Platelet immunology from the inside out

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Platelets are the critical cellular mediators of hemostasis; however, many studies have now suggested that these tiny offspring of megakaryocytes can also perform multiple immune-like functions that significantly affect both innate and adaptive immunity. For example, like whole blood, platelets can mediate transfusion-related immunomodulation (TRIM) and it appears that activated platelets are more immunoregulatory than their resting counterparts. In addition, platelets express and secrete a wide variety of critical immune molecules such as TGF-β, CD40/CD40L and MHC class I molecules. This allows platelets to directly influence adaptive immune mechanisms and a variety of immune disease processes. It has also been demonstrated that both platelets and megakaryocytes can process and present both foreign and self-antigens to CD8+ T cells. This manuscript will highlight several non-haemostatic attributes of platelets that deservedly categorize them as integral players in immunity.

Key words: antigen presentation, CD40L, immunity, platelets, TGF-β, TRIM.

Introduction

Platelets are the smallest circulating cellular elements in the blood, and their primary physiological role is to maintain the integrity of the vasculature. When a vascular insult occurs, platelet adhesion and activation receptors recognize the exposed subendothelium and this initiates a series of reactions eventually leading to thrombus formation which arrests bleeding [1-3]. In addition to hemostasis, however, platelets can also elicit important immunological functions. This is due to the platelet’s capability to express and secrete a multitude of immune molecules which are capable of regulating immune responses. An in-depth discussion of how platelets utilize these molecules to affect inflammatory and immune reactions has been previously comprehensively reviewed [4-8], and this paper will only briefly outline some of the major ways in which platelets can act as immune cells and regulate immunological functions.

Evolution and platelet immune activities

Trying to understand why platelets have immune properties may be linked to evolutionary processes. Anucleate platelets are derived from polyploid megakaryocytes in the bone marrow, and these cells are only found in mammals [9]. In all other animal species, cells involved in hemostasis and blood coagulation are nucleated, and in all non-mammalian vertebrates, these cells are termed thrombocytes [9]. In many invertebrates, for example, only one cell type circulates in the haemolymph and this cell is typically involved in multiple defence mechanisms of the organism [9]. These cells are termed haemocytes or amebocytes and are not only responsible for host defence but they are also capable of aggregating and sealing wounds. In addition to their obvious haemostatic capabilities, platelets also possess many of the properties that haemocytes have. Thus, it is possible that as platelets evolved in mammals they may have retained some of the defence properties of early haemocytes [9].

Platelets and transfusion-related immunomodulation (TRIM)

One of the earliest effects observed during the transfusion of whole blood was the so-called transfusion effect; an apparent immunosuppression that occurs after allogeneic
Platelets and their effects on immune disease processes

There are many examples of how platelets can alter immunity in different disease settings, and the reader is referred to more comprehensive reviews on the subject [4–8]. We will only highlight a few examples here. In 2009, McMorran et al. [18] demonstrated that platelets played a critical role in the pathogenesis of malarial infections by encouraging the sequestration of infected red blood cells within the cerebral vasculature. They found that purified human platelets killed Plasmodium falciparum parasites cultured in red blood cells and inhibition of platelet function by aspirin, for example, abrogated the lethal effect on the parasites. These results indicate a protective function for platelets in the early stages of erythrocytic infection distinct from their role in cerebral malaria [18].

In 2010, Boillard et al. [19] published a seminal paper showing a role for platelets in the pathogenesis of the autoimmune disease rheumatoid arthritis (RA). They identified platelet microparticles (MP) in joint fluid from patients with RA, and these MP were pro-inflammatory, eliciting cytokine responses from synovial fibroblasts via interleukin-1. They identified platelet glycoprotein VI and the Fc receptor γ-chain as activators for platelet microparticle production in patients with RA and microparticle shedding appeared to be stimulated by fibroblast-like cells in the joint cavity. These findings were one of the first to demonstrate a previously unappreciated role for platelets and their activation-induced microparticles in inflammatory joint diseases [19].

In autoimmune diseases such as immune thrombocytopenia (ITP), during active disease, there is a concomitant loss of the highly immunosuppressive T regulatory (Treg) cells and when platelet counts increase either spontaneously or because of treatment, the Treg numbers and activity normalize [20–23]. This has been a consistent finding with many different therapies including steroids, intravenous immunoglobulin (IVlg), Rituxan and thrombopoietin receptor agonists (TPO-RA) [20–23] (Fig. 1). Since these therapies have very different mechanisms of action, it seems unlikely that they are actually responsible for the reversal of the Treg defects. A more plausible explanation may be that it is the increase in the platelet mass that is responsible for the Treg normalization (Fig. 1). One possibility of why the increase in platelets reverses the Treg defects is their ability to secrete large quantities of TGF-β1, a critical molecular switch for inducing Tregs [24,25].

More recently, Rachidi et al. [26] demonstrated that platelets promote malignancy and resistance to cancer therapy by dampening host immunity. They showed that platelet-derived transforming growth factor beta (TGF-β) and lactate were major soluble factors that obliterated both CD4+ and CD8+ T-cell functions. It appears that the platelets secreted large quantities of TGF-β in the tumour microenvironment and secretion was under the control of the TGF-β-docking receptor glycoprotein A repetitions predominant (GARP) [26]. They suggested that platelets constrain T-cell immunity through the GARP-TGF-β axis and that a combination of immunotherapy and platelet inhibitors may be a therapeutic strategy against cancer. Taken together, there is ample evidence in the literature to suggest that platelets play a significant role in modulating immunity and affecting disease processes.

Platelets and innate immunity

There have been numerous papers published on how platelets can affect the innate immune system, and for brevity, a few examples will be given. The readers are referred to the cited reviews for a more comprehensive discussion of this subject [4–8]. It is now well known that platelets...
can directly interact with neutrophils and cause them to, for example, secrete neutrophil extracellular traps (NETs) [27]. Formation of NETs has been suggested to play a role in adverse transfusion reactions such as transfusion-related acute lung injury (TRALI) [28]. On the other hand, there are numerous reports that have demonstrated that platelets can interact with and potentially affect the differentiation of dendritic cells and these interactions can indirectly affect adaptive T-cell responses [4-8].

Platelets and their roles in modulating adaptive immunity

There is increasing evidence that platelets can interact with B lymphocytes and affect immunoglobulin production. For example, Elzey et al. [29] showed that activated platelets, via CD154 expression, induced B cell isotype switching and augmented CD8+ T-cell responses both in vitro and in vivo. They demonstrated that platelet-derived CD154 alone was sufficient to induce isotype switching and augment T lymphocyte function during viral infections, leading to enhanced protection against viral rechallenge. Similar results were corroborated by others [30].

In another study, Solanilla et al. [31] showed that platelets and megakaryocytes had increased expression of CD154 in patients with immune thrombocytopenia (ITP). Of interest, they showed that the platelet-associated CD154 was competent to induce the CD40-dependent proliferation of B lymphocytes and observed elevated in vitro production of autoantibodies against the platelet GPIIb/IIIa complex [31]. Thus, platelet-associated CD154 expression is able to drive the activation of autoreactive B lymphocytes in this disease suggesting that the platelets themselves may participate in their own demise in ITP (Fig. 2).

Antigen processing and peptide presentation by molecules encoded by the major histocompatibility complex (MHC) to T lymphocytes is a key event in immunity against foreign protein antigens [32]. MHC class I molecules on the platelet plasma membrane are mainly adsorbed from plasma and consist of denatured heavy chains [15-16,33-35]. This makes the platelet membrane MHC class I molecules appear to be somewhat unstable since they can passively dissociate from the platelet during blood bank storage or can be eluted from the membrane by, for example, citric acid treatment [33-35]. On the other hand, intracellular platelet MHC class I is associated with the α granules and generally consist of functional intact integral membrane proteins associated with β2-microglobulin [33-35]. It has also been demonstrated that platelets contain the entire proteasome system, including TAP molecules associated with the alpha-granules [36-38]. In syngeneic settings, platelet activation can lead to the membrane expression of nascent MHC class I molecules which are capable of presenting antigens to CD8+ T cells [39]. Activated platelets were shown to present malarial peptides to malaria-specific CD8+ T cells resulting in enhanced immunity against the parasite [39] (Fig. 2). Therefore, the type of platelet MHC class I (denatured platelet plasma membrane-bound or intact intracellular) may determine the effect on T cells (suppression or activation).

The immunological role of MKs has not been completely elucidated; however, early MK progenitors (CD34+/CD41-/MHC class II+) have been shown to support heterologous non-cognate CD4+ T-cell activation [40]. In contrast, fully mature CD34− CD41+ MKs lose their MHC Class II expression but do express MHC class I molecules [41,42]. It was recently demonstrated that mature CD34− CD41+ MKs were able to uptake exogenous protein antigens and process and present their peptides on MHC class I molecules which effectively triggered antigen-specific CD8+ T-cell activation in vitro and
The MKs could also present endogenous self-antigens and induce CD8+ T cells to cause ITP in vivo [42]. Related to this, it was shown that while the MKs were presenting antigen, they were also able to transfer immunological MHC class I/peptide complexes to their progeny, the platelets and this correlated to their ability to present self-antigens and mediate ITP [42]. These results suggest an important role for MK as potent APC that present antigens to CD8+ T cells.

Summary

Besides their classically recognized role as mediators of hemostasis, platelets have significant immune properties and these attributes closely link them to pathophysiological inflammatory processes. It also appears that activated platelets have a greater ability to enhance or reduce many different immune responses. With respect to transfusion medicine, platelet transusions are known to cause many adverse reactions and at least some of these unwanted events may be explained by the ability of activated platelets to act as immune cells. In light of these unwanted adverse reactions, it appears time to begin a larger and more rational discussion is devoted to the platelet activation status in blood products. Understanding why platelets have these immune properties and how they modulate immune responses will ultimately shed light on developing platelet-directed therapeutics that have the ability to modulate adverse reactions.

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Conflict of interests

The authors declare no conflict of interests.

Author contributions

J.W.S. provided financial resources and wrote the original draft. R.K. edited the manuscript.

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