microbial peptides and
- carries out phagocytosis.

Hämostaseologie 4/2011
tributes to the severity of diseases caused by pathogens, which also supports a role for platelets in immune defence against these organisms. Some authors have argued for an important role for reactive oxygen species in platelet defenses against pathogens, while others have provided evidence for a strong anti-pathogen effect due to the major participation of the activated platelet in thrombin generation (25).

The last few years have seen increasing evidence for an overall role of platelets in innate immunity (17). Not only do platelets carry critical receptors for the recognition of bacteria, fungi, viruses, and parasites, but there is increasing evidence that they do indeed have important roles in defence against these pathogens. In addition, platelets interact with many of the major immune cells, including monocytes, dendritic cells and T-lymphocytes and platelet storage granules also contain a wide variety of immunologically active substances such as cytokines, chemokines, and related defensins. They themselves also have chemokine receptors, allowing their activation by chemokines released from other platelets or immune cells (5).

CD154 (CD40L) is an important platelet component released on activation that influences the adaptive immune system and interactions with lymphocytes. Recent studies utilizing CD154 knockout mice extended the role of platelet-derived CD154 to the modulation of adaptive immune response by enhancing antigen presentation, improving CD86 T cell responses, and playing a critical function in T-dependent humoral immunity under physiological conditions (19).

For a long time much of the interest in platelets and pathogens was due to possible risk of contamination of products used for transfusion and ways of protection against this. This became even more acute when HIV became a major health problem in the 1980s and much effort has gone into avoiding or eliminating these dangers.

In the past few decades there has been a lot more interest in the role of platelets in pathogen-induced diseases both in their propagation and in defence against them. A further development has been the use of autologous platelet gel to treat persistant, non-healing wounds.

**Platelets and bacteria**

Bacteria are among the many pathogens that interact with platelets, both influenced by platelets and also affecting platelets. Kerrigan and Cox (11) have reviewed platelet-bacterial interactions and their role in various diseases, particularly those of the cardiovascular system. Several studies have shown that proteins secreted by or expressed on the surface of specific bacteria can bind to platelets and in some cases activate them. A wide range of different activities have been shown, and it seems likely that interactions such as these have a critical role in bacterial growth and aggression within the cardiovascular system, such as in colonization and destruction of heart valves. It was suggested many years ago that the early decrease in cardiovascular diseases, observed in the USA from the 1950s on, and long before any decrease in smoking, coincided with the onset of advanced dental hygiene and the systematic removal of dental plaque – a major source of bacteria by trained hygienists.

This link between dental hygiene and circulatory infections has been strengthened by further research, but gum disease remains seriously underestimated as a preventable cause of cardiovascular disease. Platelets have an important role in helping neutrophils to phagocytose periodontopathogens efficiently (1). In some cases bacteria seem able to subvert platelet responses for their own ends. Platelets activated by bacteria are able to activate endothelial cells and thus exacerbate sepsis.

The role of platelets in defence against bacteria and the mechanisms they use to achieve this have been reviewed by Yeaman (24). As well as using innate immunity mechanisms, platelets secrete a large number of granule components that affect bacterial viability. Most of these are cationic, which plays an important role in their binding to and disrupting bacterial membranes. Anti-bacterial peptides, including those from platelets, have been extensively reviewed by Wiesner and Völkenskas (23).

A classic example is the chemokine family member PF4, a strongly basic protein that binds tightly to negatively charged bacteria and induces strong immunological responses. The immunological response to PF4/heparin complexes seen in heparin-induced thrombocytopenia may be an unforeseen consequence of this antibacterial mechanism when heparin and heparin-like molecules are given as anti-thrombotic under conditions that can be considered non-physiological. Additional platelet antibacterial peptides are derived from other chemokine family members by proteolysis and are mainly highly negatively charged molecules. There is evidence that different molecules are stored in different granules, and can be specifically released, allowing platelets to respond appropriately to different pathogen challenges. While a number of molecules released from activated platelets have toxic effects on bacteria, activated platelets themselves also affect bacteria by a contact mechanism that may involve internalisation.

Among the molecules released from activated platelets are defensins and other microbiocidal peptides. These include the kinocidins and thrombocidins, members of the chemokine family, such as platelet factor 4 (PF4, CXCL4) and related structures, that have bacteriocidal characteristics (12). Other molecules synthesized by activated platelets are hydrogen peroxide and reactive oxygen species, also toxic for bacteria. However, some authors consider that these are minor mechanisms and that the role of platelets in formation of thrombin has a far greater effect on bacteria by generating a strong bactericidal complex next to activated platelet membranes (21).

A further way in which platelets contribute to anti-bacterial activity is via TLR4, by assisting neutrophils to lyse, distributing DNA in platelet–neutrophil extracellular traps (NETS), "spider's webs" that entangle and stick to bacteria (4).

**Platelets and fungi**

As with bacteria there is considerable evidence that platelets help in defence against fungi. However, the fungi that have been investigated are limited to the most common types. For example, platelets attenuate the virulence of Aspergillus spp. in vitro. Little is known, though, about the antifungal effects of platelets in the presence of antimi-

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Hämostaseologie 4/2011
Platelets contribute to inflammation and promote the thrombosis, characteristically seen in aspergillosis, and might be involved both in antifungal defense and in the histopathological process (Fig. 1).

In vitro activation of platelets by conidia, swollen conidia, and hyphae from A. fumigatus has been assessed by flow cytometry and enzyme immunoassays (16). THP-1 monocytes and human monocytes with and without platelets were cultured with hyphae from A. fumigatus, and the release of interleukin-8 (IL-8) measured by enzyme immunoassays. A. fumigatus potently induced the expression of CD62-p and CD63 and the release of CD154, RANTES, and Dickkopf homologue 1 in platelets, particularly enhancing the effects of hyphae compared with conidia. The hypha-mediated activation of platelets further enhanced the release of IL-8 both in THP-1 monocytes and in human adherent monocytes. Thus, A. fumigatus is a potent inducer of platelet-mediated inflammation, and could promote protection as well as damage during aspergillosis. Platelet-derived thrombocidins were also fungicidal for Cryptococcus neoformans (12).

**Platelets and viruses**

Viruses are an important class of pathogens. Thrombocytopenia commonly accompanies virus infections that indeed may lead to idiopathic thrombocytopenia purpura (ITP) by mechanisms that, as the name suggests, remain obscure. It has proved difficult to demonstrate a direct...
connection, but research in this area is making progress. Flaujac et al. (7) reviewed the relationship between platelets and viruses, dealing particularly with HIV where interactions with platelets can have serious consequences for public health. Increasing evidence points to platelets playing an important role in anti-viral defence by poorly understood mechanisms. As noted above, platelets are equipped with many of the receptors and immunologically active systems that are critical in immune cells properly. That these also have important functions in platelets is therefore hardly surprising. It should also be noted that severe thrombocytopenia affects many aspects of health other than simply haemostasis.

Recently, there has been increased interest in the role of platelets in dengue, a severe tropical and subtropical viral disease transmitted via a mosquito vector, and with climate warming also more and more a threat to temperate regions. Several papers have provided evidence for dengue virus interactions with platelets, implying that dengue might also be transmittable by platelet transfusions and leaving open the question whether platelets can and do kill dengue virus as part of the host defence (8).

Platelets and parasites

Throughout most of evolution, and until comparatively recently in some societies, humans have been exposed to high parasite loads, and these diseases still remain major causes of mortality in the developing world. Defences against parasites are still poorly understood, although considerable advances have been made over the last few decades.

The major parasitic disease affecting humans is malaria: Despite advances in treatment, between one and three million people die each year, many of them children.

One of the most virulent forms is cerebral malaria, which has a poor prognosis. Thus, any advances in understanding its basic pathology may be useful in devising better treatment methods. Cox and McConkey (6) have described the role of platelets in the pathogenesis of cerebral malaria. In a recent important study, purified human platelets have been shown to kill Plasmodium falciparum parasites cultured in red blood cells (13).

Platelet inhibition by aspirin and other platelet inhibitors blocked the lethal effect of human platelets exert on P. falciparum parasites. These results indicate a protective function for platelets in the early stages of erythrocytic infection distinct from their role in cerebral malaria where they assist infected red blood cells to bind within the cerebral vasculature.

However, platelets may well play a role in defense against other parasites, although this has been poorly studied. Some examples are briefly mentioned. In Lyme disease caused by Borrelia burgdorferi, the platelet count drops, implying a defensive role. The major platelet integrin αIIbβ3 is the main receptor for the spirochete (2).

Platelets have also been shown to be capable of killing trypanosomes, though less efficiently than macrophages or neutrophils (18). Evidence has been obtained for a role for platelets in innate defence against Schistosoma mansoni infections in mice. In replicate experiments, worm burdens were significantly increased in mice thrombocytopenic at the time of infection compared with untreated mice. Induction of thrombocytopenia four days after infection had no effect on worm count. Platelets from non-immune mice adhere to and kill mechanically transformed schistosomula in vitro. Platelets may thus provide an innate defence against schistosome infection, and the thrombocytopenia that occurs during schistosome infections may be a strategy that helps secondary parasites evade this type of host defence or could reflect platelet losses during the defence reaction (20).

There is also evidence that platelets are involved in patients infected with Echinococcus granulosus. The concentrations of β-thromboglobulin and platelet factor 4 in infected patients were elevated compared with controls, indicating that platelets had been activated (14).

In many of these parasitic diseases, it has been suggested that the platelet IgE receptor has an important role. Flow cytometry analysis showed that, like monocytes and eosinophils, about 10–20% of platelets in normal individuals are IgE-positive and that this increases to up to 50% in patients with parasitic diseases, leading to increased levels of circulating IgE, particularly in filariasis (10). This study also showed that binding of IgE was reduced by anti-IgE receptor antibodies and that about 800–1000 FcεRI molecules per platelet were present. Parasite-specific IgE binding to platelets followed by platelet activation when these are cross-linked by binding to the parasites is thought to be the major mechanism involved. As a result, platelets produce oxygen metabolites and release granule contents toxic for the parasite.

Platelets, parasites and allergies

Before the industrial era, all mammals were ‘normally’ infected with helminth parasites from shortly after birth until adulthood. Only since the 19th century have people living in developed countries organised sophisticated water purification (chlorination) and sewage disposal systems that have allowed them to escape helminth infection. Allergies are present at high levels in these human populations whereas they are absent in populations still regularly exposed to helminths. In addition, populations with endemic helminth infections show little allergic pathology that could be connected to the acquisition or elimination of helminth parasites.

It has therefore been suggested that endemic helminth infections activate the Th2 system, particularly at mucosal surfaces, to provide a different level of immunological homeostasis than occurs at present in developed societies. Under these conditions, mast cells, eosinophils and IgE rarely participate in allergic reactions, although they obviously participate in the control of helminth infections. Allergic reactions can thus be considered to be a pathologic consequence of the change in this homeostatic mechanism and have no protective consequences for the individual (3). The realization that this reciprocal relationship exists may open new approaches to prevention of allergic disorders, which are becoming an increasingly major problem in developed countries.
Some recent studies suggest that platelets may play an important role in allergic inflammation through the
- high affinity IgE receptor (FceRI), the
- low affinity IgE receptor (FceRII/CD23)
and
- low affinity IgG receptor (FcγRIIA/CD32) expressed on the cell surface.

Human platelets released serotonin and the chemokine RANTES (regulated upon activation, normal T expressed and presumably secreted), induced via FceRI, but the biological implication of human platelets in type I allergy is not yet clear. The levels of RANTES released from platelets from allergic patients and healthy individuals stimulated with monoclonal antibody (Ab) to human FcεRI α-chain or human myeloma IgE and anti-human IgE Ab were compared. The level of RANTES released from platelets of allergic patients stimulated with human IgE and anti-human IgE was significantly higher than that of healthy individuals. While the surface expression levels of FceRI on the platelets from allergic patients and healthy individuals were not significantly different, the platelets from allergic patients were more activated by the IgE–FceRII pathway than those from healthy controls, suggesting a novel and important role for human platelets in perpetuating allergic inflammation through IgE and the FceRI (9).

As other studies indicated, crucial events for a sustained allergic inflammatory response are both the
- presence of functional, intact platelets and
- release of inflammatory mediators from platelets.

Recently, platelets were shown to be critical for leukocyte recruitment in chronic skin inflammation in a mouse model of chronic contact dermatitis. Platelet depletion and restitution in an IgE-mediated intermediate hypersensitivity reaction in skin, was used to investigate the effect of mediators released from activated platelets on leukocyte recruitment (22).

References