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Review Article

Schistosomes versus platelets

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ABSTRACT

Schistosomes are parasitic platyhelminths that currently infect >200 million people and cause the chronic debilitating disease schistosomiasis. While these large intravascular parasites can disturb blood flow, they do not appear to activate platelets and provoke thrombus formation. Host-interactive tegumental molecules have been proposed to be important in this regard. For example, tegumental apyrase, SmATPDase1 can degrade the platelet-activating molecule ADP in the extracellular environment. The parasites themselves can produce prostaglandins (or may induce prostaglandin production by host cells) which could inhibit platelet aggregation. Additional tegumental proteins have been proposed to impede the coagulation cascade and to promote fibrinolysis. Platelets have been shown to be directly toxic to schistosomes. Platelets recovered from infected rats are able to kill larval parasites in culture and platelets obtained at later times post-infection are generally better at killing. Even platelets from uninfected rats can rapidly kill larval schistosomes if first exposed to a variety of activators (such as: serum from infected rats, the IgE fraction of that serum, C-reactive protein, cytokines (TNFα or TNFβ)). Passive transfer of stimulated platelets can protect rats against a challenge schistosome infection. Cytokines (TNFα, TNFβ, IFNγ or IL-6) have been shown to similarly promote normal human platelet killing of schistosomes *in vitro*. Platelet antimicrobial effector molecules (e.g. platelet microbicidal proteins) may mediate such killing. While platelets can be protective against schistosomes following infection of humans and mice, platelet numbers decline (but not so in the non-permissive rat host) and coagulopathy becomes more apparent as schistosome-induced pathology increases.

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Introduction

Schistosomes are parasitic platyhelminths that constitute an extremely important public health problem globally. The worms infect over 200 million people and cause the chronic debilitating disease schistosomiasis (or bilharzia) [1]. People become infected when larval forms called cercariae (that emerge from infected freshwater snail hosts) penetrate human skin. The larvae then transform into the intra-mammalian life forms called schistosomula. These invade a blood vessel and migrate to the hepatic vasculature where they mature into adult males and females. The worms pair up and the couples then migrate to their preferred egg laying sites: for *Schistosoma mansoni* and *S. japonicum*, the mesenteric venules and for *S. hematobium*, the vesical venous plexus.

As intravascular parasites, schistosomes interact with all components of host blood, including platelets. These small, non-nucleated fragments of large bone marrow cells (megakaryocytes) are abundant in the human blood stream (at ~150,000 – 400,000/μl). Platelets play a central role in hemostasis in addition to helping to fight infection [2–4]. The relationship between schistosome parasites and platelets is the subject of this review.

Schistosomes, platelets and hemostasis

Schistosomes can live in the bloodstream for years, sometimes decades, yet they appear to elicit little if any response from the host's

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hemostatic system [5]. Adult schistosomes are relatively large (~0.5 mm in diameter and up to 10 mm long) in comparison to the size of e.g. the mesenteric veins (1–4 mm diameter) where they commonly reside. Obstructions in blood vessels, such as those caused by the presence of schistosomes, should lead to disturbances of blood flow. This situation predisposes to thrombotic complications [6]. For instance, high fluid shear can trigger the activation of platelets and their subsequent aggregation [7,8]. This process involves the shear-induced binding of von Willebrand factor (VWF) to the platelet membrane GpIb/IX/V complex followed by platelet activation and degranulation [9,10]. Altered fluid dynamics in the case of schistosome infection may lead to activation of host hemostatic defenses and aggregation of hemostatic mediators around the worms. However, an assessment of the condition of the parasites *in situ*, within the blood vessels of infected hosts, shows that this is not the case: the worms appear unmolested by host mediators of thrombosis, such as platelets [11,12]. Indeed, human platelets do not even adhere to adult schistosomes *in vitro* [5]. Furthermore, no adherence of platelets is observed after perfusion of whole blood over surfaces coated with isolated schistosome tegumental outer surface membranes. In contrast, perfusion of whole blood over surfaces coated with homogenates of entire adults results in substantial platelet adherence [5]. These results show that the non-adhesive properties towards platelets are specific for the tegumental outer surface. It has been suggested that the action of schistosome ecto-enzymes, expressed at the surface of the parasites' tegumental membranes, are important in this regard. These enzymes are alkaline phosphatase (SmAP), phosphodiesterase (SmNPP-5) and ATP diphosphohydrolase (SmATPDase1) [13,14]. By rapidly hydrolyzing extracellular platelet activators like ATP, and its cleavage product ADP, the parasite ecto-enzymes may temper platelet activation around them. It is well established that vertebrates express similar membrane-bound, nucleotide-metabolizing ecto-enzymes to help regulate their extracellular nucleotide di- and tri-phosphate levels [15]. By knocking down expression of the genes encoding the schistosome enzymes, the abilities of these parasites to then hydrolyze exogenously added ATP and ADP was recently compared [16]. It was found that only SmATPDase1-suppressed parasites were significantly impaired in their ability to degrade these nucleotides. Suppression of the SmAP or SmNPP-5 genes did not appreciably affect the worms' ability to catabolize exogenous ATP or ADP [16]. Unlike their vertebrate hosts, schistosomes display no redundancy with regard to the exogenous ATP hydrolysis pathway [16]. These findings were confirmed by the functional characterization of the enzymatically active, full-length recombinant SmATPDase1 expressed in CHO-S cells [16]. The results support a role for tegumental SmATPDase1 in the degradation of exogenous pro-thrombotic nucleotides by live intravascular schistosomes. By degrading host pro-thrombotic chemical signals like ATP and ADP, SmATPDase1 likely helps block platelet activation and blood coagulation around the worms and helps promote schistosome survival. A second schistosome tegumental ATPDase homolog (SmATPDase2) is not involved in regulating extracellular ATP and ADP levels [17].

Another way that intravascular schistosomes may impact platelet function is via the action of enzymes with kallikrein-like activity [18]. Vertebrate kallikrein is able to cleave the plasma protein kininogen to generate the small vasoactive peptide bradykinin and bradykinin can induce the release of prostacyclin (PGI_2) from endothelial cells [19]. PGI_2 inhibits platelet degranulation. In schistosome homogenates, kallikrein-like activity was associated with a 66 kDa enzyme (designated sK1) which cleaved bradykinin from purified rat plasma kininogen and hydrolysed the kallikrein synthetic substrate d-Pro-Phe-Arg-*p*-nitroanilide [18]. In other work, an *S. mansoni* cDNA encoding a mouse plasma kallikrein homolog was identified (and designated SmSP1) [20]. Since the cloned DNA encodes a ~35 kDa protein, this molecule clearly differs from the larger, active sK1 enzyme described above. SmSP1 was detected in schistosomula released products and in male dorsal spines. The sK1 protein was found at the tegumental surface of the parasite [18]. While these localization sites are consistent with a proposed role for the proteins in regulating host platelet activation, it is important to point out that intact, living schistosomes have not been directly demonstrated to exhibit kallikrein activity. Indeed, since the parasites themselves can generate and release prostaglandins [21,22] (including PGD_2 – a potent inhibitor of platelet aggregation [23]), it is unclear why they would need to engage a kallikrein activity to act on host endothelia for the same end.

Schistosomes can also impair additional coagulation and vascular function [24,25]. In addition to the surface SmATPDase1 mentioned above, other potential coagulation inhibitors have been identified that could contribute to their refractoriness to thrombosis [26,27]. These include the tegumental protein Sm22.6 which, in recombinant form, can inhibit the proteolytic activity of thrombin, a central component in the coagulation cascade [26]. In addition, since thrombin can activate platelets, Sm22.6-mediated inhibition of the protein may impede thrombin-driven platelet activation. Earlier work reported the presence in whole adult worm homogenates of an inhibitor acting on factor XIIa, early in the coagulation cascade, but this has not been identified or further characterized [27]. Schistosome tegument fractions possess heparin-like glycosaminoglycans [28] which could conceivably interfere with hemostasis. In more recent work, plasminogen – the plasma glycoprotein that is a central component of the fibrinolytic system - was shown to bind to the surface of adult male (but not adult female) *Schistosoma bovis* worms [29]. Tegument extracts were found to contain several plasminogen-binding proteins including the enzyme enolase [29]. In recombinant form *S. bovis* enolase also bound plasminogen and increased its activation in the presence of tissue plasminogen activator [30]. *S. bovis* annexin has been shown to similarly bind plasminogen to promote its activation and, when tested in recombinant form, displays additional anti-coagulant activity [31]. These data suggest that surface-associated schistosome proteins play an important role in the activation of the host fibrinolytic system to help prevent debilitating blood clots forming around the worms.

In addition to the action of the anti-thrombotic molecules mentioned above, in schistosome-infected mice there is a diminution in total platelet numbers (thrombocytopenia) as the infection progresses. Such platelet scarcity may also act to limit thrombus formation around the worms [32]. Platelet counts in infected CBA mice are approximately half that of control mice by ~day 40 post infection [32]. The fall in platelet numbers is not observed in T-cell deprived mice (i.e. those whose thymus has been removed) [33]. It has been suggested that this is because intact mice make anti-schistosome antibodies that cross react with platelets and this leads to platelet clearance and thrombocytopenia [33].

An overview of the ways that schistosome juveniles (schistosomula) have been proposed to impact platelets is summarized in Fig. 1.

The protective role of platelets in schistosome infection

In addition to being a critical component of coagulation, platelets also play an important role in host defense against pathogens. Results of experiments in which anti-platelet antiserum is administered to mice show that platelets can play a protective role in the response to schistosomes [34]. Giving anti-platelet antiserum to mice transiently depletes circulating platelet numbers (by ~9 fold) [33]. When this therapy is used one day before and two days after infecting mice with schistosomes, significantly greater numbers of worms are recovered 6 weeks later, compared to untreated control mice [34]. Since diminished platelet numbers in mice leads to greater worm survival, the conclusion is that platelets help fight infection. Indeed, incubating schistosomula with platelets *in vitro* leads to 100% death of the parasites in 2–3 days compared to schistosomula incubated in the absence of platelets (~20% dead) [34]. It appears that only very young schistosomula are susceptible to killing *in vivo* since anti-platelet therapy administered to mice after infection (on day 4, 6 and 8) has no significant impact on subsequent worm recovery [34].