[Frontiers in Bioscience S3, 1089-1100, June 1, 2011]

Fire within the vessels: Interactions between blood cells and inflammatory vascular injury

Maria Nacher, Andes Hidalgo

Department of Epidemiology, Atherothrombosis and Cardiovascular Imaging, Centro Nacional de Investigaciones Cardiovasculares, 28029 Madrid, Spain

TABLE OF CONTENTS

1. ABSTRACT

Inflammation involves multiple molecular, humoral and cellular mechanisms that aim to protect the body from injury or infection by the tight orchestration of immune cell trafficking, interactions and activation. Platelets and neutrophils are major players in the initial thromboinflammatory response by rapidly responding to a variety of danger signals. When the intensity, timing or locations of these responses are uncontrolled, however, they can trigger localized or systemic injury. Studies over the past decades have revealed that during the normal inflammatory response, blood elements frequently interact with each other to form heterotypic aggregates. The formation of these aggregates within blood vessels, in turn, underlies injury in several models of acute inflammation and can in some instances lead to death. These phenomena are likely to have a bigger contribution to the outcome of inflammatory processes than previously expected, not only in acute scenarios but also in those that involve chronic vascular damage, such as atherosclerosis. We will review here the molecular mediators of these interactions and their consequences in the context of cardiovascular injury.

2. INTRODUCTION

Inflammation, from the latin *to set on fire*, is the protective response that cellular and humoral elements of the organism mount in response to an injury or infection. It probably evolved not only as a means to remove the originating insult in multicellular organisms, but also to promote the restoration of normal organ function and homeostasis (1).

The primary response of inflammation is very fast and involves immune cells carrying noxious agents ready to be released into the injured milieu. The main players of this initial phase of inflammation are neutrophils, a subset of leukocytes of myeloid origin. Neutrophils have a very short half life (6-8 h) and constitute about 10% of leukocytes in murine blood and up to 70% in humans, but their numbers in circulation can dramatically and rapidly increase during inflammatory conditions. The importance of neutrophils for the defense against infecting pathogens is highlighted by the moderate to severe susceptibility to infections observed in animals or humans with mutations in genes involved in neutrophil trafficking (such as leukocyte

adhesion deficiency types I, II and III, which affect integrins, selectin ligands and activators of integrins, respectively) (2), neutrophil generation or survival (3-5) or microbicidal capacity (such as that present in Chronic Granulomatous Disease (6)).

The other major players involved in the initial response to injury are platelets. These are anucleated fragments released from bone marrow megakariocytes with a short half-life (approximately 4 days in humans). Similar to neutrophils, they are prominently characterized by the presence of numerous granules filled with a cargo (including nucleotides, secretagogues, adhesion receptors, cytokines, mitogenic and coagulation factors and proteases) that participates in different aspects of hemostasis and immunity (7, 8). Consistent with their primary role in maintaining hemostasis, defects in genes related to platelet function normally result in bleeding disorders.

Platelets are, however, growingly considered as an important component of the immune response (9). This is in part due to the observation that platelets can bind other blood components: binding to lymphocytes controls their homing to lymph nodes and thus modulates the adaptive immune response (10, 11), while binding to monocytes and neutrophils has been shown to enhance their function as well as recruitment to extravascular areas. Platelet interactions with infected erythrocytes (RBC) have also revealed a direct function of platelets in eliminating malarial parasites (12).

Neutrophils and platelets are thus essential for a primary immune response and allow the elimination of the originating stimuli (e.g., by release of microbicidal agents or phagocytosis) and contribute to repairing the damaged tissue (e.g., by secretion of growth factors and recruitment of progenitor cells). An increasing body of work has revealed that beyond their individual functions, the physical interaction between neutrophils and platelets can enhance the protective inflammatory response (8, 13). This phenomenon, however, is a double-edged sword because the untimely activation of this response within the blood vessels can alter the vascular integrity and damage the tissues they irrigate. Only recently we are beginning to learn how these processes shape the inflammatory response in acute (e.g., sepsis, acute organ injury or pain crises in patients with sickle cell disease) or chronic (e.g., atherosclerosis) settings.

Because cardiovascular diseases are by far the major cause of morbidity and mortality in modern societies, accounting for over 20% of deaths world-wide (14), a better understanding of the temporal and spatial interactions among these cells may offer attractive targets for therapeutic intervention. Interactions among other cellular components of the innate immune system (e.g., monocytes, macrophages and neutrophils) in mounting and resolving inflammation has been extensively studied (reviewed in (15)) and will not be reviewed here, but can give the reader a broader understanding of the importance of heterocellular interactions in the biology of inflammation.

In this review we will consider the mechanisms that allow the recruitment of neutrophils and platelets to areas of inflammation, the distinct morphological and molecular rearrangements that neutrophils undergo in these areas, and the formation of intravascular heterotypic aggregates. Finally, we will review evidence indicating that as a result of these interactions neutrophils can become abnormally activated and contribute to inflammatory injury, and discuss the potential implications of these phenomena in the context of cardiovascular disease.

3. INITIATION OF INFLAMMATION AND NEUTROPHIL RECRUITMENT

Inflammation originates in response to the presence of antigens, foreign organisms (e.g., bacteria or fungi) or trauma. Danger signals, detected by tissue-resident sentinel cells (e.g., macrophages or dendritic cells) through various pattern-recognition receptors, trigger the production of pro-inflammatory cytokines such as interferons, interleukins or tumor necrosis factor-alpha (TNF)-alpha (1). These cytokines then induce activation of the endothelium lining the vessels that irrigate the affected tissues, and result in the expression of adhesion receptors, more cytokines or chemokines that eventually allow the recruitment of circulating leukocytes (16). A subset of monocytes identified by intravital imaging was shown to *patrol* the vascular lumen under steady-state conditions and might well be the first leukocyte subset to accumulate after injury (17).

Neutrophils are the next cell subset to rapidly and massively accumulate at the site of injury (15). In contrast with other leukocyte subsets involved in later stages of inflammation (e.g., lymphocytes), neutrophils are constitutively equipped with receptors that mediate interactions with the inflamed microvasculature. Induction of adhesive molecules on the endothelial cell surface is thus sufficient to initiate contacts between neutrophils and endothelial cells. These initial contacts are mediated by P- and E-selectins on the endothelium and their ligands on neutrophils (18) (Figure 1). P-selectin, which is also present in the alpha granules of platelets, is stored in the Weibel-Palade bodies of endothelial cells and is rapidly displayed on the cell surface following activation (16), thus allowing a first wave of leukocyte recruitment. E-selectin expression, in contrast, requires transcriptional activation and appears together with counter-receptors for integrins (mainly ICAM-1 and VCAM-1) on the endothelium to maximize leukocyte recruitment. Because of the optimal on-off binding rates at physiological shear forces, selectin-mediated interactions result in a rolling-like motion (19) that allows exposure of leukocytes to chemokines present on the endothelial surface.

P-selectin is involved in the initial capture or tethering of circulating neutrophils through engagement of P-selectin glycoprotein ligand-1 (PSGL-1) (20). E-selectin in turn mediates a slow rolling motion and promotes leukocyte arrest though engagement of a number of different glycoconjugate ligands present on the neutrophil surface (21, 22). Although the full repertoire of these

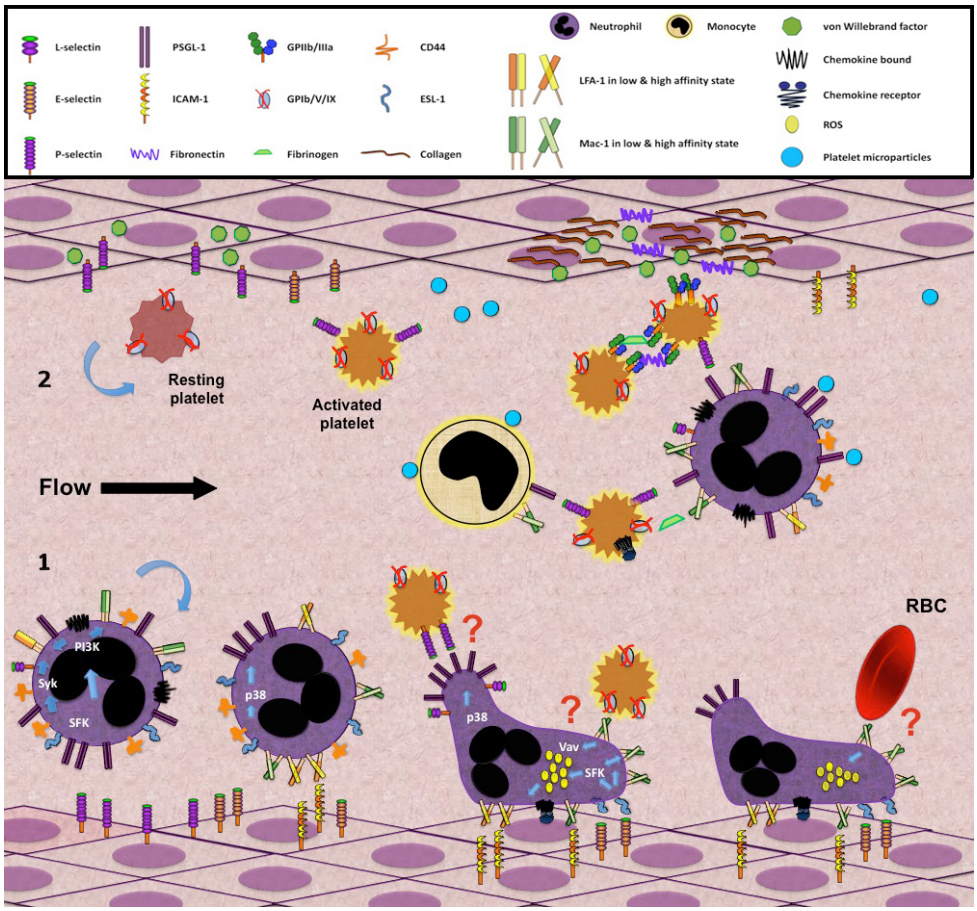

Figure 1. 1. Leukocyte interactions and microdomain formation. Leukocytes tether and roll on P- and E-selectins and their ligands PSGL-1, CD44 and ESL-1. These interactions induce activity of members of the Src kinase family (SFK) and partial LFA-1 and Mac-1 integrin activation. CD44 and ESL-1 mediate slow rolling, resulting in neutrophil activation that leads to polarization of molecules to the uropod or the leading edge. Presence of activated adhesion receptors on intravascular leukocytes allows interactions among different blood components. On polarized cells, interactions with platelets are putatively mediated by PSGL-1 at the uropod and by Mac-1 at the leading edge. The leading edge also mediates interactions with circulating RBC. The ligands on platelets and RBCs that mediate these captures are yet unidentified. As a result of these interactions, outside-in signals delivered by engaged integrins further activate adherent neutrophils and promote ROS release. 2. Platelet interactions. Resting or activated platelets roll on stimulated or damaged endothelium and can firmly arrest. Resting platelets express GPIbα and PSGL-1 which mediate the tethering on vWf and P-selectin on the endothelium, respectively. During inflammation, fibrinogen or fibrin promote aggregation of activated platelets or adhesion to the vessel wall through GPIIb/IIIa. Adherent platelets promote recruitment of leukocytes through P-selectin and can enhance pathological states. Binding to leukocytes in the circulation is mediated by P-selectin and GPIbα, uncharacterized receptor/ligand pairs.

ligands remains to be characterized, it has been reported that PSGL-1, CD44 and E-selectin ligand-1 (ESL-1) account for most of the E-selectin ligand activity on mouse neutrophils (23, 24). Additional ligands, such as CD43 on inflammatory T lymphocytes and lipids and O-glycans on neutrophils, have been reported but their functions or identities are poorly defined (25-28).

Beyond their role in mediating leukocyte tethering and rolling, engagement of both P- and E-selectin also contributes to activation of the rolling neutrophil. This is an important and underappreciated aspect of inflammation because selectins promote integrin activation and initiate the formation of microdomains that further trigger inflammatory phenomena mediated by these cells, as we discuss below. Thus, engagement of PSGL-1 by endothelial or platelet-derived P-selectin induces tyrosine phosphorylation and Src-family kinase (SFK) activation, resulting in activation of the integrin Mac-1 (αMβ2 or CD11bCD18) on neutrophils (29-31). Engagement of PSGL-1 or CD44 by E-selectin, in contrast, sequentially signals through correceptors containing immunoreceptor tyrosine-based activation motifs (ITAM), SFK, the spleen tyrosine kinase (Syk), the Bruton tyrosine kinase and the p38 MAP kinase to promote the partial activation of the other beta 2 integrin present on neutrophils, LFA-1 (22, 32, 33). Activated LFA-1 in turn engages endothelial ICAM-1, allows the effective reduction in rolling velocities, and

promotes arrest and leukocyte recruitment. In addition, activation of the integrin Mac-1 in specific microdomains of the adherent neutrophil is also induced through engagement of a different receptor for E-selectin, ESL-1, in a process that also requires SFK signaling (34). All these studies point towards a complex and rich signaling crosstalk between selectin ligands and the pathways leading to integrin activation, indicating a functionally important link between these two adhesive pathways.

Following selectin engagement, chemokine-triggered signaling mediates the full activation of rolling neutrophils and promotes firm arrest and polarization. The chemokine CXCL1/KC (IL-8 in humans), through engagement of its receptor CXCR2, is a major inducer of these events in neutrophils (35, 36). Other bacterial peptides, chemoattractants and several CXC-chemokines have similar effects on neutrophils through G alpha-i-mediated signaling (reviewed in (37)).

The classical view of leukocyte recruitment considered firm adhesion to be the last event of these cells within the vasculature. Use of *in vivo* imaging, however, has revealed that most leukocytes do not immediately extravasate but rather *crawl* on the endothelial layer, a process also referred to as locomotion and mediated by the integrin Mac-1 (38-40). This crawling process can take from several seconds to minutes and may have an important impact in the inflammatory response because it increases the time that activated neutrophils (and as we will discuss later, associated platelets) spend inside the blood vessels and consequently the time during which they can inflict vascular injury if abnormally activated.

4. PLATELET RECRUITMENT AND HETEROTYPIC AGGREGATES

The interactions of platelets with extracellular components or other cells are central to their hemostatic, immune and inflammatory functions. Depending on the surface, platelet adhesion is mediated by one or more extracellular components, including collagen, von Willebrand factor (vWf), fibrin(ogen), or fibronectin and by cellular receptors including PSGL-1 or Mac-1 (9, 41-44) (Figure1).

4.1. Platelet-Platelet interactions

Platelet aggregation requires activation by exogenous agonists such as thrombin, ADP, Thromboxane A2 or collagen and is regulated by activation of the platelet glycoprotein (GP) GPIIb/IIIa receptor (integrin alphaIIbbeta3; CD41/CD61), the most abundant protein on the platelet surface. Fibrinogen (of plasma or platelet origin) is the ligand recognized by this integrin and its dimeric structure allows favors platelet aggregation. In absence of added exogenous stimuli, platelet aggregation occurs through a pathway requiring vWf binding to two platelet receptors: GPIb and GPIIb/IIIa (45, 46). Other ligands that also participate in the interaction among platelets include fibronectin, vitronectin, and the CD40 ligand (9, 42). Although all ligands have similar affinities to the activated form of GPIIb/IIIa, fibrinogen is the dominant ligand supporting platelet aggregation under low shear conditions (47). In the final phase of thrombus formation, fibrinogen is converted to fibrin by thrombin, and this leads to stabilization and anchoring of platelet aggregates. Collagen also supports the formation of multiple layers of platelets and fibrinogen formation (48, 49). Blockade of GPIIb/IIIa prevents deposition of additional platelets onto adherent platelets and lateral cohesion, platelet-collagen adhesion and alters thrombus formation (49).

4.2. Platelet–vessel wall interactions

Platelets activated with thrombin, collagen or calcium ionophores were shown by intravital microscopy analyses to roll on the vessel wall (50). Similarly to leukocytes, platelet rolling increases depending on the venule shear rates and is mediated by P-selectin or vWf expressed in the endothelium and their counterreceptors on platelets; PSGL-1 or GPIbalpha (51, 52) (Figure 1). In the absence of additional stimulation, platelets disengage from the vessel wall within minutes and return to circulation. Their release is mediated by cleavage of vWf complexes by the plasma metalloprotease ADAMTS13 (a disintegrin-like and metalloprotease with thrombospondin type I repeats 13) (53, 54).

Under conditions of vascular damage, the glycoprotein complex (GPIb/IX/V) mediates the initial interactions of platelets with components of the exposed subendothelial membrane, including vWf and collagen (55). Recognition of collagen through GPVI participates in adhesion to the vessel wall and platelet aggregation (56, 57). GPIIb/IIIa then mediates binding to the vascular wall through engagement of fibrinogen, vWf and fibronectin (58). Due to its pro-thrombotic functions, the activation of platelet GPIIb/IIIa is tightly regulated and triggered by GPIb ligation or by G protein coupled receptors (59, 60).

4.3. Platelet–leukocyte interactions

Both platelets and platelet-derived microparticles interact with a variety of leukocytes like granulocytes, monocytes, lymphocytes and even blood progenitor cells (Figure 1). Since the density of P-selectin on activated platelets after its release from platelet alpha-granules is much higher than on endothelium, leukocytes are avidly bound by activated platelets. Leukocytes can roll on deposited platelets and transmigrate much like they do with endothelial cells and in this manner facilitate leukocyte recruitment to inflamed or injured vessel wall (61, 62).

The interactions of platelets with leukocytes have varying consequences depending on the cell subset involved. Monocytes display a high propensity for platelet binging (63) and this favors both their activation and recruitment to injured areas (64), as well as their differentiation into macrophages and transformation into foam cells (65, 66), thus promoting atherogenic processes (67).

Platelet-lymphocyte aggregates constitute around 3% of circulating lymphocytes. Platelet activation slightly

increase the formation of platelet-T lymphocyte aggregates proportional to the P-selectin-binding capacity of T cell subsets. P-selectin is essential in for these interactions, but CD41, CD40L, and CD11b also contribute to the formation of heterotypic aggregates (68). These interactions have been described to facilitate the entry of naïve lymphocytes to peripheral lymph nodes by P-selectin recognition of carbohydrate ligands present on high endothelial venules, although this was evidenced mostly in the absence of L-selectin (10, 11).

Several studies have uncovered that platelets can also bind hematopoietic and endothelial progenitor cells (HPC). Since HPC are a promising source of regeneration in atherosclerosis or ischemic heart disease the implications of these reports may be clinically significant. Adhered platelets can recruit circulating progenitors to areas of injury through presentation of P-seletin and GPIIb and deposition of chemokines, in particular CXCL12 (69). In addition, in circulating progenitors, platelets provide additional receptors that may enhance their recruitment to inflamed areas or homing to the bone marrow, thus enhancing hematopoietic reconstitution in transplantation settings (70). Beyond leukocyte trafficking, platelets may modulate inflammation by affecting the differentiation program of progenitor and monocyte subsets (reviewed in (67)).

In the remaining sections we will discuss the formation of heterotypic aggregates between platelets and myeloid leukocytes, with a special emphasis on the mechanisms by which platelets modulate neutrophil activity during acute or chronic inflammatory responses.

5. HETEROTYPIC INTERACTIONS AT SITES OF INFLAMMATION

5.1 Leukocyte polarization

Leukocyte polarization is a well described phenomenon that involves not only fast (in the order of seconds) and marked morphological changes from a round to an elongated shape, but also the segregation of receptors at the cell surface and signaling and structural molecules in the cytoplasm (71) (Figure 1). Leukocyte polarization is important for the directed migration of leukocytes during extravasation and tissue migration (72, 73) as it allows the cell to sense and respond to gradients of chemoattracting agents. Polarization is typically triggered when chemoattractants or chemokines engage G protein-coupled receptors, resulting in the formation of an elongated, non-adhesive structure at the rear end of the cell (with regard to the direction of movement) termed the trailing edge or *uropod*, and a flat area at the front characterized by the formation of membrane protrusions, termed the *leading edge*. Polymerization of F-actin at the leading edge provides the force required for membrane protrusion and is enabled by the specific accumulation and activation of Rho GTPases, particularly Cdc42, Rap1 and Rac (74-77), as well as proteins that contribute to regulating their activity, such as the guanine nucleotide exchange factor DOCK2 or phosphoinositide kinases (78, 79). Formation of the uropod, in contrast, requires Rho-Rock signaling (80) and the phosphatase and tensin homolog (PTEN) (81) to induce an anti-adhesive state at the rear and lateral sides of the polarized leukocyte. Paralleling this cytoplasmic segregation, transmembrane receptors also translocate at the cell surface: active integrins and chemokine receptors accumulate at the leading edge, whereas ligands for integrins and selectins (including PSGL-1, CD43, CD44, L-selectin, ICAM-1 and 3) are preferentially found at the uropod (38, 76, 82, 83).

Importantly, additional factors other than chemokines can induce polarization. Engagement of CD44 by E-selectin can induce clustering of receptors, such as L-selectin or PSGL-1, on rolling neutrophils (23, 84). Although not proven, it is conceivable that these structures in fact constitute “primordial” uropods and that leukocyte polarization is specified early during leukocyte recruitment.

Leukocyte polarization may be of particular relevance for the formation of heterotypic aggregates because the receptors mediating these intercellular interactions segregate and accumulate in small microdomains at higher densities, and thus might be more efficient at binding ligands under conditions of flow.

5.2. Heterotypic interactions mediated by leukocyte microdomains

Neutrophils recruited to the inflamed microvasculature can act as “anchors” to which circulating cells bind and thus represent a variation of the aforementioned scenario, where platelets recruited to sites of vascular damage allow the subsequent rolling and arrest of circulating leukocytes (Figure 1).

A recent study used high-speed multichannel intravital microscopy to demonstrate that neutrophils (identified on the basis of high levels of Ly6G/C expression) firmly arrested to or actively crawling on the inflamed endothelium captured circulating platelets with high frequency (34). A detailed microscopic analysis of these interactions showed that these took place mainly in areas of active pseudopodia formation, i.e. at the leading edge of neutrophils. This suggested that one or more receptors specifically expressed and active in this microdomain were responsible for platelet capture. Interactions with the rear side of the crawling leukocyte also occur but these represent a minor fraction under TNF alpha-induced inflammation (34). In non-inflamed vessels interactions among leukocytes and platelets were also detected, but the number of these interactions was lower and were mediated by both the leading edge and the uropod with similar frequencies (34). Thus, the formation of neutrophil-platelet aggregates at inflammatory sites is distinct and different from that found in the circulating blood in that it is initiated by recruited leukocytes and in that it involves specific microdomains on the polarized, adherent neutrophil.

While neutrophil interactions with platelets are well described, *in vitro* and *in vivo* imaging have revealed that neutrophils also establish frequent interactions with circulating RBC (34, 85) and that these interactions are also

induced under inflammatory conditions (see below). In contrast to those with platelets, these interactions are exclusively mediated by the leading edge (34), implying that a partially distinct set of receptors is involved in the interactions with platelets or RBC.

Finally, interactions of adherent neutrophils with other circulating leukocytes have also been described, and are thought to enhance leukocyte recruitment. These are mediated by binding of L-selectin and PSGL-1 (86), and are therefore mediated by the uropod of adherent cells.

5.3 Neutrophil-platelet interactions and inflammatory injury

In vitro studies have shown that unpolarized neutrophils tether on platelet-coated surfaces through interactions mediated by platelet P-selectin and leukocyte PSGL-1 (87-89). These initial contacts are followed by activation of the leukocyte integrin Mac-1 which engages the platelet glycoprotein GPIbalpha (89). Similar receptor-ligand pairs appear to be involved in the interactions between polarized, adherent neutrophils and circulating platelets as demonstrated by analysis of mice deficient in Mac-1, P-selectin or PSGL-1. Absence of Mac-1 results in a marked reduction in interactions at the leading edge, while those mediated by the uropod remain unaffected in these mice (34). By contrast, absence of P-selectin or PSGL-1 specifically reduces the interactions at the uropod (A.H., unpublished observations). It remains to be determined whether GPIbalpha or other platelet ligand(s) are the physiologically relevant ligands for Mac-1 in these types of interactions (see Figure 1).

The physiological significance of the segregation of receptors for platelets to different microdomains of adherent neutrophils (Mac-1 to the leading edge and PSGL-1 to the uropod) in the context of heterotypic interactions is intriguing; also intriguing are the functional consequences that platelet interactions with each domain may have for neutrophil activation.

What are the pathophysiological consequences of these interactions? Maugeri and colleagues have proposed that the "capture" of activated platelets by neutrophils may serve a protective role of the cardiovascular system by promoting their removal from circulation through phagocytosis (90).

Several animal models have helped to further elucidate the pathological consequences of neutrophil-platelet interactions. In a mouse model of antibody-induced transfusion-related acute lung injury (TRALI), a leading cause of transfusion-related morbidity and mortality (91), platelets rapidly (in seconds to minutes) rosette around adherent neutrophils in an E-selectin and Mac-1 dependent manner (34). Using oxidation-sensitive probes, adherent neutrophils were shown to produce radical oxygen species (ROS) within minutes of challenge, and these in turn caused endothelial injury, as evidenced by the prevention of endothelial permeability and lung injury by ROS-scavenging molecules (34). Inhibition of E-selectin or depletion of neutrophils or platelets significantly reduces lung injury in antibody-treated mice (34, 92). Likewise, treatment with aspirin to inhibit platelet cyclooxygenases also prevents neutrophil-mediated injury (92), suggesting that a physical contact between activated platelets and neutrophils within the lung microvessels is required for vascular injury in this model. These findings are in agreement with the previously described ability of activated platelets to trigger the oxidative burst on neutrophils (93, 94). In these studies inhibition of P-selectin failed to protect mice from injury, whereas the effect of Mac-1 blockade remains controversial (34, 92).

In a model of acid-induced lung injury, a marked increase in neutrophil-platelet aggregates was also detected both in the pulmonary microvasculature and in the circulation. These interactions were mediated by P-selectin and triggered endothelial damage and lung injury (95). Although in this model platelets probably bind unpolarized neutrophils in the circulation, it illustrates how different microdomains and intercellular adhesive pathways can contribute to vascular injury.

A more prominent example where these interactions play a role in vascular injury is sepsis, which is responsible for the death of about half a million people in the United States only (96). Kubes and colleagues demonstrated that bacterial products, such as lypopolysaccharide (LPS), induced TLR4-dependent platelet activation and binding to neutrophils in liver sinusoids (13). Neutrophils mediated platelet trapping in postsinusoidal liver venules, and these interactions induced endothelial cell death and organ damage. As in the case of TRALI, these interactions also appeared to be independent of P-selectin (13). Although ROS production was not measured in this study, exacerbated neutrophil activation was evidenced by the production of neutrophil extracellular traps or NETs, webs of nuclear DNA containing granule and nuclear proteins that efficiently ensnare and kill circulating bacteria (97).

These experimental models evidence that platelet engagement by neutrophils result in the production and release of toxic substances in the vascular milieu, which in turn produce organ damage. Neutrophil activation in this context is probably the result of "outside-in" signaling events triggered by engaged integrins, a process that is mediated by adaptor proteins such as Vav-1 and 3 or Kindlin-3 (98, 99). These and other proteins responsible for neutrophil activation at sites of vascular injury thus represent potential targets for the development of therapeutic drugs.

5.4 Neutrophil-erythrocyte interactions

Under controlled *in vitro* conditions, interactions between RBC and adherent neutrophils have been detected and shown to be sensitive to the applied shear rates, with very few interactions at higher shear rates, and this was confirmed *in vivo* (34, 100). This reduced capture rate may be due to the higher volume of these cells compared to platelets (and concomitant drag force under flow conditions) and/or different affinities for the ligand(s) that mediate binding, and suggest that a physiological function for neutrophil-RBC interactions may be restricted to

inflamed venules or other vessel types with reduced shear values. Use of blocking antibodies or gene-targeted mice demonstrated that the leukocyte integrin Mac-1 is an absolute requirement for these interactions (34, 100). Engagement of neutrophil ESL-1 by endothelial E-selectin enhanced the activity of Mac-1 at the leading edge of leukocytes through signaling by SFK, and consequently the frequency of these interactions (34) (Figure 1). Although the identity of the ligands on the surface of RBC that might function as a ligand for Mac-1 are not known, ICAM-4 and the complement protein C3 appear as potential candidates (100, 101). Mice deficient in C3 do indeed present significant reductions in neutrophil-RBC interactions (34). One interpretation of this data is that these interactions serve to remove aged or damaged RBC that are opsonized and targeted for phagocytosis.

The pathophysiological consequences of these interactions become evident in a murine model of sickle-cell vasoocclussion. Sickle cell disease (SCD) is a common inherited blood disorder with an elevated morbidity and mortality rate due to acute pain crises, chronic inflammation and ischemic end-organ damage such as pulmonary hypertension, renal failure and cerebrovascular injury (102, 103). Intravascular trapping of sickled RBC within the vasculature is directly responsible for endothelial activation, vasoocclusion and organ damage, and several mouse models of SCD have been established that faithfully replicate the major features of the human disease, including anemia and chronic inflammation (104). Using these "sickle" mice, Frenette and colleagues observed that sickle-shaped RBC interact with high frequency with neutrophils recruited to inflamed venules of the cremaster muscle after treatment with the cytokine TNFα, and that this phenomenon was responsible for the vaso-occlusive episodes (85). These interactions were very sensitive to signals initiated downstream of E-selectin, because absence of this selectin completely abolished the formation of neutrophil-RBC aggregates (34). Use of inhibitory antibodies to Mac-1 (34) or its genetic deletion (our unpublished observations) also demonstrated the absolute requirement of this receptor in mediating these interactions in "sickle" animals. Additionally, the elevated levels of C3 bound to deoxygenated RBC from SCD patients suggests a role for this complement protein in the pathogenesis of vasooclussion (105).

These findings suggest that the pathways identified under acute inflammatory conditions in healthy animals are also at work in a model of chronic inflammation and identify attractive targets for therapeutic intervention. Recent work using synthetic pan-selectin inhibitors have indeed proved the feasibility of reducing intravascular interactions, with a concomitant improvement of blood flow and survival of challenged "sickle" mice (106). It has been proposed that this type of heterotypic interactions may also contribute to vascular occlusion and pain during deep vein thrombosis (100).

6. IMPLICATIONS IN CARDIOVASCULAR DISEASE

The disease models described above underscore the importance of neutrophil-platelet interactions during acute inflammatory injury. What is the involvement of these heterotypic interactions in chronic vascular disease? There are a surprisingly low number of scientific reports related to the mechanisms by which neutrophils contribute to chronic inflammatory disease. Several clinical reports have nonetheless found a significant correlation between blood leukocyte counts and the incidence of long-term vascular disease associated with atherosclerosis, diabetes or SCD. Interestingly, these correlations were often more significant in the case of neutrophils than for other leukocyte subsets (reviewed in (107)).

While the initiation of these inflammatory disorders clearly involves other myeloid leukocytes (monocytes, macrophages and dendritic cells) and cells of the adaptive immune arm (108, 109), it is likely that neutrophil activation in affected vascular beds exacerbates endothelial injury and preserves inflammation. Increased numbers of leukocyte-platelet aggregates have indeed been associated with patients with an increased risk of cardiovascular disease (reviewed in (110)). Correlating with these data, the number of primed neutrophils and the levels of neutrophil-derived myeloperoxidase are found elevated in hyperlipidemic patients (111).

One plausible mechanism by which leukocyte-platelet interactions promote atherosclerosis in the *ApoE*-null mouse model was elegantly uncovered by Klaus Ley and colleagues (64). They demonstrated that activated platelets bind circulating monocytes in a P-selectin-dependent manner and enhance their recruitment to inflamed or atherosclerotic endothelium through local delivery of proinflammatory chemokines. This, in turn, increased the lesion size in aortas of *ApoE* null mice (64). A number of recent studies have described that neutrophils also accumulate in significant numbers both in the luminal and intraplaque areas of atherosclerotic lesions (112, 113). Importantly, Christian Weber's group reported that the enforced egress of bone marrow neutrophils aggravated, whereas their depletion attenuated, diet-induced atherosclerosis in *ApoE*–null mice (114).

Although these observations suggest that selectin-dependent, local activation of neutrophils that engage circulating platelets might augment endothelial injury and contribute to plaque generation or growth, this remains largely unexplored. This contention is further supported by a number of observations, including the expression of E-selectin in endothelial beds associated with human atherosclerotic plaques (115), its contribution to plaque formation alone or in combination with P-selectin (116, 117), and the significant role of platelet-derived P-selectin (118) and beta2-integrins on leukocytes in promoting long-term vascular injury, including atherosclerotic plaque formation and growth (119, 120).

Future research using improved imaging technologies and relevant animal models will likely help to unravel the mechanisms by which interactions among blood cells contribute to acute and chronic cardiovascular disease, and may lead to the rational design of therapies to

target this group of complications that so overwhelmingly threat our quality of life.

7. ACKNOWLEDGEMENTS

We thank Dr. Linnea Weiss for critically reading and editing the text. Our laboratory is supported by a Scientist Development Grant from the American Heart Association. (0735165N), a Marie Curie FP7 Reintegration Grant (246655) and a Grant for the Spanish Ministry of Science and Innovation (SAF2009-11037). A.H. is supported by a Ramón y Cajal fellowship from the Spanish Ministry of Science and Innovation. The Centro Nacional de Investigaciones Cardiovasculares is supported by the Spanish Ministry of Science and Innovation and the Pro-CNIC Foundation.

8. REFERENCES

1. R. Medzhitov: Origin and physiological roles of inflammation. *Nature*, 454(7203), 428-35 (2008)

2. A. Etzioni: Genetic etiologies of leukocyte adhesion defects. *Curr Opin Immunol*, 21(5), 481-6 (2009)

3. G. A. Diaz: CXCR4 mutations in WHIM syndrome: a misguided immune system? *Immunol Rev*, 203, 235-43 (2005)

4. F. Liu, H. Y. Wu, R. Wesselschmidt, T. Kornaga and D. C. Link: Impaired production and increased apoptosis of neutrophils in granulocyte colony-stimulating factor receptor-deficient mice. *Immunity*, 5(5), 491-501 (1996)

5. J. Palmblad and H. A. Papadaki: Chronic idiopathic neutropenias and severe congenital neutropenia. *Curr Opin Hematol*, 15(1), 8-14 (2008)

6. S. D. Rosenzweig and S. M. Holland: Phagocyte immunodeficiencies and their infections. *J Allergy Clin Immunol*, 113(4), 620-6 (2004)

7. Q. Ren, S. Ye and S. W. Whiteheart: The platelet release reaction: just when you thought platelet secretion was simple. *Curr Opin Hematol*, 15(5), 537-41 (2008)

8. A. Zarbock, R. K. Polanowska-Grabowska and K. Ley: Platelet-neutrophil-interactions: linking hemostasis and inflammation. *Blood Rev*, 21(2), 99-111 (2007)

9. J. W. Semple and J. Freedman: Platelets and innate immunity. *Cell Mol Life Sci*, 67(4), 499-511 (2010)

10. T. G. Diacovo, M. D. Catalina, M. H. Siegelman and U. H. von Andrian: Circulating activated platelets reconstitute lymphocyte homing and immunity in L-selectin-deficient mice. *J Exp Med*, 187(2), 197-204 (1998)

11. T. G. Diacovo, K. D. Puri, R. A. Warnock, T. A. Springer and U. H. von Andrian: Platelet-mediated lymphocyte delivery to high endothelial venules. *Science*, 273(5272), 252-5 (1996)

12. B. J. McMorran, V. M. Marshall, C. de Graaf, K. E. Drysdale, M. Shabbar, G. K. Smyth, J. E. Corbin, W. S. Alexander and S. J. Foote: Platelets kill intraerythrocytic malarial parasites and mediate survival to infection. *Science*, 323(5915), 797-800 (2009)

13. S. R. Clark, A. C. Ma, S. A. Tavener, B. McDonald, Z. Goodarzi, M. M. Kelly, K. D. Patel, S. Chakrabarti, E. McAvoy, G. D. Sinclair, E. M. Keys, E. Allen-Vercoe, R. Devinney, C. J. Doig, F. H. Green and P. Kubes: Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat Med*, 13(4), 463-9 (2007)

14. World-Health-Organization: http://www.who.int/whosis/whostat/en/. In, (2008)

15. O. Soehnlein and L. Lindbom: Phagocyte partnership during the onset and resolution of inflammation. *Nat Rev Immunol*, 10(6), 427-39

16. D. D. Wagner and P. S. Frenette: The vessel wall and its interactions. *Blood*, 111(11), 5271-81 (2008)

17. C. Auffray, D. Fogg, M. Garfa, G. Elain, O. Join-Lambert, S. Kayal, S. Sarnacki, A. Cumano, G. Lauvau and F. Geissmann: Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior. *Science*, 317(5838), 666-70 (2007)

18. A. Zarbock and K. Ley: Neutrophil adhesion and activation under flow. *Microcirculation*, 16(1), 31-42 (2009)

19. R. P. McEver and C. Zhu: Rolling Cell Adhesion. *Annu Rev Cell Dev Biol* (2010)

20. J. Yang, T. Hirata, K. Croce, G. Merrill-Skoloff, B. Tchernychev, E. Williams, R. Flaumenhaft, B. C. Furie and B. Furie: Targeted gene disruption demonstrates that P-selectin glycoprotein ligand 1 (PSGL-1) is required for P-selectin-mediated but not E- selectin-mediated neutrophil rolling and migration. *J Exp Med*, 190(12), 1769-82. (1999)

21. K. Ley, M. Allietta, D. C. Bullard and S. Morgan: Importance of E-selectin for firm leukocyte adhesion *in vivo*. *Circ Res*, 83(3), 287-94. (1998)

22. A. Zarbock, C. A. Lowell and K. Ley: Spleen tyrosine kinase Syk is necessary for E-selectin-induced alpha(L)beta(2) integrin-mediated rolling on intercellular adhesion molecule-1. *Immunity*, 26(6), 773-83 (2007)

23. A. Hidalgo, A. J. Peired, M. K. Wild, D. Vestweber and P. S. Frenette: Complete identification of E-selectin ligands on neutrophils reveals distinct functions of PSGL-1, ESL-1, and CD44. *Immunity*, 26(4), 477-89 (2007)

24. Y. Katayama, A. Hidalgo, J. Chang, A. Peired and P. S. Frenette: CD44 is a physiological E-selectin ligand on neutrophils. *J Exp Med*, 201(8), 1183-9 (2005)

25. R. Alon, T. Feizi, C. T. Yuen, R. C. Fuhlbrigge and T. A. Springer: Glycolipid ligands for selectins support

leukocyte tethering and rolling under physiologic flow conditions. *J Immunol*, 154(10), 5356-66 (1995)

26. M. Matsumoto, K. Atarashi, E. Umemoto, Y. Furukawa, A. Shigeta, M. Miyasaka and T. Hirata: CD43 functions as a ligand for E-Selectin on activated T cells. *J Immunol*, 175(12), 8042-50 (2005)

27. L. Nimrichter, M. M. Burdick, K. Aoki, W. Laroy, M. A. Fierro, S. A. Hudson, C. E. Von Seggern, R. J. Cotter, B. S. Bochner, M. Tiemeyer, K. Konstantopoulos and R. L. Schnaar: E-selectin receptors on human leukocytes. *Blood*, 112(9), 3744-52 (2008)

28. T. Yago, J. Fu, J. M. McDaniel, J. J. Miner, R. P. McEver and L. Xia: Core 1-derived O-glycans are essential E-selectin ligands on neutrophils. *Proc Natl Acad Sci U S A*, 107(20), 9204-9 (2010)

29. V. Evangelista, S. Manarini, B. S. Coller and S. S. Smyth: Role of P-selectin, beta2-integrins, and Src tyrosine kinases in mouse neutrophil-platelet adhesion. *J Thromb Haemost*, 1(5), 1048-54 (2003)

30. Y. Q. Ma, E. F. Plow and J. G. Geng: P-selectin binding to P-selectin glycoprotein ligand-1 induces an intermediate state of alphaMbeta2 activation and acts cooperatively with extracellular stimuli to support maximal adhesion of human neutrophils. *Blood*, 104(8), 2549-56 (2004)

31. H. B. Wang, J. T. Wang, L. Zhang, Z. H. Geng, W. L. Xu, T. Xu, Y. Huo, X. Zhu, E. F. Plow, M. Chen and J. G. Geng: P-selectin primes leukocyte integrin activation during inflammation. *Nat Immunol*, 8(8), 882-92 (2007)

32. T. Yago, B. Shao, J. J. Miner, L. Yao, A. G. Klopocki, K. Maeda, K. M. Coggeshall and R. P. McEver: E-selectin engages PSGL-1 and CD44 through a common signaling pathway to induce integrin alphaLbeta2-mediated slow leukocyte rolling. *Blood*, 116(3), 485-94 (2010)

33. A. Zarbock, C. L. Abram, M. Hundt, A. Altman, C. A. Lowell and K. Ley: PSGL-1 engagement by E-selectin signals through Src kinase Fgr and ITAM adapters DAP12 and FcR gamma to induce slow leukocyte rolling. *J Exp Med*, 205(10), 2339-47 (2008)

34. A. Hidalgo, J. Chang, J. E. Jang, A. J. Peired, E. Y. Chiang and P. S. Frenette: Heterotypic interactions enabled by polarized neutrophil microdomains mediate thromboinflammatory injury. *Nat Med*, 15(4), 384-91 (2009)

35. J. Lee, G. Cacalano, T. Camerato, K. Toy, M. W. Moore and W. I. Wood: Chemokine binding and activities mediated by the mouse IL-8 receptor. *J Immunol*, 155(4), 2158-64 (1995)

36. S. J. Morgan, M. W. Moore, G. Cacalano and K. Ley: Reduced leukocyte adhesion response and absence of slow leukocyte rolling in interleukin-8 receptor-deficient mice. *Microvasc Res*, 54(2), 188-91 (1997)

37. A. Zarbock and K. Ley: Mechanisms and consequences of neutrophil interaction with the endothelium. *Am J Pathol*, 172(1), 1-7 (2008)

38. E. Y. Chiang, A. Hidalgo, J. Chang and P. S. Frenette: Imaging receptor microdomains on leukocyte subsets in live mice. *Nat Methods*, 4(3), 219-22 (2007)

39. M. Phillipson, B. Heit, P. Colarusso, L. Liu, C. M. Ballantyne and P. Kubes: Intraluminal crawling of neutrophils to emigration sites: a molecularly distinct process from adhesion in the recruitment cascade. *J Exp Med*, 203(12), 2569-75 (2006)

40. A. R. Schenkel, Z. Mamdouh and W. A. Muller: Locomotion of monocytes on endothelium is a critical step during extravasation. *Nat Immunol*, 5(4), 393-400 (2004)

41. V. Evangelista, Z. Pamuklar, A. Piccoli, S. Manarini, G. Dell'elba, R. Pecce, N. Martelli, L. Federico, M. Rojas, G. Berton, C. A. Lowell, L. Totani and S. S. Smyth: Src family kinases mediate neutrophil adhesion to adherent platelets. *Blood*, 109(6), 2461-9 (2007)

42. S. P. Jackson: The growing complexity of platelet aggregation. *Blood*, 109(12), 5087-95 (2007)

43. H. F. Langer and T. Chavakis: Leukocyte-endothelial interactions in inflammation. *J Cell Mol Med*, 13(7), 1211-20 (2009)

44. R. P. McEver: Adhesive interactions of leukocytes, platelets, and the vessel wall during hemostasis and inflammation. *Thromb Haemost*, 86(3), 746-56 (2001)

45. W. S. Nesbitt, S. Kulkarni, S. Giuliano, I. Goncalves, S. M. Dopheide, C. L. Yap, I. S. Harper, H. H. Salem and S. P. Jackson: Distinct glycoprotein Ib/V/IX and integrin alpha IIbbeta 3-dependent calcium signals cooperatively regulate platelet adhesion under flow. *J Biol Chem*, 277(4), 2965-72 (2002)

46. B. Savage, S. J. Shattil and Z. M. Ruggeri: Modulation of platelet function through adhesion receptors. A dual role for glycoprotein IIb-IIIa (integrin alpha IIb beta 3) mediated by fibrinogen and glycoprotein Ib-von Willebrand factor. *J Biol Chem*, 267(16), 11300-6 (1992)

47. B. Savage, E. Saldivar and Z. M. Ruggeri: Initiation of platelet adhesion by arrest onto fibrinogen or translocation on von Willebrand factor. *Cell*, 84(2), 289-97 (1996)

48. D. Kirchhofer, T. B. Tschopp, B. Steiner and H. R. Baumgartner: Role of collagen-adherent platelets in mediating fibrin formation in flowing whole blood. *Blood*, 86(10), 3815-22 (1995)

49. D. Patel, H. Vaananen, M. Jirouskova, T. Hoffmann, C. Bodian and B. S. Coller: Dynamics of GPIIb/IIIa-mediated platelet-platelet interactions in platelet adhesion/thrombus formation on collagen *in vitro* as revealed by videomicroscopy. *Blood*, 101(3), 929-36 (2003)

50. P. S. Frenette, R. C. Johnson, R. O. Hynes and D. D. Wagner: Platelets roll on stimulated endothelium *in vivo*: an interaction mediated by endothelial P-selectin. *Proc Natl Acad Sci U S A*, 92(16), 7450-4 (1995)

51. P. Andre, C. V. Denis, J. Ware, S. Saffaripour, R. O. Hynes, Z. M. Ruggeri and D. D. Wagner: Platelets adhere to and translocate on von Willebrand factor presented by endothelium in stimulated veins. *Blood*, 96(10), 3322-8 (2000)

52. P. S. Frenette, C. V. Denis, L. Weiss, K. Jurk, S. Subbarao, B. Kehrel, J. H. Hartwig, D. Vestweber and D. D. Wagner: P-Selectin glycoprotein ligand 1 (PSGL-1) is expressed on platelets and can mediate platelet-endothelial interactions *in vivo*. *J Exp Med*, 191(8), 1413-22 (2000)

53. G. G. Levy, W. C. Nichols, E. C. Lian, T. Foroud, J. N. McClintick, B. M. McGee, A. Y. Yang, D. R. Siemieniak, K. R. Stark, R. Gruppo, R. Sarode, S. B. Shurin, V. Chandrasekaran, S. P. Stabler, H. Sabio, E. E. Bouhassira, J. D. Upshaw, Jr., D. Ginsburg and H. M. Tsai: Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. *Nature*, 413(6855), 488-94 (2001)

54. D. G. Motto, A. K. Chauhan, G. Zhu, J. Homeister, C. B. Lamb, K. C. Desch, W. Zhang, H. M. Tsai, D. D. Wagner and D. Ginsburg: Shigatoxin triggers thrombotic thrombocytopenic purpura in genetically susceptible ADAMTS13-deficient mice. *J Clin Invest*, 115(10), 2752-61 (2005)

55. J. Yip, Y. Shen, M. C. Berndt and R. K. Andrews: Primary platelet adhesion receptors. *IUBMB Life*, 57(2), 103-8 (2005)

56. S. Massberg, M. Gawaz, S. Gruner, V. Schulte, I. Konrad, D. Zohlnhofer, U. Heinzmann and B. Nieswandt: A crucial role of glycoprotein VI for platelet recruitment to the injured arterial wall *in vivo*. *J Exp Med*, 197(1), 41-9 (2003)

57. S. Massberg, I. Konrad, A. Bultmann, C. Schulz, G. Munch, M. Peluso, M. Lorenz, S. Schneider, F. Besta, I. Muller, B. Hu, H. Langer, E. Kremmer, M. Rudelius, U. Heinzmann, M. Ungerer and M. Gawaz: Soluble glycoprotein VI dimer inhibits platelet adhesion and aggregation to the injured vessel wall *in vivo*. *FASEB J*, 18(2), 397-9 (2004)

58. D. D. Wagner and P. C. Burger: Platelets in inflammation and thrombosis. *Arterioscler Thromb Vasc Biol*, 23(12), 2131-7 (2003)

59. D. R. Phillips, I. F. Charo and R. M. Scarborough: GPIIb-IIIa: the responsive integrin. *Cell*, 65(3), 359-62 (1991)

60. S. J. Shattil and M. H. Ginsberg: Perspectives series: cell adhesion in vascular biology. Integrin signaling in vascular biology. *J Clin Invest*, 100(1), 1-5 (1997)

61. S. M. Buttrum, R. Hatton and G. B. Nash: Selectin-mediated rolling of neutrophils on immobilized platelets. *Blood*, 82(4), 1165-74 (1993)

62. T. G. Diacovo, S. J. Roth, J. M. Buccola, D. F. Bainton and T. A. Springer: Neutrophil rolling, arrest, and transmigration across activated, surface-adherent platelets via sequential action of P-selectin and the beta 2-integrin CD11b/CD18. *Blood*, 88(1), 146-57 (1996)

63. H. M. Rinder, J. L. Bonan, C. S. Rinder, K. A. Ault and B. R. Smith: Dynamics of leukocyte-platelet adhesion in whole blood. *Blood*, 78(7), 1730-7 (1991)

64. Y. Huo, A. Schober, S. B. Forlow, D. F. Smith, M. C. Hyman, S. Jung, D. R. Littman, C. Weber and K. Ley: Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. *Nat Med*, 9(1), 61-7 (2003)

65. K. Daub, H. Langer, P. Seizer, K. Stellos, A. E. May, P. Goyal, B. Bigalke, T. Schonberger, T. Geisler, D. Siegel-Axel, R. A. Oostendorp, S. Lindemann and M. Gawaz: Platelets induce differentiation of human CD34+ progenitor cells into foam cells and endothelial cells. *FASEB J*, 20(14), 2559-61 (2006)

66. K. Stellos, S. Gnerlich, B. Kraemer, S. Lindemann and M. Gawaz: Platelet interaction with progenitor cells: vascular regeneration or inquiry? *Pharmacol Rep*, 60(1), 101-8 (2008)

67. H. F. Langer, B. Bigalke, P. Seizer, K. Stellos, S. Fateh-Moghadam and M. Gawaz: Interaction of platelets and inflammatory endothelium in the development and progression of coronary artery disease. *Semin Thromb Hemost*, 36(2), 131-8 (2010)

68. N. Li, Q. Ji and P. Hjemdahl: Platelet-lymphocyte conjugation differs between lymphocyte subpopulations. *J Thromb Haemost*, 4(4), 874-81 (2006)

69. S. Massberg, I. Konrad, K. Schurzinger, M. Lorenz, S. Schneider, D. Zohlnhoefer, K. Hoppe, M. Schiemann, E. Kennerknecht, S. Sauer, C. Schulz, S. Kerstan, M. Rudelius, S. Seidl, F. Sorge, H. Langer, M. Peluso, P. Goyal, D. Vestweber, N. R. Emambokus, D. H. Busch, J. Frampton and M. Gawaz: Platelets secrete stromal cell-derived factor 1alpha and recruit bone marrow-derived progenitor cells to arterial thrombi *in vivo*. *J Exp Med*, 203(5), 1221-33 (2006)

70. A. Janowska-Wieczorek, M. Majka, J. Kijowski, M. Baj-Krzyworzeka, R. Reca, A. R. Turner, J. Ratajczak, S. G. Emerson, M. A. Kowalska and M. Z. Ratajczak: Platelet-derived microparticles bind to hematopoietic stem/progenitor cells and enhance their engraftment. *Blood*, 98(10), 3143-9 (2001)

71. O. Barreiro, H. de la Fuente, M. Mittelbrunn and F. Sanchez-Madrid: Functional insights on the polarized redistribution of leukocyte integrins and their ligands

during leukocyte migration and immune interactions. *Immunol Rev*, 218, 147-64 (2007)

72. T. Kinashi: Intracellular signalling controlling integrin activation in lymphocytes. *Nat Rev Immunol*, 5(7), 546-59 (2005)

73. A. J. Ridley, M. A. Schwartz, K. Burridge, R. A. Firtel, M. H. Ginsberg, G. Borisy, J. T. Parsons and A. R. Horwitz: Cell migration: integrating signals from front to back. *Science*, 302(5651), 1704-9 (2003)

74. M. D. Filippi, C. E. Harris, J. Meller, Y. Gu, Y. Zheng and D. A. Williams: Localization of Rac2 via the C terminus and aspartic acid 150 specifies superoxide generation, actin polarity and chemotaxis in neutrophils. *Nat Immunol*, 5(7), 744-51 (2004)

75. M. D. Filippi, K. Szczur, C. E. Harris and P. Y. Berclaz: Rho GTPase Rac1 is critical for neutrophil migration into the lung. *Blood*, 109(3), 1257-64 (2007)

76. K. Katagiri, A. Maeda, M. Shimonaka and T. Kinashi: RAPL, a Rap1-binding molecule that mediates Rap1-induced adhesion through spatial regulation of LFA-1. *Nat Immunol*, 4(8), 741-8 (2003)

77. K. Szczur, H. Xu, S. Atkinson, Y. Zheng and M. D. Filippi: Rho GTPase CDC42 regulates directionality and random movement via distinct MAPK pathways in neutrophils. *Blood*, 108(13), 4205-13 (2006)

78. Y. Kunisaki, A. Nishikimi, Y. Tanaka, R. Takii, M. Noda, A. Inayoshi, K. Watanabe, F. Sanematsu, T. Sasazuki, T. Sasaki and Y. Fukui: DOCK2 is a Rac activator that regulates motility and polarity during neutrophil chemotaxis. *J Cell Biol*, 174(5), 647-52 (2006)

79. A. Nishikimi, H. Fukuhara, W. Su, T. Hongu, S. Takasuga, H. Mihara, Q. Cao, F. Sanematsu, M. Kanai, H. Hasegawa, Y. Tanaka, M. Shibasaki, Y. Kanaho, T. Sasaki, M. A. Frohman and Y. Fukui: Sequential regulation of DOCK2 dynamics by two phospholipids during neutrophil chemotaxis. *Science*, 324(5925), 384-7 (2009)

80. J. H. Lee, T. Katakai, T. Hara, H. Gonda, M. Sugai and A. Shimizu: Roles of p-ERM and Rho-ROCK signaling in lymphocyte polarity and uropod formation. *J Cell Biol*, 167(2), 327-37 (2004)

81. B. Heit, S. M. Robbins, C. M. Downey, Z. Guan, P. Colarusso, B. J. Miller, F. R. Jirik and P. Kubes: PTEN functions to 'prioritize' chemotactic cues and prevent 'distraction' in migrating neutrophils. *Nat Immunol*, 9(7), 743-52 (2008)

82. J. L. Alonso-Lebrero, J. M. Serrador, C. Dominguez-Jimenez, O. Barreiro, A. Luque, M. A. del Pozo, K. Snapp, G. Kansas, R. Schwartz-Albiez, H. Furthmayr, F. Lozano and F. Sanchez-Madrid: Polarization and interaction of adhesion molecules P-selectin glycoprotein ligand 1 and intercellular adhesion molecule 3 with moesin and ezrin in myeloid cells. *Blood*, 95(7), 2413-9 (2000)

83. M. Nieto, J. M. Frade, D. Sancho, M. Mellado, A. C. Martinez and F. Sanchez-Madrid: Polarization of chemokine receptors to the leading edge during lymphocyte chemotaxis. *J Exp Med*, 186(1), 153-8 (1997)

84. S. I. Simon, Y. Hu, D. Vestweber and C. W. Smith: Neutrophil tethering on E-selectin activates beta 2 integrin binding to ICAM-1 through a mitogen-activated protein kinase signal transduction pathway. *J Immunol*, 164(8), 4348-58 (2000)

85. A. Turhan, L. A. Weiss, N. Mohandas, B. S. Coller and P. S. Frenette: Primary role for adherent leukocytes in sickle cell vascular occlusion: a new paradigm. *Proc Natl Acad Sci U S A*, 99(5), 3047-51 (2002)

86. M. Sperandio, M. L. Smith, S. B. Forlow, T. S. Olson, L. Xia, R. P. McEver and K. Ley: P-selectin glycoprotein ligand-1 mediates L-selectin-dependent leukocyte rolling in venules. *J Exp Med*, 197(10), 1355-63 (2003)

87. V. Evangelista, S. Manarini, S. Rotondo, N. Martelli, R. Polischuk, J. L. McGregor, G. de Gaetano and C. Cerletti: Platelet/polymorphonuclear leukocyte interaction in dynamic conditions: evidence of adhesion cascade and cross talk between P-selectin and the beta 2 integrin CD11b/CD18. *Blood*, 88(11), 4183-94 (1996)

88. T. Palabrica, R. Lobb, B. C. Furie, M. Aronovitz, C. Benjamin, Y. M. Hsu, S. A. Sajer and B. Furie: Leukocyte accumulation promoting fibrin deposition is mediated *in vivo* by P-selectin on adherent platelets. *Nature*, 359(6398), 848-51 (1992)

89. Y. Wang, M. Sakuma, Z. Chen, V. Ustinov, C. Shi, K. Croce, A. C. Zago, J. Lopez, P. Andre, E. Plow and D. I. Simon: Leukocyte engagement of platelet glycoprotein Ibalpha via the integrin Mac-1 is critical for the biological response to vascular injury. *Circulation*, 112(19), 2993-3000 (2005)

90. N. Maugeri, P. Rovere-Querini, V. Evangelista, C. Covino, A. Capobianco, M. T. Bertilaccio, A. Piccoli, L. Totani, D. Cianflone, A. Maseri and A. A. Manfredi: Neutrophils phagocytose activated platelets *in vivo*: a phosphatidylserine, P-selectin, and {beta}2 integrin-dependent cell clearance program. *Blood*, 113(21), 5254-65 (2009)

91. M. R. Looney, B. M. Gilliss and M. A. Matthay: Pathophysiology of transfusion-related acute lung injury. *Curr Opin Hematol*, 17(5), 418-23 (2010)

92. M. R. Looney, J. X. Nguyen, Y. Hu, J. A. Van Ziffle, C. A. Lowell and M. A. Matthay: Platelet depletion and aspirin treatment protect mice in a two-event model of transfusion-related acute lung injury. *J Clin Invest*, 119(11), 3450-61 (2009)

93. A. Ruf and H. Patscheke: Platelet-induced neutrophil activation: platelet-expressed fibrinogen induces the oxidative burst in neutrophils by an interaction with CD11C/CD18. *Br J Haematol*, 90(4), 791-6 (1995)

94. A. Ruf, R. F. Schlenk, A. Maras, E. Morgenstern and H. Patscheke: Contact-induced neutrophil activation by platelets in human cell suspensions and whole blood. *Blood*, 80(5), 1238-46 (1992)

95. A. Zarbock, K. Singbartl and K. Ley: Complete reversal of acid-induced acute lung injury by blocking of platelet-neutrophil aggregation. *J Clin Invest*, 116(12), 3211-9 (2006)

96. R. C. Bone: The pathogenesis of sepsis. *Ann Intern Med*, 115(6), 457-69 (1991)

97. V. Brinkmann, U. Reichard, C. Goosmann, B. Fauler, Y. Uhlemann, D. S. Weiss, Y. Weinrauch and A. Zychlinsky: Neutrophil extracellular traps kill bacteria. *Science*, 303(5663), 1532-5 (2004)

98. M. A. Gakidis, X. Cullere, T. Olson, J. L. Wilsbacher, B. Zhang, S. L. Moores, K. Ley, W. Swat, T. Mayadas and J. S. Brugge: Vav GEFs are required for beta2 integrin-dependent functions of neutrophils. *J Cell Biol*, 166(2), 273-82 (2004)

99. M. Moser, M. Bauer, S. Schmid, R. Ruppert, S. Schmidt, M. Sixt, H. V. Wang, M. Sperandio and R. Fassler: Kindlin-3 is required for beta2 integrin-mediated leukocyte adhesion to endothelial cells. *Nat Med*, 15(3), 300-5 (2009)

100. M. S. Goel and S. L. Diamond: Adhesion of normal erythrocytes at depressed venous shear rates to activated neutrophils, activated platelets, and fibrin polymerized from plasma. *Blood*, 100(10), 3797-803 (2002)

101. P. Hermand, M. Huet, I. Callebaut, P. Gane, E. Ihanus, C. G. Gahmberg, J. P. Cartron and P. Bailly: Binding sites of leukocyte beta 2 integrins (LFA-1, Mac-1) on the human ICAM-4/LW blood group protein. *J Biol Chem*, 275(34), 26002-10 (2000)

102. P. S. Frenette: Sickle cell vasoocclusion: heterotypic, multicellular aggregations driven by leukocyte adhesion. *Microcirculation*, 11(2), 167-77 (2004)

103. O. S. Platt: Sickle cell anemia as an inflammatory disease. *J Clin Invest*, 106(3), 337-8 (2000)

104. P. S. Frenette and G. F. Atweh: Sickle cell disease: old discoveries, new concepts, and future promise. *J Clin Invest*, 117(4), 850-8 (2007)

105. R. H. Wang, G. Phillips, Jr., M. E. Medof and C. Mold: Activation of the alternative complement pathway by exposure of phosphatidylethanolamine and phosphatidylserine on erythrocytes from sickle cell disease patients. *J Clin Invest*, 92(3), 1326-35 (1993)

106. J. Chang, J. T. Patton, A. Sarkar, B. Ernst, J. L. Magnani and P. S. Frenette: GMI-1070, a novel pan-selectin antagonist, reverses acute vascular occlusions in sickle cell mice. *Blood*, 116(10), 1779-86 (2010)

107. B. S. Coller: Leukocytosis and ischemic vascular disease morbidity and mortality: is it time to intervene? *Arterioscler Thromb Vasc Biol*, 25(4), 658-70 (2005)

108. E. Galkina and K. Ley: Immune and inflammatory mechanisms of atherosclerosis (*). *Annu Rev Immunol*, 27, 165-97 (2009)

109. K. J. Woollard and F. Geissmann: Monocytes in atherosclerosis: subsets and functions. *Nat Rev Cardiol*, 7(2), 77-86 (2010)

110. L. McGregor, J. Martin and J. L. McGregor: Platelet-leukocyte aggregates and derived microparticles in inflammation, vascular remodelling and thrombosis. *Front Biosci*, 11, 830-7 (2006)

111. R. Mazor, R. Shurtz-Swirski, R. Farah, B. Kristal, G. Shapiro, F. Dorlechter, M. Cohen-Mazor, E. Meilin, S. Tamara and S. Sela: Primed polymorphonuclear leukocytes constitute a possible link between inflammation and oxidative stress in hyperlipidemic patients. *Atherosclerosis*, 197(2), 937-43 (2008)

112. P. Rotzius, S. Thams, O. Soehnlein, E. Kenne, C. N. Tseng, N. K. Bjorkstrom, K. J. Malmberg, L. Lindbom and E. E. Eriksson: Distinct infiltration of neutrophils in lesion shoulders in ApoE-/- mice. *Am J Pathol*, 177(1), 493-500 (2010)

113. L. Yvan-Charvet, C. Welch, T. A. Pagler, M. Ranalletta, M. Lamkanfi, S. Han, M. Ishibashi, R. Li, N. Wang and A. R. Tall: Increased inflammatory gene expression in ABC transporter-deficient macrophages: free cholesterol accumulation, increased signaling via toll-like receptors, and neutrophil infiltration of atherosclerotic lesions. *Circulation*, 118(18), 1837-47 (2008)

114. A. Zernecke, I. Bot, Y. Djalali-Talab, E. Shagdarsuren, K. Bidzhekov, S. Meiler, R. Krohn, A. Schober, M. Sperandio, O. Soehnlein, J. Bornemann, F. Tacke, E. A. Biessen and C. Weber: Protective role of CXC receptor 4/CXC ligand 12 unveils the importance of neutrophils in atherosclerosis. *Circ Res*, 102(2), 209-17 (2008)

115. M. J. Davies, J. L. Gordon, A. J. Gearing, R. Pigott, N. Woolf, D. Katz and A. Kyriakopoulos: The expression of the adhesion molecules ICAM-1, VCAM-1, PECAM, and E-selectin in human atherosclerosis. *J Pathol*, 171(3), 223-9 (1993)

116. R. G. Collins, R. Velji, N. V. Guevara, M. J. Hicks, L. Chan and A. L. Beaudet: P-Selectin or intercellular adhesion molecule (ICAM)-1 deficiency substantially protects against atherosclerosis in apolipoprotein E-deficient mice. *J Exp Med*, 191(1), 189-94 (2000)

117. Z. M. Dong, S. M. Chapman, A. A. Brown, P. S. Frenette, R. O. Hynes and D. D. Wagner: The combined role of P- and E-selectins in atherosclerosis. *J Clin Invest*, 102(1), 145-52 (1998)

118. P. C. Burger and D. D. Wagner: Platelet P-selectin facilitates atherosclerotic lesion development. *Blood*, 101(7), 2661-6 (2003)

119. Y. Huo and K. Ley: Adhesion molecules and atherogenesis. *Acta Physiol Scand*, 173(1), 35-43 (2001)

120. M. F. Nageh, E. T. Sandberg, K. R. Marotti, A. H. Lin, E. P. Melchior, D. C. Bullard and A. L. Beaudet: Deficiency of inflammatory cell adhesion molecules protects against atherosclerosis in mice. *Arterioscler Thromb Vasc Biol*, 17(8), 1517-20 (1997)

Key Words: Neutrophil, Platelet, Erythrocyte, Adhesion, Inflammation, Vascular Injury, Selectin, Integrin, Microdomains, Review

Send correspondence to: Andres Hidalgo, Centro Nacional de Investigaciones Cardiovasculares. Calle Melchor Fernandez Almagro, 3 Madrid, 28029 Madrid, Spain, Tel: 34-91 453 1200, Fax: 34- 91-4531245. E-mail: ahidalgo@cnic.es

http://www.bioscience.org/current/vol3S.htm