IUBMB *Life*, 63(7): 521–527, July 2011

Critical Review

Identification of Galectins as Novel Regulators of Platelet Signaling and Function

Maria Albertina Romaniuk[1], Soledad Negrotto[1], Oscar Campetella[2], Gabriel Adrian Rabinovich[3] and Mirta Schattner[1]

[1]*Thrombosis I Laboratory, National Academy of Medicine, CONICET, Argentina*
[2]*Institute for Research in Biotechnology, University of San Martín, CONICET, Argentina*
[3]*Laboratory of Immunopathology, Institute of Biology and Experimental Medicine (IBYME), CONICET, Argentina*

Summary

Platelet activation at sites of vascular injury leads to the formation of a hemostatic plug. Activation of platelets is therefore crucial for normal hemostasis. However, uncontrolled platelet activation may also lead to the formation of occlusive thrombi that can cause ischemic events. Platelets can be activated by soluble molecules including thrombin, TXA_2, adenosine diphosphate (ADP), and serotonin or by adhesive extracellular matrix (ECM) proteins such as von Willebrand factor and collagen. In this article, we review recent advances on the role of galectins in platelet physiology. By acting in either soluble or immobilized form, these glycan-binding proteins trigger platelet activation through modulation of discrete signaling pathways. We also offer new hypotheses and some speculations about the role of platelet–galectin interactions not only in hemostasis and thrombosis but also in inflammation and related diseases such as atherosclerosis and cancer.
IUBMB *Life*, 63(7): 521–527, 2011

Keywords glycobiology; membrane proteins; platelets; thrombosis; galectin; cancer; atherosclerosis.

INTRODUCTION

Platelets are enucleated blood cells derived from megakaryocytes that are essential for proper hemostasis and thrombosis and also play critical roles in inflammatory processes, atherosclerosis, tumor metastasis, and host defense. When platelets perceive activating signals through their cell surface receptors, they undergo dramatic structural and chemical changes, involving a complex interplay of cell adhesion and signaling molecules. Activated platelets rapidly bind circulating platelets via membrane integrin $\alpha_{IIb}\beta_3$ (GPIIb/IIIa) and fibrinogen, to form a thrombus to prevent bleeding at the sites of vascular injury. However, platelet aggregation can also occlude atherosclerotic arteries causing cardiac and cerebrovascular diseases (*1*).

Galectins are structurally related carbohydrate-binding proteins, which are defined by their affinity for poly-*N*-acetyllactosamine-enriched glycoconjugates and sequence similarities in the carbohydrate recognition domain (CRD) (*2*). Although experimental and clinical studies have extensively implicated galectins in the regulation of immune cell homeostasis and host–pathogens interactions, there is an increasing evidence that these proteins are also involved in the pathogenesis of cardiovascular diseases, in particular the development of atherosclerosis (*3, 4*). Moreover, recent work from our laboratory has shown that galectin (Gal)-1 and Gal-8 can trigger platelet activation and promote the formation of platelet–leukocyte aggregates suggesting a novel mechanism of thrombus formation mediated by galectin–glycan interactions (*5, 6*).

In this review, we summarize recent knowledge on the role of galectins in platelet signaling and physiology and the critical implications of these emerging findings in thrombosis, inflammation, atherosclerosis, and cancer.

BIOLOGY OF GALECTINS

Galectins constitute a family of animal lectins that bind beta-galactoside residues through their CRD composed of a sequence of 130 amino acids (*7, 8*). These lectins are found in almost all organisms and to date 15 galectins have been found in mammals although only 12 galectin genes are found in humans. According to their structure, galectins are classified into three groups: 1) "proto-type" galectins, containing a single CRD including galectin-1, -2, -7, -10, -13, and -14; 2) chimeric galectins which contain a single CRD and a large amino-terminal

Received 4 March 2011; accepted 31 March 2011
Address correspondence to: M. Schattner, National Academy of Medicine, Pacheco de Melo 3081, Buenos Aires 1425, Argentina. Tel: +54-11-4805-5759. Fax: +54-11-4805-0712.
E-mail: mschattner@hematologia.anm.edu.ar

ISSN 1521-6543 print/ISSN 1521-6551 online
DOI: 10.1002/iub.483

nonlectin domain being Gal-3 its only representative; and 3) tandem-repeat type galectins that have two CRD linked by a peptide sequence of variable length and this subgroup includes galectin-4, -8, -9, and -12 (*2*). Although the CRD sequence is highly conserved among galectins, each member of this family recognizes subtly different glycan structures and shows individual affinities for them (*9*). Recent evidence, using different approaches including glycan arrays and frontal affinity chromatography, defined significant divergences in glycan-binding preferences of individual members of the galectin family (*9, 10*). For example, galectin-8 has much higher affinity for 3′O-sulfated or 3′O-sialylated glycoconjugates and a Lewis X-containing glycan (*11*) in contrast to galectin-1 which displays high affinity for oligosaccharides terminating in Galβ_1-3/4GlcNAc, and this specificity is mainly attributed to the N-terminal CRD (*12*).

Although galectins that contain one CRD can exist as dimers, Gal-3 can form pentamers and most galectins can form ordered arrays named lattices when they bind to multivalent glycoconjugates (*7*). Regarding their subcellular localization, galectins exhibit a unique pattern as they can be found in the nucleus, cytoplasm, outer plasma membrane, and extracellular matrices shuttling between different compartments and displaying a combination of intra- and extracellular activities (*9, 13*).

Galectins are pleiotropic regulators of cell physiology that participate in many essential processes such as cell adhesion, proliferation, differentiation, and apoptosis (*7–9, 14*). They also play unique roles in intracellular signaling pathways and cell cycle control (*9, 15*). Moreover, galectins participate in wound healing and development as well (*13*). Among their multiple biological effects, galectins play key roles in the tuning of both innate and adaptive immune responses (*2, 16, 17*). Gal-1, -2, -3, -8, and -9 are modulators of T cell homeostasis (*2*). Also, Gal-1 participates in the modulation of immune cell trafficking, survival, growth, chemotaxis, and release of anti-inflammatory mediators (*7*). In addition, recent evidence indicates that Gal-1 plays critical roles in the regulation of tolerogenic dendritic cell circuits (*18*) and selectively regulates the survival of Th1 and Th17 cells (*19*). Conversely, Gal-3 activates immune cells and promotes adhesion and release of proinflammatory mediators (*16*). On the other hand, Gal-9 is a potent eosinophil chemoattractant that promotes dendritic cell maturation and induces apoptosis of T cells (*16*). Besides, galectins can bind glycans on the surface of pathogenic microbes coupling recognition processes to innate immunity (*20*).

Galectins are expressed in a wide variety of tissues. Although some members, such as Gal-1, -3, -8, and -9 show a wide tissue distribution, others such as Gal-12 or Gal-7 show a more restricted localization. Gal-1, -3, -8, and -9 are found on cells of the hematopoietic lineage as well as in endothelial cells (*2, 7, 21*). Gal-2, -4, -7, and -9 are expressed in different epithelia (*7*) and Gal-12 is almost exclusively found in adipose tissue (*7*). Galectin expression is also modulated throughout cell activation, inflammation, and in pathologic processes such as atherosclerosis and cancer (*4, 16, 22*).

Most galectins are overexpressed in several types of tumors and their expression correlates with tumor progression, aggressiveness, and metastatic phenotype (*13, 22*). For example, Gal-1 overexpression contributes to tumor immune escape, modulates tumor cell migration and adhesion, and influences tumor angiogenesis (*23*). On the other hand, Gal-3 inhibits apoptosis of tumor cells to promote tumor growth (*7, 13*). High expression of Gal-8 is associated with malignancy in some type of tumors such as colon and lung and, preliminary studies showed that inhibition of Gal-8 promotes tumor regression (*24*).

THE ROLE OF PLATELETS IN HEMOSTASIS AND OTHER PATHOPHYSIOLOGIC PROCESS

The primary function of platelets is to initiate and maintain hemostasis. However, their function is not limited to thrombosis, and extends to physiological processes such as tissue repair, wound remodeling, and antimicrobial host defense, or pathologic conditions such as atherosclerosis, chronic ischemia, or cancer (*25*). In all these processes, platelets act as sentinels capable of responding rapidly to chemical changes in their environment through their cell surface receptors.

After vascular injury, the initial tethering and firm adhesion of platelets to the exposed subendothelium are mediated by the glycoprotein (GP) Ib/IX/V complex and collagen receptors, GP VI and $\alpha_2\beta_1$ integrin, in the platelet surface, and by their respective ligands, vWF, and collagen in the vascular wall. Thereafter, soluble agonists such as ADP, thrombin, and TXA_2, produced/released at the site of injury act in autocrine and paracrine manner to amplify platelet activation and to recruit circulating platelets to the developing thrombus. Specific interactions of these agonists with their G-protein-coupled receptors generate inside-out signaling leading to conformational activation of integrins, in particular $\alpha_{IIb}\beta_3$, increasing their ligand affinity. Binding of $\alpha_{IIb}\beta_3$ to its ligands, mainly fibrinogen, supports processes such as clot retraction, platelet aggregation, and secretion of the granule proteins (*1*). Platelets have four types of granules: dense, alpha, lysosomes, and peroxisomes. The great variety of molecules released from platelet granules (chemokines, growth factors, nucleotides, cytokines, fibrinogen, vWF, and cell adhesion molecules) is the reason why platelets are also critically involved in other biological process beyond hemostasis.

The contribution of platelets to the inflammatory response is an outcome of the interaction of platelets with leukocytes and endothelial cells. Stimulated "sticky" platelets express on their cell surface, P-selectin, and CD40L α-granule-stored proteins that enable recruitment and activation of leukocytes at the sites of vascular injury as well as platelet adhesion to the endothelium. The interaction between the three types of cells creates a mutual feedback loop of reciprocal activation and inhibition leading to modulation of the inflammatory process (*26*).

Activated platelets and microparticles that are released concomitantly, also have the ability to catalyze the coagulation cascade. This is accomplished by an activation-dependent mem-

brane inversion or "flip-flop" by which normally in-facing phospholipids, mainly phosphatidylserine become exposed to plasma and render the membrane procoagulant by promoting the assembly of the vitamin K-dependent coagulation factors into their active complexes to generate the thrombin that converts fibrinogen to the fibrin plug (*27*).

The expression of P-selectin is also a main mediator of platelet–tumor cell interactions that endows tumor cell physical and mechanical support to evade the immune system and facilitates microvascular arrest of tumor cells at distant sites (*28*). In addition, platelets also participate in tumor progression by facilitating delivery of angiogenesis regulators and other growth factors (*29*).

Beyond acute activation as a consequence of vascular injury, circulating platelets are actively involved in all phases of the atherogenetic process, from atherosclerotic plaque formation to plaque inflammation and rupture. In these conditions, platelet reactivity is increased by reactive oxygen species (ROS) produced as a consequence of oxidative stress, by reduction of endothelial antithrombotic properties and by the increased availability of proinflammatory mediators, such as cytokines and chemokines (*30*).

GALECTINS AND PLATELET AGGREGATION

The capacity of platelets to form a thrombus depends on their ability to aggregate. At a molecular level, platelet aggregation is mediated by a specific receptor on the platelet surface: the $\alpha_{IIb}\beta_3$ integrin (*31*). Similar to most traditional platelet agonists, both soluble Gal-1 and Gal-8 promote the transition of this integrin from a low-affinity state (resting state) to a high-affinity state (active state), which results in the unmasking of neoepitopes in the $\alpha_{IIb}\beta_3$ complex, and allows fibrinogen binding which acts as a bridging molecule between platelets to form aggregates. This conformational change of $\alpha_{IIb}\beta_3$ integrin triggered by galectins is accompanied by a raise in intracellular calcium levels as well as morphological changes of platelets involving the rearrangement of the cytoskeleton including extension of filopodia and lamellipodia (spreading) and F-actin polymerization.

Both Gal-1 and Gal-8 induce aggregation of platelets suspended in either plasma or buffer which indicates a relevant role of these lectins in physiological media (*5, 6*). Although cell agglutination was one of the first biological activities described for these lectins, this effect on platelets was only observed at high-Gal-1 and Gal-8 concentrations. Platelet aggregation at lower concentrations is absent in fixed platelets, in the presence of calcium chelating agents, or eptifibatide (an $\alpha_{IIb}\beta_3$ antagonist), implying that platelet responses mediated by galectins could be related either to cell activation or to a clustering effect of platelet surface receptors depending on their concentration. Although both galectins were capable of promoting aggregation, Gal-8 was found to be 10 times more potent than Gal-1 in this stimulatory effect. Although the dimeric structure of Gal-8 would expect a more robust effect of this galectin compared with Gal-1, the observation that only the N-terminal domain was also able to trigger platelet activation indicates that lectin bivalency is not essential to promote activating effects on platelets. The differences in the concentration required to achieve a similar effect could therefore reflect different downstream molecular signals triggered by each galectin.

The initial formation of platelet thrombus is rapidly reinforced by the generation and release of platelet TXA_2 and ADP (the main metabolite of arachidonic acid by the cyclooxigenase pathway, and a component of platelet dense granules, respectively) which acting in a paracrine and autocrine manner promote further platelet activation (*1*). Both molecules are generated on platelet stimulation by Gal-1 and Gal-8. Moreover, although the aggregation response triggered by low-Gal-8 concentrations was inhibited in the presence of aspirin (cyclooxigenase inhibitor) and/or an ADP-scavenger, a full response was obtained at higher concentrations indicating that Gal-8 is a strong agonist that activate platelets independently from TXA_2 formation or ADP release (*6*).

GALECTINS TRIGGER PROINFLAMMATORY AND PROCOAGULANT PLATELET-MEDIATED RESPONSES

The role of platelets in inflammation and vascular repair is mainly associated with the release of α-granule content which includes among others cell adhesion molecules that favor platelet/endothelial and leukocyte interaction (P-selectin, CD40L) prothrombotic substances (vWF) and pro- and antiangiogenic molecules [vascular endothelial growth factor (VEGF) or endostatin, respectively] (*26*).

Gal-1 and Gal-8 are strong inducers of P-selectin expression. The major role of P-selectin on the platelet surface is the interaction with PSGL-1, its major counter-receptor on leukocytes, to form platelet–leukocyte aggregates (*32*). In fact, the activation of platelets by Gal-1 in the presence of polymorphonuclear leukocytes results in a significant formation of heterotypic cell aggregates (*5*). This interaction promotes the activation of both cell types, which is a crucial condition for triggering inflammation, vascular remodeling, and thrombosis. Platelet–leukocyte aggregates represent an established link between inflammation and thrombosis in acute syndromes including coronary diseases and related disorders (*33, 34*). Furthermore, the interaction of P-selectin with PSGL-1 induces the upregulation of tissue factor in the leukocyte membrane and the production of procoagulant microparticles, thereby contributing to a prothrombotic state. In addition, a role of P-selectin in platelet aggregation and the formation of arterial thrombi have also been described (*35*).

vWF is a large multimeric GP that allows platelet–endothelium, platelet–subendothelium, and platelet–platelet interaction and is therefore important for the platelet adhesion and thrombus formation. Similarly to P-selectin, vWF is stored in platelet α-granules and in Weibel–Palade bodies of endothelial cells from which is released during injury or inflammation. vWF is a biomarker for endothelial dysfunction and cardiovascular risk and high levels of vWF are found in both chronic and acute

inflammation (*36*). The release of α-granule-stored vWF occurs after the activation of platelets by Gal-1 (unpublished data) or Gal-8 (*6*). Thus, P-selectin expression and vWF release mediated by galectins might play an important role in the pathogenesis of thrombus formation and the modulation of the inflammatory responses.

Vesiculation of the platelet membrane and the formation of platelet microparticles (PMPs) is a characteristic effect of certain agonists such as collagen, thrombin, or C5b-9 complement fragment (*37*). PMPs are not only released during platelet activation *in vitro* but are also detected *in vivo* (*38, 39*). They are thought to provide catalytic surface for several enzyme complexes of the coagulation system and to underlie the procoagulant responses elicited by the platelet activation. Moreover, PMPs may themselves evoke cellular responses in the immediate microenvironment including platelet adhesion to the site of endothelial injury and angiogenesis (*40*). The ability of Gal-1 (*5*) to induce phosphatidylserine-expressing PMPs suggests that this lectin not only promotes platelet activation but could also indirectly activate the coagulation cascade.

GALECTINS AND PLATELET ADHESION

Platelet adhesion to the extracellular matrix (ECM) at the sites of vascular injury represents a key step for the limiting bleeding but, if uncontrolled, can lead to occlusion. Under low-shear rate, such as that found in veins and larger arteries, platelet adhesion to the vessel wall primarily involves binding to fibrillar collagen, fibronectin, and laminin. However, at conditions of elevated shear stress, the initial tethering and firm adhesion of platelets to the exposed subendothelium are mediated by the interaction of the platelet GPIb/IX/V complex and the subendothelial bound vWF (*1*). However, as vWF-deficient mice have delayed but not absent arterial thrombus formation, it was suggested that under these conditions GPIb may bind other ligands that can mediate platelet adhesion (*41*). Interestingly, *in vitro* studies have shown that Gal-8 (*6*) and Gal-1 (unpublished data) promote platelet adhesion and spreading and GPIbα was identified as a functional Gal-8 counter-receptor (*6*). Thus, it could be conceivable that galectins may act directly as a substrate or may either behave as matricellular proteins that bind to platelet GPIb-V-IX complex thus contributing to the platelet adhesion to the ECM. Of note, GPIb-V-IX complex plays a wide role in vascular biology as it not only binds to vWF but also recognizes several ligands on leukocytes and endothelium allowing the interaction of platelets with other vascular and blood cells (*41*).

PLATELET RECEPTORS FOR GALECTINS

Most of the extracellular functions mediated by galectins involve the interaction of these proteins with cell surface glycoconjugates containing repeating units of *N*-acetyllactosamine [Galβ1,4GlcNAc]. Platelet activation mediated by Gal-1 or Gal-8 is prevented by lactose but not sucrose (*5, 6*) indicating that these effects involve the interaction of these lectins with specific carbohydrate ligands on the platelet surface. Among the different receptors, integrins are involved in Gal-1-mediated biological responses. Given the relevance of different integrins in platelet function, these molecules may represent potential binding partners for Gal-1. In fact, matrix assisted laser desorption/ionization/time of flight (MALDI/TOF) mass spectrometry analyses determined that Gal-8 binds platelet surface GPs, subunit α_{IIb} from the $\alpha_{IIb}\beta_3$ integrin and GPIb and V from GPIb-IX-V complex (*6*). However, the use of platelets derived from patients who are deficient in $\alpha_{IIb}\beta_3$ or in GPIb revealed that only GPIb is essential for Gal-8-dependent signal transduction and therefore represents a functional Gal-8 counter-receptor (*6*). Moreover, Src, PI3K/Akt, and PLCγ2 (well known downstream signaling molecules related to GPIb-IX-V complex) (*42*) are involved in platelet activation induced by Gal-8, giving additional support to the notion that GPIb is an essential receptor for transducing Gal-8 signaling (*6*).

The platelet GP backbones bearing the discrete sets of oligosaccharide ligands required for Gal-1 binding have not yet been identified. Experiments performed with monoclonal antibodies showed that blockade of αIIb, β_1, and β_3 integrins or GPIb does not interfere with the binding of Gal-1 to the surface of human platelets (*5*). However, as antibodies may recognize epitopes that are not implicated in Gal-1 binding, the participation of these molecules as potential Gal-1-binding partners is still under investigation.

PLATELETS EXPRESS GALECTINS

In addition to almost all the vascular cells, human platelets express substantial levels of Gal-1 and Gal-8 (*5, 6*). Although both galectins are secreted proteins, they are mainly found in the cytosol of different cell types. Human platelets express the two splice variants of Gal-8. Moreover, whereas Gal-8, such as P-selectin, is absent on the surface of resting platelets, it is exposed on the membrane of thrombin-stimulated platelets. By contrast, Gal-1 is not expressed on platelet surface on activation (unpublished observation). The differences between both galectins are still not clear. Interestingly, the addition of lactose and thiodigalactoside moderately inhibits the aggregation induced by classical agonists suggesting that platelet-derived galectins might contribute to platelet activation.

PATHOPHYSIOLOGIC IMPLICATIONS OF GALECTIN–PLATELET INTERACTIONS

Hemostasis, Thrombosis, and Inflammation

Given the described effects of Gal-1 and Gal-8 in platelet physiology, the exposure of these endogenous lectins in the subendothelium or in activated endothelial cells (*21*) is expected to trigger platelet adhesion, spreading, and thrombus formation (Fig. 1A). Moreover, in the vascular system, platelets are another source of Gal-8 that would be accessible on platelet activation to eventually promote further thrombus growth. Studies in galectin-null mutant mice might reveal the relevance of these lectins in physiological hemostasis.

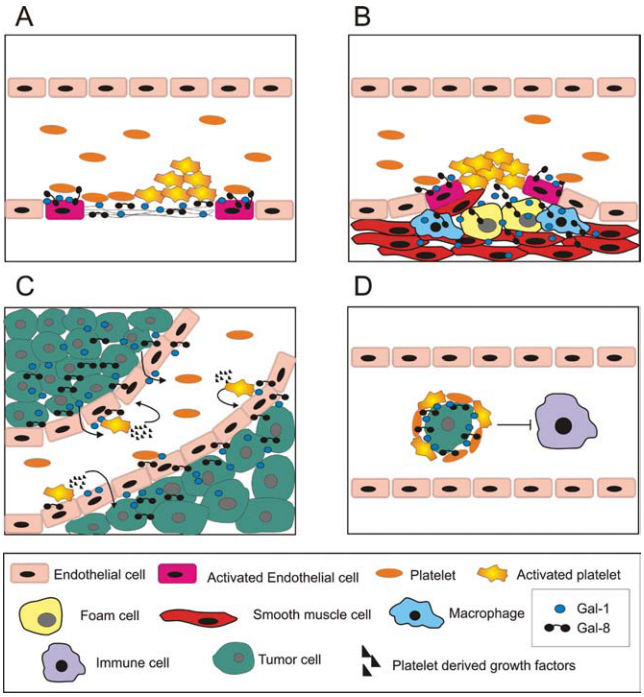

Figure 1. Potential galectin's roles in physiopathological platelet responses. (A) When endothelial injury occurs, platelets could recognize Gal-1 and Gal-8 present in the surface of the activated endothelial cells and in the exposed subendothelial ECM. Galectins would be able to trigger platelet adhesion, spreading and thrombus formation. (B) Galectins might be involved in atheroma plaque formation and progression. When plaque rupture occurs platelets may bind to galectins present in the plaque and in the activated endothelium thus enhancing thrombus formation. (C) Tumor cells overexpress Gal-1 and Gal-8. The lectins are released or exposed in the tumor cell surface and this might activate platelets which release the content of their granules that is rich in growth factors that can promote tumor development and angiogenesis. (D) A circulating tumor cell that expresses Gal-1 and Gal-8 on its surface can bind platelets, thus forming heterotypic aggregates which may possibly protect tumor cells against immune attack, favoring metastasis.

The expression of P-selectin on the platelet surface, the formation of leukocyte–platelet aggregates, and leukocyte activation are relevant events in deep vein thrombosis (DVT). Given that galectins promote all these responses, binding of these lectins to platelets might represent potentially novel mechanism involved in DVT. Interestingly, Gal-3-binding protein has been found to be upregulated in proteomics analysis of microparticles during DVT (*43*). Although Gal-8 has been linked to proinflammatory processes (*44, 45*), both, anti- and proinflammatory activities have been ascribed to Gal-1 (*7*). Whether anti-inflammatory effects occur at low concentrations of Gal-1, while proinflammatory effects prevail at high concentrations, still remains to be established. The fact that Gal-1 promotes platelet activation support to the notion that under certain circumstances, Gal-1 could also act as a proinflammatory factor.

Atherosclerosis

Although the expression and role of Gal-8 in human atherosclerotic lesions has not yet been explored, several groups demonstrated that galectins including Gal-1 participate in the initiation, progression, and rupture of an atheroma plaque. Gal-1, -3, and -8 are not only expressed by the different cellular components of the atheroma lesions (*46–48*) but also promote smooth muscle cell proliferation and transformation of macrophages into foam cells through the uptake of modified lipoproteins or advanced glycosylation end products (AGEs) (*49, 50*).

Platelets are essential mediators in atherothrombosis. Platelet adhesion and mural thrombosis are ubiquitous in the initiation and generation of the lesions of atherosclerosis in animals and humans. Platelets can adhere to dysfunctional endothelium, exposed collagen, and macrophages. When activated, platelets release their granules, which contain cytokines and growth factors that contribute to the migration, activation, and proliferation of smooth-muscle cells, monocytes, and T-leukocytes (*51*).

The expression of galectins in the inner of atheroma plaque may not only represent strong amplifiers of platelet activation but also be key components of the extremely thrombogenic core exposed after plaque rupture, the main trigger for acute thrombus formation and cause of unstable angina, myocardial infarction, transient ischemic attack, and stroke (Fig. 1B). Evidence for a role of galectins in atherosclerosis has also emerged from whole-genome association studies for myocardial infarction in Asian populations (*46*) although these findings were not reproduced in studies of populations with other ancestries (*52*).

Tumor Progression

It has been extensively shown that depletion or functional inactivation of platelets through a variety of genetic and pharmacological manipulations markedly reduces tumor progression and metastasis. Platelets may influence the metastatic potential of tumor cells via several mechanisms: (*a*) through the release of a variety of inflammatory mediators which may influence tumor growth and stroma formation, (*b*) through the expression of P-selectin, platelets may contribute to the stable adhesion to endothelium and/or transmigration of tumor cells outside the vasculature, and (*c*) through the formation of heterotypic aggregates between mucins in circulating cancer and P-selectin in activated platelets which may protect tumor cells against immune attack (*28*).

The overexpression of galectins in tumor cells might also be a trigger for the platelet activation allowing the release of α-granule contents that include growth factors which can promote tumor development and angiogenesis (Fig. 1C). Furthermore, the formation of mixed-cell aggregates between tumor cells expressing high levels of galectins and platelets might also represent a complementary molecular mechanism to mucins and

P-selectin interaction by which platelets contribute to tumor progression and metastasis (Fig. 1D).

Cancer-Associated Thrombosis

Thrombosis and disseminated intravascular coagulation are common complications in cancer patients. A hypercoagulable or prothrombotic state of malignancy occurs because of the ability of tumor cells to activate platelets and the coagulation system. Prothrombotic factors in cancer include the ability of tumor cells to produce and secrete procoagulant/fibrinolitic substances and inflammatory cytokines and the physical interaction between tumor cell and platelets (*53*). However, the mechanisms allowing the occurrence of prothrombotic states in cancer patients are not completely understood.

The observed increased levels of Gal-1 and Gal-8 in tumoral endothelial cells as well as in other malignant cells could represent a pathogenic mechanism involved in thrombosis and disseminated intravascular coagulation complications, commonly present in cancer patients. Given the pivotal role of Gal-1 and Gal-8 in tumor progression, it could be conceivable that galectin-induced platelet activation might contribute to the pathogenesis of thrombosis in cancer patients.

CONCLUSIONS

Over the last decade, we have witnessed impressive advances regarding the biology of galectins and their role in cell homeostasis, in particular as regulators of the immune response. The information currently available indicates that galectins are expressed and secreted by several cell types in normal and pathological conditions.

The emerging evidence showing that galectins are also capable of triggering platelet activation opens a completely new field of research where so far there are more questions than answers. The study of platelet activation mediated by galectins will certainly provide further insight into the mechanisms linking inflammatory mediators to thrombus formation and could expand our view of the role of platelets much beyond homeostasis to their pathophysiologic role during inflammation and cancer.

ACKNOWLEDGEMENTS

The authors apologize many authors whose articles could not be cited and instead referred to other reviews owing to space limitations. Supported by grants from Agencia Nacional de Promoción Científica y Tecnológica, SN, OC, GAR, and MS are researchers from the "Consejo Nacional de Investigaciones Científicas y Técnicas" (CONICET). MAR is a fellow from CONICET.

REFERENCES

1. Rivera, J., Lozano, M. L., Navarro-Nunez, L., and Vicente, V. (2009) Platelet receptors and signaling in the dynamics of thrombus formation. *Haematologica* **94**, 700–711.
2. Rabinovich, G. A., Baum, L. G., Tinari, N., Paganelli, R., Natoli, C., et al. (2002) Galectins and their ligands: amplifiers, silencers or tuners of the inflammatory response? *Trends Immunol.* **23**, 313–320.
3. Papaspyridonos, M., McNeill, E., de Bono, J. P., Smith, A., Burnand, K. G., et al. (2008) Galectin-3 is an amplifier of inflammation in atherosclerotic plaque progression through macrophage activation and monocyte chemoattraction. *Arterioscler. Thromb. Vasc. Biol.* **28**, 433–440.
4. Al-Ansari, S., Zeebregts, C. J., Slart, R. H., Peppelenbosch, M., and Tio, R. A. (2009) Galectins in atherosclerotic disease. *Trends Cardiovasc. Med.* **19**, 164–169.
5. Pacienza, N., Pozner, R. G., Bianco, G. A., D'Atri, L. P., Croci, D. O., et al. (2008) The immunoregulatory glycan-binding protein galectin-1 triggers human platelet activation. *FASEB J.* **22**, 1113–1123.
6. Romaniuk, M. A., Tribulatti, M. V., Cattaneo, V., Lapponi, M. J., Molinas, F. C., et al. (2010) Human platelets express and are activated by galectin-8. *Biochem. J.* **432**, 535–547.
7. Yang, R. Y., Rabinovich, G. A., and Liu, F. T. (2008) Galectins: structure, function and therapeutic potential. *Expert Rev. Mol. Med.* **10**, e17.
8. Barondes, S. H., Castronovo, V., Cooper, D. N., Cummings, R. D., Drickamer, K., et al. (1994) Galectins: a family of animal beta-galactoside-binding lectins. *Cell* **76**, 597–598.
9. Leffler, H. (2001) Galectins structure and function—a synopsis. Results Probl. *Cell Differ.* **33**, 57–83.
10. Ramkumar, R., and Podder, S. K. (2000) Elucidation of the mechanism of interaction of sheep spleen galectin-1 with splenocytes and its role in cell-matrix adhesion. *J. Mol. Recognit.* **13**, 299–309.
11. Ideo, H., Matsuzaka, T., Nonaka, T., Seko, A., and Yamashita, K. (2011) Galectin-8-N-domain recognition mechanism for sialylated and sulfated glycans. *J. Biol. Chem.* **286**, 11346–11355
12. Leppanen, A., Stowell, S., Blixt, O., and Cummings, R. D. (2005) Dimeric galectin-1 binds with high affinity to alpha2,3-sialylated and non-sialylated terminal *N*-acetyllactosamine units on surface-bound extended glycans. *J. Biol. Chem.* **280**, 5549–5562.
13. Laderach, D. J., Compagno, D., Toscano, M. A., Croci, D. O., Dergan-Dylon, S., et al. (2010) Dissecting the signal transduction pathways triggered by galectin–glycan interactions in physiological and pathological settings. *IUBMB Life* **62**, 1–13.
14. Elola, M. T., Wolfenstein-Todel, C., Troncoso, M. F., Vasta, G. R., and Rabinovich, G. A. (2007) Galectins: matricellular glycan-binding proteins linking cell adhesion, migration, and survival. *Cell. Mol. Life Sci.* **64**, 1679–1700.
15. Liu, F. T., Patterson, R. J., and Wang, J. L. (2002) Intracellular functions of galectins. *Biochim. Biophys. Acta* **1572**, 263–273.
16. Norling, L. V., Perretti, M., and Cooper, D. (2009) Endogenous galectins and the control of the host inflammatory response. *J. Endocrinol.* **201**, 169–184.
17. Rabinovich, G. A., and Toscano, M. A. (2009) Turning 'sweet' on immunity: galectin–glycan interactions in immune tolerance and inflammation. *Nat. Rev. Immunol.* **9**, 338–352.
18. Ilarregui, J. M., Croci, D. O., Bianco, G. A., Toscano, M. A., Salatino, M., et al. (2009) Tolerogenic signals delivered by dendritic cells to T cells through a galectin-1-driven immunoregulatory circuit involving interleukin 27 and interleukin 10. *Nat. Immunol.* **10**, 981–991.
19. Toscano, M. A., Bianco, G. A., Ilarregui, J. M., Croci, D. O., Correale, J., et al. (2007) Differential glycosylation of Th1, Th2 and Th17 effector cells selectively regulates susceptibility to cell death. *Nat. Immunol.* **8**, 825–834.
20. Vasta, G. R. (2009) Roles of galectins in infection. *Nat. Rev. Microbiol.* **7**, 424–438.
21. Thijssen, V. L., Hulsmans, S., and Griffioen, A. W. (2008) The galectin profile of the endothelium: altered expression and localization in activated and tumor endothelial cells. *Am. J. Pathol.* **172**, 545–553.
22. Liu, F. T., and Rabinovich, G. A. (2005) Galectins as modulators of tumour progression. *Nat. Rev. Cancer* **5**, 29–41.
23. Thijssen, V. L., Postel, R., Brandwijk, R. J., Dings, R. P., Nesmelova, I., et al. (2006) Galectin-1 is essential in tumor angiogenesis and is a

target for antiangiogenesis therapy. *Proc. Natl. Acad. Sci. USA* **103**, 15975–15980.
24. Bidon-Wagner, N., and Le Pennec, J. P. (2004) Human galectin-8 isoforms and cancer. *Glycoconj. J.* **19**, 557–563.
25. Smyth, S. S., McEver, R. P., Weyrich, A. S., Morrell, C. N., Hoffman, M. R., et al. (2009) Platelet functions beyond hemostasis. *J. Thromb. Haemost.* **7**, 1759–1766.
26. Dole, V. S., Bergmeier, W., Mitchell, H. A., Eichenberger, S. C., and Wagner, D. D. (2005) Activated platelets induce Weibel–Palade-body secretion and leukocyte rolling in vivo: role of P-selectin. *Blood* **106**, 2334–2339.
27. Freyssinet, J. M., and Toti, F. (2010) Formation of procoagulant microparticles and properties. *Thromb. Res.* **125** Suppl 1, S46–S48.
28. Borsig, L. (2008) The role of platelet activation in tumor metastasis. *Expert Rev. Anticancer Ther.* **8**, 1247–1255.
29. Jain, S., Harris, J., and Ware, J. (2010) Platelets: linking hemostasis and cancer. *Arterioscler. Thromb. Vasc. Biol.* **30**, 2362–2367.
30. Langer, H. F., and Gawaz, M. (2008) Platelet–vessel wall interactions in atherosclerotic disease. *Thromb. Haemost.* **99**, 480–486.
31. Shattil, S. J., Kim, C., and Ginsberg, M. H. The final steps of integrin activation: the end game. *Nat. Rev. Mol. Cell Biol.* **11**, 288–300.
32. Furie, B., and Furie, B. C. (2004) Role of platelet P-selectin and microparticle PSGL-1 in thrombus formation. *Trends Mol. Med.* **10**, 171–178.
33. Michelson, A. D., Barnard, M. R., Krueger, L. A., Valeri, C. R., and Furman, M. I. (2001) Circulating monocyte–platelet aggregates are a more sensitive marker of in vivo platelet activation than platelet surface P-selectin: studies in baboons, human coronary intervention, and human acute myocardial infarction. *Circulation* **104**, 1533–1537.
34. Dotsenko, O., Chaturvedi, N., Thom, S. A., Wright, A. R., Mayet, J., et al. (2007) Platelet and leukocyte activation, atherosclerosis and inflammation in European and South Asian men. *J. Thromb. Haemost.* **5**, 2036–2042.
35. Celi, A., Lorenzet, R., Furie, B. C., and Furie, B. (2004) Microparticles and a P-selectin-mediated pathway of blood coagulation. *Dis. Markers* **20**, 347–352.
36. Vischer, U. M. (2006) von Willebrand factor, endothelial dysfunction, and cardiovascular disease. *J. Thromb. Haemost.* **4**, 1186–1193.
37. Bode, A. P., Sandberg, H., Dombrose, F. A., and Lentz, B. R. (1985) Association of factor V activity with membranous vesicles released from human platelets: requirement for platelet stimulation. *Thromb. Res.* **39**, 49–61.
38. Pereira, J., Alfaro, G., Goycoolea, M., Quiroga, T., Ocqueteau, M., et al. (2006) Circulating platelet-derived microparticles in systemic lupus erythematosus. Association with increased thrombin generation and procoagulant state. *Thromb. Haemost.* **95**, 94–99.
39. van den Goor, J. M., van den Brink, A., Nieuwland, R., van Oeveren, W., Rutten, P. M., et al. (2003) Generation of platelet-derived microparticles in patients undergoing cardiac surgery is not affected by complement activation. *J. Thorac. Cardiovasc. Surg.* **126**, 1101–1106.
40. Brill, A., Dashevsky, O., Rivo, J., Gozal, Y., and Varon, D. (2005) Platelet-derived microparticles induce angiogenesis and stimulate post-ischemic revascularization. *Cardiovasc. Res.* **67**, 30–38.
41. Bergmeier, W., Piffath, C. L., Goerge, T., Cifuni, S. M., Ruggeri, Z. M., et al. (2006) The role of platelet adhesion receptor GPIbalpha far exceeds that of its main ligand, von Willebrand factor, in arterial thrombosis. *Proc. Natl. Acad. Sci. USA* **103**, 16900–16905.
42. Ozaki, Y., Asazuma, N., Suzuki-Inoue, K., and Berndt, M. C. (2005) Platelet GPIb-IX-V-dependent signaling. *J. Thromb. Haemost.* **3**, 1745–1751.
43. Diaz, J. A., Ramacciotti, E., and Wakefield, T. W. (2009) Do galectins play a role in venous thrombosis? A review. *Thromb. Res.* **125**, 373–376.
44. Tribulatti, M. V., Cattaneo, V., Hellman, U., Mucci, J., and Campetella, O. (2009) Galectin-8 provides costimulatory and proliferative signals to T lymphocytes. *J. Leukoc. Biol.* **86**, 371–380.
45. Cattaneo, V., Tribulatti, M. V., and Campetella, O. (2011) Galectin-8 tandem-repeat structure is essential for T-cell proliferation but not for co-stimulation. *Biochem. J.* **434**, 153–160.
46. Ozaki, K., Inoue, K., Sato, H., Iida, A., Ohnishi, Y., et al. (2004) Functional variation in LGALS2 confers risk of myocardial infarction and regulates lymphotoxin-alpha secretion in vitro. *Nature* **429**, 72–75.
47. Nachtigal, M., Al-Assaad, Z., Mayer, E. P., Kim, K., and Monsigny, M. (1998) Galectin-3 expression in human atherosclerotic lesions. *Am. J. Pathol.* **152**, 1199–1208.
48. Chellan, B., Narayani, J., and Appukuttan, P. S. (2007) Galectin-1, an endogenous lectin produced by arterial cells, binds lipoprotein(a) [Lp(a)] in situ: relevance to atherogenesis. *Exp. Mol. Pathol.* **83**, 399–404.
49. Papaspyridonos, M., Smith, A., Burnand, K. G., Taylor, P., Padayachee, S., et al. (2006) Novel candidate genes in unstable areas of human atherosclerotic plaques. *Arterioscler. Thromb. Vasc. Biol.* **26**, 1837–1844.
50. Moiseeva, E. P., Javed, Q., Spring, E. L., and de Bono, D. P. (2000) Galectin 1 is involved in vascular smooth muscle cell proliferation. *Cardiovasc. Res.* **45**, 493–502.
51. Ross, R. (1999) Atherosclerosis—an inflammatory disease. *N. Engl. J. Med.* **340**, 115–126.
52. Kimura, A., Takahashi, M., Choi, B. Y., Bae, S. W., Hohta, S., et al. (2007) Lack of association between LTA and LGALS2 polymorphisms and myocardial infarction in Japanese and Korean populations. *Tissue Antigens* **69**, 265–269.
53. Noble, S., and Pasi, J. (2010) Epidemiology and pathophysiology of cancer-associated thrombosis. *Br. J. Cancer* **102** Suppl 1, S2–S9.