Seminars in Hematology 53 (2016) S2–S6

Contents lists available at ScienceDirect

Seminars in Hematology

journal homepage: www.elsevier.com/locate/enganabound

The nonhemostatic immune functions of platelets

Rick Kapur[a], John W. Semple[a,b,*]

[a] Toronto Platelet Immunobiology Group, Keenan Research Centre for Biomedical Science, St. Michael's Hospital, Canadian Blood Services, Toronto, Ontario, Canada
[b] Departments of Pharmacology, Medicine, and Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada

ARTICLE INFO

Available online 5 April 2016

Keywords:
Platelets
Inflammation
Immunity
Infections
Chemokines
Cytokines

ABSTRACT

Platelets are megakaryocyte-derived cellular fragments, which lack a nucleus and are the smallest circulating cells and are classically known to have a major role in supporting hemostasis. Apart from this well-established role, it is now becoming evident that platelets are also capable of conveying other important functions, such as during infection and inflammation. This paper will outline these non-hemostatic functions in two major sections termed "Platelets versus pathogens" and "Platelet-target cell communication". Platelets actively contribute to protection against invading pathogens and are capable of regulating immune functions in various target cells, all through sophisticated and efficient mechanisms. These relatively novel features will be highlighted, illustrating the multifunctional role of platelets in inflammation.

1. Platelets versus pathogens

Platelets are anucleate small (~2–4 μmol/L in diameter) cellular fragments derived from bone marrow resident megakaryocytes (~50–100 μmol/L in diameter) and are traditionally known to be indispensable for the regulation of hemostasis [1]. It is now also clear that platelets are involved in several other functional processes, apart from their well-known role in hemostasis, in both health and disease [1–8]. The versatile role of platelets in an inflammatory setting can be demonstrated by how platelets interact with invading pathogens. Platelets have the ability to "sense danger", such as pathogens or damage, as they express functional immune receptors called pattern recognition receptors, including Ig- or complement receptors and Toll-like receptors (TLR) [1]. Via these receptors, platelets can bind invading pathogens and their cellular contents. It is currently thought that pathogens first encounter TLRs on professional phagocytes, such as neutrophils, dendritic cells (DCs), or macrophages [1,9,10]. TLRs are germline-encoded proteins, capable of binding to several infectious molecular structures and potently stimulate innate immune mechanisms [1,9,10]. The expression of TLRs 1–9 has been described on both human and murine platelets, and some of these TLRs appear to have functional roles [1]. For instance TLR4, which has been shown to enable lipopolysaccharide (LPS, a gram-negative endotoxin)-induced thrombocytopenia and tumor necrosis factor (TNF)-α production in vivo [11–17]. Another example comes from platelet TLR9, which appears to be important as sensor of internal danger signals, rather than external signals, such as oxidative stress, innate immunity and thrombosis [18]. Significant evidence has now accumulated over the years suggesting an important role for platelets as perhaps the initial pathogen sensors within the blood, due their expression of several receptors, which have no clear association with hemostasis.

Apart from the detection of pathogens, platelets are also capable of harboring pathogens internally or on their plasma membrane [3,19], as has been demonstrated for viruses [19,20], bacteria [21–23], and parasites [4]. Activated platelets were shown to surround or encapsulate *Staphylococcus aureus*, driving the pathogens into clusters resulting in reduced bacterial growth [24]. This appeared to be dependent upon secretion of the antimicrobial peptide β-defensin and stimulation of neutrophil extracellular trap (NET) formation [24]. Bacteria (eg, methicillin-resistant *S aureus* and *Bacillus cereus*) have also been shown to be trapped on the hepatic Kupffer cells, which was shown to occur via engagement of platelet-adhesion receptor GP1b [5]. In that study, infected GP1bα-deficient mice suffered more damage to endothelial and Kupffer cells and displayed increased vascular leakage and rapid mortality [5]. In addition, during sepsis, neutrophils were also shown to be activated by platelet TLR4, causing the release of NETs, which subsequently trapped bacteria in blood vessels of primarily liver sinusoids and lung capillaries [6]. Platelets may act as circulating sentinels, sensing infectious agents and presenting them to neutrophils and/or the reticuloendothelial system [13–17]. Furthermore, it was demonstrated that neutrophils are able to

This supplement was sponsored by the Intercontinental ITP Study Group.
* Corresponding author. St. Michael's Hospital, 30 Bond St, Toronto, Ontario, Canada, M5B 1W8. Tel.: 1-416-864-5534.
E-mail address: semplej@smh.ca (J.W. Semple).

http://dx.doi.org/10.1053/j.seminhematol.2016.04.002
0037-1963/$/

scan platelets for activation in the circulation via P-selectin ligand signaling, resulting in inflammation [7]. Additionally, platelet P-selectin, whether soluble or cellular, was found to trigger NET formation in mice via binding to neutrophil P-selectin glycoprotein ligand -1 (PSGL-1) [25].

Platelets have been suggested to be involved in the clearance of bacterial infections. Thrombin-stimulated platelets, for instance, were shown to facilitate clearance of streptococci in infective endocarditis [26]. In addition, it was elegantly demonstrated that activated platelets can kill the malarial parasite Plasmodium inside red blood cells [4]. Mechanistically, this process was shown to be dependent upon platelet factor 4 (PF4 or CXCL4) and the erythrocyte Duffy-antigen receptor (Fy) [27]. Consequently, this indicates that in Duffy-negative individuals, thus lacking Fy, platelets would be incapable of eliminating this intraerythrocytic malarial parasite.

Conversely, viruses and bacteria appear to have developed counter measures to evade these immune responses elicited by platelets. This is supported by the fact that acute viral or bacterial infections often result in thrombocytopenia or low platelet counts. This has frequently been observed in patients suffering from immune thrombocytopenia (ITP), an autoimmune bleeding disease in which platelets are destroyed [28]. The pathogenesis of platelet destruction during infection and inflammation is incompletely understood, but several mechanisms have been suggested to occur. These include molecular mimicry between viral/bacterial antigens and platelet antigens, resulting in cross-reactive autoantibody generation [29–33]. Furthermore, ITP patients infected with the gram-negative bacteria *Helicobacter pylori* demonstrated increased platelet counts following *H pylori* eradication therapy [34]. Similarly, the gram-negative bacterial endotoxin LPS also enhanced anti-platelet antibody mediated platelet phagocytosis in vitro [14], as well as an increased platelet clearance in vivo, when anti-platelet antibodies and LPS were co-injected in mice [35]. Furthermore, C-reactive protein (CRP) was found to be a novel factor that potentiated antibody-mediated platelet destruction both in vitro and in vivo in mice [36]. CRP is an acute-phase protein that rapidly increases during acute bacterial or viral infections. CRP was found to be increased in children suffering from ITP and treatment with IVIg correlated significantly with increased platelet counts, decreased levels of CRP, and reduced clinical bleeding severity [36]. Interestingly, elevated CRP levels at diagnosis appeared to be predictive for slower platelet count recovery after 3 months [36].

2. Platelet–target cell communication

Platelets have developed sophisticated ways to contact target cells, such as through the release of several mediators. CD40L (CD40L/CD154) and CD40, besides their role in costimulation and perhaps thrombotic diseases [37], have been found to be relevant in platelet immune reactions. When platelets are activated, most of their expressed CD40L is released, generating the soluble form (sCD40L), which is in fact the vast majority of all sCD40L in circulation [38]. Platelet CD40L can interact with endothelial cell–CD40 (in the membrane), which results in an cascade of inflammatory reactions leading to the release of several adhesion molecules including, ICAM1, VCAM1, and CCL2 [39]. Platelet-secreted sCD40L, when interacting with $CD40^+$ vascular cells (including endothelial cells), can also enhance the expression of adhesion molecules like P-selectin and E-selectin, and stimulate the release of tissue factor and interleukin (IL)-6 [40,41]. This implies a central role for platelet CD40L–CD40 interactions between endothelium/coagulation and inflammation. Additionally, platelet-derived CD40L was shown to enhance $CD8^+$ T cell responses and to stimulate T-cell responses following infection with *Listeria monocytogenes* [42,43], demonstrating a clear link between innate and adaptive immunity. Platelet–CD40L was additionally shown to bind to DCs and thereby impair their differentiation, suppress the proinflammatory cytokines IL-12p70 and TNF by DCs, and increase IL-10 production [44]. In addition, platelets can enable B-cell differentiation and antibody class switching via their CD40L [45,46].

Platelets contain many different cytokines and chemokines in their system, all differently impacting hemostasis and wound repair [47], and also proinflammatory and anti-inflammatory reactions, for example, the immunosuppressant transforming growth factor (TGF)-β [48]. Platelets appear to control the levels of TGF-β as is evident from ITP patients, who displayed low levels of TGF-β during active disease; however, those levels normalized again upon treatment, which increased platelet counts [49,50]. Most of the platelet chemokines and cytokines are located within the different platelet granules. The α granules contain several immunomodulatory soluble factors, like chemokines, which included PF (CXCL4), RANTES (CCL5), β-tromboglobulin (β-TG, an isoform of CXCL7), and MIP-1α (CCL3) [51]. Platelet activation triggers release of these chemokines causing a diverse reaction of cellular interactions and responses.

Platelets are also known to release microparticles (also referred to as microvesicles), which are small extracellular vesicles produced by cell cytoplasmic blebbing and fission. The size of microparticles ranges from ~100–1,000 nm in diameter, although mostly they are ~200 nm, and they are distinct from exosomes, which are ~50–100 nm in diameter and thus smaller in size and originating from multivesicular bodies via exocytosis [52]. Platelet microparticles have been described in various inflammatory conditions, in which platelets become activated [53,54], and clinically their levels were often found to be associated with disease progression. For example, in blood and synovial fluid of patients suffering from rheumatoid arthritis (RA), platelet microparticles were found to be increased [8,55–58]. Using a murine model of RA, it was demonstrated that depletion of platelets attenuates inflammation [8,59]. However, microparticles are observed in sterile, as well as in inflammatory diseases, making it unclear what exactly triggers the platelets to shed off microparticles. Several activation pathways may be driving the production of microparticles during inflammation, such as apoptosis, high shear forces, or platelet receptor signaling. The disease setting, at least partly, determines the route of microparticle production, as in RA activation of the collagen receptor glycoprotein VI (GPVI) is a trigger, while in sepsis the trigger appears to be TLR-4 signaling through LPS [8,60]. Both of these signals, however, are accompanied by an increase in IL-1, indicating their common link to enhancing inflammation. Additionally, signaling through immune complexes, consisting of bacterial components and well-conserved epitopes expressed by influenza viruses, via the platelet FcγRIIA [61,62], was shown to lead to the formation of microparticles. From a functional perspective, platelet microparticles are thought to mediate cell–cell communication. The platelet microparticle cargo is substantial and can consist of various cytokines and chemokines (eg, IL-1, RANTES), surface receptors (eg, CD40L), potent lipid mediators (eg, thromboxane A2), enzymes (eg, inducible NO synthase), autoantigens (eg, citrullinated fibrinogen), nucleic acids (eg, micro RNA), transcription factors (eg, PPARγ, RuvB-like2, STAT3, STAT5a), and interestingly even respiratory competent mitochondria, all of them potentially targeting and impacting a cell [53,54,63–67]. As the microparticles can express phosphatidylserine (PS) and surface receptors, they interact with other cells through integrin and via the PS-binding proteins lactaderhin [68] and developmental endothelial locus-1 (Del-1) [69]. Transcription factors transported within platelet microparticles can enable transcellular effects, such as PPARγ, which was demonstrated to be transported inside