Review Article

The Mighty Role Of Platelets In Immunity, Inflammation, Cancer And Angiogenesis

Hassan A. Hamali, BSc, MSc, PhD,

Assistant Professor, Department of Medical Laboratory Technology,
College of Applied Medical Sciences, Jazan University, Gizan, Saudi Arabia

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Corresponding Author: Dr. Hassan A Hamali, BSc, MSc, PhD. P.O. Box 1906
Gizan, 45142, Saudi Arabia. Email: hhamali@jazanu.edu.sa

Abstract

Platelets are the smallest anucleated cells, in the circulation. Platelets are produced from the megakaryocytes in the bone marrow. Platelets participates in maintaining vascular integrity and hemostasis. However, their role extends beyond hemostasis to thrombosis, inflammation, immunity, wound healing, cancer, and angiogenesis. The multifunctional roles of platelets are achieved through the expression of diverse adhesive and immune receptors, a wide array of bioactive proteins stored in their granules, and the elaboration of membrane lipid mediators upon activation. There is an obvious involvement of platelets in inflammation, immunity, cancer, and angiogenesis. Thus, the concept arises that platelets, at the intersection of these conditions, are multifunctional cells. A better understanding of the exact, as well as the diverse roles and mechanisms of platelets in pathologic conditions is a promising research area and may open a window for new strategies and prevention methods of diseases.

Key words: Platelets, hemostasis, thrombosis, angiogenesis, immunity, inflammation, cancer

الملخص

الصفائح الدموية تعتبر أصغر الخلايا الدموية بدون نواة في الدورة الدموية ويتم إنتاجها من الخلايا الكبيرة «الميجاكاريوسايت»(إحدى سلالات الصفائح الدموية) في النخاع العظمي. الصفائح الدموية تشارك في الحفاظ على سلامة الأوعية الدموية والإرقاء. ومع ذلك، فإن دور الصفائح الدموية يمتد إلى ما وراء الارقاء إلى تجلط الدم والالتهابات والمناعة واستشفاء الجروح والسرطان وتولد الأوعية. يتم تحقيق الأدوار متعددة الوظائف للصفائح الدموية من خلال مستقبلات متعددة منها المناعية، ومجموعة واسعة من البروتينات النشطة بيولوجيا المخزنة في حبيباتها. مما يعطي الصفائح الدموية صفة خلايا متعددة الوظائف. ويعد الفهم الأفضل للوظائف الدقيقة، بالإضافة إلى الأدوار والآليات المتنوعة للصفائح الدموية في الحالات المرضية، مجال بحث واعد، وقد يفتح نافذة لاستراتيجيات جديدة وأساليب الوقاية من الأمراض.

Introduction

Platelets were first identified and observed by Schultze in 1865 and Osler in 1874, and Bizzozero discovered their role in hemostasis in 1882 (1–3). However, the nature and other functions of these small cells have been important areas for research and discussion for the past several decades (3–6)Platelets are small (2-4 μm in diameter) anucleated discoid cells in the circulation. Their production from the cytoplasm of megakaryocytes in the bone marrow (7), involves the role of thrombopoietin (a hormone produced by the liver and kidneys) (5,6,8)James Homer Wright described how platelets formed from megakaryocytes and entered the circulation. Subsequent clinical and animal studies have enumerated a number of principles of platelet physiology: the platelet count is constant in any one individual but varies greatly between individuals; an inverse relationship exists between the platelet count and platelet size; the body conserves the mass, not the number, of platelets; and megakaryocyte number, size and ploidy vary in

response to changing demands for platelets. With the discovery of thrombopoietin (TPO and lineage-specific transcription factors (9). The lifespan of a platelet is 7–10 days. Platelets normally circulate in their inactive state with no interaction with the endothelium or other cells. Platelets become activated if they encounter vascular injury (damage, or at the site of inflammation) to maintain hemostasis and vascular integrity. Otherwise, they undergo apoptosis at the end of their life cycle and are removed by the spleen (6,7)a systematic review of published randomized clinical trials was conducted through extensive searches in Medline, Embase and Current Contents from 1966 till 1996 as well as manual reviews of 28 journals. The methodological quality of all trials was assessed by guidelines of the Cochrane Collaboration. Thirty-one trials were included, only one of which had no serious methodological flaws. The mean score of parasitological examination was 4.8 out of a possible 15. There was a considerable effect in cure rate of treatment versus placebo (odds 9.3, 95% CI 4.69-18.4.

The main role of platelets is associated with hemostasis, primarily to form a platelet plug to stop bleeding and to support blood coagulation (10–12). However, this is not their sole function as platelets are involved in, and contribute to, wound healing, (13)inflammation,(14)(15)immunity,(16,17) angiogenesis,(18) and cancer.(16,19)Platelets are the cellular elements that are general to these interrelated processes, indicating their role in the pathophysiology of these conditions (11–19). While the role of platelets in the pathophysiology of thrombosis is well-established, their contribution to the immune system, inflammatory pathways, angiogenesis, and cancer are yet to be clearly defined (17,20–25). A better understanding of the exact, as well as the diverse roles and mechanisms of platelet involvement in these conditions, will open new strategies and prevention methods of diseases. This short review summarizes the role of platelets in inflammation, immunity, angiogenesis, and cancer.

Structure of platelets

Circulating platelet is considered a featureless cell from the outside; with the open canalicular system (OCS) connecting the platelet's membrane with the internal membrane system. The OCS is a pathway to transport platelets' contents upon activation to their surface. In activated platelets, the content of the platelets' granules fuse with the OCS, and are released into the surrounding environment (26,27). Platelets have other components such as glycogen and organelles (like endoplasmic reticulum, lysosomes, mitochondria, and dense tubular system) (26,28). The structure of platelets is organized into three distinct zones: the platelet plasma membrane, cytoskeleton, and granular structure (27,29,30).

Platelet plasma membrane

The membrane structure of any cell, including platelets, is a standard bilayer composed of proteins (glycoproteins [GPs]) and lipids

(phospholipids and cholesterol). Phospholipids form the basic structure, while cholesterol is distributed asymmetrically and arranged throughout the phospholipids. Negatively charged phospholipids (phosphatidylserine and phosphatidylinositol) are retained in the inner layer, and neutrally charged phospholipids (phosphatidylcholine) in the outer layer thus maintaining polar heads on both sides of the membrane (i.e., towards the plasma side and towards the cytoplasm). In addition, various GPs are found on the surface of the platelet including GPIb-IX-V complex, GPIIB-IIIa, and/or GPIa-IIa, which are essential for their appropriate and normal functioning (7,31). The platelet membrane also contains glycocalyx that provides a dock to transfer proteins (such as fibrinogen) from blood to platelets (7). Overall, the membrane structure serves diverse functions in platelets, including agonist stimulation, permeability, adhesion, secretion, and aggregation (7,31).

Cytoskeletal zone

The cytoskeletal system supports the discoid shape of platelets. It consists of spectrin protein (2000 molecules per platelet), actin filaments (2 million molecules per platelet) and microtubules. In resting platelets, spectrin is an elongated protein strand interconnected by actin filaments. Spectrin and actin filaments support the platelet membrane, while microtubules located under the spectrin protein, and actin filaments, maintain platelet shape (7,29).

Granular zone

Platelets have three distinct cytoplasmic granules (alpha granules, dense granules, and lysosomal granules) that store bioactive molecules (32,33). Alpha [α-] granules (50–80 per platelet) contain high molecular weight factors (i.e., soluble and membrane-bound proteins including adhesion molecules, coagulation factor V, P-selectin, integrins, von Willebrand factor (vWF), platelet factor 4 (PF4: CXCL4), fibrinogen, fibronectin, vitronectin, protein S, plasminogen activator inhibitor-1, β-thromboglobulin, thrombospondin (TSP), proteoglycans, multimerin, α2-plasmin inhibitor, α1-protease inhibitor, C-1 inhibitor, chemokines such as CXCL7, CXCL1, and CXCL1 (GROa), CCL5 (regulated upon activation, normal T cell expressed and secreted [RANTES]), CCL3 (MIP1a), platelet-derived growth factor (PDGF), and transforming growth factor beta (TGF-β).(7,33) Dense [δ-] granules (3–8 per platelet) contain low molecular weight factors such as serotonin, ADP, ATP, Ca^{2+}, and histamine. Lysosomal [λ] granules contain glycohydrolases and proteases such as cathepsin, acid phosphatase, collagenase, and elastase (7,17,30,33,34).

Platelets and immunity

The main function of platelets is to limit blood loss at the site of injured endothelium. However, platelets also rapidly respond to the invasion of microbial pathogens in the bloodstream (34). The rapid response of platelets to infections at the site of colonization and in-

flammation not only maintains blood hemostasis and vascular integrity, but also releases chemokines to fight microbes; and trap and engulf them, indicating an important role for platelets in the immune response (35,36). This role is achieved with the help of inflammatory mediators released by activated platelets (14,37,38). Platelets store and release bioactive molecules (mediators and chemokines) and express functional immunoreceptors that modulate the immune system (17,24). Furthermore, platelets communicate with and modulate other cells to participate in innate as well as adaptive immunity by expressing receptors (36).

Innate immunity

Platelets exhibit diverse immunoreceptor attributes and have antimicrobial properties by interacting with viruses, bacteria, and fungi (14,39,40). Platelets are activated when bound to certain bacteria; this is mediated via direct and indirect interactions between bacteria and platelets. These interactions induce full responses of platelets (adhesion, degranulation, shape change, and aggregation). Platelets also participate in defense against microbial products and the products of inflamed tissues. The platelet-bacteria interaction is a complex process mediated through diverse receptors including complement receptors, such as Fc gamma receptor type 2, pattern recognition receptors, toll-like receptors (TLRs), and GPIIb-IIIa and GPIb on platelets (41).

Platelets express TLRs that modulate the release of different cytokines from platelets upon engaging with bacterial lipopolysaccharide (42–44). The presence of TLRs on platelets suggests direct engulfment of pathogens similar to leukocytes. TLRs also recognize pathogen-associated molecular patterns (PAMPs; i.e., lipids, lipoproteins, proteins, or nucleic acids) that are derived primarily from bacteria, viruses, fungi, or parasites (45,46). PAMPs in various cellular compartments, such as plasma membrane, endosomes, endolysosomes, and lysosomes, can be recognized by TLRs expressed by platelets (47).

TLRs initiate inflammation against microbial products or the products of inflamed tissues. TLRs are the primary sensors of infection because they recognize conserved structures in PAMPs. TLR2, TLR4, TLR6, TLR7, and TLR9 are shown to be expressed in human platelets. TLR activation on platelets triggers different biological responses,(43) leading to the release of acute phase reactant tumor necrosis factor-α (48) and interleukin-1 (49,50). Furthermore, platelets recognize different isoforms of lipopolysaccharide, eliciting a distinct response and release of specific chemokine and cytokine types (51).

Platelets also express various chemokine (CC and CXC) and kinocidin receptors. These have the ability to recognize signals from all types of chemokines and kinocidins in the infected area, eliciting a specific host defense response. This ensures the rapid recruitment and accumulation of platelets at the site of infection (52,53). Furthermore, platelets not

only have receptors to recognize chemokines and kinocidins, but also release platelet microbicidal proteins such as kinocidins (CXCL4, CXCL7, and CCL5), defensins (human β defensin 2), thymosin β4, and some derivatives (thrombocidins and fibrinopeptide A or B) (52,54). Moreover, platelets can modulate and coordinate the actions of other cells; i.e., immune cells to release chemokines and kinocidins including PF4 (CXCL4) and RANTES (CCL5) (52).

RANTES, derived from activated platelets, has an immune-modulator function and enhances the cytotoxic ability of T cells, as well as the production of cytokines. The expression of RANTES mRNA increases immunoglobulin synthesis from cells upon the platelet-B cell interaction. Other platelet products, such as PF4, kill erythrocytes infected with parasites, suggesting that PF4 is anti-parasitic. In addition, thrombocidins (chemokine-derived peptides) have antibacterial and antifungal properties, making platelets a player in innate immunity. The contents of lysosomal granules in platelets, such as cathepsin, also break down microbes, though non-specifically (55) (56).

Platelets not only internalize pathogens (microbes and viruses), but also kill various internalized pathogens including bacterial species (e.g., *Escherichia coli* and *Staphylococcus aureus*) and viruses (e.g., human immunodeficiency virus) to promote their clearance from the bloodstream and tissues (57,58). The exact mechanism of the phagocytic role of platelets in pathogen defense requires further investigations. Platelets are also known to fight microbes by generating and releasing reactive oxygen species as part of their antimicrobial role (59,60).

Platelets, as mentioned previously, modulate other immune cells. Specifically, they regulate the maturation and activation of cells that play crucial roles in innate immunity, such as macrophages, neutrophils, and dendritic cells (61,62). Indeed, platelets can interact with neutrophils to form a neutrophil extracellular trap (NET) (63,64). A NET can also be formed by platelet-derived β-defensin, which has a novel antibacterial activity. Activated platelets surround and trap *S. aureus* and use β-defensin 1 to force them into clusters, thereby diminishing their growth. Neutrophils trap bacteria using a NET comprised of the neutrophil nucleus and other intracellular contents that ensnare and kill bacteria. NET formation (NETosis) is an important mechanism of neutrophil killing, and inhibition of NETosis can increase the development of infections (64).

Adaptive immunity

Mobilization of the adaptive immune response is chiefly mediated by CD154. CD154 is a member of the tumor necrosis factor superfamily, which significantly enhances the presentation of antigens as well as the innate immune response. Platelets express CD154 and, thereby, have a role in adaptive immunity. This is achieved by platelets communicating with B- and T-cells (65,66). Elzey *et al.*

(2005) showed enhancement of B- and T-cells in CD154 $^{-/-}$ mice mediated by CD154-derived from platelets. Furthermore, platelet-derived CD154 includes CD8+ T-cell responses and is essential for IgG production in a murine model. These emerging data suggest a role of platelets in adaptive immunity (66). However, their exact role and mechanism in adaptive immunity is not well-established and needs more research.

Platelets and inflammation

Platelets are highly responsive to agonists, tissue injuries, and inflammation. It is suggested that platelets initiate and propagate the inflammatory response (35,67,68). The involvement of platelet in inflammation is proposed in inflammation; with data from literature suggest the effectiveness of antiplatelet drugs in inflammatory conditions (69–72). This role was recognized long ago, as demonstrated by platelets' involvement in atherosclerosis (73–75). Atherosclerosis is a chronic inflammatory process involving a well-observed interaction of platelets with leukocytes and endothelium. This leads platelets to secrete inflammatory molecules that can alter the proteolytic properties of endothelial cells. Such a sequence promotes the migration and adhesion of monocytes to the site, facilitating the formation of atherosclerotic plaque (76). This illustrates an important association between inflammation and atherogenesis.

Platelets contain, express, and release various inflammatory mediators, mostly stored in granules, mainly α-granules (33,77). Examples of these mediators include arachidonic acid metabolites, serotonin, adhesive proteins (fibrinogen, vWF), cell growth factors (PDGF, TGF-α and -β, platelet-derived angiogenesis factor, and basic fibroblast growth factor), and other plasma proteins and protease inhibitors. These mediators facilitate the role of platelets in inflammation either by adhesion to other cells via immunoreceptors, or the release of chemokines. In addition, platelets interact with many cells, including leukocytes (lymphocytes, neutrophils, and monocytes) dendritic cells, endothelial cells, erythrocytes, and tumor cells (23,78).

The most abundant protein secreted from activated platelet α–granules is PF4, a member of the CXC chemokine family. PF4 is chemotactic for neutrophils. In addition, PF4 contributes to atherosclerosis by accelerating atherogenesis, resulting in vascular inflammation. Even though, the retention of lipoproteins in the vascular wall is promoted by PF4 and prevents the catabolism of low-density lipoproteins by hindering the interaction between low-density lipoproteins and their receptor, increasing the retention of low-density lipoproteins on the cell surface; (79,80) PF4 also increases the release of neutrophil granules and adhesion to endothelial cells. This adhesion is facilitated by L-selectin receptor and leukocyte function-associated molecule-1 (81). In addition, PF4 influences monocytes, where it inhibits monocyte apoptosis, increases monocyte differentiation into macrophages, and induces reactive oxygen

species generation (54)

Platelets and cancer

Armand Trousseau observed an increased risk of thrombotic "hypercoagulability" in certain cancer patients that was accompanied with hemostatic and platelet abnormalities (82,83). He also identified a coagulant state in his own blood before his death from pancreatic cancer, leading to the term "Trousseau's syndrome". The molecular mechanism of Trousseau's syndrome was established in the 20th century following studies on tumor cell-induced platelet activation, mainly platelet aggregation (82,83).

The role of platelets in tumor cell proliferation has been demonstrated in animal studies (84–86). Their contribution to metastasis and angiogenesis in various types of tumors, such as colon, breast, lung, ovarian carcinomas, and melanoma, is well-recognized (87,88). The obvious connection between platelets and tumor angiogenesis and growth predicts their potential impact on malignancies (89). Platelets form complex bidirectional interactions with tumor cells, leading to the pathogenesis of malignancies (84). In addition, surface molecules, proteins, and platelet lipids contribute to the progression of inflammation in cancer, and cancer itself (90–92). Indeed, tumor cells express a variety of membrane receptors that bind directly to platelets (85,86).

Platelets contribute to the pathophysiology of cancer in terms of the increase and progression of tumor growth, and offer physical and mechanical support for tumor cells to escape the immune system and move to secondary organs (84). This is achieved by platelet-tumor cell aggregates that mask cancer cells and allow cancer metastasis. Research in this area led to the identification of a wide range of platelet receptors from the α-granules that are involved in malignancy, including cancer cells binding to P-selectin on platelet surface. P- selectin is transported from the α-granule to the surface of activated platelets (93). In P-selectin-deficient mice, tumor growth was reduced in colon carcinoma and melanoma cells (94,95).

Other platelet membrane receptors are involved in cancer metastasis, further supporting the importance of platelets in cancer development (84). This is clearly demonstrated with GPIb-IX-V that has a role in platelet adhesion with injured endothelium. GPIb-IX -V-deficient mice have a 15-fold reduction in melanoma cell metastasis (96). Other membrane receptors, such as GPVI(97) and GPIb-IIIa,(98–100) are also involved in cancer pathophysiology.

PDGF have been shown to be rich and play a role in tumor environment, this role is achieved by autocrine stimulation of the cancer cell, and by the effect on the tumor's interstitial pressure (101,102). PDGF is also expressed by tumor and stromal cells, and drives their carcinogenesis and tumor progression. Targeting PDGF had shown effectiveness on tumors' environment interstitial pressure and augments the effect of chemotherapy in ex-

perimental models (103). In addition, promising results have been found when PDGF signaling was blocked in inhibiting tumor growth in ovarian cancer (104,105), prostate cancer (106), and gliomas cell lines (107).

As platelets contribute to tumor growth and environment, antiplatelet drugs have shown a potential therapeutic strategy as anticancer drugs. Antiplatelet drugs reduce tumor growth, cancer metastasis and angiogenesis in many conditions (108).

Platelets and angiogenesis

Angiogenesis broadly refers to the formation of new blood vessels. This process occurs in physiological (e.g., wound healing, menstrual cycle, and embryonic development) and pathological (e.g., diabetic retinopathy, rheumatoid arthritis, and all types of malignancies) condition (109). Angiogenesis is essential for vascular development and organ growth,(110) and has been linked to tumor formation and prognosis because the newly formed vessels feed the tumor mass by providing it with oxygen and nutrition.

Tumor growth is an angiogenesis-dependent process (109), where platelets contribute to the regulation, modulation, and control of angiogenesis through several pathways, (111) as well as in atherosclerotic plaque formation (79). Activated platelets, at the site of an atherosclerotic lesion, release modulators to recruit endothelial progenitors and monocytes to promote angiogenesis (79). Platelets are rich sources of angiogenesis modulators, but their exact role is not fully elucidated (111). The wide array of pro- and anti-angiogenic factors stored in platelets participate directly and indirectly in physiological and pathological angiogenesis, and tumor growth (109,111).

Platelets are involved in promoting angiogenesis in diverse conditions (80,112,113) including cancer (96,114). Platelets contain pro-angiogenic (activators including vascular endothelial growth factor (VEGF), PDGF, and fibroblast growth factor-2, and matrix metallopeptidase-9) and anti-angiogenic (inhibitors such as endostatin, TSP-1, PF4, plasminogen activation inhibitor-1, and TGF-β) proteins (111). These factors are organized in platelets and megakaryocytes in distinct α-granule populations. These pro- and anti-angiogenic factors are released selectively from platelets in response to different stimuli. This suggests potential clinical implications to target the release of specific factors. Therefore, the role of platelets in controlling angiogenic switches may be defined by the activation of specific cell surface receptors (109).

The role of platelets in regulating angiogenesis has been clearly shown from studies of tumor angiogenesis. VEGF is found abundantly in platelet α-granules. It plays a role in endothelial cell proliferation, migration, and tube formation. VEGF released from platelets is increased in the serum of cancer patients and has been associated with the prognosis of several cancers. Importantly, inhibition of VEGF is associated with a reduction in tumor growth (90).

Another example of a pro-angiogenic factor in platelets is platelet-derived endothelial cell growth factor (PD-ECGF). PD-ECGF is an important angiogenic growth factor and its presence indicates a poor prognosis (91). Both VEGF and PD-ECGF have shown to augment neovascularization in diabetic retinopathy (91,92,115). The release of angiogenic molecules from platelets is agonist dependent. A study of the angiogenic effect of platelets on human umbilical vein endothelial cells showed that ADP activated platelet stimulate the release of VEGF but not endostatin, and this promoted the migration of human umbilical vein endothelial cells and the formation of capillary structures. In contrast, TxA2 stimulated the release of endostatin but not VEGF from platelets, which inhibited cell migration and capillary-like tube formation (111).

Conclusions

Platelets exert a role not only in hemostasis and thrombosis, but also in other conditions. There is an obvious involvement of platelets in inflammation, immunity, cancer, and angiogenesis. Thus, the concept that platelets are at the intersection of these conditions makes them a multifunction cell. Achieving a better understanding of the exact as well as diverse roles and mechanisms of platelet involvement in these conditions is a promising research area and will open a window for new strategies and prevention methods of diseases.

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