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The Platelet as an Immunomodulator: The Old Thespian with New Roles in Atherosclerosis, Sepsis and Autoimmune Disease

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1. Introduction

Models of the inflammatory process depict scenes of a drama that has being studied for millenniums. A drama that began at the macroscopic level, with Celsus and the aphoristic comment "*Vero notae inflammationis sunt quatuor: rubor et tumor cum calore et dolore*"[1] ; with the four cardinal signs of inflammation, followed by Virchow at the microscopic level by proposing a response to insult model, and one that continues to unfold as we develop molecular representations of this process, in an attempt to complete the story of a drama.

> "*If therefore we speak of an irritament*[2]*, we cannot properly intend to attach any other meaning to it, than that, in consequence of some cause or other external to the part which falls into a state of irritation, and acting upon it either directly or through the medium of the blood the composition and constitution of this part undergo alterations which at the same time alter its relations to the neighboring parts (whether they be blood-vessels or other structures) and enable it to attract to itself and absorb from them a larger quantity of matter than usual, and to transform it according to circumstances. Every form of inflammation with which we are acquainted, may be naturally explained in this way*".
>
> Rudolf Virchow 1858

It's an open secret, and we continue to publish it as if it is a surprise or new knowledge even. Whether we realize it or not we have evolved a new field; a field that is defined by the intersection of immunology and hemostasis. Platelets whose main purpose has traditionally been considered as a plug forming device, is currently participating in new paradigms of disease, as a sophisticated mediator in a milieu of chemokines and adhesion molecules which modulate the immune response and consequently inflammation. Studies from

[1] "*But the signs of inflammation are four; redness, and swelling, with heat & pain*"
[2] Inflammatory stimulus

inflammatory diseases such as sepsis, rheumatoid arthritis, and acute lung injury set the stage for modification of the thespian paradigm. One that helps us complete the continuum from coagulation to inflammation and back to coagulation again. Here we propose to name this field of study "Immunohemostasis". No better model to appreciate the crosstalk between coagulation and inflammation than atherosclerosis.

2. Platelets have a role early in the development of atherosclerosis

Although platelets are not solely responsible for the development of atherosclerosis, their contribution to the inception of the vascular lesion, up until to atherothrombosis - its most critical consequence - is conceptually best understood as a model of inflammation. This is somewhat amusingly explained by Rudolf Virchow on a footnote in, *Cellular Pathology* (1865), "Suppose three people were sitting quietly on a bench, and suddenly a stone came and injured one of them, the others would be excited, not only by the sudden appearance of the stone, but also by the injury done to their companion, to whose help they would feel bound to hasten. Here the stone would be the irritant, the injury the irritament, the help an expression of the irritation called forth in the bystanders". Following Dr. Virchow's thought process, modern science not only has documented many different stones but also acknowledges that at times, these bystanders can hasten the *irritament* (inflammatory stimulus), therefore as we will understand an overzealous and excited bystander could prove to be, vessel hardening.

If we look at atherosclerosis as a model of inflammatory disease, platelet adhesion could similarly be regarded as a model of platelet induced disease (Langer & Gawaz, 2008). Atherogenesis is influenced by platelets that adhere to activated vascular endothelial cells and feed chemotactic mediators to adjoining cells. Although the underlying mechanism of atherosclerosis is attributed to endothelial impairment due to insults from genetic and environmental factors (Lusis, 2000), it needs platelet firm adhesion to the endothelium for inception of the atheromatous plaque (Spagnoli et al., 2007). Genetic and environmental factors that trigger injurious events, which include the formation of reactive oxygen species, reduce the bioavailability of nitric oxide (Lowenstein et al., 2005). Then the non-denuded, but aggravated endothelium fails to inhibit control over Weibel–Palade body exocytosis translocating P-selectin and von Willebrand Factor (vWF) from within the granules to the outer cellular surface (Wagner & Frenette, 2008). These two proteins allow the adhesion of platelets to the vascular endothelium in a multistep process. First platelets are tethered to the vascular wall with assistance by endothelial selectins. Platelets then roll on the vascular endothelial cells (Polgar et al., 2005). Depending on further activation of the endothelial cell and expression of endothelial integrins, the platelet adheres firmly to the vascular wall (May et al., 2008), or in the absence of further endothelial activation, the platelet, disengages from the vessel wall and returns to circulation (White, 2007). Remarkably this can occur due to the fact that platelet activation is not required for platelet rolling (Harrison, 2005). In contrast, experimental models of mice infused with activated platelets also stimulate Weibel–Palade body exocytosis, promoting the development of atherosclerosis which is attributed to platelet P-selectin – mediated delivery of platelet-derived proinflammatory factors to monocytes/leukocytes and the vessel wall (Delvaeye & Conway, 2009).

Molecule	Tissue Distribution	Function	Ligand
Selectins			
P-selectin	Endothelium & platelets	Rolling of leukocytes on endothelium and platelets & of platelets on endothelium	PSGL-1
E-selectin	Endothelium	Rolling of leukocytes on endothelium	PSGL-1 ESL-1 CD44
Immunoglobulins			
ICAM-1	Endothelium	Firm adhesion and transmigration of leukocytes	β2-integrins
ICAM-2	Endothelium, dendritic cells	Firm adhesion & transmigration of leukocytes; platelet adhesion to leukocytes	β2-integrins
VCAM-1	Endothelium	Firm adhesion & transmigration of leukocytes	α4-integrins
PECAM-1	Leukocytes, endothelial cell-cell junctions	Transmigration	PECAM-1
Glycoprotein			
von Willebrand Factor	Endothelium, α-granules of platelets, & subendothelial connective tissue	Binding to other proteins, most efficient under high shear stress	Factor VIII, Collagen, Platelet GPIb
Glycoprotein IV	Platelets & monocytes	Scavenger receptor, implicated in hemostasis, thrombosis, inflammation, lipid metabolism & atherogenesis	Collagen, thrombospondin, platelet-agglutinating protein p37, oxLDL & long-chain fatty acids
Integrins			
β2-integrins	Leukocytes	Firm adhesion to endothelium & platelets	ICAMs VCAM fibrinogen
MAC-1	Polymorphonuclear leukocytes, NK cells, & mononuclear phagocytes	Pattern recognition receptor causes phagocytosis	various
β3-integrins	Platelets & neutrophils/endothelium	Firm cell adhesion	Fibrinogen; ECM
IIβIIIα	Platelets	Aids in platelet activation	Fibrinogen, vWF and fibronectin
Tumor necrosis factor family			
CD40 **CD40L**	endothelium, leukocyte & platelet	Activates different endothelium, leukocyte & platelet function	CD40L CD40; αIIbβ3 on platelets

Table 1. Major Receptor molecules in endothelium, platelet, and leukocyte interactions (Modified from Harrison, 2005)

Activated platelets propel inflammation, by forming platelet-leukocyte complexes which facilitate leukocyte migration into the arterial wall. Since the density of P-selectin on platelets after activation is much higher than on endothelium, leukocytes are easily recruited to the adherent activated platelets (White, 2007). In these dynamics, the balance between homeostasis and inflammation is easily shifted to inflammation in a vicious cycle as soluble P-selectin, shed from activated platelets and endothelium, stimulate leukocytes to produce tissue factor which subsequently activates more platelets (Vicic & Weiss, 1983). In further interaction with leukocytes; polymorphonuclear cells adhere to platelets in a Mac-1–dependent manner, inducing complex activation cascades in monocytes that promote monocyte or neutrophil adhesion, thrombosis, monocytic chemokine and cytokine release, or the oxidative burst of neutrophils (Rivera et al., 2009). See table 1. Among the many receptor pairs, that contribute to neutrophil activation in platelet-neutrophil interactions, there is neutrophil surface triggering receptor expressed on myeloid cells 1 (TREM-1) and platelet surface TREM-1 ligand, although not required for platelet-neutrophil aggregate formation, cellular interactions involving this receptor pair induce respiratory burst activity and IL-8 secretion in neutrophils which attract leukocytes and further activate other platelets (Minors, 2007).

By no means are platelets, endothelial cells and leukocytes innocent bystanders; through years of evolution these eager to protect cells fight infection, hemorrhage and constant insults. In the theater of inflammation, as it often happens, we undergo friendly fire, which make our vessels harder but not tougher. Without regard to its etiology, the interactions between platelets, endothelial cells, and leukocytes as a result of platelet activation, endothelial dysfunction and leukocyte adhesion, causes inflammatory responses which lead to the origin and establishment of atherosclerosis.

3. Platelets resourceful machinery in function

Platelets are the smallest of the many types of cells in circulating blood, with an average size of only 2.0 to 5.0 μm in diameter, 0.5 μm in thickness, with a mean cell volume of 6 to 10 femtoliters (Riddel, Jr. et al., 2007). Platelets are anucleate, discoid shaped blood cells that partake in pathophysiological roles like: hemostasis, thrombosis, clot retraction, vessel constriction/repair, inflammation and other aspects of host defense (Austin S.K., 2008). They are critical players in immune oversight and an important key to cellular interactions during the coagulation and inflammation process (Brass, 2010). Their sheer number, shape and small size make possible for platelets to localize to the vessel wall under flow, making possible a constant survey of the wall integrity (Coughlin, 2000). Platelets contain scarce functional mitochondria, glycogen, an intricate membranous system, and three major morphologically different, secretory organelles which are: α-granules, dense core granules & lysosomes (Coughlin, 2005). Inside the α-granules can be found a wide variety of coagulation/adhesive proteins, growth factors and protease inhibitors involved in both primary and secondary hemostatic mechanisms (Kahn et al., 1999). The platelet membrane is covered by a wide variety of mobile transmembrane receptors, including integrins (e.g., αIIbβ3: IIβ IIIα), leucine-rich repeated receptors (e.g., GPIb/IX/V), G-protein coupled seven transmembrane receptors (e.g., PAR-1 and PAR-4 thrombin receptors) and C-type lectin receptors (e.g., P-selectin) (Coughlin, 2000).

Under normal conditions, hemostasis occurs by two independent, but related processes: the platelet activation pathway and the coagulation cascade (Sivaraman & Latour, 2011).

The primary role of platelets in hemostasis is the formation of an initial plug at the site of the vascular injury or as commonly known, primary hemostasis. The formation of a stable plug consists of three principal events: adhesion, activation and aggregation. When the vessel wall is damaged, it exposes the blood to subendothelial collagen and microfibrilis, which stimulate the initial step that allows platelet adhesion (Delvaeye & Conway, 2009). Adhesion is also mediated via von Willebrand Factor (vWF), a multimeric protein synthesized by endothelial cells that serves as a bridge between the tissue and platelets. vWF binds to both exposed collagen at sites of vascular injury and the platelet membrane glycoprotein Ib-V-IX (GPIb-V-IX) receptor complex (Romney & Glick, 2009). Platelet activation is caused by exposure of various agonists, such as thrombin, thromboxane A2 (TxA_2), adenosine diphosphate (ADP), collagen, and arachidionic acid to their particular receptors (Perez-Gomez & Bover, 2007). One example is the G protein-coupled receptors on the platelet surface whose ligand are TxA_2 , ADP, and thrombin (Satran & Almog, 2003).

The PARs are G-protein-coupled receptors that use a unique mechanism to convert an extracellular proteolytic cleavage of the receptor into a transmembrane signal (Eyre L. & Gamlin F., 2010), hence the derivation of the thrombin receptor activating peptide (TRAP). Human platelets express two different G-coupled protease activated receptors, PAR-1 and PAR-4, while mouse platelets express PAR-3 and PAR-4 (Brass, 2010). In human platelets, PAR-1 is a high-affinity receptor that is activated at low concentrations of thrombin, PAR-4 is the lower-affinity receptor that mediates thrombin signaling at higher concentrations of thrombin than PAR-1 (Johns, 2004), but it initiates signaling for a more extended duration that its PAR-1 counterpart. The activation of both PAR-1 and PAR-4 is enough to trigger platelet secretion and aggregation (Cambien & Wagner, 2004).

Activation of platelets results in a conformational change from normal disc shape to a compact sphere with long dendritic extensions called pseudopods, which facilitate platelet-platelet interaction. This process alters the membrane permeability and allows the entry of calcium into the platelet cytosol, leading to integrin activation. Following platelet activation, the membrane receptor glycoprotein IIβ/IIIα (IIβ IIIα) undergoes a conformational change, which takes us to the adhesion phase mediated by platelet IIβ IIIα binding to fibrin(ogen), allowing the formation of multiple stable crosslinks between adjacent platelets. The GPIb-IX-V receptor complex and IIβ IIIα are two unique platelets receptors responsible of platelet adhesion and thrombus formation to regulate hemostasis (Furie & Furie, 2004).

A revised model of hemostasis described by Satran & Almog (Satran & Almog, 2003), emphasizes the role of different cell surfaces in the localization and control of the coagulation processes, this includes three overlapping phases: initiation, amplification, and propagation. Tissue Factor (TF) is the key initiator of coagulation and is expressed primarily by subendothelial mural cells and adventitial fibroblast in and around the vessel wall (Filice & Niewoehner, 1987). The initiation phase starts when exposed collagen causes accumulation and activation of platelets, while exposed TF initiates the process of generating thrombin through binding to factor VII, creating the tissue factor-factor VIIa complex (TF/FVIIa) (Spitznagel & Shafer, 1985). TF/FVIIa complex activates factor X, either directly or indirectly via factor IX, and transforms prothrombin into thrombin in small amounts that are insufficient to complete the process of fibrin formation. The amplification phase occurs after platelet adhesion to the initiation site while in a state of partial activation. At that point thrombin, generated in the initiation phase and responsible for activating coagulation factors V, VIII, and IX, binds to platelets enhancing both their binding to the

vessel injury and activation. In the propagation phase the generation of thrombin is continuous. Factor X_a production is maintained by intrinsic tenase complex which, consist of factor $VIII_a$ the cofactor for factor IX_a (Spitznagel & Shafer, 1985). These three overlapping phases allow a fibrin platelet clot to form over an area of injury; this clotting process is limited by negative feedback by activated protein C which inactivates factors V_a and $VIII_a$ to avoid thrombotic occlusion in surrounding normal areas of the vasculature. The formation of a stable hemostatic plug is possible by important platelets properties which include their shape, the secretory granules, high density regulatory and adhesion receptors, and the ability to promote thrombin generation (Goncalves et al., 2011). The coagulation cascade, although important to platelet activation, is outside of the scope of this review

Platelet disorders can be divided into two categories: quantitative and qualitative. Quantitative defects are abnormalities in platelet number, whereas qualitative defects are abnormalities in platelet function (Siljander, 2011). This classification is somewhat random as some platelet disorders are characterized by both decreased number and function (Heijnen et al., 1999). Platelet defects such as storage granule diseases like Hermansky–Pudlak & Grey platelet syndromes or Glansmann thrombasthenia in which the platelets lack IIβIIIα are rare qualitative diseases.

An important receptor in platelet interactions which has being studied in models of qualitative platelets defects is P-selectin (CD62P), formerly known as PADGEM or GMP-140, is a member of the selectin family of cell adhesion receptors, and as its name implies, it is a lectin that binds various sugar moieties (Castaman et al., 1996). P-selectin , is stored premade in the Weibel-Palade bodies of endothelial cells and the platelet α-granules (Berckmans et al., 2001) and remains inaccessible when these cells are in the resting state (Wolf et al., 1999).

Activation of either/or both platelets and endothelial cells brings P-selectin to the cell surface. Thus control of P-selection dependant interactions is conditional of the presence of P-selectin on the cell that is in the active state. Upon cell activation, P-selectin is translocated to the plasma membrane, mediating the interaction of stimulated platelets and endothelial cells with leukocytes though its interaction with P-selectin glycosylated ligand (PSGL-1). PSGL-1 is a constitutively expressed receptor on platelets, leukocytes, and a subset of lymphocytes (Heemskerk et al., 2002) (psgl-1 does bind E- and L selectin as well). P-selectin mediates the formation of preliminary platelet-leukocyte aggregates (Yang & Wilson, 1996). Early studies on P-selectin demonstrated that P-selectin mediated *in vitro* platelet-leukocyte interactions and induced leukocyte rolling in a flow chamber system (Elzey et al., 2003). These studies were confirmed in the P-selectin null mouse where leukocyte rolling was attenuated and neutrophil migration to the peritoneum was delayed (Amabile et al., 2010). The implications of these findings have been profound and have set the stage for us to discern the relationship between hemostasis and inflammation.

4. Platelets as dedicated players in immune function

The inflammatory process in the classical sense has been defined by the innate and adaptive systems where early contact to foreign invaders is mediated by the innate compartment and if the danger persists the adaptive compartment will assist. There is an intricate series of communication behaviors within and between these compartments that include physical interaction and a series of chemical signals which regulate the migration of the white blood

cell population. Although we understand many of the individual parts, the system as a whole gets very complicated. The innate immune response is designed as our first response by identifying pieces of our puzzle that just don't fit. Using a series of basic pattern recognition receptors, the innate immune system identifies foreign substances and seeks to destroy or remove them from our body. The cells of the innate immune system have a complicated system of chemical communications that act as sentinels attractants or differentiation agents that push the limits of the innate immune system to adapt to the problem at hand. Traditionally, natural killer cells, monocytes, monocyte derived cells and polymorphonuclear cells (a vast majority of which are neutrophils) form the major portion of our innate immune system. Complement is a series of soluble factors that form a cascade, similar to the serine proteases of the coagulation cascade, that results in lysis of microbial and apoptotic cells. Platelets not having the ability to rearrange receptors to acquire antigen specificity and having the ability to respond quickly to insult would be considered a member of the innate immune system.

4.1 Platelet regulation of neutrophil function

Foreign invaders are recognized by neutrophils and neutrophils are considered to form our first line of defense (Puddu et al., 2010). Next to platelets, neutrophils are the most numerous white blood cells in our body and they are innate immune effectors. Neutrophils are dangerous to foreign substances as well as to ourselves if left unchecked. When they identify foreign invaders they use one of several killing mechanisms to dispose of the identified threat. Neutrophils will phagocytose bacteria or when the conditions are right they will release their granule contents causing non-specific damage to the quarry as well as the host (Chen et al., 1995). Here we will argue that even though neutrophils MAY be our first line of defense, they need platelets to regulate their decision making at point of endothelial contact (figure 1). Platelet regulation of neutrophil function is best introduced by evaluation of platelet depletion studies.

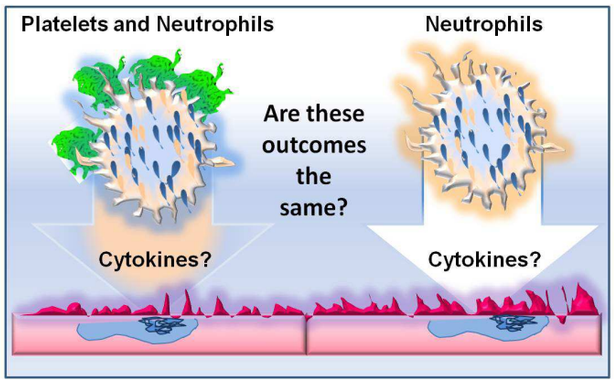

Fig. 1. Do platelets maintain vascular integrity preemptively by regulating neutrophil function?

Over the past five years, a wealth of investigators has used platelet depletion in mouse based disease models to better understand the platelet's role in inflammation. These studies have begun to show that the depletion of platelets leads to abbreviated neutrophil derived inflammation and neutrophil transendothelial migration. For example, using a corneal epithelium abrasion model, (Lam et al., 2011) demonstrated that platelet depletion lowers

neutrophil transmigration across corneal endothelium at six and twelve hours. Using P-selectin glycosylated ligand (psgl)-1 null mice Zarbock et al. implicate the psgl-1/P-selectin ligand pair as a key regulator in the connection between platelets and neutrophils. In models of acute lung injury, platelet depletion reduced neutrophil influx (Zarbock et al., 2006). Zarbock and colleagues show that platelets recruit neutrophils to the lungs and use P-selectin and TxA_2 dependant mechanisms to mediate damage. TxA_2 is an arachadonic acid derived inflammatory mediator librated from activated platelets (Zarbock et al., 2006). Using a two hit lung injury model Looney et al., demonstrated that platelet depletion actually seemed to improve survival outcomes (Looney et al., 2009). The two hit model was shown to be P-selectin independent, but the investigator's use of aspirin, which lowered lung injury and improved survivorship supports a role for TxA_2. Aspirin inhibits TxA_2 production from platelets. Kornerup et al., used two different models (LPS inhalation and xymosan induced peritonitis) to show that platelet depletion reduces neutrophil recruitment to the inflammatory site in the lungs and the liver (Kornerup et al., 2010). Their data suggests that platelets activate the neutrophils forming platelet/neutrophil complexes which aid in neutrophil transmigration. Depletion of platelets was also shown to lower recruitment of eosinophils and lymphocytes in allergic inflammation (Pitchford et al., 2005), and apparently increases the efficacy of breast cancer chemotherapy (Demers et al., 2011). Taken together, therapeutic targeting of inflammation may need to focus on platelet function in addition, if not totally, to control the immune response.

A recent platelet derived mechanism that was identified is the neutrophil extracellular traps (NETs: (Clark et al., 2007), in which the toll like receptor on platelets recognizes its ligand and mediates platelets to influence to neutrophils to release their DNA. The DNA is used to ensnare bacteria in the lungs and liver sinusoids. Neutrophils are born to die protecting their host, in many ways they are like larger nucleated platelets performing many of the same functions including: aggregation, microparticle release, and phagocytosis, with the addition of the ability to extravate into the tissue. There are two points to be made here: first, in the neutrophil NET study, they demonstrate that platelet recruitment to the lungs during sepsis is neutrophil dependent, a finding that is in stark contrast to the work by Zarbock, where they show the opposite. Secondly, they clearly show a different outcome as a result of platelet-neutrophil interactions. Why were these outcomes so very different, although they used similar stimulants (LPS)?

4.2 Monocytes and platelets line up to work against infection

The second part of the innate immune system is monocytes and monocyte derived cells (macrophages). Monocytes are innate immune regulators with the responsibility to clean up the debris left from dying and apoptotic neutrophils that have engaged in battle. Monocytes usually arrive to scenes of inflammation after neutrophils and platelets and will persist in the inflamed tissue until the infection subsides. The expression of tissue factor by monocytes will attract the coagulation factor VII_a and is believed to be a primary cause for the initiation of the coagulation cascade and subsequent deregulation of hemostasis during sepsis. Monocytes, however, are also the primary link between the innate and the adaptive immune system (Ziegler-Heitbrock, 2007). Monocytes maintain the ability to differentiate into tissue macrophages or dendritic cells which present antigen to lymphocytes and prime the adaptive immune system. Once in the tissue, monocytes quickly mature into tissue macrophages and they are often referred to by a different name, dependant on the tissue.

For example the resident macrophage of the liver is called the Kupffer cell. Tissue macrophages release a battery of cytokines and chemokines that gage the enormity of the task at hand using chemoattractants to call in neutrophils, platelets, or more macrophages if deemed necessary. In a model of sepsis, where LPS from *Klebsiella* was given intravenously, Kupffer cells were shown to be responsible for the recruitment of platelets to the liver (Yamaguchi et al., 2006). However, in a model of *Leishmaina major*, platelets were demonstrated to recruit monocytes indirectly through the release of platelet derived growth factor (PDGF) (Goncalves et al., 2011). The model that the Goncalves et al., puts forth suggests that the release of PDGF from activated platelets cause a release of the cytokine CCL2 from a multitude of cell types which in turn attracts monocytes. Platelet depletion reduced the accumulation of effector monocytes and reduced clearance of *Leishmanina*, therefore demonstrating the importance of platelets in the removal of parasitic infections. Interestingly, platelet activation was dependent on complement factor 3 (C3), allowing us to address in the next scene in our drama, platelet interaction with complement.

4.3 Complement and new pathways in platelet function

Complement is a major immune regulation pathway. The complement system is ancient, abundant, and redundant (Ricklin et al., 2010). Not only is the system ancient, so has been our understanding of complement until recently. The biochemical origin of our understanding of complement is revealed by the terminology used with complement components, which are called factors. Much like the coagulation system where fractions of blood components with activity were isolated and its activity was given a number; our understanding of the complement system has grown from these obscure beginnings. Molecular genetics has breathed new life into our understanding this, one of our oldest and most conserved systems. In immunity, complement is a major component in the control of bacterial infection. In a process called, opsonization, a cascade involving a series serine proteases and proteins result in a membrane attack complex or MAC that forms pores on foreign cells such as bacteria and apoptotic cells and is chemoattractant to white blood cells.

Complement in its most basic sense is focused around factor C3 (Lambris et al., 2008). There are three major pathways that lead to the formation of C3 activators or convertases. These pathways are the lectin, classical, and alternative pathways. The lectin pathway is activated by the mannose binding protein leading to the formation of the C3 convertase 4b2b. However, to date not much information in regards to the lectin pathway and platelets is published and we will focus this portion of the review on the classical and alternative pathways of complement activation.

The classical pathway is most commonly activated when complement factor C1q interacts with IgG or IgM (MacKenzie et al., 1971). The binding of C1q to immunoglobulin allows the complement/Ig complex to activate components C1r and C1s, leading to the cleavage of C4 and C2. C4 is cleaved into C4a and C4b. While C4a diffuses away, C4b is momentarily enzymatically active and may form covalent bonds with the complement/Ig complex or bind to endothelial cells. In the event that neither of these options happen, the interaction of C4b with the surrounding water converts C4b into a ligand for C2 allowing C2's conversion into C2a and C2b by C1s. C2b bound to C4b forms the C3 convertase C4b2b cleaving the central complement factor C3 into C3a and C3b. (there is controversy in the literature to nomenclature of the C4b2b convertase, in some references it is called C4b2a). Platelets contain C1q and have demonstrated activity of the classical pathway (Nayak et al., 2010; Yin

et al., 2007). Platelet activation of the classical pathway is associated with anti-phospholipid syndrome and immune thrombocytopenia purpura (Peerschke et al., 2010).

The alternative pathway is responsible for up to 95% of the activated C3b (Bexborn et al., 2008). The tick over theory provides a model for the activation of the alternative pathway. C3 is relatively dormant in circulation; however a small amount is spontaneously activated to $C3_{H2O}$, and provides windows of opportunity for C3 to behave essentially as a pattern receptor recognizing potentially harmful substances. $C3_{H2O}$ is primed to bind factor B and subsequently cleaved by factor D into C3a which is chemoattractive and the C3 convertase, C3b (Fearon et al., 1973; Fearon & Austen, 1975). Under the correct circumstances C3b will insert into cells causing an increase in deposition of C3b eventually tipping the scales toward complement opsonization of cells and initiation of the complement cascade (Bexborn et al., 2008). Increased C3b activates C5 leading to the deposition of the C5-9 membrane attack complex (MAC), which functions to produce pores in cells eventually leading to cell destruction.

Opsonization of cells by C1, C3b and C5b can also lead to phagocytosis when complement bound cells are recognized by their corresponding receptors (Ricklin et al., 2010). CD35 (CR1), which is found on many white blood cells and in the granules of neutrophils, recognizes C3b and C4b deposited on cells mediating phagocytosis. The integrins CD11b (CR3) and CD11c (CR4: both of these integrins are complexed with CD18 (Gahmberg, 1997)) mediate binding to complement iC3b which is the inactivated form of C3b, and mediates phagocytosis as well (Arnaout, 1990; Ross, 2000). Although CD11c is constitutively expressed on neutrophils both CD11b/CD18 complexes and CD35 are stored in neutrophil granules and are upregulated on activated neutrophils. When deposition of complement is in the endothelium, neutrophils are prompted to release their granules and phagocytize opsonized cells (Yin et al., 2007). It is suspected that complement deposition is a major cause of loss of vascular integrity, edema, and bleeding associated with inflammation.

Tight regulation is maintained on complement. An overzealous complement system will lead to self-attack of endothelium and may be an initiation factor of pathways leading to hemorrhage. Factor I (fI) is a major inhibitor of C3b and in conjunction with Factor H (fH) mediate the deceleration of the MAC formation. Factor H is a cofactor in fI binding and deactivating C3b that has bound the cell surface (Paixao-Cavalcante et al., 2009). Once bound, active C3b is transformed to iC3b which stops further opsonization, iC3b becomes the ligand of neutrophils and monocytes expressing CD11b or CD11c. Oddly enough, CD35 while initiating phagocytosis also mediates inactivation by fI (Ricklin et al., 2010).

Mutations in factor H are associated with hemolytic uremic sysdrome (HUS) (Licht et al., 2009; Stahl et al., 2008). Studies in fH deficient mice show that platelets are in large part responsible for this association. Platelets are a sink for fH (Vaziri-Sani et al., 2005). Platelets uptake fH from the plasma and store fH in various locations including the α-granules (Devine & Rosse, 1987; Licht et al., 2009). Mutations in fH lead to increased complement deposition on platelets and increased platelet activation (Stahl et al., 2008). These findings are consistent with the thrombocytopenia and thrombus formation seen in the kidneys of HUS patients.

Platelets are not immune to opsonization and there are numerous reports of complement binding to platelets, but these waters remain murky. Upon platelet activation there is a drastic increase in the binding of each of the anaphalaxins (C3b, C4b and C5 - 9) as well as C1q (Peerschke & Ghebrehiwet, 1997). Furthermore, P-selectin has recently been shown to propagate C3 activation opening a point of possible crosstalk between the hemostatic and

innate immune systems (Del Conde et al., 2005). Subsequent studies, however, suggest that even though C3 binds to activated platelets it doesn't necessitate proteolytic activation. The C3 associated with platelets was estimated to be $C3_{H2O}$ containing an exposed thioester, which in the presence of fH and fI is inactivated rapidly (Martel et al., 2011). Coincidently, the C3 associated with the platelets facilitated binding of a soluble CR1, consistent with the idea that platelets facilitate the inactivation of the $C3_{H2O}$. In fact, in support of this idea, Martel et al. demonstrate that the C5-9 complex will form on less than 15% of platelets indicating that the MAC pathway is not a major form of platelet destruction or mechanism of platelet immune crosstalk (Martel et al., 2011). They suggest that the binding of the C5–9 complex may contribute to micropartical formation. Thus the binding and activation of C3b by P-selectin may need additional triggers such as sheer to induce C3 activation.

The studies in patients with aHUS strongly suggest that the regulation of C3b is a major mechanistic contact point between the immune and hemostatic systems. Platelets from patients lacking a functional fH, have increased deposits of C3 and C5–9 and are subject to complement mediated activation. As mentioned above, aHUS patients have the tendency to develop microthrombi in their kidneys that lead to kidney damage and accordingly they suffer from thrombocytopenia (Hirahashi et al., 2009; Stave & Croker, 1984). Thus, the ratio between C3 and fH represent the delicate balance that has to be overcome for complement to involve platelet participation. Early sepsis studies demonstrated that C3 depletion alleviated the thrombocytopenia associated with the sepsis response (Ulevitch & Cochrane, 1978), supporting the notion that from the immune system, it is C3 that initiates the participation of platelets. Here we submit that the complement cascade is the immunological system's major pathway to platelet participation (see figure 2). Complement activates platelets in a manner similar to the coagulation system, but with less of a robust reaction. A complement derived platelet activation cascade would not be to form an occlusive clot but localized thrombi, such as that to contain bacterial infection. Complement activation may lead to differential release of specific subsets of platelet α-granules (Italiano, Jr. et al., 2008). When platelets are depleted, complement activation leads to hemorrhage (Goerge et al., 2008).

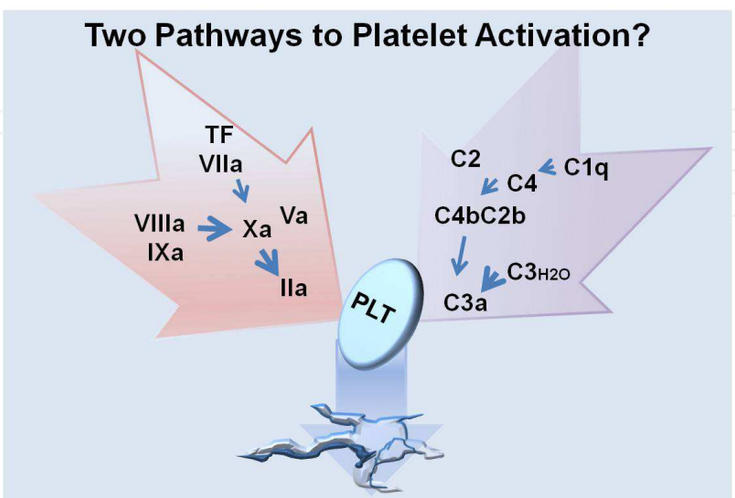

Fig. 2. Platelets are activated by both coagulation and complement cascades. The complement cascade represents the immune systems access to platelet function.

4.4 Adaptive immunity

Although we will not focus on adaptive immunity in this review, we will mention that lymphocytes comprise our adaptive immune system. Lymphocytes produce molecules that are derived to specifically recognize foreign potentially dangerous substances. Humoral immunity, mediated by B-cells, involves the production of antibodies. B-Cells will produce classes of antibodies that specifically recognize regional markers on foreign substances and cause clustering of the identified antigen into immune complexes (ICs). ICs can be bound by complement and targeted for destruction, or are directly removed by the cells of our innate immune system which include neutrophils and monocytes.

Oddly enough, the antibodies produced by B-cells are made in a sequential manner. For example, the pentametric IgM class is made first. IgM is subsequently changed to dimeric IgG in a process called class switching. Class switching is regulated by the CD40-CD40 ligand (CD40L) interaction. Mice lacking CD40L develop a hyper IgM syndrome, where the B-cells fail to change from production of IgM to IgG. CD40L is expressed on platelets and is believed to be a major source of CD40L. Transfer of wild type platelets to CD40L null mice rescues this syndrome, demonstrating an important regulatory role for platelets in the adaptive immune system (Elzey et al., 2003).

T-lymphocytes or T-cells mediate cellular immunity through a T-cell receptor that is made to specifically recognize one antigen. The T-cell receptor directs the T-cell to its antigen where it mediates a supportive function (T-helper cells) or can mediate cytotoxicity (cytotoxic T-cells). Cytotoxic T-cells recognize cells in distress such as cancer or virally infected cells. Using two different models of hepatic viral infection Iannacone et al. demonstrated that platelet depletion during a hepatic viral infection lowered the amount of cytotoxic T-cells that entered the liver and lowering hepatic cell damage. However, even though there was reduced liver damage, viral clearance was also reduced. Reintroduction of activated platelets restored the hepatic damage, but also allowed the viral load to be removed (Iannacone et al., 2005). In the theatrical setting of adaptive immunity, platelets demonstrate an indispensible role.

5. Platelet derived microparticles fuel the inflammatory response

Microparticles are plasma membrane-derived vesicles shed from stimulated cells which include leukocytes, erythrocytes, endothelial cells, various cells in the central nervous system (CNS), adipocytes and platelets (Siljander, 2011). Platelet microparticles (PMP) are small membrane-coated vesicles which circulate in the blood stream. PMP are estimated to vary in size from 0.1–1.0 μm with the smaller particles originating from α-granules and the larger particles being derived from the plasma membrane (Heijnen et al., 1999). There is increasing evidence that these submicron fragments, termed microparticles, have important physiological roles. A good example is Castaman defect, which is a deficiency in the ability to generate platelet microparticles from platelets that is associated with a bleeding tendency (Castaman et al., 1996).

PMP constitute for approximately 70-90% of all microparticles (Berckmans et al., 2001). Recently it was found, that megakaryocytes generate microparticles. Using a system where particles bearing CD62 or LAMP-1 were considered to be platelet derived and those that expressed filamin A were megakaryocyte derived. Flaumenhaft et al., determined that the microparticles circulating in healthy individuals are surprisingly predominantly megakaryocyte derived (Flaumenhaft et al., 2009). Characterization of microparticles, using

proteomics and functional approaches, has shown that PMP can be separated into different size classes. Each of these size classes can differ in protein components, protein/lipid ratio, and functional effects on neighboring platelets and endothelial cells. When separated by centrifugation, PMP can be separated into four fractions: two of which (fractions 2 and 3) the phospholipid/protein ratios are typical of the plasma membranes, while the composition of fractions 1 and 4 indicate a greater proportion of lipid (Dean et al., 2009). The study of PMP has evolved rapidly, putting them as central mediators of many physiological processes such as inflammation. This part of the chapter will describe the origin of PMP and discuss their role in the inflammatory process under different conditions.

PMP are mainly released by the α-granules and multivesicular bodies of activated platelets, having exposed phosphatidylserine (PS), and resembling apoptotic cells. In a cell, aminophospholipids such as PS and phosphatidylethanolamine (PEA) are specifically segregated on the internal leaflet of the plasma membrane, whereas phosphatidylcholine and sphingomyelin are enriched on the external one. The greater proportion of PS exposed on the surface of activated platelets is reflected in PMP derived from the platelet membrane. A significant and sustained increase of cytosolic $Ca2^{+}$ accompanying cell stimulation may lead to the collapse of the membrane asymmetry by stimulating scramblase and floppase enzymatic activities and concomitantly inhibiting the flippase. The mechanisms involved in PMP release include platelet activation, which is calcium-dependant and Bak/Bax/caspase mediated release, which is independent of platelet activation (Schoenwaelder et al., 2009). The calcium dependant mechanisms involve membrane fragmentation or blebbing, loss of membrane integrity, microvesiculation, and the exposure of PS on the platelet surface followed by release of the PMP caused by cytoskeleton degradation of filamin 1A and gelsolin by Ca2+-dependent calpalin (Wolf et al., 1999). Bak/Bax mechanisms described by Schoenwaelder *et al.*, showed that prosurvival Bcl-xL maintains platelet viability, primarily by restraining the pro-apoptotic function of Bak (Schoenwaelder et al., 2009). It has been reported that Bcl-xL levels decline in stored platelets. This is consistent with an increase of PS exposure seen in platelets, a process which requires the Bak/Bax proteins, which play a central role in apoptosis. The release of PS expressing PMPs increases platelet procoagulant activity. Accordingly, the mechanisms of PMP release affect the size, constituents and function of the PMP.

5.1 Functional importance of platelet microparticles

PMP function is largely defined by the receptors and any molecules trapped in and on the vesicle at the time of release. PMP reflects a subset of the total receptors on the platelet surface or from the platelet α-granules at any given time. The major functional significance of these bioactive vesicles is associated with its procoagulant activity. For example, PMPs are enriched with membrane receptors for coagulation factor V_a and the fibrinogen receptor IIβ IIIα. When PS is exposed on the surface of activated platelets, membrane assembly of coagulation factor complexes occurs (Heemskerk et al., 2002). The presence of factor Va and PS form the foundation of the tenase complex and facilitate the assembly of the prothombinase complex. PMPs with IIβ IIIα facilitate polymerization though the binding of fibrin forming mini scaffolding stations that support the generation of thrombin. Together these molecules provide a catalytic surface for the prothrombinase reaction, thus contributing to the acceleration of thrombin generation (Ando et al., 2002). PMPs can also become membrane templates for fibrinolytic and proteolytic factors as well.

5.2 PMP and disease

CD40 is a receptor expressed on a wide range of cells such as B cells, neutrophils, monocytes, macrophages, platelets, dendritic cells, endothelial cells, fibroblast, keratinocytes and smooth muscle cells; modulating humoral and cellular immunity. As a member of the tumor necrosis factor (TNF) superfamily, the activation of CD40 by CD40L (CD40L/CD154) plays a crucial role for the development and progression of a variety of inflammatory processes including T-cell priming, enhanced T-cell cytotoxicity, and T-cell depended B-cell responses (Yang & Wilson, 1996). As mentioned above, platelets express CD40L. Matrix metalloproteinase-2 (MMP-2) releases CD40L from the platelet membrane creating the bioactive soluble fragment, sCD40L. Platelets are responsible for the production of approximately 95% of sCD40L in the peripheral blood (Elzey et al., 2003). Furthermore, CD40L is found on PMP which implicates PMPs in several diseases. Multiple sclerosis (MS), for example, is characterized by the formation sclerotic plaques in the central nervous system. During MS, the cells responsible for the production of myelin sheaths, oligodendrocytes, are destroyed. Auto reactive T cells attack the sheaths, causing neuronal destruction. In the case of MS, chronic platelet activation leads to the release of CD40L, implicating PMP as a potential effector in the development of MS.

The autoimmune disease, rheumatoid arthritis (RA), manifests as a chronic inflammation of the synovial lining in the joints, which results in pain, swelling and in the most severe cases a destruction of the bone and cartilage. In an elegant study, Boilard et al., implicate PMPs in the pathology of RA. They were investigating the presence of platelets in the synovial fluid of RA patients. Using flow cytometry and the platelet specific marker, CD41 to detect the platelet specific integrin IIβ IIIα, they demonstrated an average of $2x10^5$ PMP/μl in RA patients compared to non-detectable levels in patients with osteoarthritis. Interestingly enough there were a subset of platelets that rosetted the leukocytes that were present in the synovial fluid (Boilard et al., 2010). RA can be induced in mice by the passive transfer of immunoglobulin G (IgG) auto-antibodies from K/BxN mice to wild type. Using the K/BxN system, the authors demonstrated that platelet depletion greatly reduced arthritic symptoms, supporting a role for platelets in the progression of RA. They subsequently determined using glycoprotein VI (GPVI) deficient mice that the microparticles were generated by activation by collagen and that these PMPs contained the ability to release IL-1. IL-1 subsequently induced proinflammatory cytokines IL-6 and neutrophil chemoattractant IL-8 production leading to the recruitment of neutrophils. Thus, PMP are major regulators of RA. The finding answered several nuances found in patients with RA. For instance how come patients with RA have platelet proteins in their synovial fluid but no platelets? Here the aerosolized platelets in the form of PMP delivered discrete packets of platelet function resulting in bone damage and the recruitment of neutrophils by an indirect method.

Therefore, aerosolized PMPs allow the delivery of skewed platelet function as opposed to the complete regulatory balance of platelet function. The recruitment of inflammatory cells at the sites of vessels injury can drive the development of arthrosclerosis, in which the participation of PMP is mostly the formation of the plaque and its progression (Amabile et al., 2010). PMP participate by amplifying and sustaining coagulation and inflammatory response after the rupture of the plaque (Puddu et al., 2010). β-amyloid, for example, is found in PMP and is implicated in the progression of Alzheimer disease. Platelets are the major source of β-amyloid in the peripheral blood (Chen et al., 1995). In fact, it was shown

that β-amyloid secretion supersedes that of all other proteins shed from the platelet surface upon activation (Fong et al., 2011). Accordingly, PMPs have been cited as carriers of soluble Alzheimer's beta amyloid (Matsubara et al., 2002). In the case of cancer PMP contributes to the metastasis by producing MMP and VEGF (vascular endothelial growth factor) among other important factors. Following a paradigm where platelets are strictly hemostatic vessels, the delivery of PMP would strictly cause coagulatory disarray. However, here we show that while PMP does play a coagulatory role in situations like atherosclerosis, they also play diverse role like those seen with activation of T-cells or exacerbation or RA in the synovial fluid of joints. PMPs may be important force working toward unresolved inflammation as described by Khatami, where two opposing inflammatory forces (wound healing and apoptotic) are shifted leading to a chronic inflammatory state (Khatami, 2011).

6. Immunohemostasis

Our laboratory's interest in the intersection of inflammation and hemostasis is derived from the study of a platelet specific gene that mediates platelet involvement in inflammation called TREM-Like transcript (TLT)-1 (Washington et al., 2002). TLT-1 is a single Ig domain receptor found on platelets and megakaryocytes (Barrow et al., 2004; Washington et al., 2004). Like the adhesion molecule p-selectin, TLT-1 is stored in the platelet α-granules until activation when it is quickly bought to the surface (Washington et al., 2004).

6.1 TLT-1

The TLT-1 gene (*treml-1*) is situated in the TREM cluster, amongst a group of immunoregulatory receptors that are found on leukocytes and endothelial cells (Allcock et al., 2003). TLT-1 is the first member of the cluster found on platelets giving it a unique role among the TREM family of genes (note; TLT-2 has recently been found in platelet releasates suggesting it is present on platelets; (Fong et al., 2011)). There are two published forms of TLT-1 which include a long form that has several interesting motifs in the cytoplasmic domain and a splice variant form that only has a 16 amino acid tail (Allcock et al., 2003; Washington et al., 2002). Interactions with the phosphatases SHP-1, -2 and moesin have been described but the significance of these interactions have not been uncovered (Barrow et al., 2004; Washington et al., 2002). Nevertheless, an activation role for TLT-1 was supported when single chain antibodies specific to TLT-1 were shown to inhibit platelet aggregation in the presence of low levels of agonist in vitro (Giomarelli et al., 2007). These results support an enhancing role for TLT-1 and suggest that TLT-1 may play an important role in maintaining platelet activation.

To better understand TLT-1's potential in vivo, a null mouse model was developed (Washington et al., 2009). The TLT-1 mouse is viable with negligible differences seen in platelet counts. During platelet aggregation assays we found that the null platelets show reduced capacity for aggregation with the secondary platelet agonists ADP and the TxA_2 mimetic U46619 compared to wild type *(wt)*. Consistent with these results, tail bleeding assays demonstrate and overall increase in time to secession of bleeding compared to wild type mice, however the difference between null and wt mice were not as substantial as a PAR1 or GPVI null mouse suggesting that TLT-1's role in hemostasis was not of primary hemostasis, but one that was "unique".

To gain a clearer picture of TLT-1 function we used an acute sepsis model where we inject LPS intraperitoneally and monitored hemostatic and immunological parameters as well as

survival (Washington et al., 2009). Sepsis is an interesting model because it starts with a strictly immunological challenge and mortality is a direct response to rife platelet activation and microthrombi. Platelets play an indispensible role during hemostasis and an often unappreciated role during inflammation (Levi & van der Poll, 2004). The involvement of platelets in the immune response and sepsis is undeniable, but never the less not completely understood.

Treatment of null and wt mice with LPS causes the TLT-1$^{-/-}$ mice to succumb faster and in greater numbers to the immunological challenge. Null mice display a distortion of both hemostatic and immunological parameters. Plasma levels of one of the major inflammatory mediators, tissue necrosis factor- α (TNF- α), is also elevated in the null mouse, demonstrating TLT-1 either directly or indirectly modulates immune function. Platelet counts in the null mouse are significantly lower, while the levels of d-dimers are elevated. These two important markers of sepsis, platelet count and d-dimers, suggest the presence of a more severe DIC in the null mouse than *wt* (Washington et al., 2009).

The severity of DIC in the null mouse compared to the wild type mouse was further supported in the localized Shwartzman model of vasculitis. The localized Shwartzman reaction, which correlates well with the presence of DIC in humans, challenges the animal subcutaneously with the inflammatory mediators LPS and TNF-α and subsequently the lesion is evaluated for hemorrhage, clotting, and leukocyte infiltration. Null mice showed slightly increased amounts of microclots, and neutrophil infiltration, however the quantity of hemorrhage was twofold and the area of the lesion was almost three fold greater than in *wt* mice (Washington et al., 2009). These results are indicative of a role in the integration of inflammation with hemostasis; however at the time, this idea, although reported in numerous publications, was not quite accepted.

6.2 Immune – Hemostasis

It was in a series of articles originating from the Wagner laboratory that light was shed on the connection between inflammation and hemorrhage. In essence they asked, "When you are thrombocytopenic, where do you bleed?" A surprising answer was in inflamed tissue (Goerge et al., 2008). In a series of eloquent experiments they provide convincing evidence that platelets are necessary to control immune derived bleeding, and what's more they (platelets) use other than the classical hemostatic mechanisms involved in plug formation. Using the reverse arthus model significant hemorrhage is witnessed only in the group of mice that are thrombocytopenic, confirming the importance of platelets to **immune-hemostasis**. The authors go on to show that deletion mutants for IIβ IIIα, GPIV, GP1b, or p-selectin, like *wt* mice, maintained their ability to control hemorrhage, demonstrating that the members of the classical pathways of platelet function are not necessary to mandate an **immune-hemostasis**; signifying that there are alternate pathways to hemostasis. They also used a LPS inhalation model to show that mice that are thrombocytopenic bleed in the lungs demonstrating the hemostatic effect at the organ level (Goerge et al., 2008). Both of these models induce complement deposition, neutrophil activation, and endothelial damage that ultimately recruit platelet involvement. It is easy to imagine that the bleeding diathesis could be reproduced by the inhibition of only a handful of specific molecules important to the cause, which in essence is what they were testing with the deficient mice in the reverse arthus model.

The mechanisms that regulate the hemorrhage seen at sites of inflammation and in cancer seem to be similar. In a cancer model, it was shown that the addition of resting platelets, but

not activated platelets, rescues the hemorrhage seen during inflammation in cancer. One of the major differences between resting and activated platelets is that resting platelets maintain the contents of their α-granules and accordingly, they go on to show that supernatants from activated platelets will rescue the bleeding seen at a tumor. These results indicate that platelet α-granules contain a soluble factor or factors that have the ability to maintain vascular integrity at sites of hemorrhage induced by inflammation. Although they were able to show distinct changes of various culprit α-granule proteins, their work did not reveal the protein or proteins responsible for the control of hemorrhage (Ho-Tin-Noe et al., 2008). Their conclusion is that platelets continually maintain hemostasis in the face of inflammation using mechanisms other than those well described during plug formation. This opens the idea that platelets may work preemptively to stop hemorrhage by regulating leukocyte activity at the vessel wall. So the question remains, is there a molecule or a hand full of molecules responsible for maintaining hemostasis in the face of inflammation?

These series of experiments clearly define what we have known since the time of Celsius, but have ignored. In light of these experiments, our interpretation of TLT-1's "unique" role becomes plausible. TLT-1's purpose on platelets appears to be that of transition. In conjunction with molecules like p-selectin, GpIb, and TREM-1, TLT-1 mediates the integration of inflammatory signals with platelet function, mediating coagulation when only secondary agonists like TxA_2 or low levels of thrombin are present. TLT-1 may be one of a handful of molecules regulating immuno-hemostasis. These eloquent experiments (Goerge et al., 2008; Ho-Tin-Noe et al., 2008; Ho-Tin-Noe et al., 2009) also call for an official beginning of a new field that defines the intersection between immunology and hemostasis, one that we refer to as Immunohemostasis.

It is no longer adequate to state that platelets are linked to the inflammatory response. Here we have outlined numerous publications that demonstrate: how platelets influence neutrophil function (Clark et al., 2007; Looney et al., 2009; Zarbock et al., 2006), monocyte function (Goncalves et al., 2011), both T and B cell function (Elzey et al., 2003; Iannacone et al., 2005), the interrelation with complement and the effect of microparticles on the immune system (Boilard et al., 2010). These studies clearly demonstrate that platelets play an important role in inflammation. What is called for now is the kinetics of interactions and outcomes from studies using enhanced or decreased platelet count in immune reactions.

While all of the studies mentioned point out that platelets influence immune function, very few point out the outcomes from having increased or decreased platelet involvement. We pointed out earlier in this discussion, seemingly conflicting results between studies with similar stimuli but that had with different outcomes. Using LPS, Zarbock *et al.,* show that platelet depletion decreases inflammation. Clark et al., uses LPS and show that platelets are critical for NET formation. Why are they different and do they connect? Zarbock *et al*., looks at a 2 hour time point (Zarbock et al., 2006) where Clark *et al*., uses longer time points (Clark et al., 2007). NETS are notoriously slow in formation (Massberg et al., 2010); therefore these two results could be different stages of the same continuum. Neither of the articles describes outcomes, but leave open for debate which comes first, the platelet or the neutrophil. Looney *et al*., (Looney et al., 2009) show an acute lung injury model where platelet depletion decreases mortality, but they used a two hit model where LPS was followed by antibodies to MHC. It is easy to see how platelet depletion could be life preserving. Their studies however, suggest that it is the neutrophils that recruit the platelets.

In reports that do report outcome, they show that even though platelet depletion reduces inflammation, they also point out that without the platelets, the immune response was

inadequate. In the *Leishmania* study for example, platelet depletion lowered monocyte recruitment and inflammation, but at the same time the *Leishmania* infection was not cleared (Goncalves et al., 2011). In the thrombotic glomerular nephritis model, platelet depletion increased lethality of the treatment suggesting that platelets play a protective role. Similar outcomes were seen with viral models, where platelets caused hepatic damage and removal of platelets reduced the damage. Iannacone *et al*., demonstrated that although there was less damage without platelets, the T-cells were unable to remove the viral infection, demonstrating that platelets are a critical part of the inflammatory response (Iannacone et al., 2005).

In a final note, it was shown that coagulation was important in bacterial immune response to help contain the infection (Massberg et al., 2010). It was ascertained that neutrophils release nucleosomes containing serine proteases. These nucleosomes function to make NETs and also that degrade platelet derived tissue factor pathway inhibitor, thus shifting the hemostatic balance at the platelet neutrophil interface toward coagulation. The authors point out the conserved nature of coagulation's role in controlling infection in stating that insects don't have an adaptive immune system and use coagulation as a mechanism to control infection in the hemolymph. Therefore they maintain that coagulation is an evolutionally efficient mechanism to control infection. Thus coagulation and platelets play a critical role in maintaining disease during process of **immuno-hemostasis**.

In conclusion, platelets are key regulators of the immune system and immune function cannot be considered complete without considering platelet function. It may be hard for those who prescribe to the self non-self theory of immune function to swallow platelets as playing more than a bystander a role in immune function. If we look at platelets as derived from megakaryocytes, recent studies show that bacterial infection changes the profile of what transcripts platelets store and therefore produce after activation (Freishtat et al., 2009). Thus, maybe it is not the platelets that are in control, but feedback to and from the megakaryocyte. Alternatively, if we subscribe to the newly derived Danger theory of immune function (Matzinger, 2001; Matzinger, 2002), platelets as well as neutrophils fit the bill as perfect detectors of danger and mediators of immune response. We can easily see how over activation of platelets and neutrophils could signal danger and elicit a more robust immune response. Either way, platelets play an indispensible role in the immune system and the hemostatic response to immune challenges and we propose the beginning of a new scene in our studies, a scene where the platelet is the immunomodulator; in a scene called **Immunohemostasis**.

6.3 Acknowledgements

This publication was possible by Grant Number 1U54RR026139-01A1 and G12RR-03035 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH) and grant number SC2GM081237 from the National Heart Lung and Blood Institute

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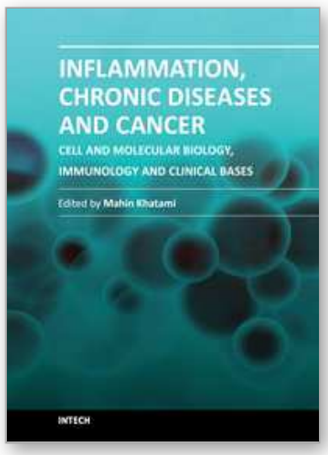

Inflammation, Chronic Diseases and Cancer - Cell and Molecular Biology, Immunology and Clinical Bases

Edited by Dr Mahin Khatami

ISBN 978-953-51-0102-4
Hard cover, 430 pages
Publisher InTech
Published online 09, March, 2012
Published in print edition March, 2012

This book is a collection of excellent reviews and perspectives contributed by experts in the multidisciplinary field of basic science, clinical studies and treatment options for a wide range of acute and chronic inflammatory diseases or cancer. The goal has been to demonstrate that persistent or chronic (unresolved or subclinical) inflammation is a common denominator in the genesis, progression and manifestation of many illnesses and/or cancers, particularly during the aging process. Understanding the fundamental basis of shared and interrelated immunological features of unresolved inflammation in initiation and progression of chronic diseases or cancer are expected to hold real promises when the designs of cost-effective strategies are considered for diagnosis, prevention or treatment of a number of age-associated illnesses such as autoimmune and neurodegenerative diseases as well as many cancers.

How to reference

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Omar L. Esponda, Yaliz Loperena, Gladiany Ramos and A. Valance Washington (2012). The Platelet as an Immunomodulator: The Old Thespian with New Roles in Atherosclerosis, Sepsis and Autoimmune Disease, Inflammation, Chronic Diseases and Cancer - Cell and Molecular Biology, Immunology and Clinical Bases, Dr Mahin Khatami (Ed.), ISBN: 978-953-51-0102-4, InTech, Available from: http://www.intechopen.com/books/inflammation-chronic-diseases-and-cancer-cell-and-molecular-biology-immunology-and-clinical-bases/the-platelet-as-an-immunomodulator-the-old-thespian-with-new-roles-in-atherosclerosis-sepsis-and-aut

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