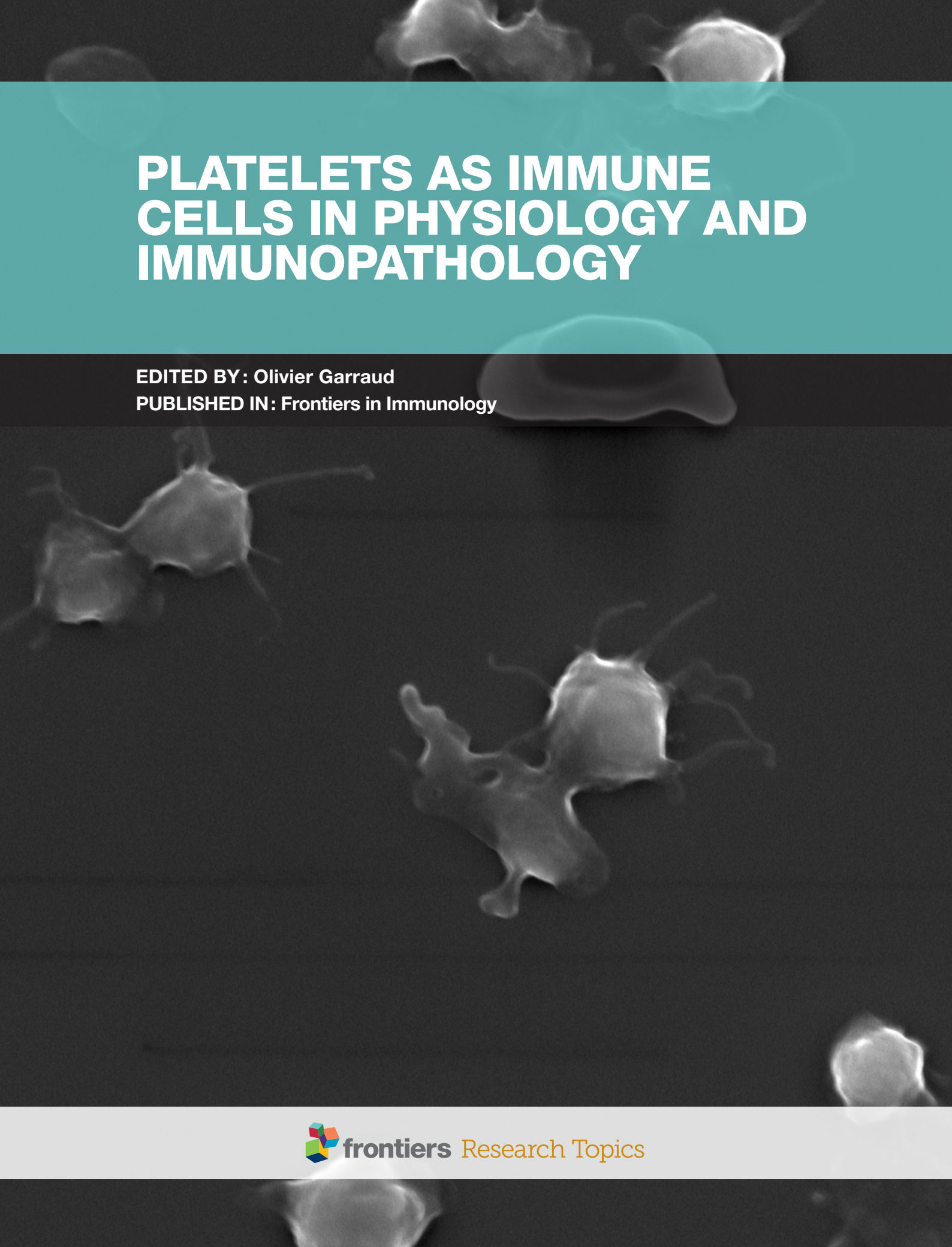
PLATELETS AS IMMUNE CELLS IN PHYSIOLOGY AND IMMUNOPATHOLOGY

EDITED BY: Olivier Garraud

PUBLISHED IN: Frontiers in Immunology



frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88919-740-8
DOI 10.3389/978-2-88919-740-8

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

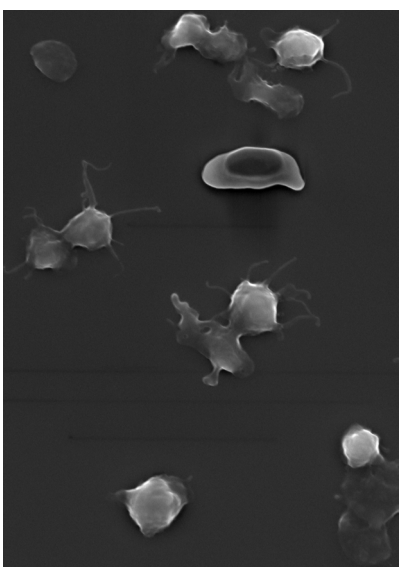
Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view.
By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: **researchtopics@frontiersin.org**

PLATELETS AS IMMUNE CELLS IN PHYSIOLOGY AND IMMUNOPATHOLOGY

Topic Editor:
Olivier Garraud, Institut National de la Transfusion Sanguine, France



Platelets possess important secretory functions, express internal membrane proteins, and release adhesive proteins, coagulation, and growth factors. Certain of the proteins facilitate the cross-talk of platelets with immune (e.g., leukocytes) and non-immune cells (e.g., endothelial cells). Thus, platelets play and important role in inflammatory and proliferative events and play a critical role for tissue remodeling and wound healing.

Image by Olivier Garraud

Are platelets cells? (Not everyone agrees, since they are non-nucleate). And if platelets are cells - which all specialists consider at the time being - are they immune cells? The issue that platelets participate in immunity is no longer debated; however, the issue that they are key cells in immunity is challenged. It has even been proposed a couple of years ago that platelets can present antigen to T-lymphocytes by using their HLA class I molecules. No one has the same functional definition of platelets. The 'Frontiers Research Topic'-coordinators' own view is that platelets are primarily repairing cells, what they do in deploying tools of physiological inflammation. This function is better acknowledged as primary hemostasis, i.e. platelet adherence to injured or wounded vessels, followed by activation, aggregation, and constitution of the initial clot. Platelets would thus repair damaged vascular endothelium; so doing, as they patrol to detect damages, they sense danger along the vascular arborescence. As the latter is immense, platelets get close to tissues, which are not allowed to them under 'physiological' conditions but are readily accessible in pathology. Platelets are equipped with a variety of Pathogen Recognition Receptors such as TLRs; they have a complete signalosome, which is functional until the phosphorylation of NFkB; they have been proved to retro-transcribe RNA and synthesize de novo proteins; etc. Platelets participate to inflammation along the whole spectrum: from physiological (tissue repair, healing) to acute/severe

inflammation (as can be seen in e.g. sepsis). In general, platelets engage complex interactions with most infectious pathogens.

We propose there to cover those topics - from physiology to pathology, that put platelets within cells that not only take place in-, but also are key players of-, innate immunity. The relation of platelets with adaptive immunity is even more complex. Not everyone is convinced that platelets present antigens; however, platelets influence adaptive immunity since they have mutual interactions with Dendritic cells, Monocytes/Macrophages, and B-lymphocytes (the key players of antigen presentation); they also have mutual interactions with T-lymphocytes, though is issue is less clearly deciphered. We propose to also cover these topics—or to present the forum. There is another issue which is medically relevant—speaking of physiology/physiopathology—: this is fetal maternal incompatibility of platelet specific antigens (the HPA system) and the likely formation of maternal antibodies that often injure the newborn with risks of severe thrombocytopenia and intracranial hemorrhage. We propose an update on this issue as well. Last, platelets are very special because they can be directly therapeutic (by transfusion), even when being offered by a generous blood donor displaying given genetic and phenotypic parameters to a patient/recipient in need, who also display his/her own genetic and phenotypic parameters, which—for a large part—differ from the donor's ones. Besides immunization—via mechanisms probably close to the fetal maternal platelet incompatibility, but likely not similar—, transfusion has allowed the identification of the tremendous capacity of platelets to mediate inflammation: we propose to conclude the Topics with this item/forum.

Citation: Garraud, O., ed. (2015). Platelets as Immune Cells in Physiology and Immunopathology. Lausanne: Frontiers Media. doi: 10.3389/978-2-88919-740-8

Table of Contents

frontiers in Immunology

EDITORIAL
published: 03 June 2015
doi: 10.3389/fimmu.2015.00274

Editorial: Platelets as immune cells in physiology and immunopathology

Olivier Garraud[1,2]*

[1] EA3064, University of Lyon Saint-Etienne, Saint-Etienne, France, [2] Institut National de la Transfusion Sanguine (INTS), Paris, France

Keywords: platelets, inflammation, infection, transfusion, immunity

Blood platelets are essential for the earliest stages of coagulation, namely, primary hemostasis. They adhere to damaged vessel endothelium, stick to each other (aggregate), and form clots; this prevents bleeding. For most physicians, those attributes of platelets are exactly what they learned in medical school years ago, and this basic knowledge seems quite enough to allow a valid therapeutic strategy when numbers or hemostatic functions of platelets are aberrant. In some cases, this consists in prescribing anti-platelet drugs (aspirin or more sophisticated drugs) to prevent overly active clotting in cardiovascular and metabolic dysfunctions. In other instances, this consists in prescribing platelet transfusions when the platelet count is dangerously low (or – in exceptional occasions – when platelets are dysfunctional). It could be as simple as that, but in fact, it is often not, because platelets are more versatile than initially thought (or expected) and some modification is needed in many cases (1). To cite only one example, anti-viral treatment of HIV infection causes atheroma and platelet deposition, emphasizing the recently recognized inflammatory function of platelets (2, 3); anti-platelet therapy seems a likely approach, but this is not current practice yet.

Thus, let us imagine that a scientific magazine writer decides to contribute a paper emphasizing novel advances in platelet research; the journal's instructions are: no more than four key points, a concise style, and only issues that can be understood by a large community; and – icing on the cake – a translation into today's or tomorrow's therapeutics. What would he/she insist on?

The proposed four points, which in our opinion, are either really new or newly rediscovered (after having been buried for decades and perhaps completely forgotten) would be:

- Not only are platelets genuine cells but also are they intelligent cells, as they can sense dangers differentially (4, 5).
- Despite platelets have been suspected to be inflammatory cells as soon as in the early 70s', this opinion either has been ignored or faded (6). Platelets indeed participate in innate immunity and they can influence adaptive immunity (7–9); they are "licensed" as highly potent pro-inflammatory cells (10).
- Platelets have a remarkable ability to sense and bind microbial agents, in particular, pathogenic viruses, and foremost bacteria (11, 12); interestingly enough, this property has been recognized in the early 70s' (13) but not exploited since recently (14).
- Platelets have more than one cell partner (the endothelial cell) as they intimately interact with the leukocyte, not only at different phases of the clot formation but also in tissue pathology (15, 16); this is also an issue, which is rediscovered after having been under-acknowledged according to its importance (17).

Why are all four key points really interesting for the medical community, and – beyond – to the patient community?

OPEN ACCESS

Edited by:
Pietro Ghezzi, Brighton and Sussex Medical School, UK

Reviewed by:
Rolando Rumbaut, Baylor College of Medicine, USA

***Correspondence:**
Olivier Garraud
ogarraud@ints.fr

Specialty section:
This article was submitted to Inflammation, a section of the journal Frontiers in Immunology

Received: *04 May 2015*
Accepted: *18 May 2015*
Published: *03 June 2015*

Citation:
Garraud O (2015) Editorial: Platelets as immune cells in physiology and immunopathology. Front. Immunol. 6:274. doi: 10.3389/fimmu.2015.00274

Concisely, we suggest three reasons:

– First, these findings lead to revisiting the essential functions of platelets. While platelets were principally considered relevant to vascular pathology (vessel injury and bleeding), today they are also considered as sentinels along the vascular tree, detecting insults and making daily repairs. Importantly, platelets perform an immune function as danger sensors, detecting circulating viruses and bacteria.
– Second, because they are non-nucleated, and mere fragments of the megakaryocyte, platelets were thought to be terminally differentiated cells, limited in function, fully equipped with static content; only one option remained to enrich their functions: to borrow glycoproteins from the environment. Recent evidence is that platelets can give rise to progeny platelets (18). Do these daughter cells possess identical capabilities to those of the mother cell? And are there no distinct subsets of platelets with different functions *in vivo*, as might be indicated by varying capacity for differential cytokine/chemokine secretion? Further, platelets are capable of using RNA to make secreted proteins (19, 20), an issue, which was suspected as in the late 60s' (21) and then disregarded (it is not fully consensual yet): not so a static dead-end cell after all!
– Lastly, platelets have been considered for some time as sentinels in severe clinical infection and particularly in sepsis (14). Maybe platelets are not just sentinels, but one among the primary targets of infectious pathogens, contributing to severe organ failure, especially because of their intimate relationship with leukocytes (22). Platelets were recently shown to infiltrate joints and cause serious inflammatory damage. Collectively, these observations call for revisiting at least partly the therapy of certain auto-inflammatory and infectious disease: what about anti-platelet drug use? Oh yes, some are very cheap such as aspirin: but is this effective and safe? In all, anti-platelet therapy reveals itself far more complex and nuanced than previously considered (23).

In aggregate, platelets span the classic field of hemostasis and thrombosis and the novel field of immunology and inflammation. Even transfusion medicine gurus are confused. Most were taught that low platelet counts below a given threshold (ranging from 10,000 to 30,000/μL of blood) require a platelet transfusion; now, they are kindly advised that all platelet transfusions are not equal and some transfusions may well be more pro-inflammatory than others (24, 25). How can they choose? They cannot, because the blood bank has made the choice for them: they are left with some confusion and concerns for their patients. Of note, this last paragraph would certainly not have been appropriate for the hypothetical science writer, because it does not speak to current reality of medical practice. Well, maybe not yet; but physicians and scientists are making significant progress in rendering platelet transfusion definitely much safer in terms of reduction of immunological hazards, and detecting genetic predisposition to harm (26, 27). Thus, we have the new general understanding that platelets are not that simple, after all, and that there may well be much more to learn again.

Acknowledgments

The author wishes to thank Dr. Neil Blumberg, University of Rochester, NY, USA, for sharing thoughts and for having given kind advice on this Editorial.

References

1. Kumar A, Mhaskar R, Grossman BJ, Kaufman RM, Tobian AA, Kleinman S, et al. Platelet Transfusion Guidelines Panel transfusion: a systematic review of the clinical evidence. *Transfusion* (2015) **55**(5):1116–27. doi:10.1111/trf.12943
2. Nording HM, Seizer P, Langer HF. Platelets in inflammation and atherogenesis. *Front Immunol* (2015) **6**:98. doi:10.3389/fimmu.2015.00098
3. Damien P, Cognasse F, Lucht F, Suy F, Pozzetto B, Garraud O, et al. Highly active antiretroviral therapy alters inflammation linked to platelet cytokines in HIV-1-infected patients. *J Infect Dis* (2013) **208**:868–70. doi:10.1093/infdis/jit260
4. Garraud O, Cognasse F. Are platelets cells? And if yes, are they immune cells? *Front Immunol* (2015) **6**:70. doi:10.3389/fimmu.2015.0007
5. Rondina MT, Garraud O. Emerging evidence for platelets as immune and inflammatory effector cells. *Front Immunol* (2014) **5**:653. doi:10.3389/fimmu.2014.00653
6. Nachman RL, Weksler B. The platelet as an inflammatory cell. *Ann N Y Acad Sci* (1972) **201**:131–7. doi:10.1111/j.1749-6632.1972.tb16294.x
7. Yeaman MR. Platelets: at the nexus of antimicrobial defence. *Nat Rev Microbiol* (2014) **12**:426–37. doi:10.1038/nrmicro3269
8. Morrell CN, Aggrey AA, Chapman LM, Modjeski KL. Emerging roles for platelets as immune and inflammatory cells. *Blood* (2014) **123**:2759–67. doi:10.1182/blood-2013-11-462432
9. Cognasse F, Nguyen KA, Damien P, McNicol A, Pozzetto B, Hamzeh-Cognasse H, et al. The Inflammatory role of platelets via their TLRs and Siglec receptors. *Front Immunol* (2015) **6**:83. doi:10.3389/fimmu.2015.00083
10. Semple JW, Italiano JE Jr., Freedman J. Platelets and the immune continuum. *Nat Rev Immunol* (2011) **11**:264–74. doi:10.1038/nri2956
11. Hamzeh-Cognasse H, Damien P, Chabert A, Pozzetto B, Cognasse F, Garraud O. Platelets and infections – complex interactions with bacteria. *Front Immunol* (2015) **6**:82. doi:10.3389/fimmu.2015.00082
12. Assinger A. Platelets and infection – an emerging role of platelets in viral infection. *Front Immunol* (2014) **5**:649. doi:10.3389/fimmu.2014.00649
13. Clawson CC, White JG. Platelet interaction with bacteria. II. Fate of the bacteria. *Am J Pathol* (1971) **65**:381–97.
14. Franks Z, Carlisle M, Rondina MT. Current challenges in understanding immune cell functions during septic syndromes. *BMC Immunol* (2015) **16**:11. doi:10.1186/s12865-015-0073-4
15. Gros A, Ollivier V, Ho-Tin-Noé B. Platelets in inflammation: regulation of leukocyte activities and vascular repair. *Front Immunol* (2015) **5**:678. doi:10.3389/fimmu.2014.00678
16. Goldenberg NM, Kuebler W. Endothelial cell regulation of pulmonary vascular tone, inflammation, and coagulation. *Compr Physiol* (2015) **5**:531–59. doi:10.1002/cphy.c140024
17. Hobbs JB, Cliff WJ. A study of allograft kidney rejection occurring simultaneously in whole organ and ear chamber grafts in the rabbit. *J Exp Med* (1973) **137**:776–98. doi:10.1084/jem.137.3.776
18. Schwertz H, Köster S, Kahr WH, Michetti N, Kraemer BF, Weitz DA, et al. Anucleate platelets generate progeny. *Blood* (2010) **115**:3801–9. doi:10.1182/blood-2009-08-239558
19. Lannan KL, Sahler J, Kim N, Spinelli SL, Maggirwar SB, Garraud O, et al. Breaking the mold: transcription factors in the anucleate platelet and platelet-derived microparticles. *Front Immunol* (2015) **6**:48. doi:10.3389/fimmu.2015.00048
20. Schubert S, Weyrich AS, Rowley JW. A tour through the transcriptional landscape of platelets. *Blood* (2014) **124**:493–502. doi:10.1182/blood-2014-04-512756

21. Warshaw AL, Laster L, Shulman NR. Protein synthesis by human platelets. *J Biol Chem* (1967) **242**:2094–7.
22. Rossaint J, Zarbock A. Platelets in leucocyte recruitment and function. *Cardiovasc Res* (2015). doi:10.1093/cvr/cvv048
23. Gachet C. P2 receptors, platelet function and pharmacological implications. *Thromb Haemost* (2008) **99**:466–72. doi:10.1160/TH07-11-0673
24. Katus MC, Szczepiorkowski ZM, Dumont LJ, Dunbar NM. Safety of platelet transfusion: past, present and future. *Vox Sang* (2014) **107**:103–13. doi:10.1111/vox.12146
25. Stolla M, Refaai MA, Heal JM, Spinelli SL, Garraud O, Phipps RP, et al. Platelet transfusion – the new immunology of an old therapy. *Front Immunol* (2015) **6**:28. doi:10.3389/fimmu.2015.00028
26. Garraud O, Cognasse F, Hamzeh-Cognasse H, Spinelli S, Phipps RP, Blumberg N. Removal of biologic response modifiers associated with platelet transfusion reactions: strategies worth considering? *Transfusion* (2014) **54**:2583. doi:10.1111/trf.12811
27. Aloui C, Sut C, Prigent A, Fagan J, Cognasse F, Granados-Herbepin V, et al. Are polymorphisms of the immunoregulatory factor CD40LG implicated in acute transfusion reactions? *Sci Rep* (2014) **4**:7239. doi:10.1038/srep07239

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 20 February 2015
doi: 10.3389/fimmu.2015.00070

Are platelets cells? And if yes, are they immune cells?

***Olivier Garraud*[1,2]* and *Fabrice Cognasse*[2,3]**

[1] *Institut National de la Transfusion Sanguine, Paris, France*
[2] *EA3064, Université de Lyon, Saint-Etienne, France*
[3] *Etablissement Français du Sang Auvergne-Loire, Saint-Etienne, France*

Edited by:
Masaaki Murakami, Hokkaido University, Japan

Reviewed by:
Daisuke Kamimura, Hokkaido University, Japan
Bo Shen, University of Illinois at Chicago, USA

***Correspondence:**
Olivier Garraud, Institut National de la Transfusion Sanguine, 6 rue Alexandre-Cabanel, Paris 75015, France
e-mail: ogarraud@ints.fr

Small fragments circulating in the blood were formally identified by the end of the nineteenth century, and it was suggested that they assisted coagulation via interactions with vessel endothelia. Wright, at the beginning of the twentieth century, identified their bone-marrow origin. For long, platelets have been considered sticky assistants of hemostasis and pollutants of blood or tissue samples; they were just cell fragments. As such, however, they were acknowledged as immunizing (to specific HPA and HLA markers): the platelet's dark face. The enlightened face showed that besides hemostasis, platelets contained factors involved in healing. As early as 1930s, platelets entered the arsenal of medicines were transfused, and were soon manipulated to become a kind of glue to repair damaged tissues. Some gladly categorized platelets as cells but they were certainly not fully licensed as such for cell physiologists. Actually, platelets possess almost every characteristic of cells, apart from being capable of organizing their genes: they have neither a nucleus nor genes. This view prevailed until it became evident that platelets play a role in homeostasis and interact with cells other than with vascular endothelial cells; then began the era of physiological and also pathological inflammation. Platelets have now entered the field of immunity as inflammatory cells. Does assistance to immune cells itself suffice to license a cell as an "immune cell"? Platelets prove capable of sensing different types of signals and organizing an appropriate response. Many cells can do that. However, platelets can use a complete signalosome (apart from the last transcription step, though it is likely that this step can be circumvented by retrotranscribing RNA messages). The question has also arisen as to whether platelets can present antigen via their abundantly expressed MHC class I molecules. In combination, these properties argue in favor of allowing platelets the title of immune cells.

Keywords: platelets, hemostasis, cell physiology, cell biology, immunophysiology, immunopathology, cytokines

INTRODUCTION

It has become usual in the medicine and physiology community to present platelets as "cells" that are indispensable to primary hemostasis (their function in thrombosis is sometimes ignored); but still today, it is not uncommon that representatives of the pathologist community refute use of the word "cells" for platelets. Platelets, for many, are dust or pollutants at worse, and cell debris at best, though no one doubts their hemostatic functions. Two distinct arguments may be considered in an attempt to explain their reluctance to recognize platelets as cells:

1. *Ex vivo*, platelets usually "contaminate" histological preparations; because they have no nucleus and they apparently are "solely" cytoplasm fractions, and platelets differ from cells forming tissues and also from the regular blood cells: they resemble impurities.
2. *In vivo*, platelet "puree," and not mandatorily fresh, alive, platelets within platelet components (PCs) for transfusion purposes, can also achieve hemostasis in emergency conditions and stop bleeding. This is how a large proportion of thawed PCs [a type of blood component (BC) rarely used] behaves.

DISCOVERY OF PLATELETS AND THEIR FUNCTIONS

The initial discovery of platelets is disputed. Was Boyle (end of the seventeenth century), Donné (early nineteenth century), or Bizzozzero (end of the nineteenth century) the real discoverer? It is generally considered that the acknowledgment there is a third cellular element in blood besides, and independent of, erythrocytes and leukocytes, is attributed to Bizzozzero, around 1881–1882. This Italian (Lombardy) physician and researcher also very elegantly acknowledged the role of platelets not only in hemostasis but also in thrombosis. Prior to Bizzozzero, and indeed as early as the seventeenth century, platelets had been suspected. For instance, van Leewenhoek, the Dutch microscopist who delivered amazing, seminal observations of natural life, reported precise observations of platelets around 1675. Hewson (around 1780) reported undefined blood particles. At that time, platelets bore names such as particles, corpuscles, and globules. Important information on their size, form change, granular content, and ability to emit filaments was thus available quite early. However, at this time, platelets were often considered to be of leukocyte origin and to be degenerate or degraded. Some observers favored an erythrocyte origin (such as erythrocyte precursors or haemoblasts). Bizzozzero set up

experiments to see platelets in veins, and also as circulating in the mesentery of living animals. He found that they were unrelated to erythrocytes and leukocytes, showed their role in hemostasis and thrombosis, and predicted the intimate relationship of platelets and leukocytes in the latter (and in other functions), as previously only leukocytes were visible in clots. The "only" discovery that Bizzozzero did not make is the acknowledgment of the bone marrow megakaryocyte ancestry of platelets, which Wright did in 1910 (1, 2).

WHY ARE PLATELETS SOMETIMES NOT RECOGNIZED AS CELLS?

Platelets are non-nucleated cell elements that, clearly, result from fractionation of bone marrow megakaryocytes (MKs). During differentiation, MKs are exposed to constantly increasing pH and pO_2 until reaching the sinuses, where platelets are released from proplatelets supported by shear from the blood flow. Expression of CD34 decreases as MKs mature, while expression of CD41 and CD42b increases (3). In the final step of MK development, platelets are released, and MK cytoskeletal reorganization is an important intracellular process for these morphologic changes. The correlation between cytoskeleton reorganization and proplatelet formation has not been completely clarified. Serotonin is thought to modulate cell migration and remodeling through activation of cytoskeleton reorganization, depending on the Rho/ROCK and Erk1/2 pathways, and $5HT_{1A/1B/1D}$ and 5HT4 receptors (4).

As such, platelets contain (inherit) MK cytoplasm complete with granules, mitochondria, and mRNA. Indeed, anucleate platelets lack genomic DNA but inherit a diverse array of functional coding or non-coding RNAs and translational machinery from their parent cells, enabling activated platelets to synthesize proteins, which suggests the possibility of post transcriptional gene regulation in platelets (5–8). The soluble proteins stored in the α-granule matrix, such as von Willebrand factor and thrombospondin, are derived via exclusive synthesis in the MKs, or for proteins such as fibrinogen and albumin, through endocytosis of plasma proteins (9).

Thus, platelets resemble cell fragments rather than fully licensed cells. One of the strongest arguments is probably that platelets have no genes to reorganize, because they have no nucleus and supporting DNA material (apart from the mitochondrial genome).

What about comparison with erythrocytes? Erythrocytes are not denied the qualification of cells though they have neither a nucleus nor genes to reorganize. However, the non-nucleated status of erythrocytes only pertains to mammals, not birds or reptiles. Erythrocytes in mammals evolved from erythroblasts, which are nucleated, and the process of enucleation is finite: one nucleated erythroblast gives rise to one erythrocyte with no intermediate division or transformation (10, 11). MKs, when forced to terminal maturation, separate into a degraded nucleus and platelets, emphasizing the nature of platelets, which are "only" cytoplasm fragments. An MK may produce 10–20 proplatelets, each of which starts as a blunt protrusion that over time elongates, thins, and branches repeatedly. The proplatelets extend into sinusoidal spaces, where they detach and fragment into individual platelets, giving rise to about 2000–5000 new platelets. Each day, in every human, approximately 1×10^{11} platelets are produced by the cytoplasmic fragmentation of MKs (12–15).

Although it is well established that platelets originate from MKs, the mechanisms by which they are formed and released remains controversial. Three models of platelet formation have been proposed: (1) cytoplasmic fragmentation, (2) platelet budding, and (3) proplatelet formation (13, 16, 17).

One must note that this classical picture of thrombopoiesis is unique (if one accepts the long-debated possibility that there is extramedullary thrombopoiesis, possibly in the lung). It has, however, recently been shown that there is a modest but definite thrombopoiesis in the circulation, as platelets give rise themselves to buds and extrusions distinct from platelet micro-particles (PMPs), turning into pro- and then pre-platelets (5–8, 18, 19). This is not a characteristic of a cell fragment without autonomy. This is, if further evidence is needed, a very strong argument in favor of platelets' classification as cells. Platelets also form the link between thrombosis and inflammation through the production of microparticles (MPs). PMPs are phospholipid vesicles (100–1000 nm) released after budding from the platelet plasma membrane. Platelets shed these membrane vesicles after stimulation with physiological agonists such as thrombin or collagen, in response to high shear stress (e.g., in severe stenosis) or in the presence of danger signals. As a result, PMP express the same antigens as their parent cells, i.e., GPIIb–IIIa, GPIb, CD31, CD61, and CD62P. This distinguishes them from MPs derived from other cell types (red blood cells, leukocytes, monocytes, endothelial cells). PMPs thus make up between 70 and 90% of the circulating vesicles. PMPs differ from exosomes by their size, and also due to the fact that they are not derived from exocytosis of multivesicular bodies (20–24).

PLATELETS BEHAVE AS CELLS

Platelets share some very important properties of cells. We list nine here – in physiological order of appearance – but there are many more.

1. Platelets display receptors for a variety of moieties, collectively termed "stimuli," but individually are quite different in nature. Platelets have glycoproteins (GPs) that sense exposed vascular sub-endothelium structures after vessel stress, insult, or attrition (including mechanical erosion and aging). Platelets can sense non-self infectious danger signals via a panoply of receptors detailed in a companion article. Platelets also display alarmins that can sense self-injury (25–27).
2. Platelets can respond to soluble molecules, via their receptors, and this can be particularly evidenced for thrombin or thrombin-derived peptides; hence, platelets are reactive to agonists and also to antagonists of such hemostatic/thrombotic factors. Additionally, platelets can react to biological response modifiers (BRMs) such as cytokines and chemokines, and become activated or inhibited. Some new drugs and biologics exploit those discerning properties (28–36).
3. Platelets comprise a complete and functional signalosome. Upon stimulation, they can phosphorylate a cascade of signaling molecules upstream of NFkB. NFkB is a crucial molecule in platelet physiology (37–42).

4. The platelet proteome is currently being investigated to determine the effects of treatments inflicted on platelets, such as for PC processing prior to transfusion. It is quite impressive, with more than 1000 proteins currently identified, and more being discovered regularly; 300–350 of those proteins have been proven as secreted by platelets (43). Platelet originating proteins come from three origins: (i) they may be inherited from the MK, (ii) they are absorbed from neighboring fluids and especially plasma [platelets have been described as "sponges" (44)], and (iii) they can be produced *de novo*, using a retrotranscription RNA process and spliceosome. This latter property further justifies the qualification of "cell" for platelets (45–51).
5. Platelets are docked with proteins/GPs for several, and distinct, purposes, including hemostasis, thrombosis, sensing, natural anti-infection (bacterial, viral, perhaps fungal) defense, chemo-attraction, cell communication, angiogenesis, healing, and tissue repair. It is clear, though under-evaluated in terms of physiological value, that platelets make distinction among the "dangers" they face. They can decipher vascular insult and presence of (circulating) infectious pathogens or components of the microbiota (infectious or not, an area that has not yet been examined in-depth), and secrete discrete, probably best-fitted, assortments of "products," likely coming into "profiles" of BRMs (34, 52–55). This has been well-studied in the case of innate immunity to bacterial products (and live bacteria as well), using the TLR1, TLR2, TLR3, TLR4, TLR6, TLR7, and TLR9 pattern recognition receptors (PRRs) (33, 35, 56–65): platelets can sense distinct natures of danger and secrete different patterns of BRMs accordingly. For instance, LPS, targeting TLR4, was shown to stimulate a distinct intracellular signaling pathway, and elicit the secretion of distinct profiles of BRMs (54, 55). Therefore, platelets are "intelligent" in that they effectively sense the nature of a given danger and respond accordingly.
6. It has been reported recently that platelets have novel functions in vascular permeability. Certain among these have been revealed in pathology; hence, platelets and their PMPs occasionally infiltrate tissues and cause attrition and inflammation (66). Platelets have recently been identified as being largely responsible for the discerned control of vascular permeability, with extravasation of lymphocytes, activated as effectors of immunity, but not erythrocytes because they otherwise warrant the sealing of the vascular arborescence (which is the primary role of platelets) (67). Platelets have also been reported novel roles in pathology: they make leukocytes prone to release neutrophil extracellular traps (NETs) with functions in infection (e.g., sepsis) and also in cancer, favoring thrombosis in either case (68–70).
7. Platelets can bind pathogens. Platelets can sense infectious pathogens and in certain occasion bind to them, sometimes tightly, either directly or indirectly as immune complexes with antibodies or complement factors such as C5a. *In vitro*, C5b–9 is capable of inducing P-selectin expression on platelets, and both C5a and C5b–9 induce surface expression of P-selectin on endothelial cells (71–74).
8. For reasons yet unclear, some infectious pathogens can enter platelets and at least reside in them. The issue of platelet "infection" is largely unstudied, as well as the outcome of the infected platelets. The relationship between platelets and infectious pathogens has been described in recent reviews (29, 30, 35, 75–81).
9. Platelets have one of the shortest lifespan among all human cells (only certain epithelial cells are comparable to them, if one excepts the granulocytes, which dye shortly from functioning as phagocytes): this property has probably contributed to deny the "cellularity" of platelets. Platelets die as a consequence of different causes/mechanisms. Leytin (82) clearly described all events of platelet death by apoptosis, but Jackson and Schoenwaelder (83) uses the term senescent platelet death rather than apoptosis *stricto sensu*. All events of activation-associated death are necrosis, since activation-associated platelet death results in improved inflammatory receptors, release of BRMs and aggregation that causes immune reactions. Because platelets are anucleate, their apoptosis leading to cell death is intriguing (82). Two main pathways were reported, (i) intrinsic and (ii) extrinsic, that are highly regulated by intra-platelet signaling mechanisms (84). Platelet apoptosis might also play a role in hemostasis, thrombosis, and inflammatory processes (84, 85).

The cellular functions of platelets are cartooned in **Figure 1** (**Figure 1A** gives a broad picture of the cellular functions of platelets, while **Figure 1B** details one selected platelet activation pathway from the sensing of a danger signal on the surface to the phosphorylation of NFκB (54) [NF-κB is a protein complex that usually controls transcription of DNA in Eukaryotes. NF-κB is found in almost all cell types involved in cellular responses to an extremely large variety of stimuli; it has NF-κB a key role in regulating the immune response to infection and – in turn – incorrect regulation of NF-κB has been linked to cancer, inflammatory, and autoimmune diseases, septic shock, viral infection, and improper immune development; it is also implicated in plasticity and cell survival (86)].

PLATELETS AS IMMUNE CELLS

What are "immune cells"? There is neither clear nor definite definition of an immune cell. A recent NIAID document states that: "*The immune system stockpiles a huge arsenal of cells, not only lymphocytes but also cell-devouring phagocytes and their relatives. Some immune cells take on all intruders, whereas others are trained on highly specific targets. To work effectively, most immune cells need the cooperation of their comrades. Sometimes immune cells communicate by direct physical contact, and sometimes they communicate releasing chemical messengers* [. . .]"[1]. According to that, platelets would be acknowledged as immune cells, but this document next stipulates that: "*All immune cells begin as immature stem cells in the bone marrow. They respond to different cytokines and other chemical signals to grow into specific immune cell types* [. . .]"[1]; this addition would thus next deny the attribute of "immune" cell to platelets, as they do not transform themselves. Well considered, this definition does not, either, take into consideration cells and organs that are now known as being essential to optimal immune functioning,

[1]http://www.niaid.nih.gov/topics/immunesystem/Pages/immuneCells.aspx

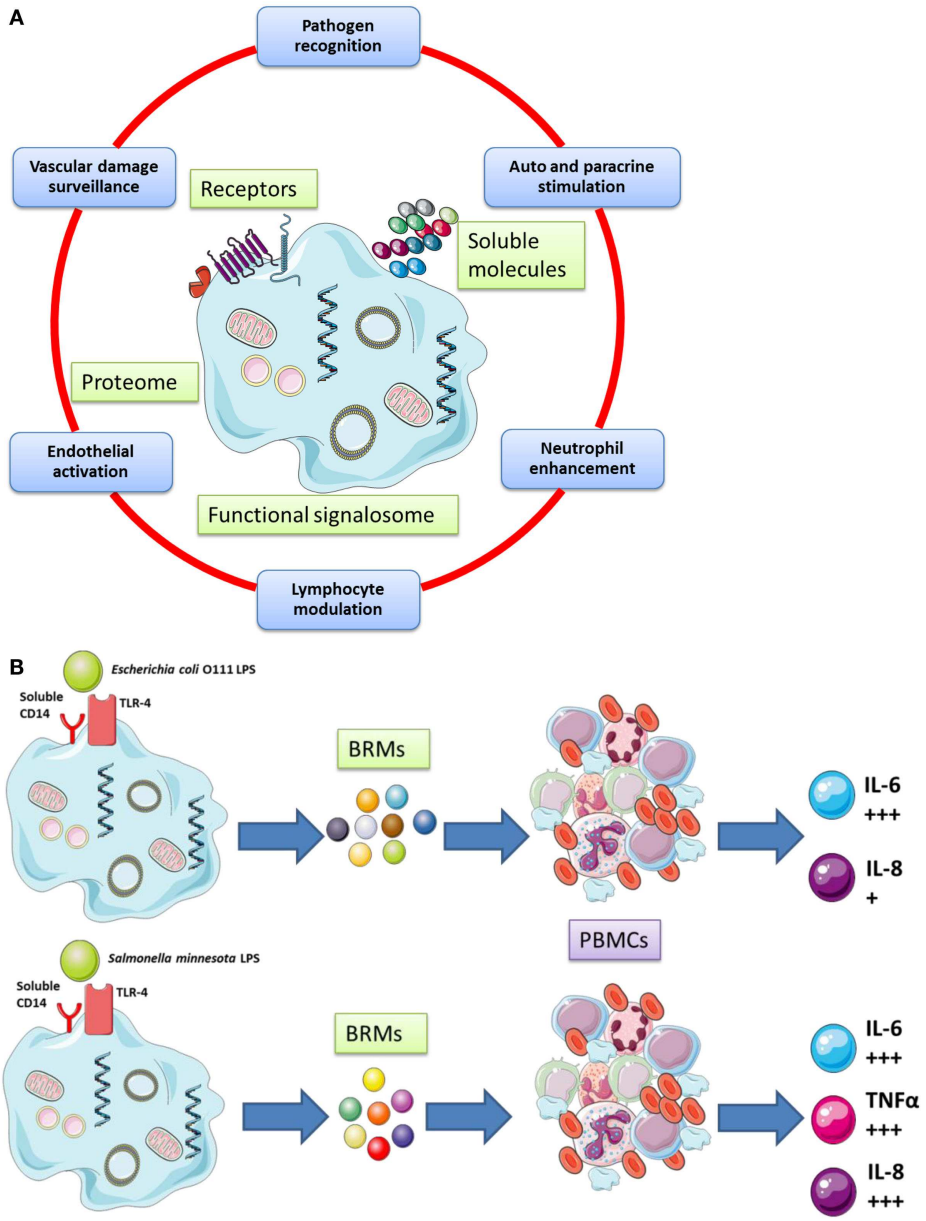


FIGURE 1 | (A) Platelets possess important secretory functions, express internal membrane proteins, and release adhesive proteins, coagulation, and growth factors. Certain of the proteins facilitate the cross-talk of platelets with immune (e.g., leukocytes) and non-immune cells (e.g., endothelial cells). Thus, platelets play and important role in inflammatory and proliferative events and play a critical role for tissue remodeling and wound healing. **(B)** Human platelets can discriminate between various bacterial LPS isoforms via TLR4 signaling and differential cytokines secretion [adapted from Berthet et al. (54) and Hamzeh-Cognasse et al. (87)].

such as the microbiota for example. One may this consider that besides to key cellular actors ("Stars") of immunity such as the lymphocytes, the phagocytes and the Ag presenting cells, there are "Supporting – though essential – roles" on stage, that are cells, which participate to immunity (such as endothelial cells, epithelial cells, and platelets).

The issue of platelets as "immune" cells has thus been not only endorsed but also extensively covered recently in a number of excellent review articles (33, 48, 88–90); therefore, we present only a brief overview and select three representative issues to address the question of platelet cellularity.

1. Platelets are innate immune sensors. As has been already presented, platelets display on their surface, and up-regulate upon stimulation, PRRs: hallmarks of innate immune functioning, beginning with the sensing of danger (28, 33, 35, 54). This property allows platelets to deal with infectious pathogens, with different outcomes depending on the nature of the invader (76,

78, 79, 81, 91–103). Platelets' relationships with germs from the microbiota at the mucosal surfaces are suspected, but not yet deciphered.

2. Doing so, the platelets initiate inflammation. We, in fact, believe platelets are definitely inflammatory cells that exert their principal role in the physiology of vessel endothelium by detecting (sensing) dangers (vascular insults and attritions) and by fixing damage on a permanent basis. This physiological intervention and repair are no less than a healing process, which itself relates to physiological inflammation. To assist this physiological inflammation, platelets produce assortments of repair tools, such as clotting factors, cytokines, and other BRMs, growth factors, and angiogenic factors (23, 35, 75, 104–108). Apart from taht, platelets can surpass their physiological role and participate in pathological inflammation, as with cardiovascular disease, severe infection and sepsis, and arthritis (18, 23, 29, 31, 32, 36, 75, 105, 106, 109–117). Platelets, when transfused as PCs, exert their physiological and repairing role, but in 2–3% of cases (118), the physiological barrier is overcome and they release significant amounts of pro-inflammatory and directly inflammatory factors from the α and δ granules, and membrane-bound as well as solubilized or cleaved molecules. They also secrete in certain cases pro-allergenic factors (from the δ granules).
3. Platelets assist innate immunity and affect adaptive immune cells. The very first interactive role of platelets with other blood cells was, again, discovered nearly 140 years ago, with the interplay between platelets and leukocytes in thrombosis. Platelets also have extensive, though complex, interplay with leukocytes and especially polymorphonuclear cells (granulocytes) in increasing, among other things, NETs (95, 119–123). Platelets also activate monocytes and macrophages (109, 115, 124–126), T cells (127–129), B cells (28, 130–135), NK cells (136–138), and dendritic cells (DCs) (87, 93, 139–144). In turn, platelets can be activated by monocytes, T cells, B cells, and DCs (87, 89, 132, 145). This mutual interaction is not anecdotic since, for example, activated platelets can alter the isotype (Ig class) switch program of differentiated B cells (132). Moreover, platelets that harbor numerous copies of HLA class I molecules (~100,000 copies per cell) have been proposed as antigen presenting cells (this discovery, however, awaits firmer confirmation) (146). It has otherwise been suggested that more MHC-I is absorbed from the plasma by the platelets, than is derived from the platelet itself (33). Chapman et al. indicated that platelets always express significant amounts of MHC-I, but that this expression significantly increases during infection (146). Of note, those HLA class I molecules, along with variant moieties harbored by the GP molecules that mediate platelet adhesion and aggregation, termed human platelet antigens or HPA, display polymorphisms that render one's platelets possibly immunizing when transfused into a recipient's body (or from the fetus to the mother) (147).

In sum, platelets are not only innate and inflammatory cells themselves, but they can also assist, depending on the circumstances, adaptive immunity. They do not only assist immunity as has long been thought, but are immune cells.

CONCLUDING REMARKS

There is no longer doubt that platelets are cells. They are also "intelligent" in that they are capable of discriminating between several types of danger and of adjusting a secretory response. This response is in general, physiologically, only that which is needed to face the danger or to fix a limited insult. This is indeed a platelet's usual function, and what they do when they repair daily the vascular endothelium that they patrol is detect cracks and erosions, and prevent breaches and leakage (bleeding). However, the nature or extent of secretory responses by activated platelets occasionally surpasses physiological conditions and becomes pathogenic. This suggests susceptibility factors in individuals or in transfused patients, along with favorable conditions linked to causal diseases and/or therapeutics. This last issue is now regarded with renewed interest because platelet actions can be easily manipulated by drugs. If correctly readdressed, platelet activation can be turned from deleterious to beneficial to the patient in a range of different infectious and inflammatory situations such as cardiovascular disease, serious infections, autoimmune disorders, autoinflammatory diseases, and cancer.

REFERENCES

1. Gazzaniga V, Ottini L. The discovery of platelets and their function. *Vesalius* (2011) **7**:22–6. Available from: http://www.biusante.parisdescartes.fr/ishm/vesalius/VESx2001x07x01x022x026.pdf
2. Kuter DJ. Milestones in understanding platelet production: a historical overview. *Br J Haematol* (2014) **165**:248–58. doi:10.1111/bjh.12781
3. Panuganti S, Schlinker AC, Lindholm PF, Papoutsakis ET, Miller WM. Three-stage ex vivo expansion of high-ploidy megakaryocytic cells: toward large-scale platelet production. *Tissue Eng Part A* (2013) **19**:998–1014. doi:10.1089/ten.TEA.2011.0111
4. Ye JY, Liang EY, Cheng YS, Chan GC, Ding Y, Meng F, et al. Serotonin enhances megakaryopoiesis and proplatelet formation via p-Erk1/2 and F-actin reorganization. *Stem Cells* (2014) **32**:2973–82. doi:10.1002/stem.1777
5. Schwertz H, Tolley ND, Foulks JM, Denis MM, Risenmay BW, Buerke M, et al. Signal-dependent splicing of tissue factor pre-mRNA modulates the thrombogenicity of human platelets. *J Exp Med* (2006) **203**:2433–40. doi:10.1084/jem.20061302
6. Zimmerman GA, Weyrich AS. Signal-dependent protein synthesis by activated platelets: new pathways to altered phenotype and function. *Arterioscler Thromb Vasc Biol* (2008) **28**:s17–24. doi:10.1161/ATVBAHA.107.160218
7. Rowley JW, Weyrich AS. Coordinate expression of transcripts and proteins in platelets. *Blood* (2013) **121**:5255–6. doi:10.1182/blood-2013-03-487991
8. Shi DS, Smith MC, Campbell RA, Zimmerman PW, Franks ZB, Kraemer BF, et al. Proteasome function is required for platelet production. *J Clin Invest* (2014) **124**:3757–66. doi:10.1172/JCI75247
9. Youssefian T, Masse JM, Rendu F, Guichard J, Cramer EM. Platelet and megakaryocyte dense granules contain glycoproteins Ib and IIb-IIIa. *Blood* (1997) **89**:4047–57.
10. Konstantinidis DG, Pushkaran S, Johnson JF, Cancelas JA, Manganaris S, Harris CE, et al. Signaling and cytoskeletal requirements in erythroblast enucleation. *Blood* (2012) **119**:6118–27. doi:10.1182/blood-2011-09-379263
11. Palis J. Losing a "nucleus" to gain a cytoplasm. *Blood* (2012) **119**:5948–9. doi:10.1182/blood-2012-04-422519
12. Hartwig J, Italiano J Jr. The birth of the platelet. *J Thromb Haemost* (2003) **1**:1580–6. doi:10.1046/j.1538-7836.2003.00331.x
13. Italiano JE Jr, Shivdasani RA. Megakaryocytes and beyond: the birth of platelets. *J Thromb Haemost* (2003) **1**:1174–82. doi:10.1046/j.1538-7836.2003.00290.x
14. Deutsch VR, Tomer A. Megakaryocyte development and platelet production. *Br J Haematol* (2006) **134**:453–66. doi:10.1111/j.1365-2141.2006.06215.x
15. Thon JN, Italiano JE. Platelets: production, morphology and ultrastructure. *Handb Exp Pharmacol* (2012) **210**:3–22. doi:10.1007/978-3-642-29423-5_1
16. Malara A, Balduini A. Blood platelet production and morphology. *Thromb Res* (2012) **129**:241–4. doi:10.1016/j.thromres.2011.11.042

17. Machlus KR, Thon JN, Italiano JE Jr. Interpreting the developmental dance of the megakaryocyte: a review of the cellular and molecular processes mediating platelet formation. *Br J Haematol* (2014) **165**:227–36. doi:10.1111/bjh.12758
18. Rondina MT, Weyrich AS, Zimmerman GA. Platelets as cellular effectors of inflammation in vascular diseases. *Circ Res* (2013) **112**:1506–19. doi:10.1161/CIRCRESAHA.113.300512
19. Weyrich AS, Zimmerman GA. Platelets in lung biology. *Annu Rev Physiol* (2013) **75**:569–91. doi:10.1146/annurev-physiol-030212-183752
20. Boilard E, Nigrovic PA, Larabee K, Watts GF, Coblyn JS, Weinblatt ME, et al. Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science* (2010) **327**:580–3. doi:10.1126/science.1181928
21. Flaumenhaft R, Mairuhu AT, Italiano JE. Platelet- and megakaryocyte-derived microparticles. *Semin Thromb Hemost* (2010) **36**:881–7. doi:10.1055/s-0030-1267042
22. Italiano JE Jr, Mairuhu AT, Flaumenhaft R. Clinical relevance of microparticles from platelets and megakaryocytes. *Curr Opin Hematol* (2010) **17**:578–84. doi:10.1097/MOH.0b013e32833e77ee
23. Nurden AT. Platelets, inflammation and tissue regeneration. *Thromb Haemost* (2011) **105**(Suppl 1):S13–33. doi:10.1160/THS10-11-0720
24. Boilard E, Blanco P, Nigrovic PA. Platelets: active players in the pathogenesis of arthritis and SLE. *Nat Rev Rheumatol* (2012) **8**:534–42. doi:10.1038/nrrheum.2012.118
25. Varki A. Since there are PAMPs and DAMPs, there must be SAMPs? Glycan "self-associated molecular patterns" dampen innate immunity, but pathogens can mimic them. *Glycobiology* (2011) **21**:1121–4. doi:10.1093/glycob/cwr087
26. Varki A, Gagneux P. Multifarious roles of sialic acids in immunity. *Ann N Y Acad Sci* (2012) **1253**:16–36. doi:10.1111/j.1749-6632.2012.06517.x
27. Nguyen KA, Hamzeh-Cognasse H, Palle S, Anselme-Bertrand I, Arthaud CA, Chavarin P, et al. Role of siglec-7 in apoptosis in human platelets. *PLoS One* (2014) **9**:e106239. doi:10.1371/journal.pone.0106239
28. Elzey BD, Tian J, Jensen RJ, Swanson AK, Lees JR, Lentz SR, et al. Platelet-mediated modulation of adaptive immunity. A communication link between innate and adaptive immune compartments. *Immunity* (2003) **19**:9–19. doi:10.1016/S1074-7613(03)00177-8
29. Garraud O, Cognasse F. Platelet toll-like receptor expression: the link between "danger" ligands and inflammation. *Inflamm Allergy Drug Targets* (2010) **9**:322–33. doi:10.2174/187152810793937991
30. Garraud O, Berthet J, Hamzeh-Cognasse H, Cognasse F. Pathogen sensing, subsequent signalling, and signalosome in human platelets. *Thromb Res* (2011) **127**:283–6. doi:10.1016/j.thromres.2010.10.015
31. Phipps RP. Platelets at the interface between thrombosis, inflammation and immunity. *Thromb Res* (2011) **127**:179. doi:10.1016/j.thromres.2010.10.014
32. Refaai MA, Phipps RP, Spinelli SL, Blumberg N. Platelet transfusions: impact on hemostasis, thrombosis, inflammation and clinical outcomes. *Thromb Res* (2011) **127**:287–91. doi:10.1016/j.thromres.2010.10.012
33. Semple JW, Italiano JE Jr, Freedman J. Platelets and the immune continuum. *Nat Rev Immunol* (2011) **11**:264–74. doi:10.1038/nri2956
34. Garraud O, Hamzeh-Cognasse H, Cognasse F. Platelets and cytokines: how and why? *Transfus Clin Biol* (2012) **19**:104–8. doi:10.1016/j.tracli.2012.02.004
35. Garraud O, Hamzeh-Cognasse H, Pozzetto B, Cavaillon JM, Cognasse F. Bench-to-bedside review: platelets and active immune functions – new clues for immunopathology? *Crit Care* (2013) **17**:236. doi:10.1186/cc12716
36. Zamora C, Canto E, Nieto JC, Ortiz MA, Diaz-Torne C, Diaz-Lopez C, et al. Functional consequences of platelet binding to T lymphocytes in inflammation. *J Leukoc Biol* (2013) **94**(3):521–9. doi:10.1189/jlb.0213074
37. Liu F, Morris S, Epps J, Carroll R. Demonstration of an activation regulated NF-kappaB/I-kappaBalpha complex in human platelets. *Thromb Res* (2002) **106**:199–203. doi:10.1016/S0049-3848(02)00130-5
38. Malaver E, Romaniuk MA, D'Atri LP, Pozner RG, Negrotto S, Benzadon R, et al. NF-kappaB inhibitors impair platelet activation responses. *J Thromb Haemost* (2009) **7**:1333–43. doi:10.1111/j.1538-7836.2009.03492.x
39. Spinelli SL, Casey AE, Pollock SJ, Gertz JM, McMillan DH, Narasipura SD, et al. Platelets and megakaryocytes contain functional nuclear factor-{kappa}B. *Arterioscler Thromb Vasc Biol* (2010) **30**:591–8. doi:10.1161/ATVBAHA.109.197343
40. Spinelli SL, Maggirwar SB, Blumberg N, Phipps RP. Nuclear emancipation: a platelet tour de force. *Sci Signal* (2010) **3**:e37. doi:10.1126/scisignal.3144pe37
41. Schattner M. Role of NF-kappaB pathway on platelet activation. *Circ Res* (2013) **113**:e92. doi:10.1161/CIRCRESAHA.113.302333
42. Rivadeneyra L, Carestia A, Etulain J, Pozner RG, Fondevila C, Negrotto S, et al. Regulation of platelet responses triggered by toll-like receptor 2 and 4 ligands is another non-genomic role of nuclear factor-kappaB. *Thromb Res* (2014) **133**:235–43. doi:10.1016/j.thromres.2013.11.028
43. Fong KP, Barry C, Tran AN, Traxler EA, Wannemacher KM, Tang HY, et al. Deciphering the human platelet sheddome. *Blood* (2011) **117**:e15–26. doi:10.1182/blood-2010-05-283838
44. Adelson E, Rheingold JJ, Crosby WH. The platelet as a sponge: a review. *Blood* (1961) **17**:767–74.
45. McRedmond JP, Park SD, Reilly DF, Coppinger JA, Maguire PB, Shields DC, et al. Integration of proteomics and genomics in platelets: a profile of platelet proteins and platelet-specific genes. *Mol Cell Proteomics* (2004) **3**:133–44. doi:10.1074/mcp.M300063-MCP200
46. Denis MM, Tolley ND, Bunting M, Schwertz H, Jiang H, Lindemann S, et al. Escaping the nuclear confines: signal-dependent pre-mRNA splicing in anucleate platelets. *Cell* (2005) **122**:379–91. doi:10.1016/j.cell.2005.06.015
47. Gnatenko DV, Perrotta PL, Bahou WF. Proteomic approaches to dissect platelet function: half the story. *Blood* (2006) **108**:3983–91. doi:10.1182/blood-2006-06-026518
48. Smyth SS, McEver RP, Weyrich AS, Morrell CN, Hoffman MR, Arepally GM, et al. Platelet functions beyond haemostasis. *J Thromb Haemost* (2009) **7**:1759–66. doi:10.1111/j.1538-7836.2009.03586.x
49. Weyrich AS, Schwertz H, Kraiss LW, Zimmerman GA. Protein synthesis by platelets: historical and new perspectives. *J Thromb Haemost* (2009) **7**:241–6. doi:10.1111/j.1538-7836.2008.03211.x
50. Rowley JW, Oler AJ, Tolley ND, Hunter BN, Low EN, Nix DA, et al. Genome-wide RNA-seq analysis of human and mouse platelet transcriptomes. *Blood* (2011) **118**:e101–11. doi:10.1182/blood-2011-03-339705
51. Rowley JW, Schwertz H, Weyrich AS. Platelet mRNA: the meaning behind the message. *Curr Opin Hematol* (2012) **19**:385–91. doi:10.1097/MOH.0b013e328357010e
52. Seghatchian J. Platelet storage lesion: an update on the impact of various leukoreduction processes on the biological response modifiers. *Transfus Apher Sci* (2006) **34**:125–30. doi:10.1016/j.transci.2005.09.002
53. Looney MR, Gilliss BM, Matthay MA. Pathophysiology of transfusion-related acute lung injury. *Curr Opin Hematol* (2010) **17**:418–23. doi:10.1097/MOH.0b013e32833c07d3
54. Berthet J, Damien P, Hamzeh-Cognasse H, Arthaud CA, Eyraud MA, Zeni F, et al. Human platelets can discriminate between various bacterial LPS isoforms via TLR4 signaling and differential cytokine secretion. *Clin Immunol* (2012) **145**:189–200. doi:10.1016/j.clim.2012.09.004
55. Kappelmayer J, Beke Debreceni I, Vida A, Antal-Szalmas P, Clemetson KJ, Nagy B Jr. Distinct effects of Re- and S-forms of LPS on modulating platelet activation. *J Thromb Haemost* (2013) **11**(4):775–8. doi:10.1111/jth.12151
56. Shiraki R, Inoue N, Kawasaki S, Takei A, Kadotani M, Ohnishi Y, et al. Expression of toll-like receptors on human platelets. *Thromb Res* (2004) **113**:379–85. doi:10.1016/j.thromres.2004.03.023
57. Andonegui G, Kerfoot SM, McNagny K, Ebbert KV, Patel KD, Kubes P. Platelets express functional toll-like receptor-4. *Blood* (2005) **106**:2417–23. doi:10.1182/blood-2005-03-0916
58. Cognasse F, Hamzeh H, Chavarin P, Acquart S, Genin C, Garraud O. Evidence of toll-like receptor molecules on human platelets. *Immunol Cell Biol* (2005) **83**:196–8. doi:10.1111/j.1440-1711.2005.01314.x
59. Aslam R, Speck ER, Kim M, Crow AR, Bang KW, Nestel FP, et al. Platelet toll-like receptor expression modulates lipopolysaccharide-induced thrombocytopenia and tumor necrosis factor-alpha production in vivo. *Blood* (2006) **107**:637–41. doi:10.1182/blood-2005-06-2202
60. Cognasse F, Semple JW, Garraud O. Platelets as potential immunomodulators: is there a role for platelet toll-like receptors? *Curr Immunol Rev* (2007) **3**:109–15. doi:10.2174/157339507780655522
61. Blair P, Rex S, Vitseva O, Beaulieu L, Tanriverdi K, Chakrabarti S, et al. Stimulation of toll-like receptor 2 in human platelets induces a thromboinflammatory response through activation of phosphoinositide 3-kinase. *Circ Res* (2008) **104**:346–54. doi:10.1161/CIRCRESAHA.108.185785
62. Panigrahi S, Ma Y, Hong L, Gao D, West XZ, Salomon RG, et al. Engagement of platelet toll-like receptor 9 by novel endogenous ligands promotes platelet hyper-reactivity and thrombosis. *Circ Res* (2012) **112**(1):103–12. doi:10.1161/CIRCRESAHA.112.274241

63. Thon JN, Peters CG, Machlus KR, Aslam R, Rowley J, Macleod H, et al. T granules in human platelets function in TLR9 organization and signaling. *J Cell Biol* (2012) **198**:561–74. doi:10.1083/jcb.201111136
64. Anabel AS, Eduardo PC, Antonio HC, Carlos SM, Juana NM, Honorio TA, et al. Human platelets express toll-like receptor 3 and respond to poly I:C. *Hum Immunol* (2014) **75**(12):1244–51. doi:10.1016/j.humimm.2014.09.013
65. Koupenova M, Vitseva O, Mackay CR, Beaulieu LM, Benjamin EJ, Mick E, et al. Platelet-TLR7 mediates host survival and platelet count during viral infection in the absence of platelet-dependent thrombosis. *Blood* (2014) **124**:791–802. doi:10.1182/blood-2013-11-536003
66. Cloutier N, Pare A, Farndale RW, Schumacher HR, Nigrovic PA, Lacroix S, et al. Platelets can enhance vascular permeability. *Blood* (2012) **120**:1334–43. doi:10.1182/blood-2012-02-413047
67. Herzog BH, Fu J, Wilson SJ, Hess PR, Sen A, McDaniel JM, et al. Podoplanin maintains high endothelial venule integrity by interacting with platelet CLEC-2. *Nature* (2013) **502**:105–9. doi:10.1038/nature12501
68. Demers M, Krause DS, Schatzberg D, Martinod K, Voorhees JR, Fuchs TA, et al. Cancers predispose neutrophils to release extracellular DNA traps that contribute to cancer-associated thrombosis. *Proc Natl Acad Sci U S A* (2012) **109**:13076–81. doi:10.1073/pnas.1200419109
69. Demers M, Wagner DD. NETosis: a new factor in tumor progression and cancer-associated thrombosis. *Semin Thromb Hemost* (2014) **40**:277–83. doi:10.1055/s-0034-1370765
70. Tanaka K, Koike Y, Shimura T, Okigami M, Ide S, Toiyama Y, et al. In vivo characterization of neutrophil extracellular traps in various organs of a murine sepsis model. *PLoS One* (2014) **9**:e111888. doi:10.1371/journal.pone.0111888
71. Hattori R, Hamilton KK, McEver RP, Sims PJ. Complement proteins C5b-9 induce secretion of high molecular weight multimers of endothelial von Willebrand factor and translocation of granule membrane protein GMP-140 to the cell surface. *J Biol Chem* (1989) **264**:9053–60.
72. Ferrer-Lopez P, Renesto P, Schattner M, Bassot S, Laurent P, Chignard M. Activation of human platelets by C5a-stimulated neutrophils: a role for cathepsin G. *Am J Physiol* (1990) **258**:C1100–7.
73. Wiedmer T, Sims PJ. Participation of protein kinases in complement C5b-9-induced shedding of platelet plasma membrane vesicles. *Blood* (1991) **78**:2880–6.
74. Rinder CS, Smith MJ, Rinder HM, Cortright DN, Brodbeck RM, Krause JE, et al. Leukocyte effects of C5a-receptor blockade during simulated extracorporeal circulation. *Ann Thorac Surg* (2007) **83**:146–52. doi:10.1016/j.athoracsur.2006.08.019
75. Klinger MH, Jelkmann W. Role of blood platelets in infection and inflammation. *J Interferon Cytokine Res* (2002) **22**:913–22. doi:10.1089/10799900260286623
76. Fitzgerald JR, Foster TJ, Cox D. The interaction of bacterial pathogens with platelets. *Nat Rev Microbiol* (2006) **4**:445–57. doi:10.1038/nrmicro1425
77. Beaulieu LM, Freedman JE. The role of inflammation in regulating platelet production and function: toll-like receptors in platelets and megakaryocytes. *Thromb Res* (2010) **125**:205–9. doi:10.1016/j.thromres.2009.11.004
78. Yeaman MR. Bacterial-platelet interactions: virulence meets host defense. *Future Microbiol* (2010) **5**:471–506. doi:10.2217/fmb.09.112
79. Yeaman MR. Platelets in defense against bacterial pathogens. *Cell Mol Life Sci* (2010) **67**:525–44. doi:10.1007/s00018-009-0210-4
80. Johansson D, Shannon O, Rasmussen M. Platelet and neutrophil responses to Gram positive pathogens in patients with bacteremic infection. *PLoS One* (2011) **6**:e26928. doi:10.1371/journal.pone.0026928
81. Speth C, Rambach G, Lass-Florl C. Platelet immunology in fungal infections. *Thromb Haemost* (2014) **112**:632–9. doi:10.1160/TH14-01-0074
82. Leytin V. Apoptosis in the anucleate platelet. *Blood Rev* (2012) **26**:51–63. doi:10.1016/j.blre.2011.10.002
83. Jackson SP, Schoenwaelder SM. Procoagulant platelets: are they necrotic? *Blood* (2010) **116**:2011–8. doi:10.1182/blood-2010-01-261669
84. Gyulkhandanyan AV, Mutlu A, Freedman J, Leytin V. Selective triggering of platelet apoptosis, platelet activation or both. *Br J Haematol* (2013) **161**(2):245–54. doi:10.1111/bjh.12237
85. Leytin V, Allen DJ, Mykhaylov S, Lyubimov E, Freedman J. Thrombin-triggered platelet apoptosis. *J Thromb Haemost* (2006) **4**:2656–63. doi:10.1111/j.1538-7836.2006.02200.x
86. Mantovani A. Molecular pathways linking inflammation and cancer. *Curr Mol Med* (2010) **10**:369–73. doi:10.2174/156652410791316968
87. Hamzeh-Cognasse H, Cognasse F, Palle S, Chavarin P, Olivier T, Delezay O, et al. Direct contact of platelets and their released products exert different effects on human dendritic cell maturation. *BMC Immunol* (2008) **9**:54. doi:10.1186/1471-2172-9-54
88. Li C, Li J, Li Y, Lang S, Yougbare I, Zhu G, et al. Crosstalk between platelets and the immune system: old systems with new discoveries. *Adv Hematol* (2012) **2012**:384685. doi:10.1155/2012/384685
89. Duerschmied D, Bode C, Ahrens I. Immune functions of platelets. *Thromb Haemost* (2014) **112**:678–91. doi:10.1160/TH14-02-0146
90. Ghoshal K, Bhattacharyya M. Overview of platelet physiology: its hemostatic and nonhemostatic role in disease pathogenesis. *ScientificWorldJournal* (2014) **2014**:781857. doi:10.1155/2014/781857
91. Bik T, Sarov I, Livne A. Interaction between vaccinia virus and human blood platelets. *Blood* (1982) **59**:482–7.
92. Polack B, Peyron F, Auriault C. Platelet cytotoxicity against parasites. *Nouv Rev Fr Hematol* (1991) **33**:317–22.
93. Chaipan C, Soilleux EJ, Simpson P, Hofmann H, Gramberg T, Marzi A, et al. DC-SIGN and CLEC-2 mediate human immunodeficiency virus type 1 capture by platelets. *J Virol* (2006) **80**:8951–60. doi:10.1128/JVI.00136-06
94. White JG. Why human platelets fail to kill bacteria. *Platelets* (2006) **17**:191–200. doi:10.1080/09537100500441234
95. Clark SR, Ma AC, Tavener SA, McDonald B, Goodarzi Z, Kelly MM, et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat Med* (2007) **13**:463–9. doi:10.1038/nm1565
96. Cox D. Bacteria-platelet interactions. *J Thromb Haemost* (2009) **7**:1865–6. doi:10.1111/j.1538-7836.2009.03611.x
97. Pleass RJ. Platelet power: sticky problems for sticky parasites? *Trends Parasitol* (2009) **25**:296–9. doi:10.1016/j.pt.2009.04.002
98. Flaujac C, Boukour S, Cramer-Borde E. Platelets and viruses: an ambivalent relationship. *Cell Mol Life Sci* (2010) **67**:545–56. doi:10.1007/s00018-009-0209-x
99. Kerrigan SW, Cox D. Platelet-bacterial interactions. *Cell Mol Life Sci* (2010) **67**:513–23. doi:10.1007/s00018-009-0207-z
100. McNicol A, Israels SJ. Mechanisms of oral bacteria-induced platelet activation. *Can J Physiol Pharmacol* (2010) **88**:510–24. doi:10.1139/y10-029
101. Cox D, Kerrigan SW, Watson SP. Platelets and the innate immune system: mechanisms of bacterial-induced platelet activation. *J Thromb Haemost* (2011) **9**:1097–107. doi:10.1111/j.1538-7836.2011.04264.x
102. Alonzo MT, Lacuesta TL, Dimaano EM, Kurosu T, Suarez LA, Mapua CA, et al. Platelet apoptosis and apoptotic platelet clearance by macrophages in secondary dengue virus infections. *J Infect Dis* (2012) **205**:1321–9. doi:10.1093/infdis/jis180
103. Wong CH, Jenne CN, Petri B, Chrobok NL, Kubes P. Nucleation of platelets with blood-borne pathogens on Kupffer cells precedes other innate immunity and contributes to bacterial clearance. *Nat Immunol* (2013) **14**:785–92. doi:10.1038/ni.2631
104. Brandt E, Ludwig A, Petersen F, Flad HD. Platelet-derived CXC chemokines: old players in new games. *Immunol Rev* (2000) **177**:204–16. doi:10.1034/j.1600-065X.2000.17705.x
105. von Hundelshausen P, Weber C. Platelets as immune cells: bridging inflammation and cardiovascular disease. *Circ Res* (2007) **100**:27–40. doi:10.1161/01.RES.0000252802.25497.b7
106. Bozza FA, Shah AM, Weyrich AS, Zimmerman GA. Amicus or adversary: platelets in lung biology, acute injury, and inflammation. *Am J Respir Cell Mol Biol* (2009) **40**:123–34. doi:10.1165/rcmb.2008-0241TR
107. Schubert P, Devine DV. De novo protein synthesis in mature platelets: a consideration for transfusion medicine. *Vox Sang* (2010) **99**:112–22. doi:10.1111/j.1423-0410.2010.01333.x
108. Verschoor A, Langer HF. Crosstalk between platelets and the complement system in immune protection and disease. *Thromb Haemost* (2013) **110**:910–9. doi:10.1160/TH13-02-0102
109. Gear AR, Camerini D. Platelet chemokines and chemokine receptors: linking hemostasis, inflammation, and host defense. *Microcirculation* (2003) **10**:335–50. doi:10.1038/sj.mn.7800198
110. Cognasse F, Garraud O. Human platelets exhibit infectious-pathogen binding ligands and participate to inflammation (and more?). *Exp Hematol* (2005) **33**:1081–2. doi:10.1016/j.exphem.2005.06.033
111. Gawaz M, Langer H, May AE. Platelets in inflammation and atherogenesis. *J Clin Invest* (2005) **115**:3378–84. doi:10.1172/JCI27196

112. Steinhubl SR. Platelets as mediators of inflammation. *Hematol Oncol Clin North Am* (2007) **21**:115–21. doi:10.1016/j.hoc.2006.11.015
113. McNicol A, Israels SJ. Beyond hemostasis: the role of platelets in inflammation, malignancy and infection. *Cardiovasc Hematol Disord Drug Targets* (2008) **8**:99–117. doi:10.2174/187152908784533739
114. Projahn D, Koenen RR. Platelets: key players in vascular inflammation. *J Leukoc Biol* (2012) **92**:1167–75. doi:10.1189/jlb.0312151
115. Mantovani A, Garlanda C. Platelet-macrophage partnership in innate immunity and inflammation. *Nat Immunol* (2013) **14**:768–70. doi:10.1038/ni.2666
116. Yan SL, Russell J, Harris NR, Senchenkova EY, Yildirim A, Granger DN. Platelet abnormalities during colonic inflammation. *Inflamm Bowel Dis* (2013) **19**(6):1245–53. doi:10.1097/MIB.0b013e318281f3df
117. Boudreau LH, Duchez AC, Cloutier N, Soulet D, Martin N, Bollinger J, et al. Platelets release mitochondria serving as substrate for bactericidal group IIA-secreted phospholipase A2 to promote inflammation. *Blood* (2014) **124**:2173–83. doi:10.1182/blood-2014-05-573543
118. ANSM. Onzième rapport des données nationales d'hémovigilance. *Agence Nationale de Sécurité du Médicament et des produits de santé (ANSM)*. Saint-Denis: ANSM (2013). p. 1–91.
119. Zarbock A, Polanowska-Grabowska RK, Ley K. Platelet-neutrophil-interactions: linking hemostasis and inflammation. *Blood Rev* (2007) **21**:99–111. doi:10.1016/j.blre.2006.06.001
120. Ma AC, Kubes P. Platelets, neutrophils, and neutrophil extracellular traps (NETs) in sepsis. *J Thromb Haemost* (2008) **6**:415–20. doi:10.1111/j.1538-7836.2007.02865.x
121. Kornerup KN, Salmon GP, Pitchford SC, Liu WL, Page CP. Circulating platelet-neutrophil complexes are important for subsequent neutrophil activation and migration. *J Appl Physiol* (2010) **109**:758–67. doi:10.1152/japplphysiol.01086.2009
122. Manfredi AA, Rovere-Querini P, Maugeri N. Dangerous connections: neutrophils and the phagocytic clearance of activated platelets. *Curr Opin Hematol* (2010) **17**:3–8. doi:10.1097/MOH.0b013e3283324f97
123. Caudrillier A, Kessenbrock K, Gilliss BM, Nguyen JX, Marques MB, Monestier M, et al. Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury. *J Clin Invest* (2012) **122**:2661–71. doi:10.1172/JCI61303
124. Clemetson KJ, Clemetson JM, Proudfoot AE, Power CA, Baggiolini M, Wells TN. Functional expression of CCR1, CCR3, CCR4, and CXCR4 chemokine receptors on human platelets. *Blood* (2000) **96**:4046–54.
125. Menezes GB, McAvoy EF, Kubes P. Hyaluronan, platelets, and monocytes: a novel pro-inflammatory triad. *Am J Pathol* (2009) **174**:1993–5. doi:10.2353/ajpath.2009.081138
126. Hottz ED, Medeiros-De-Moraes IM, Vieira-De-Abreu A, De Assis EF, Vals-De-Souza R, Castro-Faria-Neto HC, et al. Platelet activation and apoptosis modulate monocyte inflammatory responses in dengue. *J Immunol* (2014) **193**:1864–72. doi:10.4049/jimmunol.1400091
127. Buchner K, Henn V, Grafe M, De Boer OJ, Becker AE, Kroczek RA. CD40 ligand is selectively expressed on CD4+ T cells and platelets: implications for CD40-CD40L signalling in atherosclerosis. *J Pathol* (2003) **201**:288–95. doi:10.1002/path.1425
128. Danese S, De La Motte C, Reyes BM, Sans M, Levine AD, Fiocchi C. Cutting edge: T cells trigger CD40-dependent platelet activation and granular RANTES release: a novel pathway for immune response amplification. *J Immunol* (2004) **172**:2011–5. doi:10.4049/jimmunol.172.4.2011
129. Elzey BD, Grant JF, Sinn HW, Nieswandt B, Waldschmidt TJ, Ratliff TL. Cooperation between platelet-derived CD154 and CD4+ T cells for enhanced germinal center formation. *J Leukoc Biol* (2005) **78**:80–4. doi:10.1189/jlb.1104669
130. Elzey BD, Sprague DL, Ratliff TL. The emerging role of platelets in adaptive immunity. *Cell Immunol* (2005) **238**:1–9. doi:10.1016/j.cellimm.2005.12.005
131. Lee SP, Ataga KI, Orringer EP, Phillips DR, Parise LV. Biologically active CD40 ligand is elevated in sickle cell anemia: potential role for platelet-mediated inflammation. *Arterioscler Thromb Vasc Biol* (2006) **26**:1626–31. doi:10.1161/01.ATV.0000220374.00602.a2
132. Cognasse F, Hamzeh-Cognasse H, Lafarge S, Chavarin P, Cogne M, Richard Y, et al. Human platelets can activate peripheral blood B cells and increase production of immunoglobulins. *Exp Hematol* (2007) **35**:1376–87. doi:10.1016/j.exphem.2007.05.021
133. Li N. Platelet-lymphocyte cross-talk. *J Leukoc Biol* (2008) **83**:1069–78. doi:10.1189/jlb.0907615
134. Sprague DL, Elzey BD, Crist SA, Waldschmidt TJ, Jensen RJ, Ratliff TL. Platelet-mediated modulation of adaptive immunity: unique delivery of CD154 signal by platelet-derived membrane vesicles. *Blood* (2008) **111**:5028–36. doi:10.1182/blood-2007-06-097410
135. Cognasse F, Hamzeh-Cognasse H, Lafarge S, Acquart S, Chavarin P, Courbil R, et al. Donor platelets stored for at least 3 days can elicit activation marker expression by the recipient's blood mononuclear cells: an in vitro study. *Transfusion* (2009) **49**:91–8. doi:10.1111/j.1537-2995.2008.01931.x
136. de Bruijne-Admiraal LG, Modderman PW, Von Dem Borne AE, Sonnenberg A. P-selectin mediates Ca(2+)-dependent adhesion of activated platelets to many different types of leukocytes: detection by flow cytometry. *Blood* (1992) **80**:134–42.
137. Palumbo JS, Talmage KE, Massari JV, La Jeunesse CM, Flick MJ, Kombrinck KW, et al. Platelets and fibrin(ogen) increase metastatic potential by impeding natural killer cell-mediated elimination of tumor cells. *Blood* (2005) **105**:178–85. doi:10.1182/blood-2004-06-2272
138. Li N, Ji Q, Hjemdahl P. Platelet-lymphocyte conjugation differs between lymphocyte subpopulations. *J Thromb Haemost* (2006) **4**:874–81. doi:10.1111/j.1538-7836.2006.01817.x
139. Hilf N, Singh-Jasuja H, Schwarzmaier P, Gouttefangeas C, Rammensee HG, Schild H. Human platelets express heat shock protein receptors and regulate dendritic cell maturation. *Blood* (2002) **99**:3676–82. doi:10.1182/blood.V99.10.3676
140. Kaneider NC, Kaser A, Tilg H, Ricevuti G, Wiedermann CJ. CD40 ligand-dependent maturation of human monocyte-derived dendritic cells by activated platelets. *Int J Immunopathol Pharmacol* (2003) **16**:225–31.
141. Hagihara M, Higuchi A, Tamura N, Ueda Y, Hirabayashi K, Ikeda Y, et al. Platelets, after exposure to a high shear stress, induce IL-10-producing, mature dendritic cells in vitro. *J Immunol* (2004) **172**:5297–303. doi:10.4049/jimmunol.172.9.5297
142. Martinson J, Bae J, Klingemann HG, Tam Y. Activated platelets rapidly up-regulate CD40L expression and can effectively mature and activate autologous ex vivo differentiated DC. *Cytotherapy* (2004) **6**:487–97. doi:10.1080/14653240410005249-1
143. Blumberg N, Spinelli SL, Francis CW, Taubman MB, Phipps RP. The platelet as an immune cell-CD40 ligand and transfusion immunomodulation. *Immunol Res* (2009) **45**(2–3):251–60. doi:10.1007/s12026-009-8106-9
144. Nomura S, Fujita S, Nakanishi T, Yokoi T, Shimamoto K, Miyamoto R, et al. Platelet-derived microparticles cause CD154-dependent activation of dendritic cells. *Platelets* (2012) **23**:81–2. doi:10.3109/09537104.2011.595850
145. Sowa JM, Crist SA, Ratliff TL, Elzey BD. Platelet influence on T- and B-cell responses. *Arch Immunol Ther Exp* (2009) **57**:235–41. doi:10.1007/s00005-009-0032-y
146. Chapman LM, Aggrey AA, Field DJ, Srivastava K, Ture S, Yui K, et al. Platelets present antigen in the context of MHC class I. *J Immunol* (2012) **189**(2):916–23. doi:10.4049/jimmunol.1200580
147. Anani Sarab G, Moss M, Barker RN, Urbaniak SJ. Naturally processed peptides spanning the HPA-1a polymorphism are efficiently generated and displayed from platelet glycoprotein by HLA-DRB3*0101-positive antigen-presenting cells. *Blood* (2009) **114**:1954–7. doi:10.1182/blood-2009-04-211839

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 24 November 2014; paper pending published: 05 January 2015; accepted: 03 February 2015; published online: 20 February 2015.

*Citation: Garraud O and Cognasse F (2015) Are platelets cells? And if yes, are they immune cells? Front. Immunol. **6**:70. doi: 10.3389/fimmu.2015.00070*

This article was submitted to Inflammation, a section of the journal Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 18 December 2014
doi: 10.3389/fimmu.2014.00653

Emerging evidence for platelets as immune and inflammatory effector cells

Matthew T. Rondina*[1,2,3] ***and Olivier Garraud*[4,5]**

[1] *Division of General Internal Medicine, University of Utah, Salt Lake City, UT, USA*
[2] *Program in Molecular Medicine, University of Utah School of Medicine, Salt Lake City, UT, USA*
[3] *Department of Internal Medicine, George E. Wahlen Department of Veterans Affairs Medical Center, Salt Lake City, UT, USA*
[4] *Faculty of Medicine of Saint-Etienne, University of Lyon, Lyon, France*
[5] *French Blood Establishment, Auvergne-Loire, Saint-Etienne, France*

Edited by:
Pierre Miossec, University of Lyon, France

Reviewed by:
Dietmar Fuchs, Innsbruck Medical University, Austria
Fulvio D'Acquisto, Queen Mary University of London, UK
Matthieu Mahévas, Unité INSERM 783 Développement du système immunitaire, France

****Correspondence:***
Matthew T. Rondina, Department of Internal Medicine, Eccles Institute of Human Genetics, University of Utah, 15 North 2030 East, Building 533, Suite 4220, Salt Lake City, UT 84112, USA
e-mail: matt.rondina@u2m2.utah.edu

While traditionally recognized for their roles in hemostatic pathways, emerging evidence demonstrates that platelets have previously unrecognized, dynamic roles that span the immune continuum. These newly recognized platelet functions, including the secretion of immune mediators, interactions with endothelial cells, monocytes, and neutrophils, toll-like receptor (TLR) mediated responses, and induction of neutrophil extracellular trap formation, bridge thrombotic and inflammatory pathways and contribute to host defense mechanisms against invading pathogens. In this focused review, we highlight several of these emerging aspects of platelet biology and their implications in clinical infectious syndromes.

Keywords: platelets, immunity, infection, sepsis, pathogens

INTRODUCTION

Platelets are small anucleate cells highly specialized for hemostasis and vascular wall repair. Emerging data demonstrate that in addition to their traditional hemostatic functions, platelets are also versatile effector cells with a repertoire of functions that span the immune continuum. These newly recognized and well-established platelet functions bridge thrombotic and inflammatory pathways and contribute to many systemic inflammatory and immune processes and diseases (1–3). Moreover, the inflammatory and immune specializations of platelets are likely evolutionarily driven adaptations that augment host defenses against invading pathogens. These newly recognized platelet activities include the release of pleiotropic immune mediators, heterotypic interactions with endothelial cells, monocytes, and neutrophils, toll-like receptor (TLR) mediated responses, and induction of neutrophil extracellular trap (NET) formation. Here, we provide a focused review on several of the emerging aspects of the biology of platelets across the inflammatory and immune continuum. For additional information on these topics, the reader is referred to several recent reviews (1, 3–5).

Abbreviations: CLEC-2, C-type lectin-like 2; CNS, central nervous system; DAMPs, danger associated molecular patterns; GPVI, glycoprotein VI; HIV-1, human immunodeficiency virus 1; IL, Interleukin; LPS, lipopolysaccharide; MCP-1, monocyte chemotactic protein-1; MPs, microparticles; NET, neutrophil extracellular trap; PF4, platelet factor 4; PMNs, polymorphonuclear leukocytes; RANTES, regulated on activation, normal T-cell expressed and secreted; ROS, reactive oxygen species; TLR, toll-like receptor; TNF-α, tumor necrosis factor alpha.

PLATELET SURFACE LIGANDS SENSE AND RESPOND TO PATHOGENS

The platelet surface is replete with numerous receptors that not only regulate hemostatic responses but also trigger proinflammatory and immune cascades (**Figure 1**). Many of these surface receptors have been called "immunoreceptors" in homage of their molecular structure and the ligands they recognize (6). For example, platelet Fc receptors bind immunoglobulins of the IgE, IgG, and IgA class and immune complexes, directly inducing immune signaling pathways. Glycoprotein VI (GPVI), which is only found on platelets, triggers platelet microvesicle release and subsequent inflammatory signals through interleukin (IL)-1 (7). GPVI may also be a receptor for hepatitis C, mediating viral transport and replication mechanisms (8). Moreover, GPVI amplifies platelet activation by thrombin, thus providing a mechanism for coordinate signaling with G-protein-coupled pathways (9). C-type lectin-like (CLEC-2) receptors, including DC-SIGN, mediate human immunodeficiency virus 1 (HIV-1) capture by platelets (10) and platelet activation in dengue infection (11).

Human platelets and megakaryocytes also express mRNA and/or protein for the TLRs 1, 2, 4–7, and 9 (2, 12–15). TLRs bind diverse ligands from many infectious pathogens, including bacteria, viruses, parasites, and protozoa. Thus, the discovery that human platelets possess TLRs established direct mechanisms by which platelets may function as pathogen "sensors" (12, 16). Moreover, by recognizing endogenous ligands as well as microbial pathogen-associated molecular pattern motifs, platelet TLRs provide pathways by which human platelets can response to danger

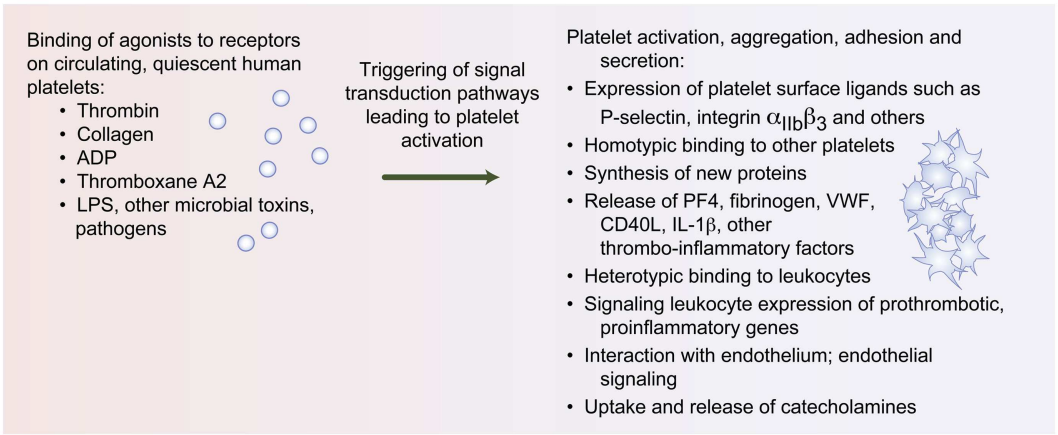


FIGURE 1 | Upon activation, platelets mediate responses central to inflammation and hemostasis. Agonists such as thrombin, collagen, adenosine diphosphate (ADP), thromboxane A2, and LPS bind to agonists on human platelets, triggering classic responses of platelet activation, aggregation, adhesion, and secretion. Platelet activation also results in protein synthetic activities, release of thrombo-inflammatory modulators, heterotypic binding to leukocytes, and interaction with the endothelium.

associated molecular patterns (DAMPs), whereby mediating both infectious and non-infectious immune syndromes (17, 18).

Among the TLRs identified on platelets at either the mRNA and/or protein level, TLR4 has been most extensively studied to date (2, 12). TLR4 is the receptor for lipopolysaccharide (LPS), an endotoxin component on the outer membrane of Gram-negative organisms that elicits strong immune responses. LPS activates human platelets both *in vitro* and during *in vivo* settings where healthy volunteers are given low doses of LPS, although activation patterns are variable (19–22). Although still incompletely understood, LPS-induced platelet responses include aggregation, degranulation, secretion, pre-mRNA splicing and protein synthesis, and alterations in surface CD40L. Moreover, LPS-induced platelet activation triggers formation of NETs by polymorphonuclear leukocytes (PMNs), enhancing bacterial capture, and killing (23) (**Figure 2**).

Emerging data have identified new and intriguing roles for platelet TLR7 during viral infections. TLR7, which localizes to the endosomal compartment of the cell (24), is a sensor for nucleic acid ligands – in particular for ssRNA. Viral pathogens such as influenza and HIV, which continue to have significant public health burden, activate the TLR7 receptor (24). These and other ssRNA viruses, which signal through TLR7, are associated with thrombocytopenia, giving rise to suppositions that platelet TLR7 mediates viral sensing and/or host defense mechanisms. In recent and elegantly performed studies (15), activation of platelet TLR7 not only induced platelet-neutrophil aggregation and a reduction in platelet count, but was also necessary for optimal survival in murine models of encephalomyocarditis (EMCV) viral infection. Engagement of platelet TLR7 under these conditions, however, did not induce platelet aggregation (15). Thus, platelet TLR7 plays a key role in host recognition of ssRNA viruses and suggests that platelets may be integral mediators of innate immune responses.

Toll-like receptor9, while less well examined currently, is intriguing given its unexpected cellular localization patterns (19, 25). TLR9 is expressed on the plasma membrane and in the cytoplasm of quiescent human platelets and its surface expression increases upon activation of platelets with agonists such as thrombin (14, 25, 26). TLR9 is a ligand for unmethylated CpG islands in viral and bacterial DNA, suggesting a previously unrecognized system of pathogen sensing by human platelets (**Figure 2**). Moreover, TLR9 binds a carboxyalkylpyrrole protein adduct that may serve as a DAMP, resulting in platelet activation, aggregation, and granule secretion and thrombosis (27). Thus, TLR9 represents another functional platelet receptor linking immune, inflammatory, and thrombotic pathways.

PLATELETS SECRETE MEDIATORS THAT AUGMENT HOST IMMUNE MECHANISMS

Activated platelets have numerous direct and indirect mechanisms for delivering signals to target cells involved in immune and inflammatory interactions (**Figure 1**). These diverse mechanisms include platelet secretion of chemokines, cytokines, and other mediators (4). There are more than 300 proteins secreted by platelets, the majority of which are stored in at least one of three types of storage organelles: alpha granules, dense granules or bodies, or lysosomes. Recently, a fourth electron-dense tubular system compartment, called the T-granule, was discovered and, intriguingly, co-localizes with TLR9 (25). Many of these proteins translocate to the platelet surface and are released upon platelet activation. Other secreted proteins are located basally on the platelet membrane and/or within other subcellular stores (**Figure 1**).

Protein products released by platelets orchestrate intercellular signaling for dynamic immune and inflammatory response (1, 2, 4). For example, quiescent human platelets possess the IL-1β pre-mRNA but almost no mature IL-1β mRNA or IL-1β protein (28). Upon activation with agonists often present in the infectious milieu, including thrombin and LPS, platelets synthesize pro-IL-1β protein (28, 29). Components of the inflammasome, which regulates host defense mechanisms to pathogens (30, 31), are present within platelets and mediate conversion of pro-IL-1β

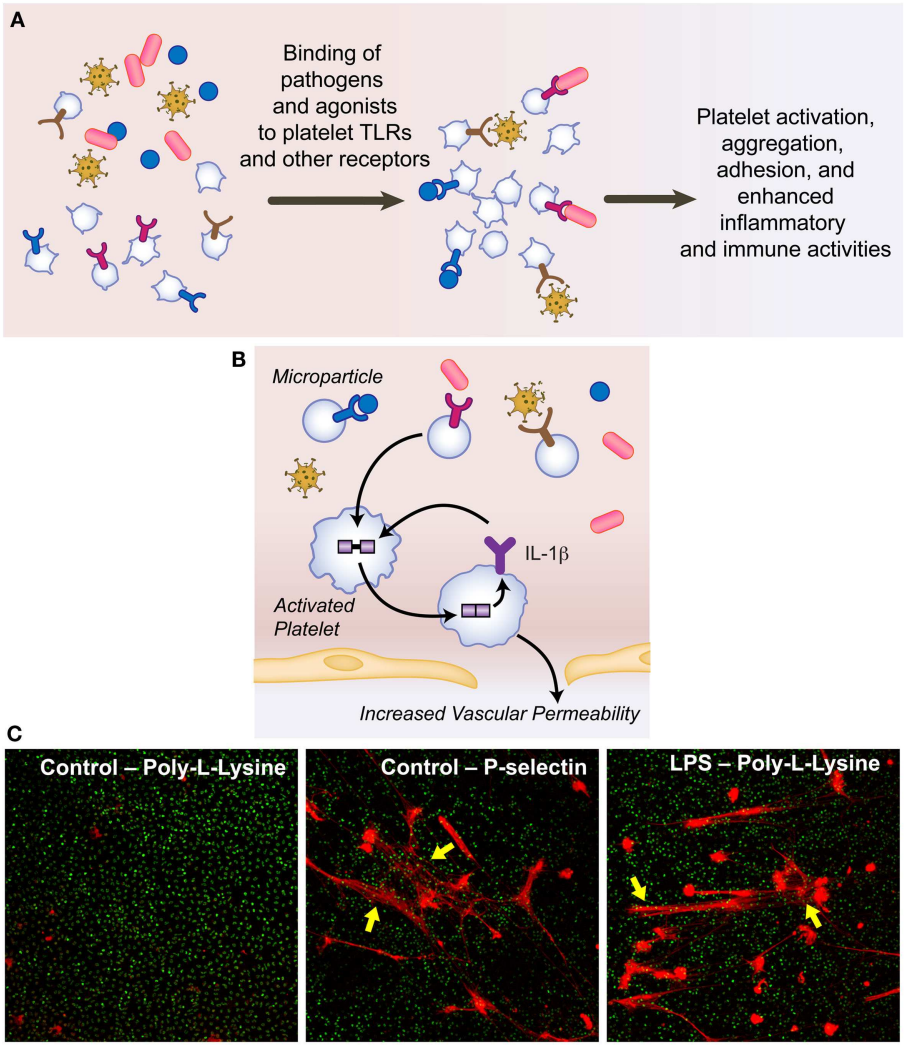


FIGURE 2 | Platelets interact with pathogens present in the infections milieu, increase vascular permeability, and mediate NET formation. **(A)** Binding of pathogens and agonists present in the infectious milieu leads to platelet activation and enhanced inflammatory and immune activities. **(B)** Platelet-derived microparticles carry IL-1β, which increases vascular permeability. **(C)** P-selectin, which is expressed on the surface of activated platelets, induces NET formation in a reductionist model of endothelial cell and platelet activation. PMNs isolated from healthy adult donors were incubated on poly-L-lysine or p-selectin coated glass coverslips and assessed qualitatively for NET formation using live cell imaging. Extracellular, NET-associated DNA is shown in red fluorescence (Sytox Orange) and intracellular, nuclear DNA is shown in green fluorescence (Syto Green). NET formation by LPS-stimulated PMNs is shown as a positive control. These images are representative of four separate experiments performed using PMNs isolated from four different healthy adult donors (used with kind permission from Drs. Nathan L. Thornton and Christian C. Yost).

to the mature IL-1β cytokine (2). Mature IL-1β, the gatekeeper of inflammation, can be released by platelets into the systemic circulation and/or packaged within platelet-derived microparticles (MPs). IL-1β within platelet MPs allows for communication with and activation of extravascular cells and induction of endothelial permeability (7, 32). Recent evidence demonstrates that IL-1β also acts through an autocrine loop to amplify platelet activation responses (33). Platelet shedding of IL-1β rich MPs also may contribute to enhanced vascular permeability in hemorrhagic viral infections such as dengue (34) (**Figure 2**). Other proteins secreted by platelets, including platelet factor 4 (PF4), RANTES (*r*egulated on *a*ctivation, *n*ormal *T*-cell *e*xpressed and *s*ecreted), CD154, transforming growth factor beta 1 (TGF-beta 1), and CD40L are involved in key pathways for innate immune regulation, inflammation, and adaptive immune responses (1, 19, 35–38).

CLINICAL IMPLICATIONS OF PLATELET IMMUNE ACTIVITIES

Emerging and established evidence demonstrates that platelets participate in diverse inflammatory and immune clinical syndromes. While a detailed discussion is beyond the scope of this review, the reader is referred to several recent articles summarizing these activities (1–3, 12, 19, 36, 37). In the section below, we briefly discuss several of these clinical conditions.

Platelets are key mediators of bacterial and viral infectious syndromes. Emerging and intriguing data also highlight that fungal pathogens such as invasive aspergillosis, also induce platelet activation *in vitro* and *in vivo*. Moreover, upon activation by fungal serine protease and the mycotoxin gliotoxin, platelets inhibit fungal growth (39). These and other data demonstrate that platelet-fungus interactions are multifaceted and complex, involving complement activation, chemotactic attraction, and activation of other immune cells such as phagocytes (40).

Among these various infectious syndromes, sepsis, malaria, and dengue are particularly important to highlight given their commonness and high risk of significant morbidity and mortality (41). Alterations in platelet number and function are integral to each of these, yet the mechanisms underlying these changes remain only incompletely defined. Moreover, the extent to which platelets augment host defense mechanisms or, contrarily, induce injurious systemic responses is unclear.

Thrombocytopenia is common in septic syndromes with clinical studies demonstrating an inverse correlation between platelet number and adverse outcomes (12, 42, 43). The precise mechanisms underlying reductions in platelet counts during infectious syndromes are not entirely clear although interferon production during infections may contribute to impairments in thrombopoiesis and resulting thrombocytopenia. The precise pathways by which interferon causes an antiproliferative effect are still incompletely understood but likely involve direct effects on megakaryocytes residing within the bone marrow niche.

While many studies have focused on alterations in "traditional" platelet activation patterns (44, 45) (e.g., adhesion, secretion, platelet-monocyte aggregation), emerging evidence highlights the new biology of platelets in septic syndromes. For example, in response to *in vitro* stimulation LPS, thrombin, bacteria, and bacterial toxins, human platelets process constitutively present tissue facture (TF) pre-mRNA, resulting in a mature TF transcript, synthesis of TF protein, and generation of TF-dependent procoagulant activity (22). Moreover, platelets from septic patients, but not healthy controls, express the mature, spliced TF mRNA, demonstrating that the septic milieu may alter the platelet transcriptome. Preliminary analyses of next-generation RNA sequencing data ("deep sequencing") performed on platelets from septic patients identified numerous transcripts that are significantly altered compared to matched, healthy control subjects. Many of these altered transcripts mediate key immune and inflammatory pathways and augment host defenses to bacterial invaders.

Dengue, a mosquito-borne viral infection, has variable clinical manifestations. Nevertheless, especially in more severe cases, marked thrombocytopenia, hemorrhage, "capillary leak," and shock may occur. While the dengue flavivirus targets endothelial cells and leukocytes, interactions of the virus with platelets and megakaryocytes is likely a central feature of the pathophysiology and course of infection (41). Cytokines synthesized by platelets or in response to platelet-dependent signaling of monocytes, including IL-1β, IL-8, tumor necrosis factor (TNF)-α, and monocyte chemotactic protein (MCP)-1 (19), are increased in plasma from dengue patients and correlate with the degree of thrombocytopenia (46). Increased IL-1β in platelets and platelet MPs was recently reported to occur through a mechanism dependent on mitochondrial reactive oxygen species (ROS)-triggered NLRP3 inflammasomes (34). Dengue virus also alters L-arginine transport and nitric oxide generation in platelets *in vitro*, resulting in impaired aggregation (47). Platelets isolated from dengue patients have evidence of mitochondrial dysfunction with activation of apoptosis pathways, mediated through DC-SIGN (11). These findings suggest that platelet dysfunction may contribute to the thrombocytopenia and associated hemorrhagic complications of dengue infection.

Platelets are also likely early responder and effector cells in malaria infections. Human platelets inhibit the growth of some malaria species and also kill the malarial pathogen in parasitized red blood cells (PRBCs), experimental evidence that platelets may serve as early host defense responses to malarial infection in the vasculature (48–50). In contrast to these protective innate immune activities, platelets may also contribute to injurious vasculopathy in severe malaria. While the mechanisms are still not entirely understood, murine studies demonstrate that platelet signaling via PF4 release mediates T-cell recruitment and monocyte activation within the central nervous system (CNS) (51). These processes may contribute to CSN injury in severe malaria. Recent studies of patients infected with *Plasmodium vivax* malaria suggest that the thrombocytopenia that commonly occurs in malaria may be due to platelet phagocytosis (52). Taken together, these studies and others provide experimental and clinical evidence that platelets are important immune effector cells in malaria (53, 54).

CONCLUSIONS

While traditionally thought of as primary hemostatic cells, platelets are emerging as dynamic effector cells that sense and respond to invading pathogens. Ongoing studies in experimental models of infection and human studies will further elucidate the mechanisms, pathways, and processes governing the platelet's broad repertoire of immune functions. These previously unrecognized findings may also lead to the development of novel therapies targeting the platelet to augment host defense mechanisms.

AUTHORS' CONTRIBUTIONS

Each author contributed to the manuscript ideas and content. Each author was involved in writing the manuscript, the decision to submit the manuscript for publication, and final editing and approval.

ACKNOWLEDGMENTS

We thank Ms. Diana Lim for figure preparation and Ms. Alexandra Greer for editorial assistance. This work was supported by the NIH and NIA (U54HL112311, R03AG040631, K23HL092161, and R01AG048022 to MTR) and a Pilot Grant from the University of Utah Center on Aging.

REFERENCES

1. Rondina MT, Weyrich AS, Zimmerman GA. Platelets as cellular effectors of inflammation in vascular diseases. *Circ Res* (2013) **112**(11):1506–19. doi:10.1161/CIRCRESAHA.113.300512
2. Vieira-de-Abreu A, Rondina MT, Weyrich AS, Zimmerman GA. Inflammation. 3rd ed. In: Michelson AD, editor. *Platelets*. New York, NY: Elsevier (2012). p. 733–66.

3. Morrell CN, Aggrey AA, Chapman LM, Modjeski KL. Emerging roles for platelets as immune and inflammatory cells. *Blood* (2014) **123**(18):2759–67. doi:10.1182/blood-2013-11-462432
4. Semple JW, Italiano JE Jr, Freedman J. Platelets and the immune continuum. *Nat Rev Immunol* (2011) **11**(4):264–74. doi:10.1038/nri2956
5. Garraud O, Berthet J, Hamzeh-Cognasse H, Cognasse F. Pathogen sensing, subsequent signalling, and signalosome in human platelets. *Thromb Res* (2011) **127**(4):283–6. doi:10.1016/j.thromres.2010.10.015
6. Kasirer-Friede A, Kahn ML, Shattil SJ. Platelet integrins and immunoreceptors. *Immunol Rev* (2007) **218**:247–64. doi:10.1111/j.1600-065X.2007.00532.x
7. Boilard E, Nigrovic PA, Larabee K, Watts GF, Coblyn JS, Weinblatt ME, et al. Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science* (2010) **327**(5965):580–3. doi:10.1126/science.1181928
8. Zahn A, Jennings N, Ouwehand WH, Allain JP. Hepatitis C virus interacts with human platelet glycoprotein VI. *J Gen Virol* (2006) **87**(Pt 8):2243–51. doi:10.1099/vir.0.81826-0
9. Hughan SC, Hughes CE, McCarty OJ, Schweighoffer E, Soultanova I, Ware J, et al. GPVI potentiation of platelet activation by thrombin and adhesion molecules independent of Src kinases and Syk. *Arterioscler Thromb Vasc Biol* (2007) **27**(2):422–9. doi:10.1161/01.ATV.0000252826.96134.21
10. Chaipan C, Soilleux EJ, Simpson P, Hofmann H, Gramberg T, Marzi A, et al. DC-SIGN and CLEC-2 mediate human immunodeficiency virus type 1 capture by platelets. *J Virol* (2006) **80**(18):8951–60. doi:10.1128/JVI.00136-06
11. Hottz ED, Oliveira MF, Nunes PC, Nogueira RM, Valls-de-Souza R, Da Poian AT, et al. Dengue induces platelet activation, mitochondrial dysfunction and cell death through mechanisms that involve DC-SIGN and caspases. *J Thromb Haemost* (2013) **11**(5):951–62. doi:10.1111/jth.12178
12. Semple JW, Freedman J. Platelets and innate immunity. *Cell Mol Life Sci* (2010) **67**(4):499–511. doi:10.1007/s00018-009-0205-1
13. Andonegui G, Kerfoot SM, McNagny K, Ebbert KV, Patel KD, Kubes P. Platelets express functional toll-like receptor-4. *Blood* (2005) **106**(7):2417–23. doi:10.1182/blood-2005-03-0916
14. Cognasse F, Hamzeh H, Chavarin P, Acquart S, Genin C, Garraud O. Evidence of toll-like receptor molecules on human platelets. *Immunol Cell Biol* (2005) **83**(2):196–8. doi:10.1111/j.1440-1711.2005.01314.x
15. Koupenova M, Vitseva O, MacKay CR, Beaulieu LM, Benjamin EJ, Mick E, et al. Platelet-TLR7 mediates host survival and platelet count during viral infection in the absence of platelet-dependent thrombosis. *Blood* (2014) **124**(5):791–802. doi:10.1182/blood-2013-11-536003
16. Garraud O, Cognasse F. Platelet toll-like receptor expression: the link between "danger" ligands and inflammation. *Inflamm Allergy Drug Targets* (2010) **9**(5):322–33. doi:10.2174/187152810793937991
17. Iwasaki A, Medzhitov R. Regulation of adaptive immunity by the innate immune system. *Science* (2010) **327**(5963):291–5. doi:10.1126/science.1183021
18. Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, et al. Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature* (2010) **464**(7285):104–7. doi:10.1038/nature08780
19. Vieira-de-Abreu A, Campbell RA, Weyrich AS, Zimmerman GA. Platelets: versatile effector cells in hemostasis, inflammation, and the immune continuum. *Semin Immunopathol* (2012) **34**(1):5–30. doi:10.1007/s00281-011-0286-4
20. Ward JR, Bingle L, Judge HM, Brown SB, Storey RF, Whyte MK, et al. Agonists of toll-like receptor (TLR)2 and TLR4 are unable to modulate platelet activation by adenosine diphosphate and platelet activating factor. *Thromb Haemost* (2005) **94**(4):831–8.
21. Shashkin PN, Brown GT, Ghosh A, Marathe GK, McIntyre TM. Lipopolysaccharide is a direct agonist for platelet RNA splicing. *J Immunol* (2008) **181**(5):3495–502. doi:10.4049/jimmunol.181.5.3495
22. Rondina MT, Schwertz H, Harris ES, Kraemer BF, Campbell RA, Mackman N, et al. The septic milieu triggers expression of spliced tissue factor mRNA in human platelets. *J Thromb Haemost* (2011) **9**(4):748–58. doi:10.1111/j.1538-7836.2011.04208.x
23. Clark SR, Ma AC, Tavener SA, McDonald B, Goodarzi Z, Kelly MM, et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat Med* (2007) **13**(4):463–9. doi:10.1038/nm1565
24. Akira S, Takeda K. Toll-like receptor signalling. *Nat Rev Immunol* (2004) **4**(7):499–511. doi:10.1038/nri1391
25. Thon JN, Peters CG, Machlus KR, Aslam R, Rowley J, Macleod H, et al. T granules in human platelets function in TLR9 organization and signaling. *J Cell Biol* (2012) **198**(4):561–74. doi:10.1083/jcb.201111136
26. Aslam R, Speck ER, Kim M, Crow AR, Bang KW, Nestel FP, et al. Platelet toll-like receptor expression modulates lipopolysaccharide-induced thrombocytopenia and tumor necrosis factor-alpha production in vivo. *Blood* (2006) **107**(2):637–41. doi:10.1182/blood-2005-06-2202
27. Panigrahi S, Ma Y, Hong L, Gao D, West XZ, Salomon RG, et al. Engagement of platelet toll-like receptor 9 by novel endogenous ligands promotes platelet hyperreactivity and thrombosis. *Circ Res* (2013) **112**(1):103–12. doi:10.1161/CIRCRESAHA.112.274241
28. Denis MM, Tolley ND, Bunting M, Schwertz H, Jiang H, Lindemann S, et al. Escaping the nuclear confines: signal-dependent pre-mRNA splicing in anucleate platelets. *Cell* (2005) **122**(3):379–91. doi:10.1016/j.cell.2005.06.015
29. Lindemann S, Tolley ND, Dixon DA, McIntyre TM, Prescott SM, Zimmerman GA, et al. Activated platelets mediate inflammatory signaling by regulated interleukin 1beta synthesis. *J Cell Biol* (2001) **154**(3):485–90. doi:10.1083/jcb.200105058
30. Lamkanfi M, Dixit VM. Modulation of inflammasome pathways by bacterial and viral pathogens. *J Immunol* (2011) **187**(2):597–602. doi:10.4049/jimmunol.1100229
31. Stutz A, Golenbock DT, Latz E. Inflammasomes: too big to miss. *J Clin Invest* (2009) **119**(12):3502–11. doi:10.1172/JCI40599
32. Cloutier N, Pare A, Farndale RW, Schumacher HR, Nigrovic PA, Lacroix S, et al. Platelets can enhance vascular permeability. *Blood* (2012) **120**(6):1334–43. doi:10.1182/blood-2012-02-413047
33. Brown GT, Narayanan P, Li W, Silverstein RL, McIntyre TM. Lipopolysaccharide stimulates platelets through an IL-1beta autocrine loop. *J Immunol* (2013) **191**(10):5196–203. doi:10.4049/jimmunol.1300354
34. Hottz ED, Lopes JF, Freitas C, Valls-de-Souza R, Oliveira MF, Bozza MT, et al. Platelets mediate increased endothelium permeability in dengue through NLRP3-inflammasome activation. *Blood* (2013) **122**(20), 3405–14. doi:10.1182/blood-2013-05-504449
35. Sprague DL, Elzey BD, Crist SA, Waldschmidt TJ, Jensen RJ, Ratliff TL. Platelet-mediated modulation of adaptive immunity: unique delivery of CD154 signal by platelet-derived membrane vesicles. *Blood* (2008) **111**(10):5028–36. doi:10.1182/blood-2007-06-097410
36. Elzey BD, Sprague DL, Ratliff TL. The emerging role of platelets in adaptive immunity. *Cell Immunol* (2005) **238**(1):1–9. doi:10.1016/j.cellimm.2005.12.005
37. von Hundelshausen P, Weber C. Platelets as immune cells: bridging inflammation and cardiovascular disease. *Circ Res* (2007) **100**(1):27–40. doi:10.1161/01.RES.0000252802.25497.b7
38. Grainger DJ, Wakefield L, Bethell HW, Farndale RW, Metcalfe JC. Release and activation of platelet latent TGF-beta in blood clots during dissolution with plasmin. *Nat Med* (1995) **1**(9):932–7. doi:10.1038/nm0995-932
39. Speth C, Hagleitner M, Ott HW, Wurzner R, Lass-Florl C, Rambach G. Aspergillus fumigatus activates thrombocytes by secretion of soluble compounds. *J Infect Dis* (2013) **207**(5):823–33. doi:10.1093/infdis/jis743
40. Speth C, Rambach G, Lass-Florl C. Platelet immunology in fungal infections. *Thromb Haemost* (2014) **112**(4):632–9. doi:10.1160/TH14-01-0074
41. Hottz ED, Tolley ND, Zimmerman GA, Weyrich AS, Bozza FA. Platelets in dengue infection. *Drug Discov Today Dis Mech* (2011) **8**:e33–8. doi:10.1016/j.ddmec.2011.09.001
42. Katz JN, Kolappa KP, Becker RC. Beyond thrombosis: the versatile platelet in critical illness. *Chest* (2011) **139**(3):658–68. doi:10.1378/chest.10-1971
43. Schwertz H, Weyrich AS, Zimmerman GA. Cellular interactions of platelets, leukocytes and endothelium in systemic inflammatory responses and sepsis. In: Castro Faria Neto H, Marcus D, editors. *Sepsis: From Bench to Bedside*. Rio de Janerio: Revinter Press (2007). p. 107–20.
44. Rondina MT, Grissom CK, Men S, Harris ES, Schwertz H, Zimmerman GA, et al. Whole blood flow cytometry measurements of in vivo platelet activation in critically-Ill patients are influenced by variability in blood sampling techniques. *Thromb Res* (2011) **129**(6):729–35. doi:10.1016/j.thromres.2011.11.031
45. Gawaz M, Dickfeld T, Bogner C, Fateh-Moghadam S, Neumann FJ. Platelet function in septic multiple organ dysfunction syndrome. *Intensive Care Med* (1997) **23**(4):379–85. doi:10.1007/s001340050344
46. Bozza FA, Cruz OG, Zagne SM, Azeredo EL, Nogueira RM, Assis EF, et al. Multiplex cytokine profile from dengue patients: MIP-1beta and IFN-gamma as predictive factors for severity. *BMC Infect Dis* (2008) **8**:86. doi:10.1186/1471-2334-8-86
47. Lee YR, Liu MT, Lei HY, Liu CC, Wu JM, Tung YC, et al. MCP-1, a highly expressed chemokine in dengue haemorrhagic fever/dengue shock syndrome

patients, may cause permeability change, possibly through reduced tight junctions of vascular endothelium cells. *J Gen Virol* (2006) **87**(Pt 12):3623–30. doi:10.1099/vir.0.82093-0
48. Greenbaum DC, FitzGerald GA. Platelets, pyrexia, and plasmodia. *N Engl J Med* (2009) **361**(5):526–8. doi:10.1056/NEJMcibr0903050
49. Peyron F, Polack B, Lamotte D, Kolodie L, Ambroise-Thomas P. Plasmodium falciparum growth inhibition by human platelets in vitro. *Parasitology* (1989) **99**(Pt 3):317–22. doi:10.1017/S0031182000059011
50. McMorran BJ, Marshall VM, de Graaf C, Drysdale KE, Shabbar M, Smyth GK, et al. Platelets kill intraerythrocytic malarial parasites and mediate survival to infection. *Science* (2009) **323**(5915):797–800. doi:10.1126/science.1166296
51. Srivastava K, Cockburn IA, Swaim A, Thompson LE, Tripathi A, Fletcher CA, et al. Platelet factor 4 mediates inflammation in experimental cerebral malaria. *Cell Host Microbe* (2008) **4**(2):179–87. doi:10.1016/j.chom.2008.07.003
52. Coelho HC, Lopes SC, Pimentel JP, Nogueira PA, Costa FT, Siqueira AM, et al. Thrombocytopenia in *Plasmodium vivax* malaria is related to platelets phagocytosis. *PLoS One* (2013) **8**(5):e63410. doi:10.1371/journal.pone.0063410
53. van der Heyde HC, Gramaglia I, Sun G, Woods C. Platelet depletion by anti-CD41 (alphaIIb) mAb injection early but not late in the course of disease protects against Plasmodium berghei pathogenesis by altering the levels of pathogenic cytokines. *Blood* (2005) **105**(5):1956–63. doi:10.1182/blood-2004-06-2206
54. Essien EM, Ebhota MI. Platelet secretory activities in acute malaria (*Plasmodium falciparum*) infection. *Acta Haematol* (1983) **70**(3):183–8. doi:10.1159/000206720

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 20 October 2014; accepted: 06 December 2014; published online: 18 December 2014.
*Citation: Rondina MT and Garraud O (2014) Emerging evidence for platelets as immune and inflammatory effector cells. Front. Immunol. **5**:653. doi: 10.3389/fimmu.2014.00653*
This article was submitted to Inflammation, a section of the journal Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 02 March 2015
doi: 10.3389/fimmu.2015.00083

The inflammatory role of platelets via their TLRs and Siglec receptors

Fabrice Cognasse[1,2]*, Kim Anh Nguyen[2], Pauline Damien[2], Archibald McNicol[3], Bruno Pozzetto[2], Hind Hamzeh-Cognasse[2] and Olivier Garraud[2,4]

[1] *Etablissement Français du Sang Auvergne-Loire, Saint-Etienne, France*
[2] *GIMAP-EA3064, Université de Lyon, Saint Etienne, France*
[3] *Faculty of Health Sciences, Colleges of Pharmacy and Medicine, University of Manitoba, Winnipeg, MB, Canada*
[4] *Institut National de Transfusion Sanguine (INTS), Paris, France*

Edited by:
Heiko Mühl, University Hospital Goethe University Frankfurt, Germany

Reviewed by:
Patrizia Rovere Querini, Ospedale San Raffaele and Vita-Salute University, Italy
Matthew Dean Linden, University of Western Australia, Australia

***Correspondence:**
Fabrice Cognasse, GIMAP-EA 3064, Faculté de Médecine, Université de Saint-Etienne, 15 rue Ambroise Paré, 42023 Saint-Etienne Cedex 2, France; Etablissement Français du Sang Auvergne-Loire, 25 Boulevard Pasteur, Saint-Etienne 42100, France
e-mail: fabrice.cognasse@efs.sante.fr

Platelets are non-nucleated cells that play central roles in the processes of hemostasis, innate immunity, and inflammation; however, several reports show that these distinct functions are more closely linked than initially thought. Platelets express numerous receptors and contain hundreds of secretory products. These receptors and secretory products are instrumental to the platelet functional responses. The capacity of platelets to secrete copious amounts of cytokines, chemokines, and related molecules appears intimately related to the role of the platelet in inflammation. Platelets exhibit non-self-infectious danger detection molecules on their surfaces, including those belonging to the "toll-like receptor" family, as well as pathogen sensors of other natures (Ig- or complement receptors, etc.). These receptors permit platelets to both bind infectious agents and deliver differential signals leading to the secretion of cytokines/chemokines, under the control of specific intracellular regulatory pathways. In contrast, dysfunctional receptors or dysregulation of the intracellular pathway may increase the susceptibility to pathological inflammation. Physiological vs. pathological inflammation is tightly controlled by the sensors of danger expressed in resting, as well as in activated, platelets. These sensors, referred to as pathogen recognition receptors, primarily sense danger signals termed pathogen associated molecular patterns. As platelets are found in inflamed tissues and are involved in auto-immune disorders, it is possible that they can also be stimulated by internal pathogens. In such cases, platelets can also sense danger signals using damage associated molecular patterns (DAMPs). Some of the most significant DAMP family members are the alarmins, to which the Siglec family of molecules belongs. This review examines the role of platelets in anti-infection immunity via their TLRs and Siglec receptors.

Keywords: platelets, innate immunity, cytokine/chemokine, inflammation, TLR, Siglec

INTRODUCTION

It is well accepted that blood platelets play a principal role in primary hemostasis. Since more than 50 years, they have been used therapeutically as transfused blood products (1), and platelet extracts have been used for their healing properties, especially in ophthalmology (2). Platelet concentrate transfusions were considered necessary in a number of situations (e.g., central thrombocytopenia in preference to peripheral thrombocytopenia; thrombopathy associated with bleeding; severe bleeding or risk of bleeding) (3). However, transfusion hazards and, at minimum, discomfort, are common (4), leading the medical community to question this therapy.

Traditionally, hemostasis and thrombosis have been considered difficult to understand for non-specialists. However, there is a general understanding that human platelet antigen (HPA) polymorphisms can complicate the post-transfusion situation in thrombopathies, such as von Willebrand disease (4) and pregnancies and deliveries [notably regarding fetal neonatal allo-immunization (FNAIT) (5)], along with allo-immunization to HLA class I antigens responsible for transfusion refractoriness (6).

Now, however, it is recognized that platelets have a complex role in the whole process of hemostasis and thrombosis (7). As the platelet proteome started to be investigated, it became clear that platelets contain proteins beyond those with hemostatic functions, including angiogenic factors, growth factors, pro-inflammatory factors, anti-inflammatory factors, and biological response modifiers (BRMs) (8).

AN OVERVIEW OF THE MAIN PLATELET RECEPTORS

Platelets play a vital role in hemostasis, especially primary hemostasis, and subsequently in vascular repair (9). Platelet membrane integrins interact with the subendothelial matrix of a damaged vascular wall, leading to their activation and subsequent creation of a platelet thrombus together by fibrinogen (Fg) to close the vascular lesion and terminate blood loss (10).

Activated platelets liberate arachidonic acid from membrane phospholipids, which in turn is converted to thromboxane A_2 (TxA_2). As a consequence, TxA_2 is secreted and acts on TP receptors on the cell surface of other platelets, leading to further platelet activation. The TP receptor belongs to the major

agonist receptor family seven transmembrane G-protein coupled receptors (GPCRs).

Granule contents are released from activated platelets. The dense granules contain important agonists like ADP and serotonin. ADP is a significant amplifier of initial platelet aggregation (11). It interacts with specific extracellular membrane receptors to induce intracellular signaling. There are two classes of receptors for ADP, P2Y1 and P2Y12, which belong to the GPCR family (12) and P2 × 1, which belongs to the ADP/ATP determined calcium channel family of purinergic receptors (13, 14). Engagement of the TP receptor by TxA_2 and P2Y1 by ADP lead to the hydrolysis of phosphatidylinositol-4,5 bisphosphate by phospholipase C (PLC), leading to the generation of diacylglycerol (DAG) and inositol triphosphate (IP_3) (15). DAG and IP_3 stimulate protein kinase C and cause release of calcium from the dense tubular system, respectively, which in turn are responsible for many of the final event associated with platelet activation, including the exocytosis of platelet granules. ADP interacts with calcium activated pathway. P2Y12 receptor has been reported to potentiate platelet secretion and be involved in "sustained irreversible platelet aggregation" (12).

Platelets express a class of cell surface protease-activated receptors (PARs), which are members of the GPCR family and activated by thrombin. Human platelets only express PAR1 and PAR4, not PAR2 and PAR3 (16). PAR1 is also the most abundant PAR, expressing about 2500 copies per platelet and is, therefore, predominant for thrombin mediated platelet activation. PAR1 is a highly glycosylated protein that consist of 425 amino acids and has a molecular weight of 70 kDa. PAR4 on the other hand, the last PAR to be discovered, consists of 397 amino acids (17). The PAR1 activating peptide SFLLRN and PAR4 activating peptide AYPGKF are synthetic peptides with the same sequence as their respectively N-terminal after cleavage. They are widely used in research because they effectively and specific activate their receptors (18).

Interestingly, several hemostatic receptors, in addition being the subject of genetic polymorphisms leading to variant HPAs, bind to infectious pathogens (**Figure 1**).

Platelets also express receptors that indirectly bind infectious pathogens: Complement (C) Rs and Fc-Ig-Rcs (**Table 1**).

The apparently complex relationships between platelets and infectious pathogens, as well as the involvement of platelets during sepsis, which have been the subject of recent reviews (10, 27–33), led a number of groups to examine the possibility that platelets have dedicated roles in innate immunity and foremost in pathogen sensing. Those groups almost simultaneously described, both in mice and humans, the presence and then the functionality of toll-like receptors (TLRs) on (TLR2/TLR1/TLR6 and TLR4 ± TLR9) and in platelets (TLR9) (19, 23, 24, 34–36). It is only recently that TLR3 (37) and TLR7 (38) have also been identified. This discovery allowed a paradigm change in the understanding of platelet physiopathology, putting platelets in a continuum of immunity, at the crossroad of innate and adaptive immunity (10, 35, 39–41).

By contrast, Montrucchio et al. (42) demonstrated that platelets neither bind FITC-LPS nor express the LPS-receptors CD14 and toll-like receptor 4 (TLR4); in contrast, LPS primed monocytes – and to a lesser extent polymorphonuclear neutrophils – proved to adhere to platelets. Next, both platelet–leukocyte interactions and platelet aggregation (in whole blood) were inhibited by blockade of CD14 and TLR4.

It must also be noticed that the Sabroe team observed no modulation in platelet response after engagement of TLR1 and TLR4 (43), no platelet aggregation, no increase in CD62p on the platelets surface, and no increase in intra-platelet $Ca2^+$ levels after stimulation by "natural" ligands of TLR2 (e.g., Pam3CSK4) or TLR4 (e.g., LPS); this allowed them to conclude that agonists of platelet

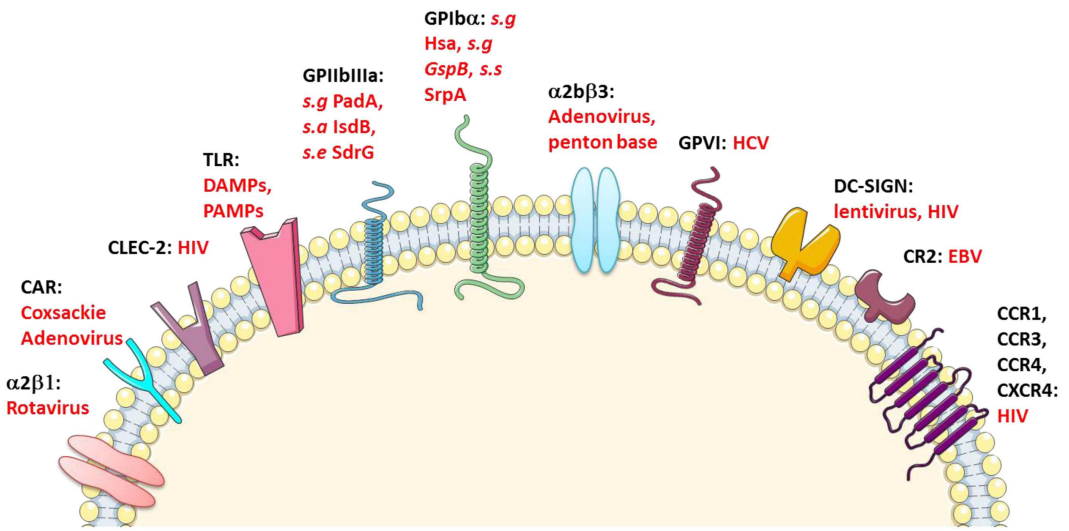


FIGURE 1 | Platelet immune receptors. CLEC-2, C-type lectin-like type II transmembrane receptor; CR-2, complement receptor type 2; CCR-1, CCR-3, and CCR-4, C–C chemokine receptor type 1, 3, and 4, respectively; CXCR-4, C-X-C chemokine receptor type 4; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; GP-VI, glycoprotein VI; CAR, coxsackie adenovirus receptor; *s.a, Staphylococcus aureus*; *s.g, Streptococcus gordonii*; *s.s, Streptococcus sanguinis*; *s.e, Staphylococcus epidermidis*. Adapted from Ref. (10, 19–23).

Table 1 | Platelet–bacteria interactions adapted from Ref. (24–26).

Platelet receptors	Bacteria/fungi	Bacterial proteins	Intermediary plasma molecules
GP-IIb–IIIa	*S. epidermidis*	SdrG	Fg
	S. Aureus	FnbpA/B	Fibronectin
	S. Aureus	FnbpA/B	Fg
	S. Aureus	ClfA	Fibronectin
	S. Aureus	ClfA	Fg
	S. Aureus	IsdB	Direct
	S pyogenes	M1	Fg
	S gordonii	PadA	Direct
	S. lugdunensis	Fbl	Fg
GP-Iba	*S. sanguis*	SrpA	Direct
	S. gordonii	GspB/Hsa	Direct
	S. Aureus	Protein A	vWF
	H. pylori	?	vWF
FCγRIIa	*S. Aureus*	FnbpA/B	IgG
	S. Aureus	ClfA	IgG
TLR2	*S. pneumoniae*	?	Direct
	?	Lipoprotein	Direct
TLR4	*E. coli*	LPS	Direct
gC1q-R	*S. sanguinis*	?	C1
TLR	Fungi	?	Complement
Protease-activated receptors	Fungi	?	Complement

ClfA, clumping factor A; Fnbp, fibronectin-binding protein; Fg, Fibrinogen; GspB, glycosylated streptococcal protein B; Hsa, hemagglutinin salivary antigen; IsdB, iron-regulated surface determinant B; LPS, lipopolysaccharide; PadA, platelet adhesion binding protein A; SdrG, serine–aspartate repeat G; SrpA, serine-rich protein A; TLR, toll-like receptor; VWF, von Willebrand factor; ?, undefined.

TLRs have no direct effect on platelet activation. This was rather puzzling because agonists of TLR2 and TLR4 are acknowledged to play a significant role in diseases such as atherosclerosis; a mechanism other than the direct activation of platelets is believed to be involved.

Similar data have been observed by Jayachandran et al. (44) who attempted to determine whether LPS affects platelet phenotype after TLR4 engagement. After injecting knockout (KO) mice for the TLR4 coding gene with a non-lethal dose of LPS (0.2 mg/kg IV), they demonstrated that the platelets in TLR4 KO mice are less abundant than in normal mice, contain less RNA, and express less CD62p upon thrombin exposure. However, platelets from TLR4 KO mice do neither aggregate nor secrete adenosine triphosphate upon thrombin stimulation. One week after injection (which is the time required for a full turnover of the platelet pool), the number of circulating platelets, the CD62p expression, and the platelet aggregation had increased; consequently, the platelet phenotype observed 1 week after exposure to LPS appears independent on TLR4. It is concluded that the 1-week lasting effect of LPS is related to the megakaryocytes themselves and the platelets they produce, rather than to the already circulating platelets (44).

Platelets, already identified as secretors of pro-inflammatory cytokines, chemokines, and BRMs, such as soluble-CD40-Ligand (sCD40L)/sCD154, were revisited for their role in inflammation (45–48).

Initially, platelet linked-inflammation was recognized in pathologies, such as transfusion associated hazards or adverse events, however, further work also identified platelets in peripheral pathologies, such as in cardio-vascular disease and atheroma-plaque formation, inflammatory bowel disease, and arthritis (20, 27, 41, 49–58). This identified the role of platelets in pathological inflammation; however, a role for platelets in physiological inflammation, as this concept potentially established in the immunology landscape (59, 60), remained ill-defined. A series of dedicated studies aimed at stimulating platelets with a variety of defined activators with small differences demonstrated that platelets, despite being non-nucleated, are capable of mobilizing a signalosome differentially, and to secrete discrete panels of BRMs upon activator-dependent stimulation. Platelets appear to have the ability to decipher between vessel endothelial cell injuries eliciting hemostatic responses and between distinct infectious pathogen moieties; however, both types of receptors can be engaged in parallel as – for example – responses to TLR2 engagement on platelets involves PAR1 (61, 62).

In summary, the newly proposed paradigm on platelet pathophysiology is that platelets are equipped with multiple receptors aimed at sensing their environment; basically, the principal danger that can be sensed by circulating platelets is created by endothelial damage. Under normal situations, platelets can repair this damage by eliciting a local micro-inflammatory response aimed at recruiting repairing and healing molecules. In addition, platelets can also sense, under certain circumstances, other potential dangers, such as infectious pathogens harboring Pathogen Associated Molecular Patterns (PAMPs). Therefore, platelets can be regarded as sentinels of danger, especially at the vascular level. Platelets are thus involved along the spectrum of inflammation, from physiology to pathology.

Platelets are engaged in complex relationships with leukocytes [all types (63–66)], notably with polymorphonuclear cells (PMNCs – formally known as neutrophils), both in physiology and pathology (67–73). It has recently been shown that platelets express a (or the) triggering receptor expressed on myeloid cells 1 (TREM1)-receptor which, when engaged by a protein expressed on neutrophils, TRIM1, increases the activation of these cells and modulates the inflammatory response by augmenting both IL-8 secretion and the production of reactive oxygen species (ROS) (74).

Further studies identified that platelets were capable of leaving the circulation (they were initially thought to be strictly contained in the blood flux), especially by being "cargoed" by leukocytes (66, 75, 76), and to participate in peripheral pathology [by themselves and by microparticles (PMPs) shed from their membrane (77–79). The capacity of platelets to sense damaged tissues was then addressed and proved to depend on platelet expression of damage associated molecular patterns (DAMP)-sensors] (80, 81). Moreover, Varki et al. (82) proposed "self-associated molecular patterns" (SAMPs), which would be recognized by intrinsic inhibitory receptors, to maintain the baseline non-activated state of innate immune cells and dampen their reactivity following an immune response. To detect such SAMPs, there must be cognate

Self-PRRs (SPPRs). The first-studied example was factor H and the second are the Siglec (sialic acid recognizing Ig-like lectins), which have N-terminal V-set Ig-like domains that recognize sialic acids and often have tyrosine-based inhibitory signaling motifs within their cytosolic tails (83–87). Siglec molecules (Siglec-7, 9, and 11) are now acknowledged as controlling platelet apoptosis (88).

THE PLATELET TOLL-LIKE RECEPTORS

BACKGROUND ON TLRs

Toll-like receptors are expressed either on the external surfaces or in the cytoplasms of a wide variety of cells involved in immune processes, and are, to a large extent, involved in natural (or innate) immunity. In particular, TLRs are membrane receptors for pathogens known to play a major role in phagocytosis and inflammation.

It should also be noted that TLRs have endogenous ligands with the capacity to cause, or accelerate, inflammation; these include heat shock proteins (HSP) 60 and 70, Fg, and a diverse products of apoptotic cells (89).

Toll-like receptor expression was initially discovered on immune cells (**Table 2**), such as macrophages, dendritics cells (DCs), and, later, in B-lymphocytes, as well as some categories of T lymphocytes. These receptors are key-players in the defense of the body, as they form the interface between recognition of the danger signal (pathogenic or endogenous) and initiation of various types of immune response (inflammation, release of molecules involved in inducing adaptive response). The example that best emphasizes their importance is the activation of TLRs present on the surface of DCs. The signaling pathways concerned lead to the release of pro-inflammatory cytokines as well as the "up-regulation" of

Table 2 | Summary of known mammalian TLRs and Siglecs.

	Name	Expression	Ligands – Sialic acid linkage specificity		Name	Expression	Ligands
Siglec-family proteins in humans	Siglec-1 (CD169)	Mac	α2,3 > α2,6	**TLR-family proteins in humans**	TLR1	B, Mo, Mac, DCs, Plt	Multiple triacyl lipopeptides
	Siglec-2 (CD22)	B	α2,6		TLR2	Mo, Mac, N, MyDCs, Mc, Plt	Multiple glycolipids Multiple lipopeptides Multiple lipoproteins Lipoteichoic acid HSP70 Zymosan (beta-glucan)
	Siglec-3 (CD33)	Mo, MyP	α2,6 > α2,3		TLR3	DC, B, Plt	Double-stranded RNA Poly I:C
	Siglec-4 (MAG)	OligoD, Schw	α2,3 > α2,6		TLR4	Mo, Mac, N, MyDCs, Mc, B, IE, Plt	Lipopolysaccharide heat shock proteins Fg Heparan sulfate fragments Hyaluronic acid fragments Nickel Various opioid drugs
	Siglec-5 (CD170)	N, Mo, B	α2,3		TLR5	Mo, Mac, DC, IE	Bacterial flagellin Profilin
	Siglec-6 (CD327)	Troph, B	α2,6		TLR6	Mo, Mac, B, Mc, Plt	Diacyl lipopeptides
	Siglec-7 (CD328)	NK, Mo, Plt	α2,8 > α2,6 > α2,3		TLR7	Mo, Mac, pDC, B, Plt	Imidazoquinoline loxoribin (a guanosine analog) Bropirimine Single-stranded RNA
	Siglec-8	Eo, Ba	α2,3 > α2,6		TLR8	Mo, Mac, DC, Mc	Small synthetic compounds; single-stranded RNA
	Siglec-9 (CD329)	Mo, N, DC, NK, Plt	α2,3 = α2,6 (prefers sulfated residues)		TLR9	Mo, Mac, pDC, B, Plt	Unmethylated CpG oligodeoxynucleotide DNA
	Siglec-10	B, Mo, Eo	α2,3 = α2,6		TLR10	Unknown	Unknown
	Siglec-11	Mac, Plt	α2,8		TLR11	Mo, Mac, LC, KC, UBE	Profilin
	Siglec-14	ND	α2,6		TLR12	NE, pDC, DC, Mac	Profilin
	Siglec-15	ND	ND		TLR13	Mo, Mac, DC	Bacterial ribosomal RNA sequence "CGGAAAGACC"

Toll-like receptors and Siglecs bind different ligands and are expressed by different types of leukocytes or other cell types. Human B, B cells; Ba, basophils; cDCs, conventional dendritic cells; Eo, eosinophils; GRB2, growth-factor-receptor-bound protein 2; ITIM, immunoreceptor tyrosine-based inhibitory motif; Mac, macrophages; Mo, monocytes; MyP, myeloid progenitors; N, neutrophils; ND, not determined; NK, natural killer cells; OligoD, oligodendrocytes; pDCs, plasmacytoid dendritic cells; Schw, Schwann cells; Troph, trophoblasts; Plt, platelets, My DC, Myeloid dendritic cells; Mc, Mast cells; IE, Intestinal epithelium; liver cells, LC; kidney cells, KC; Urinary Bladder Epithelium, UBE; Neurons, NE. Adapted from Ref. (10, 35, 84, 86–88, 91–97).

molecules that potentiate the function of Ag presentation (class II CMH, IL-12, CD80) and consequently the activation of T lymphocytes (90).

Toll-like receptors are also expressed by cells considered non-immune but which occupy the interface between the outside environment and the immune system, in particular fibroblasts and epithelial cells (98).

Toll-like receptors are type 1 transmembrane proteins possessing: (i) leucine-rich ectodomains folded into β-sheets, enabling the interaction with PAMPs; (ii) a transmembrane domain; and (iii) a cytoplasmic "Toll-interleukin-1 receptor" (TIR) domain that is fundamental to signal transduction. To date, 10 TLRs have been identified in humans and 13 in mice; TLR12 and TLR13 have not been identified in the former but the latter (89). Of note, TLR11 has the unique feature of possessing a sequence recognized as a stop codon by human transcription machinery; *Toxoplasma* profilin, a ligand of murine TLR11, is recognized by humans and a truncated, but functional, form of TLR11 is, therefore, presumed to exist in the human (99).

The PAMPs recognized by TLRs are lipids, lipoproteins, proteins, or nucleic acids derived from bacteria, viruses, fungi, or parasites. Moreover, PAMPs can be recognized by TLRs in various cellular compartments, including the plasma membrane, endosomes, lysosomes, and endolysosomes (89). After engagement, each TLR triggers its own distinctive biological response, which is specific for the PAMP recognized. These differences were identified by the discovery of various adaptive molecules that bind to the TIR domain; these include the "Myeloid differentiation primary response gene (88)" (MyD88), TIRAP, TIR-domain-containing adapter-inducing interferon-beta (TRIF), and TRAM. These adaptors activate a variety of signaling pathways. Refer to **Figure 2** for a more detailed description of TLR-signaling pathways.

High mobility group box 1 (HMGB1) is a protein that in humans that is encoded by the HMGB1 gene. Platelets bind to HMGB1 but the cell surface receptor mediating this interaction is less documented. Platelets express previously recognized HMGB1 receptors TLR2/4/9, RAGE, transmembrane proteoglycans, and anionic lipids. Whether these structures mediate HMGB1 binding to platelets has not been much studied.

Recently, Yu et al. (100) evidenced a mechanism by which platelets promote tumor cell metastasis and suggest TLR4 – and its endogenous ligand HMGB1 (alarmin HMGB1) – as targets for antimetastatic therapies. The Manfredi's team reported that activated platelets present HMGB1 to neutrophils and commit them to autophagy and neutrophil extracellular trap (NET) generation (101); further, the abundantly produced ROS dramatically increased the ability of extracellular HMGB1 to activate blood leukocytes (102). Moreover, Vogel et al. (103),

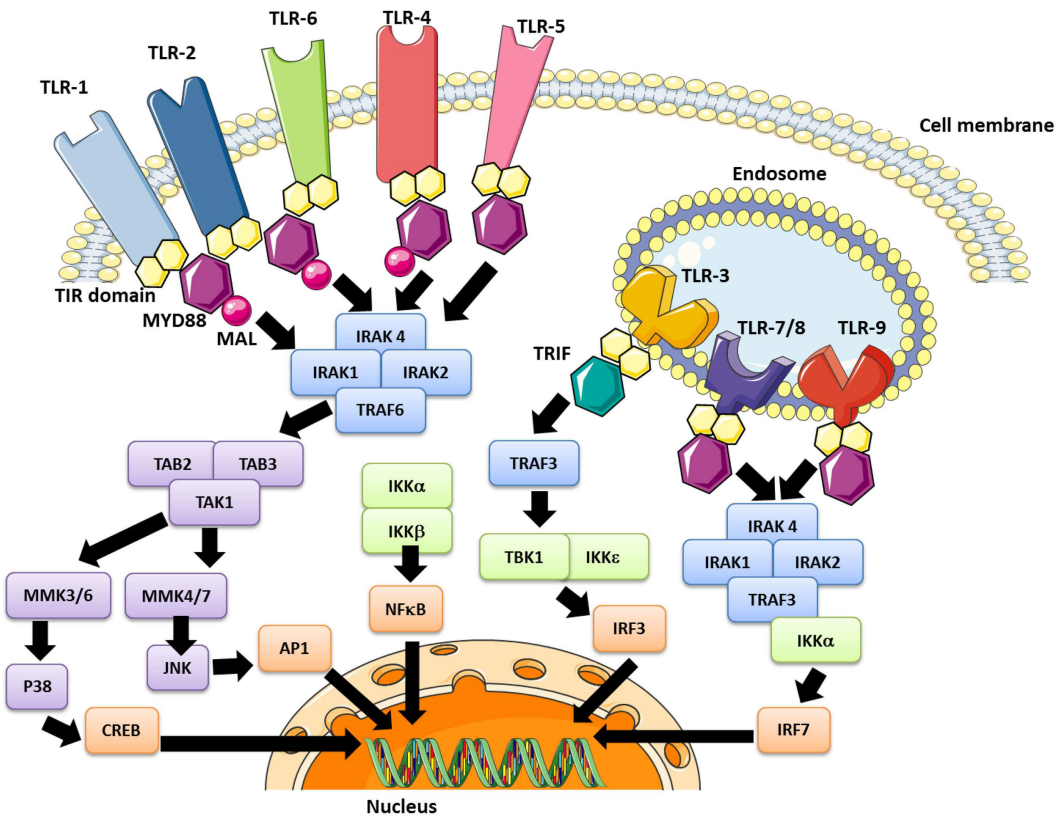


FIGURE 2 | The TLR signaling pathway and modulation effector molecules. Depending upon the TLR involved, the nuclear translocation of transcription factors occurs, including the "nuclear factor kappa B" (NFκB) in early and late stages (all TLRs), AP-1 (all except TLR 3), the "interferon regulation factor" (IRF)-3 (TLR3 and TLR4) and IRF-7 (TLR7/8/9). These pathways lead to inflammatory cytokine synthesis, or at least secretion in the case of platelets, as well as the production of interferon type 1 (IFN1).

demonstrated that migration of mesenchymal stem cells (MSC) to apoptotic cardiac myocytes and fibroblasts was driven by hepatocyte growth factor (HGF), and platelet activation was followed by HMGB1/TLR4-dependent downregulation of HGF receptor MET on MSC, thereby impairing HGF-driven MSC recruitment.

Toll-like receptors are vital to immunity. However, inappropriate responses can, alternatively, trigger chronic and acute inflammation as well as auto-immune illnesses (triggered by the recognition of endogenous ligands) (104).

TLR EXPRESSION ON/IN MEGAKARYOCYTES AND PLATELETS

Identification in megakaryocytes

Megakaryocytes (MK) have been shown to contain mRNA, which codes for TLRs, consistent with these receptors being continuously expressed in MK lineage cells rather than captured through the circulation (34, 35, 62). Moreover, several studies have shown TLR expression both on human megakaryocyte lineage cells (34) and on the MK of mice or isolated from human donors (62, 105), suggesting the origin of platelet TLR expression.

Toll-like receptor 4 expression increases during the MK maturation process. The kinetics of expression of this receptor is similar to that of CD41 (106). Similarly, TLR9 shows a considerable increase in the number of transcripts from day five of MK differentiation in pro-platelets (105).

In contrast to the burgeoning studies into the role of platelet TLRs, few studies have been conducted on the functional role of TLRs on MKs. Two studies (44, 106) have shown that TLR4$^{-/-}$ mice have defects in their circulating and reticulated platelet counts compared with wild-type mice, suggesting that TLR4 could play a role in thrombocytopoiesis. Recent studies have shown that hematopoiesis is not a stereotypical phenomenon; rather, it can be activated by an inflammatory environment (107). The TLR4 of hematopoietic precursors may be involved in this regulation. This hypothesis appears to be confirmed by a study showing that mice stimulated by a TLR4 ligand, at a non-lethal dose, have a higher number of platelets compared with untreated mice (44). An increase in the number of encoding transcripts has also been noted for TLR1 and TLR6 in MKs grown in the presence of IFNγ in both a dose-dependent and a time-dependent manner (107). This could be a mechanism that enables newly formed platelets to be more numerous, augments the amount of their TLRs and plays a more active anti-infectious role as a result.

Early identification on/in platelets

In 2004, Shiraki et al. demonstrated the presence of TLR1 and TLR6 on platelet surfaces and their possible involvement in the process of atherosclerosis (34). Studies conducted that same year by our team complemented these findings with the discovery of TLR2, TLR4, and TLR9 on both the human platelet surface membrane and in the cytoplasm; further these TLRs could be modulated, based on the state of platelet activation (106, 108, 109) (**Figure 3**).

Platelet TLR expression has also been observed in rodents (62), which are a widely used model for the study of these molecules; the

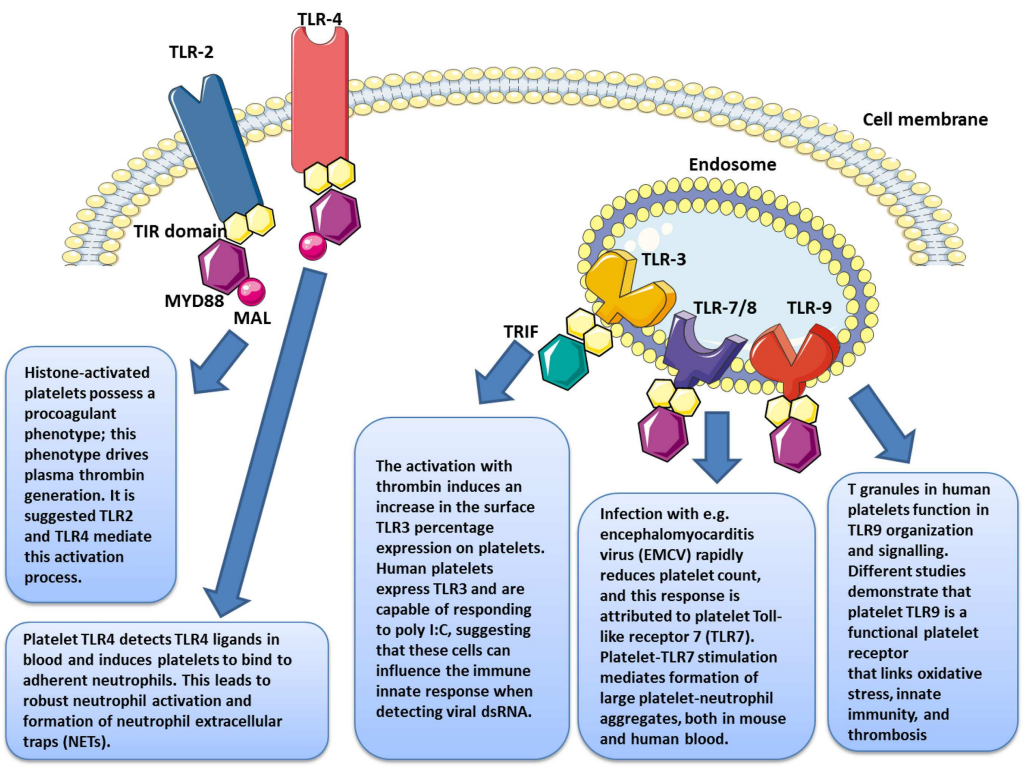


FIGURE 3 | Platelets express several functional toll-like receptors (TLRs), such as TLR2, TLR3, TLR4, TLR7, and TLR9, which may potentially link innate immunity.

characterization of TLRs has also been investigated on the surface of in chicken thrombocytes (110).

Function of TLR4 in platelets

Toll-like receptor 4 is the most abundantly expressed TLR on platelets (111). Several groups have examined its function in humans and mice (10, 19, 35, 106, 109).

Lipopolysaccharides (LPS), a major component of the membrane of Gram-negative bacteria, are ligands of TLR4. On eukaryotic cells, TLR4 forms a complex with the MD-2 molecule, thus enabling binding to LPS. Other proteins contribute to LPS binding, such as the "LPS Binding Protein" (LBP) and CD14 (98, 112).

The engagement of platelet TLR4 by LPS significantly increases the number of spliced mRNA encoding tissue factor (TF), which are then translated into functional proteins (113). LPS has been shown to cause severe thrombocytopenia in murine models, with a 60% decrease in the platelet count 4 h after treatment with LPS, compared with a 20% decrease in TLR4 KO mice (106). These results, therefore, suggest that platelet TLR4 is functional *in vivo*. The reduction in circulating platelets after LPS binding can have two causes: (i) an accumulation of platelets in the lungs (along with the otherwise described neutrophil sequestration) (52) and (ii) an increase in their destruction by phagocytosis (114, 115).

The signaling through TLR in general and TLR4 in particular, in platelets has also been examined. Specifically in platelets, the associated intracellular signaling pathways are less clear than in nucleated cells, as the purpose and function of these transcription factors has to be further elucidated. However, the engagement of platelet TLR4 potentiates signaling pathways traditionally detected in platelets. A notable example is the LPS dose-responsive increase in cGMP (116). Inhibition of PKG signaling blocks TLR4-dependent platelet responses, suggesting that cGMP is involved in TRL4-dependent activation (117). MyD88 expression has also been demonstrated in platelets at levels comparable to that obtained with leukocytes (117). Moreover, LPS-induced aggregation was not observed in MyD88-deficient mice or using salting-out processes in immunomodulatory factors (117). As is the case with other cell types, MyD88 is required for transmission of the signal emitted by TLR4 in platelets. It may be proposed, therefore, that either there is the creation of a link with other signaling pathways or the initiation of a TLR pathway specific to platelets. This suggestion is based on our observations that in addition to MyD88, platelets express most of the molecules traditionally involved in signal transduction, such as TRIF, MyD88, TBK-1, IRAK-1, JNKs, MAPk, TRAF3, TRAF6, IRF-3, IKK-i, IκB-α, and NF-kB p65 (111). For an identical amount of protein extracts, the expression of certain molecules is at times even stronger in platelets than in PBMCs. The level of signal transduction molecules in platelets is quite abundant, in amount that would be usable for intracellular signaling in platelets; the precise meaning of such abundant levels of, i.e., NF-kB in platelets is questioned. This would explain why we showed a difference in platelet activation depending on whether a smooth-type or a rough-type LPS is used as the stimulant. Studies in leukocytes revealed that smooth-type LPS stimulates only the MyD88 pathway while rough-type LPS, owing to its high affinity, has the ability to activate both pathways. While this distinguishing mechanism is present in platelets, the activation of each of these pathways proved to be associated with differential cytokine release (118). TRAF6 has a key role in signaling via platelet TLR4, leading possibly to de novo synthesis of IL1-β. In fact, direct stimulation of TRAF-6 produces splicing five times thicker than is obtained in platelets stimulated by LPS. TRAF-6 activation is followed by phosphorylation of Akt and JNK (119). These phosphorylation events are sufficient to initiate the splicing of mRNA encoding IL-1β. Consequently, a derivation of the TLR4 pathway by TRAF6 occurs, allowing the signal to pass through the Akt and MAP kinase pathways.

Several studies have reported that TLR4 expression varies depending on the state of activation of the platelet (23, 109, 120) (**Figure 3**). Platelet stimulation by thrombin leads to increased TLR4 expression, which, in synergistic combination with membrane CD62P, enables improved LPS binding (120). The fact that platelets have an intracellular pool of TLRs (108) may account for the receptor's translocation to the surface during activation. Even while the LPS does not apparently induce platelet aggregation, it nonetheless potentiates the response triggered by very small concentrations of thrombin and collagen. Our group has shown that TLR4 engagement potentiates the activation of cGMP, bridging activation and aggregation; however, when used at high doses, LPS appears to induce aggregation by itself (57).

As in eukaryotic cells, the function of platelet TLR4 is supported by one or more plasma molecules; the addition of recombinant CD14 to washed platelets, as well as LBP, allows optimal activation to be restored (119–121). CD14, and possibly also MD-2, could be borrowed by platelets in the environment. This is, however, somehow disputed (57).

Several studies have examined the effect of platelet TLR4 engagement on the salting-out of immunomodulatory molecules. For example, Aslam et al. demonstrated that the administration of *Escherichia coli* O111 LPS to mice leads to an increase in the serum levels of "Tumor necrosis factor" (TNF)-α, mainly derived from platelets (109). Our team used similar LPS to stimulate *in vitro* purified platelets obtained from healthy blood donors; we observed a modulation of certain molecules: some were increased (sCD40L and PF4) while others remained unchanged (sCD62P, IL-8, EGF, and TGF) or were even reduced (RANTES, angiogenin, and PDGF-AB) (122). Platelets thus have a highly regulated system for the release of cytokines.

Furthermore, it has been shown that platelets can distinguish between two types of LPS, referred to as "smooth" and "rough," and to adapt their cytokine response accordingly. In this way, only the smooth-type LPS molecules significantly inhibit the secretion of PDGF-AB (118). In contrast, rough-type LPS, unlike smooth-type LPS, potentiates the production of PMPs as well as platelet aggregation induced by the PAR1 agonists SFLLRN (123). The differences in activation can also be seen in the ability of the supernatants obtained to activate peripheral blood mononuclear cells (PBMCs) (118).

Function of TLR2 in platelets

The functional role of platelet TLR2 has not been as widely examined as TLR4. However, TLR2 is a highly inflammatory receptor that can recognize a very large number of PAMPs.

Among these ligands are, *inter alia*, bacterial lipopeptides, peptidoglycan, and lipoteichoic acid from Gram-positive bacteria, microbacterial lipoarabinomannan, yeast zymosan, and even viral hemagglutinin (89). TLR2 forms heterodimers with TLR1 or TLR6: the TLR2/TLR1 complex preferentially binds triacylated lipopeptides (Gram-negative bacteria and mycoplasma), and the TLR2-TLR6 complex binds diacylated lipopeptides of Gram-positive bacteria and mycoplasma.

Structural studies are consistent with the mechanism for such discrimination involving the presence of a hydrophobic channel present on the TLR1 but not on the TLR6 (89).

The engagement of platelet TLR2 has also been reported to stimulate the splicing of mRNA encoding IL-1β (121). However, this synthetic lipopeptide is less effective than LPS, though just as effective as thrombin. The splicing of mRNA encoding IL-1β can, therefore, be regulated differently depending on the TLR activated.

Regarding the signaling pathway, TLR2 uses MyD88 to commence its signaling cascade (124). Platelet TLR2 also has the ability to trigger the activation of signaling proteins normally involved downstream of hemostatic receptors. Morello et al. demonstrated that platelet TLR2s were functional and showed that the PI3K/Akt pathway (already detected in platelets, downstream of αIIbβ3) plays a role in platelet aggregation, adhesion, and secretion (125). They further demonstrated that in immune cells, TLR2 has the ability to interact with the p85 sub-unit of PI3K (126), suggesting the involvement of this pathway after TLR2 engagement, independently of the MyD88 pathway. The use of a specific inhibitor of PI3K (LY294002) prior to stimulation by Pam3CSK4 significantly reduces the effects generated by the engagement of platelet TLR2 (i.e., the aggregation, adhesion and membrane expression of CD62P, platelet-neutrophil complex formation, αIIbβ3 activation, as well as oxygen radicals) (127). LY294002 also significantly diminishes, but does not completely inhibit, the formation of platelet:neutrophil aggregates, suggesting that platelets can use alternate pathways besides TLR2 and 4. Other studies showed that during platelet stimulation by *Aggregatibacter actinomycetem comitans Y4* and *Porphyromonas gingivalis*, the salting-out of sCD40L resulting from TLR2 engagement is regulated by PI3K (128). However, PI3K is not the only pathway involved in the release of soluble factors induced by TLR2 engagement. In fact, the use of a PLC inhibitor, U73122, prevents CD40L from being expressed on the platelet surface in a manner similar to that observed when the PI3K pathway is blocked (128). PLC is classically identified in the platelets as acting downstream of PAR and GP-VI receptors, leading to degranulation and the generation of TxA_2.

Phospholipase C and P3IK pathways are, therefore, not only activated by hemostatic stimulation but also following TLR2 engagement. However, phosphorylation kinetics differs depending on the type of stimulation involved (129). In this way, stimulation by thrombin induces a rapid and substantial phosphorylation of Akt, p38, and Erk. In contrast, stimulation by Pam_3CSK_4 induces a more gradual phosphorylation of Akt and p38, suggesting that it takes longer for the mechanisms to occur and that platelet inflammatory responses take place over time rather than rapidly. The large majority of experimental studies to stimulate TLR2 on platelets use Pam_3CSK_4, a triacylated ligand that causes the dimerization of TLR2 with TLR1. The use of MALP-2, a diacylated ligand that makes use of the TLR2/6 heterodimer, does not allow platelet activation (124). In addition, the preincubation of platelets with MALP-2 reduces and even inhibits the effect of Pam_3CSK_4. The engagement of TLR2 and TLR6 is believed rather to have an antagonist effect on platelet activation and could act as a regulator in platelet activation during bacterial invasion by simply blocking access to TLR2.

The first demonstration of platelet TLR2 function, *in vivo* as well as *ex vivo*, was reported in 2009 by Blair et al. (127). The stimulation of platelets by Pam_3CSK_4, a synthetic ligand mimicking bacterial lipopeptide, caused activation involving an "inside-out" αIIbβ3 signaling, aggregation, and platelet adhesion to collagen, CD62P release and generation of reactive oxygen derivatives. These phenomena were either blocked by a TLR2 antagonist antibody or absent in *tlr2*$^{-/-}$ KO mice, demonstrating the engagement of the receptor. Another study on the functional role of platelet TLR2 showed that Pam_3CSK_4 also controls increases in platelet intracellular Ca^{2+} concentrations, the release of ATP, and the synthesis of TxA_2 (124). These observations are consistent with a role for platelet TLR2 as a thrombo-inflammatory receptor. Even where the ultimate thrombotic purpose is similar to that of classic stimulation (ADP, Fg, collagen, etc.), the intracellular mechanisms activated are quite distinct, particularly with regard to the phosphorylation of signaling molecules (PI3K and MAPK pathways) and protein–protein interactions (mainly in the case of the FXIIIa protein involved in platelet remodeling). Compared with hemostatic stimulation, there are also differences in the release of granular proteins (Fg, thrombospondin, PF4) which can be released or sequestered during TLR2 stimulation (130).

Apart from the thrombotic function of the platelet TLR2, this receptor can also induce an inflammatory response by platelets. Bacterial stimulation of platelets by periodontopathogens (*A. actinomycetemcomitans* and *P. gingivalis*) demonstrated that sCD40L is released independently of TLR2. Furthermore, patients who have undergone restorative dental procedures had a significantly higher level of circulating sCD40L than healthy control subjects (128).

It has been observed, moreover, that mice infected with *P. gingivalis* displayed a higher proportion of platelet-neutrophil complexes than uninfected mice or TLR2$^{(-/-)}$ mice, suggesting that platelet TLR2 is involved in the formation of platelet-neutrophil aggregates (127).

Assinger et al. confirmed these observations and showed that this increase in platelet–neutrophil complexes is accompanied by a rise in neutrophil-mediated phagocytosis of periodontopathogens, which requires TLR2 to be functional (131). Platelet TLR2 is thought to be a prerequisite to the activation of the latter, which are then believed to transmit a signal activating the neutrophils and making them suitable for phagocytosis. The particular signal can involve ligand:receptor pairs (CD62P/PSGL-1, CD40L/CD40, GP-IIb–IIIa/CD11b) or platelet cytokines.

Similarly, it has recently been observed that platelets promote the clearance of *Staphylococcus aureus* and *Bacillus cereus* by Kupffer cells.

When absorbed by these liver macrophages, such bacteria are thought to be rapidly engulfed by a platelet aggregate, predisposing them to destruction. The formation of aggregates would involve the participation of platelet GP-Ib and vWF on Kupffer cells (32).

The release of histones from cells in apoptosis is associated with microvascular thrombosis. In citrated PRP, the presence of histones has been proven to stimulate thrombin generation, independent of dose, with noticeable effects from 10 μg/ml (132). When an anti-TLR2 monoclonal antibody is used prior to stimulation by histones, the procoagulant profile of platelets [membrane expression of phosphatidylserine (PS), CD62P, and coagulation factor V] is reduced. Under the same conditions, TLR2 blockade leads to a reduction of about 50% in the salting-out of thrombin and increases the salting-out time for the remaining 50% by 40 minutes (**Figure 3**).

Platelet TLR2 is, therefore, involved in histone-induced thrombin generation. These results reaffirm the importance of platelet TLR2 in a thrombo-inflammatory response. Inversely, this response can be considered beneficial to the host in cases where the agent that induces cell apoptosis becomes trapped in the fibrin network.

Function of TLR9 in platelets

Studies conducted on the role of platelet TLRs rarely address the subject of TLR9. In eukaryotic cells, TLR9 recognizes unmethylated 2′-deoxyribo (cytidine-phosphate guanosine) DNA motifs, commonly referred to as CpG motifs, which are found specifically in bacteria, parasites, and viruses. The location of TLR9 is limited to the endosomes in eukaryotic cells, enabling it to recognize the internal constituents of pathogens, which are often released after they are endocytosed (89, 133).

Platelet TLR9 is also found in cell cytosol and cell membranes (108). Activated platelets overexpress this receptor, either after stimulation by a CpG motif (105) or by thrombin (109).

A recent study on human and murine platelets showed that intra-platelet TLR9 is distributed in a specific, previously unidentified, sub-compartment known as the T granule, which has dense appearance under an electron micrograph (105, 134). This distribution of TLR9 is thought to occur during pro-platelet formation. T granules also contain VAMP7 and VAMP8 proteins, which are involved in directing TLR9 to the membrane. The process by which platelets internalize the CpG/TLR9 complex appears to be similar to that described for other cell types (105, 134).

Platelet TLR9 was recently reported as binding carboxy-alkyl-pyrrole, a product derived from the combination of polyunsaturated fatty acid oxidation products and protein products, considered a danger signal in cases of oxidative stress (135). In platelets, this joining together triggers aggregation and degranulation. Therefore, in platelets TLR9 appears to function as sensors of internal danger signals rather than external ones (**Figure 3**).

It is only recently that TLR3 (37) and TLR7 (38) have also been identified (**Figure 3**). Human platelets express TLR3 and are capable of responding to poly I:C, suggesting that these cells might influence the immune innate response when detecting viral dsRNA (37). Infection with encephalomyocarditis virus (EMCV) rapidly reduces platelet count, and this response is attributed to platelet Toll-like receptor 7 (TLR7) (38).

THE SIGLEC: SENSORS OF PATHOGENS NEWLY ACKNOWLEDGED ON PLATELETS

BACKGROUND ON SIGLEC MOLECULES

Siglec molecules belong to the large family of Ig-like lectins (84, 87, 96), which are categorized in two main families (**Table 2**). They are type I transmembrane proteins comprising three regions (84, 86, 136). First, the extracellular region has a "V-set" N-terminal Ig domain containing an arginine residue that forms a saline bridge with the carboxylate group of sialic acid, which enables specific binding with this molecule and a number varying between 1 and 16 in the "C2-set" domain (97, 136). Second, the transmembrane region allows the signal to be transmitted to the third region, the intracellular region of the receptor, which contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) close to the membrane, and an ITIM-like motif, which is more distal from the membrane. Certain Siglec molecules, such as sialoadhesins (137), Siglec-H (138), Siglec-14 (139), Siglec-15 (140), and Siglec-16, do not have an intracellular region (141).

The primary function of CD33r Siglec (3, 5–11, 14, 16) is to recognize "self" molecules in order to regulate host immune response by the engagement of inhibitory ITIM-like intra-cytoplasmic molecules. Siglec principally recognize and bind sialylated glycans and gangliosides.

Siglec molecules have arginine residues that can form a saline bridge with the carboxylate group of sialic acid enabling them to bind in a specific way (136). In their basal state, Siglec are bound with ligands and are, therefore, expressed on the same cell (*cis* interaction); thus their binding sites are generally masked (84, 95). The interaction is, therefore, predominantly a *cis* interaction (95). However, *trans* interaction may compete with *cis* interaction when the sialylated (*trans*) glycan ligands are attached to glycoproteins and glycolipids, which gives them higher affinity [for example, PAMP structures (LPS or bacterial peptidoglycan) or gangliosides] (96). Furthermore, the *cis* interaction sites can be cleaved by sialidase [a component of certain pathogens like *Vibrio cholerae, Clostridium perfringen*, and *Arthobacter ureafacien* (96, 97)], or unmasked following cell activation, which enables Siglec to have *trans* interactions (142).

The gangliosides formed from glycosphingolipids are bound to Siglec with a very high affinity (143). The affinity of CD33r Siglec is different for each ganglioside depending on their structure; for example, Siglec-7 and Siglec-9 are selective in targeting B-series gangliosides containing the 2,8-disialyl residue (GalNAc or GlcNAc), such as GQ1b, GT1b, GD2, and GD3, while Siglec-8 appears to have low affinity with these gangliosides (143–146).

The main known function of Siglec molecules are:

(i) the regulation of innate immune responses by balancing (down: *cis* ligation; up: *trans*-ligation) the defense against a number of infectious pathogens that use sialic acid to disguise themselves in an attempt to escape elimination by the infected body;

(ii) the negative regulation of the B-cell receptors (by co-engagement of two main Siglec: CD22 and Siglec-10 [Human; -G in the mouse; no cluster of differentiation assigned so far] (84, 87, 95, 96, 143, 147) and of T-cell receptors [by

co-engagement of three main Siglec: 7, 9 and 1 [sialoadhesin] (136)].

Siglec molecules operate by phosphorylating principally ITIM/ITIM-like motifs in their intracellular tails, or – exceptionally (Siglec-H and -15) – by mobilizing DAP12. Thus, Siglec appear in general essential to preventing excessive innate and adaptive immune responses and maintaining homeostasy and tolerance.

SIGLEC AND PLATELETS

A substantial expression of Siglec-7 has been identified very recently (by our group) on platelet surfaces, with more stored on platelet α-granule membranes. Furthermore, surface membrane expression of Siglec-7 is significantly increased after platelet activation, in a manner similar to the activation-induced membrane expression of CD62P. Indeed the kinetics of Siglec-7 expression on the platelet membrane of closely resembles that of CD62P.

There is also a significant amount of Siglec-7 in the supernatant in both control and TRAP-activated platelets, consistent with the cleavage, or direct salting-out, of Siglec-7 from platelet α-granules. However, Siglec-7 cleavage does not correlate with a reduction in its expression on the platelet membrane, as is the case for CD62P. A probable explanation for this apparent reduction in concentration over time may be the degradation induced by endogenous proteases.

The engagement of Siglec-7 by its specific ligand, GD2 ganglioside (as well as GD3 and GT1b), does not induce activation, aggregation, or platelet secretion, but leads to platelet apoptosis by the intrinsic and an extra-mitochondrial pathway. Similarly, Martini et al. demonstrated that platelet incubation with exogenous GD3 had no effect on platelet morphology, nor function, nor on ADP-induced platelet aggregation (148). The authors also indicated that GD3 functions like a second-messenger molecule that augments CD32 expression (FcgRII, platelet FcR isoform); it then binds to this receptor, leading to platelet adhesion on the subendothelial matrix (148). In our study, however, the apoptogenic effects of GD2 on platelets were independent on the engagement of CD32, whereas reduced in the presence of specific antibodies that block Siglec-7. This shows that Siglec-7 engagement is essential for GD2-induced platelet apoptosis. Inhibitors of NADPH oxidase, PI3k and PKC, but not of NF-kB prevented. The engagement of the P2Y1 platelet receptor and of the GP-IIb–IIIa integrin is required for a fully functional Siglec-7.

Platelet apoptosis induced by Siglec-7 engagement is probably a mechanism for negatively regulating platelet inflammatory responses. This mechanism would limit excessive reactions responsible for the destruction of tissues and cells due to inflammation and in this way promotes the healing of wounds. Stored platelets (with the purpose of being used for transfusion purposes) display apoptosis markers that increase independently on activation (149–151). This is a significant aspect of storage damage that reduces the viability and number of platelets in PCs following prolonged storage. Platelet apoptosis can potentially have negative effects in PC recipients, such as reduced function and altered corrected count increment (CCI) (149); probable adverse effects due to the pro-inflammatory and pro-thrombotic properties of PMPs generated during apoptosis are also likely (152).

The mechanisms of Siglec-7 translocation to human platelet membranes and its subsequent cleavage are still unaddressed (88) and require further studies. It would be interesting, first, to discover whether this increase is an advance event for triggering platelet activation or whether it is secondary, occurring after platelet activation in order to negatively control inflammatory response following this process (autoregulation). Second, it will be important to determine whether cleavage of soluble Siglec-7 is associated with such translocation. Little is known of the role of soluble Siglec-7 in inflammatory reactions, particularly in immune cells, ECs and platelets, or of the importance of the physiology and platelet interactions at work in the immune system.

In addition, it would be interesting to be able to determine whether Siglec-7 engagement is also specifically linked to platelet recognition of DAMPs. In other words, it would be valuable to examine whether platelet Siglec-7 distinguish several PAMPs (infectious pathogens). Moreover, it would be interesting to investigate platelet Siglec-7 response after DAMP activation (generated either during PAMP-induced inflammatory reactions or due to intra-platelet oxidative stress linked to preparation and/or storage conditions) and how their inflammatory response is modulated. Several studies show that by expressing a very large number of receptors, among them TLRs, GPCRs, RTKs, integrins, and CK/CH receptors, platelets appear to have the ability to differentiate between a hemostatic stimulus or an infectious

Table 3 | Modulation of TLR function by Siglecs adapted from Ref. (95, 155, 156).

Molecules	TLR ligands used	Cell type	Observed phenotype
CD22	TLR3, 4, 7, and 9	B	Enhanced proliferation of CD22 KO B cells
Siglec-G	TLR3, 4, 7, and 9	B	Enhanced proliferation of Siglec-G KO B cells
	HMGB1	DC	Enhanced TNF-α production in Siglec-G KO DCs
Siglec-E	TLR4	Mac	Reduced IL-12 production by cross-linking with Abs
Siglec-H	TLR9	pDC	Reduced IFN-α production by cross-linking with Abs
Siglec-5	TLR2, 3, 4, and 9	Mac	Reduced TNF-α and enhanced IL-10 production by over-expression
Siglec-9	TLR2, 3, 4, and 9	Mac	Reduced TNF-α and enhanced IL-10 production by over-expression
Siglec-11	TLR4	Mac	Reduced IL-1β transcript by cross-linking with Abs
Siglec-14	TLR4	Mac	Augmented TNF-α production by over-expression
CD33/Siglec-3	TLR4/CD14	imDCs	Reduced the phosphorylation of NF-κB
Siglec-9	TLR2	Mac	Siglecs exhibit lectin-dependent changes in cellular localization, which may be partly linked to its control mechanism that increases the production of IL-10

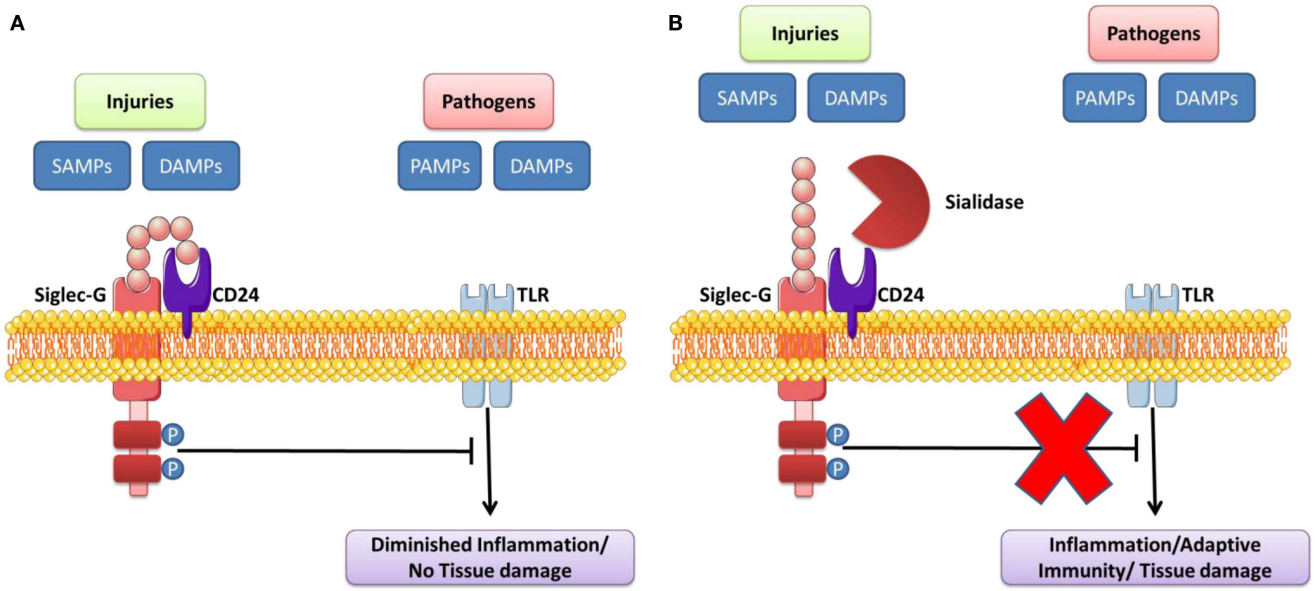


FIGURE 4 | Sialidase interrupts the Siglec-G inhibitory function that suppresses TLR signaling by DAMPs/PAMPs. **(A)** CD24 forms trimolecular complex with DAMPs/SAMPs and Siglec G that inhibits activation of TLR. **(B)** Pathogen-encoded sialidases cleave sialic acids on CD24 from interacting with Siglec G, leading to induce the inflammation/adaptive immunity/tissue damage. Adapted from Ref. (95, 142, 157).

stimulus, and endogenous danger signals (DAMPs) and exogenous ones (PAMPs). This method of differentially detecting pathogens produces variations in membrane expression of adhesion molecules, activation molecules, and especially in the salting-out of platelet granule contents and their secretion kinetics, leading to various platelet responses, such as hemostatic, inflammatory, or reparative (35, 118, 122, 153, 154). Furthermore, it would be interesting to study Siglec-7 in the platelet signalosome in the presence of various stimuli, including hemostatic stimuli (as shown in the appendix), infectious agents (PAMPs), or DAMPs. To this effect, numerous studies are considered to complement the characterization of the functional role of platelet Siglec, including Siglec-7.

Siglec molecules contribute to the negative regulation of the intracellular pathways stimulated following TLR/NLR engagement. This serves to prevent excessive immune responses after activation of these receptors (96). The regulation and activation of cells after TLR engagement is different for each cell type (95). This inhibitory function of Siglec is used by pathogens to imitate the structure of their ligands, which initiates an immune response favorable to their survival within the host. Recent studies show that, when Siglec molecules are neutralized by blocking antibodies, TLR signaling is significantly modified; this suggests that the density of Siglec expression near to TLRs can influence the function of these receptors (95). The location (on membranes or endosomes) where Siglec sensors are expressed can also modulate innate immune response in the host following TLR engagement (**Table 3**). Siglec molecules are normally expressed on immune cells that contribute to regulation of the innate immune system (96). PRRs can recognize "self" (DAMP) and "non-self" (PAMP) danger signals in order to trigger inflammatory reactions. The engagement of these receptors after engagement by DAMPs can also reduce inflammation and promote the repair and healing of wounds (142). Chen et al. originally illustrated the mechanism by which Siglec-10/Siglec-G enables innate immunity cells to distinguish DAMPs and PAMPs in order to set up an immune response that can defend against pathogens, while at the same time preserving the integrity of tissue at the end of the post-infection defense process. They showed that the interaction between CD24 and human Siglec-10 (murine Siglec-G) can reduce inflammatory response induced by DAMPs of the HMGB1 and HSP70 types, but not in the presence of certain PAMPs (LPS and PolyI:C which activate TLR4 and TLR3, respectively). The glycoprotein CD24 actually has the ability to form a complex of Siglec-10/Siglec-G and DAMP, which inhibits TLR/NLR activation by inhibiting ITIM motifs (**Figure 4**). However, certain infectious pathogens like *Vibrio cholerae, Clostridium perfringen,* and *Arthobacter ureafacien* (96, 142), which have a sialidase, can cleave the bond between Siglec sensors and CD24; in this case, the distinction between the DAMP and PAMP signals will, as a result, not be effective.

CONCLUSION

As a conclusion, analyzing platelet responses to PRR agonist stimulation in whole blood, platelet-rich plasma and in transfusion platelets concentrate would help clarify the relative contributions of platelets on inflammatory process. How can the small platelet, without a nucleus, be so intelligent?

ACKNOWLEDGMENTS

The authors are grateful to Charles Antoine Arthaud, Marie Ange Eyraud, and Jocelyne Fagand for their invaluable contribution. We would like to thank the medical staff and personnel

of Etablissement Français du Sang Auvergne-Loire, Saint-Etienne, France for their technical support throughout our studies. This work was supported by grants from the French National Blood Service – EFS (Grant APR), France; the Association for Research in Transfusion (ART), Paris, France; the Agence Nationale de la Sécurité et du Médicament et des produits de santé (ANSM – AAP-2012-011, Reference 2012S055); the "Agence Nationale de la Recherche" (ANR), reference ANR-12-JSV1-0012-01; and the Association "Les Amis de Rémi," Savigneux, France.

REFERENCES

1. Blajchman MA. Platelet transfusions: an historical perspective. *Hematology Am Soc Hematol Educ Program* (2008) **2008**:197. doi:10.1182/asheducation-2008.1.197
2. Alio JL, Arnalich-Montiel F, Rodriguez AE. The role of "eye platelet rich plasma" (E-PRP) for wound healing in ophthalmology. *Curr Pharm Biotechnol* (2012) **13**:1257–65. doi:10.2174/138920112800624355
3. McCullough J. Overview of platelet transfusion. *Semin Hematol* (2010) **47**:235–42. doi:10.1053/j.seminhematol.2010.04.001
4. MacLennan S, Williamson LM. Risks of fresh frozen plasma and platelets. *J Trauma* (2006) **60**:S46–50. doi:10.1097/01.ta.0000199546.22925.31
5. Sachs UJ. Fetal/neonatal alloimmune thrombocytopenia. *Thromb Res* (2013) **131**(Suppl 1):S42–6. doi:10.1016/S0049-3848(13)70020-3
6. Zimmermann R, Wittmann G, Zingsem J, Blasczyk R, Weisbach V, Eckstein R. Antibodies to private and public HLA class I epitopes in platelet recipients. *Transfusion* (1999) **39**:772–80. doi:10.1046/j.1537-2995.1999.39070772.x
7. Golebiewska EM, Poole AW. Platelet secretion: from haemostasis to wound healing and beyond. *Blood Rev* (2014). doi:10.1016/j.blre.2014.10.003
8. Jenne CN, Urrutia R, Kubes P. Platelets: bridging hemostasis, inflammation, and immunity. *Int J Lab Hematol* (2013) **35**:254–61. doi:10.1111/ijlh.12084
9. Smyth SS, McEver RP, Weyrich AS, Morrell CN, Hoffman MR, Arepally GM, et al. Platelet functions beyond haemostasis. *J Thromb Haemost* (2009) **7**:1759–66. doi:10.1111/j.1538-7836.2009.03586.x
10. Garraud O, Hamzeh-Cognasse H, Pozzetto B, Cavaillon JM, Cognasse F. Bench-to-bedside review: platelets and active immune functions – new clues for immunopathology? *Crit Care* (2013) **17**:236. doi:10.1186/cc12716
11. Gachet C. Regulation of platelet functions by P2 receptors. *Annu Rev Pharmacol Toxicol* (2006) **46**:277–300. doi:10.1146/annurev.pharmtox.46.120604.141207
12. Dorsam RT, Kunapuli SP. Central role of the P2Y12 receptor in platelet activation. *J Clin Invest* (2004) **113**:340–5. doi:10.1172/JCI20986
13. Hechler B, Lenain N, Marchese P, Vial C, Heim V, Freund M, et al. A role of the fast ATP-gated P2X1 cation channel in thrombosis of small arteries in vivo. *J Exp Med* (2003) **198**:661–7. doi:10.1084/jem.20030144
14. Erhardt JA, Toomey JR, Douglas SA, Johns DG. P2X1 stimulation promotes thrombin receptor-mediated platelet aggregation. *J Thromb Haemost* (2006) **4**:882–90. doi:10.1111/j.1538-7836.2006.01849.x
15. Fabre JE, Nguyen M, Latour A, Keifer JA, Audoly LP, Coffman TM, et al. Decreased platelet aggregation, increased bleeding time and resistance to thromboembolism in P2Y1-deficient mice. *Nat Med* (1999) **5**:1199–202. doi:10.1038/13522
16. Van Holten TC, Bleijerveld OB, Wijten P, De Groot PG, Heck AJ, Barendrecht AD, et al. Quantitative proteomics analysis reveals similar release profiles following specific PAR-1 or PAR-4 stimulation of platelets. *Cardiovasc Res* (2014) **103**:140–6. doi:10.1093/cvr/cvu113
17. Clemetson KJ. Platelets and primary haemostasis. *Thromb Res* (2012) **129**:220–4. doi:10.1016/j.thromres.2011.11.036
18. Ramachandran R, Hollenberg MD. Proteinases and signalling: pathophysiological and therapeutic implications via PARs and more. *Br J Pharmacol* (2008) **153**(Suppl 1):S263–82. doi:10.1038/sj.bjp.0707507
19. Cognasse F, Semple JW, Garraud O. Platelets as potential immunomodulators: is there a role for platelet toll-like receptors? *Curr Immunol Rev* (2007) **3**:109–15. doi:10.2174/157339507780655522
20. Von Hundelshausen P, Weber C. Platelets as immune cells: bridging inflammation and cardiovascular disease. *Circ Res* (2007) **100**:27–40. doi:10.1161/01.RES.0000252802.25497.b7
21. Cox D. Bacteria-platelet interactions. *J Thromb Haemost* (2009) **7**:1865–6. doi:10.1111/j.1538-7836.2009.03611.x
22. Flaujac C, Boukour S, Cramer-Borde E. Platelets and viruses: an ambivalent relationship. *Cell Mol Life Sci* (2010) **67**:545–56. doi:10.1007/s00018-009-0209-x
23. Garraud O, Cognasse F. Platelet toll-like receptor expression: the link between "danger" ligands and inflammation. *Inflamm Allergy Drug Targets* (2010) **9**:322–33. doi:10.2174/187152810793937991
24. Cox D, Kerrigan SW, Watson SP. Platelets and the innate immune system: mechanisms of bacterial-induced platelet activation. *J Thromb Haemost* (2011) **9**:1097–107. doi:10.1111/j.1538-7836.2011.04264.x
25. Arman M, Krauel K, Tilley DO, Weber C, Cox D, Greinacher A, et al. Amplification of bacteria-induced platelet activation is triggered by FcgammaRIIA, integrin alphaIIbbeta3, and platelet factor 4. *Blood* (2014) **123**:3166–74. doi:10.1182/blood-2013-11-540526
26. Speth C, Rambach G, Lass-Florl C. Platelet immunology in fungal infections. *Thromb Haemost* (2014) **112**:632–9. doi:10.1160/TH14-01-0074
27. Klinger MH, Jelkmann W. Role of blood platelets in infection and inflammation. *J Interferon Cytokine Res* (2002) **22**:913–22. doi:10.1089/10799900260286623
28. Vincent JL, Yagushi A, Pradier O. Platelet function in sepsis. *Crit Care Med* (2002) **30**:S313–7. doi:10.1097/00003246-200205001-00022
29. Yaguchi A, Lobo FL, Vincent JL, Pradier O. Platelet function in sepsis. *J Thromb Haemost* (2004) **2**:2096–102. doi:10.1111/j.1538-7836.2004.01009.x
30. Yeaman MR. Bacterial-platelet interactions: virulence meets host defense. *Future Microbiol* (2010) **5**:471–506. doi:10.2217/fmb.09.112
31. Yeaman MR. Platelets in defense against bacterial pathogens. *Cell Mol Life Sci* (2010) **67**:525–44. doi:10.1007/s00018-009-0210-4
32. Wong CH, Jenne CN, Petri B, Chrobok NL, Kubes P. Nucleation of platelets with blood-borne pathogens on Kupffer cells precedes other innate immunity and contributes to bacterial clearance. *Nat Immunol* (2013) **14**:785–92. doi:10.1038/ni.2631
33. Yeaman MR. Platelets: at the nexus of antimicrobial defence. *Nat Rev Microbiol* (2014) **12**:426–37. doi:10.1038/nrmicro3269
34. Shiraki R, Inoue N, Kawasaki S, Takei A, Kadotani M, Ohnishi Y, et al. Expression of toll-like receptors on human platelets. *Thromb Res* (2004) **113**:379–85. doi:10.1016/j.thromres.2004.03.023
35. Semple JW, Italiano JE Jr, Freedman J. Platelets and the immune continuum. *Nat Rev Immunol* (2011) **11**:264–74. doi:10.1038/nri2956
36. Li C, Li J, Li Y, Lang S, Yougbare I, Zhu G, et al. Crosstalk between platelets and the immune System: old systems with new discoveries. *Adv Hematol* (2012) **2012**:384685. doi:10.1155/2012/384685
37. Anabel AS, Eduardo PC, Antonio HC, Carlos SM, Juana NM, Honorio TA, et al. Human platelets express toll-like receptor 3 and respond to poly I:C. *Hum Immunol* (2014) **75**(12):1244–51. doi:10.1016/j.humimm.2014.09.013
38. Koupenova M, Vitseva O, Mackay CR, Beaulieu LM, Benjamin EJ, Mick E, et al. Platelet-TLR7 mediates host survival and platelet count during viral infection in the absence of platelet-dependent thrombosis. *Blood* (2014) **124**:791–802. doi:10.1182/blood-2013-11-536003
39. Elzey BD, Tian J, Jensen RJ, Swanson AK, Lees JR, Lentz SR, et al. Platelet-mediated modulation of adaptive immunity. A communication link between innate and adaptive immune compartments. *Immunity* (2003) **19**:9–19. doi:10.1016/S1074-7613(03)00177-8
40. Garraud O, Berthet J, Hamzeh-Cognasse H, Cognasse F. Pathogen sensing, subsequent signalling, and signalosome in human platelets. *Thromb Res* (2011) **127**:283–6. doi:10.1016/j.thromres.2010.10.015
41. Mantovani A, Garlanda C. Platelet-macrophage partnership in innate immunity and inflammation. *Nat Immunol* (2013) **14**:768–70. doi:10.1038/ni.2666
42. Montrucchio G, Bosco O, Del Sorbo L, Fascio Pecetto P, Lupia E, Goffi A, et al. Mechanisms of the priming effect of low doses of lipopoly-saccharides on leukocyte-dependent platelet aggregation in whole blood. *Thromb Haemost* (2003) **90**:872–81. doi:10.1267/THRO03050872
43. Ward JR, Bingle L, Judge HM, Brown SB, Storey RF, Whyte MK, et al. Agonists of toll-like receptor (TLR)2 and TLR4 are unable to modulate platelet activation by adenosine diphosphate and platelet activating factor. *Thromb Haemost* (2005) **94**:831–8. doi:10.1160/TH05-01-0009
44. Jayachandran M, Brunn GJ, Karnicki K, Miller RS, Owen WG, Miller VM. In vivo effects of lipopolysaccharide and TLR4 on platelet production and

activity: implications for thrombotic risk. *J Appl Physiol* (2007) **102**:429–33. doi:10.1152/japplphysiol.01576.2005
45. Phipps RP, Kaufman J, Blumberg N. Platelet derived CD154 (CD40 ligand) and febrile responses to transfusion. *Lancet* (2001) **357**:2023–4. doi:10.1016/S0140-6736(00)05108-4
46. Freedman JE. CD40-CD40L and platelet function: beyond hemostasis. *Circ Res* (2003) **92**:944–6. doi:10.1161/01.RES.0000074030.98009.FF
47. Davi G, Ferroni P. CD40-CD40L interactions in platelet activation. *Thromb Haemost* (2005) **93**:1011–2. doi:10.1160/TH05-04-0270
48. Elzey BD, Ratliff TL, Sowa JM, Crist SA. Platelet CD40L at the interface of adaptive immunity. *Thromb Res* (2011) **127**:180–3. doi:10.1016/j.thromres.2010.10.011
49. Henn V, Slupsky JR, Grafe M, Anagnostopoulos I, Forster R, Muller-Berghaus G, et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. *Nature* (1998) **391**:591–4. doi:10.1038/35393
50. Danese S, Fiocchi C. Platelet activation and the CD40/CD40 ligand pathway: mechanisms and implications for human disease. *Crit Rev Immunol* (2005) **25**:103–21. doi:10.1615/CritRevImmunol.v25.i2.20
51. Gawaz M, Langer H, May AE. Platelets in inflammation and atherogenesis. *J Clin Invest* (2005) **115**:3378–84. doi:10.1172/JCI27196
52. Bozza FA, Shah AM, Weyrich AS, Zimmerman GA. Amicus or adversary: platelets in lung biology, acute injury, and inflammation. *Am J Respir Cell Mol Biol* (2009) **40**:123–34. doi:10.1165/rcmb.2008-0241TR
53. Boilard E, Nigrovic PA, Larabee K, Watts GF, Coblyn JS, Weinblatt ME, et al. Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science* (2010) **327**:580–3. doi:10.1126/science.1181928
54. Phipps RP. Platelets at the interface between thrombosis, inflammation and immunity. *Thromb Res* (2011) **127**:179. doi:10.1016/j.thromres.2010.10.014
55. Rutella S, Vetrano S, Correale C, Graziani C, Sturm A, Spinelli A, et al. Enhanced platelet adhesion induces angiogenesis in intestinal inflammation and inflammatory bowel disease microvasculature. *J Cell Mol Med* (2011) **15**:625–34. doi:10.1111/j.1582-4934.2010.01033.x
56. Smith TL, Weyrich AS. Platelets as central mediators of systemic inflammatory responses. *Thromb Res* (2011) **127**:391–4. doi:10.1016/j.thromres.2010.10.013
57. Rondina MT, Weyrich AS, Zimmerman GA. Platelets as cellular effectors of inflammation in vascular diseases. *Circ Res* (2013) **112**:1506–19. doi:10.1161/CIRCRESAHA.113.300512
58. Boudreau LH, Duchez AC, Cloutier N, Soulet D, Martin N, Bollinger J, et al. Platelets release mitochondria serving as substrate for bactericidal group IIA-secreted phospholipase A2 to promote inflammation. *Blood* (2014) **124**:2173–83. doi:10.1182/blood-2014-05-573543
59. Sansonetti PJ, Medzhitov R. Learning tolerance while fighting ignorance. *Cell* (2009) **138**:416–20. doi:10.1016/j.cell.2009.07.024
60. Sansonetti PJ. To be or not to be a pathogen: that is the mucosally relevant question. *Mucosal Immunol* (2011) **4**:8–14. doi:10.1038/mi.2010.77
61. Damas JK, Jensenius M, Ueland T, Otterdal K, Yndestad A, Froland SS, et al. Increased levels of soluble CD40L in African tick bite fever: possible involvement of TLRs in the pathogenic interaction between Rickettsia africae, endothelial cells, and platelets. *J Immunol* (2006) **177**:2699–706. doi:10.4049/jimmunol.177.4.2699
62. Beaulieu LM, Freedman JE. The role of inflammation in regulating platelet production and function: toll-like receptors in platelets and megakaryocytes. *Thromb Res* (2010) **125**:205–9. doi:10.1016/j.thromres.2009.11.004
63. Ogura H, Kawasaki T, Tanaka H, Koh T, Tanaka R, Ozeki Y, et al. Activated platelets enhance microparticle formation and platelet-leukocyte interaction in severe trauma and sepsis. *J Trauma* (2001) **50**:801–9. doi:10.1097/00005373-200105000-00005
64. Pitchford SC, Yano H, Lever R, Riffo-Vasquez Y, Ciferri S, Rose MJ, et al. Platelets are essential for leukocyte recruitment in allergic inflammation. *J Allergy Clin Immunol* (2003) **112**:109–18. doi:10.1067/mai.2003.1514
65. Zeller JA, Lenz A, Eschenfelder CC, Zunker P, Deuschl G. Platelet-leukocyte interaction and platelet activation in acute stroke with and without preceding infection. *Arterioscler Thromb Vasc Biol* (2005) **25**:1519–23. doi:10.1161/01.ATV.0000167524.69092.16
66. Li N. Platelet-lymphocyte cross-talk. *J Leukoc Biol* (2008) **83**:1069–78. doi:10.1189/jlb.0907615
67. Zarbock A, Polanowska-Grabowska RK, Ley K. Platelet-neutrophil-interactions: linking hemostasis and inflammation. *Blood Rev* (2007) **21**:99–111. doi:10.1016/j.blre.2006.06.001
68. Ma AC, Kubes P. Platelets, neutrophils, and neutrophil extracellular traps (NETs) in sepsis. *J Thromb Haemost* (2008) **6**:415–20. doi:10.1111/j.1538-7836.2007.02865.x
69. Asaduzzaman M, Lavasani S, Rahman M, Zhang S, Braun OO, Jeppsson B, et al. Platelets support pulmonary recruitment of neutrophils in abdominal sepsis. *Crit Care Med* (2009) **37**:1389–96. doi:10.1097/CCM.0b013e31819ceb71
70. Kornerup KN, Salmon GP, Pitchford SC, Liu WL, Page CP. Circulating platelet-neutrophil complexes are important for subsequent neutrophil activation and migration. *J Appl Physiol* (2010) **109**:758–67. doi:10.1152/japplphysiol.01086.2009
71. Johansson D, Shannon O, Rasmussen M. Platelet and neutrophil responses to Gram positive pathogens in patients with bacteremic infection. *PLoS One* (2011) **6**:e26928. doi:10.1371/journal.pone.0026928
72. Caudrillier A, Kessenbrock K, Gilliss BM, Nguyen JX, Marques MB, Monestier M, et al. Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury. *J Clin Invest* (2012) **122**:2661–71. doi:10.1172/JCI61303
73. Duerschmied D, Suidan GL, Demers M, Herr N, Carbo C, Brill A, et al. Platelet serotonin promotes the recruitment of neutrophils to sites of acute inflammation in mice. *Blood* (2013) **121**:1008–15. doi:10.1182/blood-2012-06-437392
74. Haselmayer P, Grosse-Hovest L, Von Landenberg P, Schild H, Radsak MP. TREM-1 ligand expression on platelets enhances neutrophil activation. *Blood* (2007) **110**:1029–35. doi:10.1182/blood-2007-01-069195
75. Nash GB. Adhesion between platelets and leukocytes or endothelial cells. *Methods Mol Biol* (2004) **272**:199–213. doi:10.1385/1-59259-782-3:199
76. McGregor L, Martin J, McGregor JL. Platelet-leukocyte aggregates and derived microparticles in inflammation, vascular remodelling and thrombosis. *Front Biosci* (2006) **11**:830–7. doi:10.2741/1840
77. Flaumenhaft R, Mairuhu AT, Italiano JE. Platelet- and megakaryocyte-derived microparticles. *Semin Thromb Hemost* (2010) **36**:881–7. doi:10.1055/s-0030-1267042
78. Italiano JE Jr, Mairuhu AT, Flaumenhaft R. Clinical relevance of microparticles from platelets and megakaryocytes. *Curr Opin Hematol* (2010) **17**:578–84. doi:10.1097/MOH.0b013e32833e77ee
79. Vasina E, Heemskerk JW, Weber C, Koenen RR. Platelets and platelet-derived microparticles in vascular inflammatory disease. *Inflamm Allergy Drug Targets* (2010) **9**:346–54. doi:10.2174/187152810793938008
80. Land WG. Transfusion-related acute lung injury: the work of DAMPs. *Transfus Med Hemother* (2013) **40**:3–13. doi:10.1159/000345688
81. Lee YL, King MB, Gonzalez RP, Brevard SB, Frotan MA, Gillespie MN, et al. Blood transfusion products contain mitochondrial DNA damage-associated molecular patterns: a potential effector of transfusion-related acute lung injury. *J Surg Res* (2014) **191**:286–9. doi:10.1016/j.jss.2014.06.003
82. Varki A. Since there are PAMPs and DAMPs, there must be SAMPs? Glycan "self-associated molecular patterns" dampen innate immunity, but pathogens can mimic them. *Glycobiology* (2011) **21**:1121–4. doi:10.1093/glycob/cwr087
83. Freeman SD, Kelm S, Barber EK, Crocker PR. Characterization of CD33 as a new member of the sialoadhesin family of cellular interaction molecules. *Blood* (1995) **85**:2005–12.
84. Crocker PR, Paulson JC, Varki A. Siglecs and their roles in the immune system. *Nat Rev Immunol* (2007) **7**:255–66. doi:10.1038/nri2056
85. McMillan SJ, Crocker PR. CD33-related sialic-acid-binding immunoglobulin-like lectins in health and disease. *Carbohydr Res* (2008) **343**:2050–6. doi:10.1016/j.carres.2008.01.009
86. Cao H, Crocker PR. Evolution of CD33-related siglecs: regulating host immune functions and escaping pathogen exploitation? *Immunology* (2011) **132**:18–26. doi:10.1111/j.1365-2567.2010.03368.x
87. Crocker PR, McMillan SJ, Richards HE. CD33-related siglecs as potential modulators of inflammatory responses. *Ann N Y Acad Sci* (2012) **1253**:102–11. doi:10.1111/j.1749-6632.2011.06449.x
88. Nguyen KA, Hamzeh-Cognasse H, Palle S, Anselme-Bertrand I, Arthaud CA, Chavarin P, et al. Role of siglec-7 in apoptosis in human platelets. *PLoS One* (2014) **9**:e106239. doi:10.1371/journal.pone.0106239
89. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on toll-like receptors. *Nat Immunol* (2010) **11**:373–84. doi:10.1038/ni.1863
90. Werling D, Jungi TW. Toll-like receptors linking innate and adaptive immune response. *Vet Immunol Immunopathol* (2003) **91**:1–12. doi:10.1016/S0165-2427(02)00228-3

91. Abreu MT, Fukata M, Arditi M. TLR signaling in the gut in health and disease. *J Immunol* (2005) **174**:4453–60. doi:10.4049/jimmunol.174.8.4453
92. Kawai T, Akira S. TLR signaling. *Semin Immunol* (2007) **19**:24–32. doi:10.1016/j.smim.2006.12.004
93. Beutler BA. TLRs and innate immunity. *Blood* (2009) **113**:1399–407. doi:10.1182/blood-2008-07-019307
94. O'reilly MK, Paulson JC. Siglecs as targets for therapy in immune-cell-mediated disease. *Trends Pharmacol Sci* (2009) **30**:240–8. doi:10.1016/j.tips.2009.02.005
95. Paulson JC, Macauley MS, Kawasaki N. Siglecs as sensors of self in innate and adaptive immune responses. *Ann N Y Acad Sci* (2012) **1253**:37–48. doi:10.1111/j.1749-6632.2011.06362.x
96. Pillai S, Netravali IA, Cariappa A, Mattoo H. Siglecs and immune regulation. *Annu Rev Immunol* (2012) **30**:357–92. doi:10.1146/annurev-immunol-020711-075018
97. Varki A, Gagneux P. Multifarious roles of sialic acids in immunity. *Ann N Y Acad Sci* (2012) **1253**:16–36. doi:10.1111/j.1749-6632.2012.06517.x
98. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell* (2006) **124**:783–801. doi:10.1016/j.cell.2006.02.015
99. Lauw FN, Caffrey DR, Golenbock DT. Of mice and man: TLR11 (finally) finds profilin. *Trends Immunol* (2005) **26**:509–11. doi:10.1016/j.it.2005.08.006
100. Yu LX, Yan L, Yang W, Wu FQ, Ling Y, Chen SZ, et al. Platelets promote tumour metastasis via interaction between TLR4 and tumour cell-released high-mobility group box1 protein. *Nat Commun* (2014) **5**:5256. doi:10.1038/ncomms6256
101. Maugeri N, Campana L, Gavina M, Covino C, De Metrio M, Panciroli C, et al. Activated platelets present high mobility group box 1 to neutrophils, inducing autophagy and promoting the extrusion of neutrophil extracellular traps. *J Thromb Haemost* (2014) **12**:2074–88. doi:10.1111/jth.12710
102. Maugeri N, Rovere-Querini P, Baldini M, Baldissera E, Sabbadini MG, Bianchi ME, et al. Oxidative stress elicits platelet/leukocyte inflammatory interactions via HMGB1: a candidate for microvessel injury in sytemic sclerosis. *Antioxid Redox Signal* (2014) **20**:1060–74. doi:10.1089/ars.2013.5298
103. Vogel S, Chatterjee M, Metzger K, Borst O, Geisler T, Seizer P, et al. Activated platelets interfere with recruitment of mesenchymal stem cells to apoptotic cardiac cells via high mobility group box 1/toll-like receptor 4-mediated down-regulation of hepatocyte growth factor receptor MET. *J Biol Chem* (2014) **289**:11068–82. doi:10.1074/jbc.M113.530287
104. Szatmary Z. Molecular biology of toll-like receptors. *Gen Physiol Biophys* (2012) **31**:357–66. doi:10.4149/gpb_2012_048
105. Thon JN, Peters CG, Machlus KR, Aslam R, Rowley J, Macleod H, et al. T granules in human platelets function in TLR9 organization and signaling. *J Cell Biol* (2012) **198**:561–74. doi:10.1083/jcb.201111136
106. Andonegui G, Kerfoot SM, McNagny K, Ebbert KV, Patel KD, Kubes P. Platelets express functional toll-like receptor-4. *Blood* (2005) **106**:2417–23. doi:10.1182/blood-2005-03-0916
107. Boiko JR, Borghesi L. Hematopoiesis sculpted by pathogens: toll-like receptors and inflammatory mediators directly activate stem cells. *Cytokine* (2012) **57**:1–8. doi:10.1016/j.cyto.2011.10.005
108. Cognasse F, Hamzeh H, Chavarin P, Acquart S, Genin C, Garraud O. Evidence of toll-like receptor molecules on human platelets. *Immunol Cell Biol* (2005) **83**:196–8. doi:10.1111/j.1440-1711.2005.01314.x
109. Aslam R, Speck ER, Kim M, Crow AR, Bang KW, Nestel FP, et al. Platelet toll-like receptor expression modulates lipopolysaccharide-induced thrombocytopenia and tumor necrosis factor-alpha production in vivo. *Blood* (2006) **107**:637–41. doi:10.1182/blood-2005-06-2202
110. St Paul M, Paolucci S, Barjesteh N, Wood RD, Schat KA, Sharif S. Characterization of chicken thrombocyte responses to toll-like receptor ligands. *PLoS One* (2012) **7**:e43381. doi:10.1371/journal.pone.0043381
111. Berthet J, Damien P, Hamzeh-Cognasse H, Pozzetto B, Garraud O, Cognasse F. toll-like receptor 4 signal transduction in platelets: novel pathways. *Br J Haematol* (2010) **151**:89–92. doi:10.1111/j.1365-2141.2010.08292.x
112. Park BS, Song DH, Kim HM, Choi BS, Lee H, Lee JO. The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. *Nature* (2009) **458**:1191–5. doi:10.1038/nature07830
113. Rondina MT, Schwertz H, Harris ES, Kraemer BF, Campbell RA, Mackman N, et al. The septic milieu triggers expression of spliced tissue factor mRNA in human platelets. *J Thromb Haemost* (2011) **9**:748–58. doi:10.1111/j.1538-7836.2011.04208.x
114. Semple JW, Aslam R, Kim M, Speck ER, Freedman J. Platelet-bound lipopolysaccharide enhances Fc receptor-mediated phagocytosis of IgG-opsonized platelets. *Blood* (2007) **109**:4803–5. doi:10.1182/blood-2006-12-062695
115. Tremblay T, Aubin E, Lemieux R, Bazin R. Picogram doses of lipopolysaccharide exacerbate antibody-mediated thrombocytopenia and reduce the therapeutic efficacy of intravenous immunoglobulin in mice. *Br J Haematol* (2007) **139**:297–302. doi:10.1111/j.1365-2141.2007.06777.x
116. Li Z, Zhang G, Marjanovic JA, Ruan C, Du X. A platelet secretion pathway mediated by cGMP-dependent protein kinase. *J Biol Chem* (2004) **279**:42469–75. doi:10.1074/jbc.M401532200
117. Zhang G, Han J, Welch EJ, Ye RD, Voyno-Yasenetskaya TA, Malik AB, et al. Lipopolysaccharide stimulates platelet secretion and potentiates platelet aggregation via TLR4/MyD88 and the cGMP-dependent protein kinase pathway. *J Immunol* (2009) **182**:7997–8004. doi:10.4049/jimmunol.0802884
118. Berthet J, Damien P, Hamzeh-Cognasse H, Arthaud CA, Eyraud MA, Zeni F, et al. Human platelets can discriminate between various bacterial LPS isoforms via TLR4 signaling and differential cytokine secretion. *Clin Immunol* (2012) **145**:189–200. doi:10.1016/j.clim.2012.09.004
119. Brown GT, McIntyre TM. Lipopolysaccharide signaling without a nucleus: kinase cascades stimulate platelet shedding of proinflammatory IL-1beta-rich microparticles. *J Immunol* (2011) **186**:5489–96. doi:10.4049/jimmunol.1001623
120. Stahl AL, Svensson M, Morgelin M, Svanborg C, Tarr PI, Mooney JC, et al. Lipopolysaccharide from enterohemorrhagic *Escherichia coli* binds to platelets via TLR4 and CD62 and is detected on circulating platelets in patients with hemolytic uremic syndrome. *Blood* (2006) **108**:167–76. doi:10.1182/blood-2005-08-3219
121. Shashkin PN, Brown GT, Ghosh A, Marathe GK, McIntyre TM. Lipopolysaccharide is a direct agonist for platelet RNA splicing. *J Immunol* (2008) **181**:3495–502. doi:10.4049/jimmunol.181.5.3495
122. Cognasse F, Osselaer JC, Payrat JM, Chavarin P, Corash L, Garraud O. Release of immune modulation factors from platelet concentrates during storage after photochemical pathogen inactivation treatment. *Transfusion* (2008) **48**:809–13. doi:10.1111/j.1537-2995.2008.01655.x
123. Kappelmayer J, Beke Debreceni I, Vida A, Antal-Szalmas P, Clemetson KJ, Nagy B Jr. Distinct effects of Re- and S-forms of LPS on modulating platelet activation. *J Thromb Haemost* (2013) **11**(4):775–8. doi:10.1111/jth.12151
124. Kalvegren H, Skoglund C, Helldahl C, Lerm M, Grenegard M, Bengtsson T. toll-like receptor 2 stimulation of platelets is mediated by purinergic P2X1-dependent Ca2+ mobilisation, cyclooxygenase and purinergic P2Y1 and P2Y12 receptor activation. *Thromb Haemost* (2010) **103**:398–407. doi:10.1160/TH09-07-0442
125. Morello F, Perino A, Hirsch E. Phosphoinositide 3-kinase signalling in the vascular system. *Cardiovasc Res* (2009) **82**:261–71. doi:10.1093/cvr/cvn325
126. Ha T, Hu Y, Liu L, Lu C, McMullen JR, Kelley J, et al. TLR2 ligands induce cardioprotection against ischaemia/reperfusion injury through a PI3K/Akt-dependent mechanism. *Cardiovasc Res* (2010) **87**:694–703. doi:10.1093/cvr/cvq116
127. Blair P, Rex S, Vitseva O, Beaulieu L, Tanriverdi K, Chakrabarti S, et al. Stimulation of toll-like receptor 2 in human platelets induces a thromboinflammatory response through activation of phosphoinositide 3-kinase. *Circ Res* (2008) **104**:346–54. doi:10.1161/CIRCRESAHA.108.185785
128. Assinger A, Laky M, Badrnya S, Esfandeyari A, Volf I. Periodontopathogens induce expression of CD40L on human platelets via TLR2 and TLR4. *Thromb Res* (2012) **130**:e73–8. doi:10.1016/j.thromres.2012.04.017
129. Hoebe K, Georgel P, Rutschmann S, Du X, Mudd S, Crozat K, et al. CD36 is a sensor of diacylglycerides. *Nature* (2005) **433**:523–7. doi:10.1038/nature03253
130. Rex S, Beaulieu LM, Perlman DH, Vitseva O, Blair PS, McComb ME, et al. Immune versus thrombotic stimulation of platelets differentially regulates signalling pathways, intracellular protein-protein interactions, and alpha-granule release. *Thromb Haemost* (2009) **102**:97–110. doi:10.1160/TH08-08-0513
131. Assinger A, Laky M, Schabbauer G, Hirschl AM, Buchberger E, Binder BR, et al. Efficient phagocytosis of periodontopathogens by neutrophils requires plasma factors, platelets and TLR2. *J Thromb Haemost* (2011) **9**:799–809. doi:10.1111/j.1538-7836.2011.04193.x

132. Semeraro F, Ammollo CT, Morrissey JH, Dale GL, Friese P, Esmon NL, et al. Extracellular histones promote thrombin generation through platelet-dependent mechanisms: involvement of platelet TLR2 and TLR4. *Blood* (2011) **118**:1952–61. doi:10.1182/blood-2011-03-343061
133. Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, et al. A toll-like receptor recognizes bacterial DNA. *Nature* (2000) **408**:740–5. doi:10.1038/35047123
134. Thon JN, Italiano JE. Platelets: production, morphology and ultrastructure. *Handb Exp Pharmacol* (2012) **210**:3–22. doi:10.1007/978-3-642-29423-5_1
135. Panigrahi S, Ma Y, Hong L, Gao D, West XZ, Salomon RG, et al. Engagement of platelet toll-like receptor 9 by novel endogenous ligands promotes platelet hyper-reactivity and thrombosis. *Circ Res* (2013) **112**(1):103–12. doi:10.1161/CIRCRESAHA.112.274241
136. Varki A, Angata T. Siglecs – the major subfamily of I-type lectins. *Glycobiology* (2006) **16**:1R–27R. doi:10.1093/glycob/cwj008
137. Kelm S, Pelz A, Schauer R, Filbin MT, Tang S, De Bellard ME, et al. Sialoadhesin, myelin-associated glycoprotein and CD22 define a new family of sialic acid-dependent adhesion molecules of the immunoglobulin superfamily. *Curr Biol* (1994) **4**:965–72. doi:10.1016/S0960-9822(00)00220-7
138. Zhang J, Raper A, Sugita N, Hingorani R, Salio M, Palmowski MJ, et al. Characterization of Siglec-H as a novel endocytic receptor expressed on murine plasmacytoid dendritic cell precursors. *Blood* (2006) **107**:3600–8. doi:10.1182/blood-2005-09-3842
139. Angata T, Hayakawa T, Yamanaka M, Varki A, Nakamura M. Discovery of Siglec-14, a novel sialic acid receptor undergoing concerted evolution with Siglec-5 in primates. *FASEB J* (2006) **20**:1964–73. doi:10.1096/fj.06-5800com
140. Angata T, Tabuchi Y, Nakamura K, Nakamura M. Siglec-15: an immune system Siglec conserved throughout vertebrate evolution. *Glycobiology* (2007) **17**:838–46. doi:10.1093/glycob/cwm049
141. Cao H, Lakner U, De Bono B, Traherne JA, Trowsdale J, Barrow AD. SIGLEC16 encodes a DAP12-associated receptor expressed in macrophages that evolved from its inhibitory counterpart SIGLEC11 and has functional and non-functional alleles in humans. *Eur J Immunol* (2008) **38**:2303–15. doi:10.1002/eji.200738078
142. Liu Y, Chen GY, Zheng P. Sialoside-based pattern recognitions discriminating infections from tissue injuries. *Curr Opin Immunol* (2011) **23**:41–5. doi:10.1016/j.coi.2010.10.004
143. Rapoport E, Mikhalyov I, Zhang J, Crocker P, Bovin N. Ganglioside binding pattern of CD33-related siglecs. *Bioorg Med Chem Lett* (2003) **13**:675–8. doi:10.1016/S0960-894X(02)00998-8
144. Ito A, Handa K, Withers DA, Satoh M, Hakomori S. Binding specificity of siglec7 to disialogangliosides of renal cell carcinoma: possible role of disialogangliosides in tumor progression. *FEBS Lett* (2001) **498**:116–20. doi:10.1016/S0014-5793(01)02476-0
145. Nicoll G, Avril T, Lock K, Furukawa K, Bovin N, Crocker PR. Ganglioside GD3 expression on target cells can modulate NK cell cytotoxicity via siglec-7-dependent and -independent mechanisms. *Eur J Immunol* (2003) **33**:1642–8. doi:10.1002/eji.200323693
146. Attrill H, Imamura A, Sharma RS, Kiso M, Crocker PR, Van Aalten DM. Siglec-7 undergoes a major conformational change when complexed with the alpha(2,8)-disialylganglioside GT1b. *J Biol Chem* (2006) **281**:32774–83. doi:10.1074/jbc.M601714200
147. Kivi E, Elima K, Aalto K, Nymalm Y, Auvinen K, Koivunen E, et al. Human Siglec-10 can bind to vascular adhesion protein-1 and serves as its substrate. *Blood* (2009) **114**:5385–92. doi:10.1182/blood-2009-04-219253
148. Martini F, Riondino S, Pignatelli P, Gazzaniga PP, Ferroni P, Lenti L. Involvement of GD3 in platelet activation. A novel association with Fcgamma receptor. *Biochim Biophys Acta* (2002) **1583**:297–304. doi:10.1016/S1388-1981(02)00250-0
149. Li J, Xia Y, Bertino AM, Coburn JP, Kuter DJ. The mechanism of apoptosis in human platelets during storage. *Transfusion* (2000) **40**:1320–9. doi:10.1046/j.1537-2995.2000.40111320.x
150. Leytin V, Allen DJ, Mutlu A, Mykhaylov S, Lyubimov E, Freedman J. Platelet activation and apoptosis are different phenomena: evidence from the sequential dynamics and the magnitude of responses during platelet storage. *Br J Haematol* (2008) **142**:494–7. doi:10.1111/j.1365-2141.2008.07209.x
151. Leytin V. Apoptosis in the anucleate platelet. *Blood Rev* (2012) **26**:51–63. doi:10.1016/j.blre.2011.10.002
152. Burnier L, Fontana P, Kwak BR, Angelillo-Scherrer A. Cell-derived microparticles in haemostasis and vascular medicine. *Thromb Haemost* (2009) **101**:439–51. doi:10.1160/TH08-08-0521
153. Clemetson KJ. Platelets and pathogens. *Cell Mol Life Sci* (2010) **67**:495–8. doi:10.1007/s00018-009-0204-2
154. Gill R, Tsung A, Billiar T. Linking oxidative stress to inflammation: toll-like receptors. *Free Radic Biol Med* (2010) **48**:1121–32. doi:10.1016/j.freeradbiomed.2010.01.006
155. Ando M, Shoji T, Tu W, Higuchi H, Nishijima KI, Iijima S. Lectin-dependent localization of cell surface sialic acid-binding lectin Siglec-9. *Cytotechnology* (2014). doi:10.1007/s10616-014-9691-6
156. Ishida A, Akita K, Mori Y, Tanida S, Toda M, Inoue M, et al. Negative regulation of toll-like receptor-4 signaling through the binding of glycosylphosphatidylinositol-anchored glycoprotein, CD14, with the sialic acid-binding lectin, CD33. *J Biol Chem* (2014) **289**:25341–50. doi:10.1074/jbc.M113.523480
157. Paulson JC, Kawasaki N. Sialidase inhibitors DAMPen sepsis. *Nat Biotechnol* (2011) **29**:406–7. doi:10.1038/nbt.1859

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 10 December 2014; accepted: 12 February 2015; published online: 02 March 2015.

*Citation: Cognasse F, Nguyen KA, Damien P, McNicol A, Pozzetto B, Hamzeh-Cognasse H and Garraud O (2015) The inflammatory role of platelets via their TLRs and Siglec receptors. Front. Immunol. **6**:83. doi: 10.3389/fimmu.2015.00083*

This article was submitted to Inflammation, a section of the journal Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 18 December 2014
doi: 10.3389/fimmu.2014.00649

Platelets and infection – an emerging role of platelets in viral infection

Alice Assinger [1,2] *

[1] Department of Physiology and Pharmacology, Medical University of Vienna, Vienna, Austria
[2] Department of Medicine, Center for Molecular Medicine, Karolinska University Hospital, Stockholm, Sweden

Edited by:
Olivier Garraud, Institut National de la Transfusion Sanguine, France

Reviewed by:
Bo Shen, University of Illinois at Chicago, USA
Mirta Schattner, Institute of Experimental Medicine, Argentina

****Correspondence:***
Alice Assinger, Department of Physiology and Pharmacology, Medical University of Vienna, Schwarzspanierstrasse 17, Vienna A-1090, Austria
e-mail: alice.assinger@meduniwien.ac.at

Platelets are anucleate blood cells that play a crucial role in the maintenance of hemostasis. While platelet activation and elevated platelet counts (thrombocytosis) are associated with increased risk of thrombotic complications, low platelet counts (thrombocytopenia) and several platelet function disorders increase the risk of bleeding. Over the last years, more and more evidence has emerged that platelets and their activation state can also modulate innate and adaptive immune responses and low platelet counts have been identified as a surrogate marker for poor prognosis in septic patients. Viral infections often coincide with platelet activation. Host inflammatory responses result in the release of platelet activating mediators and a pro-oxidative and pro-coagulant environment, which favors platelet activation. However, viruses can also directly interact with platelets and megakaryocytes and modulate their function. Furthermore, platelets can be activated by viral antigen–antibody complexes and in response to some viruses B-lymphocytes also generate anti-platelet antibodies. All these processes contributing to platelet activation result in increased platelet consumption and removal and often lead to thrombocytopenia, which is frequently observed during viral infection. However, virus-induced platelet activation does not only modulate platelet count but also shape immune responses. Platelets and their released products have been reported to directly and indirectly suppress infection and to support virus persistence in response to certain viruses, making platelets a double-edged sword during viral infections. This review aims to summarize the current knowledge on platelet interaction with different types of viruses, the viral impact on platelet activation, and platelet-mediated modulations of innate and adaptive immune responses.

Keywords: platelets, viruses, thrombocytopenia, thrombosis, immune response

INTRODUCTION

From an evolutionary perspective, the cellular mediators of hemostasis and immune defense have not always been separated. In invertebrates, a cell type called hemocyte protects the host from invading microbes and the same cell also prevents "blood" (i.e., hemolymph) loss upon injury by triggering coagulation (1, 2). In highly developed species, hemostasis and immune response have been divided and leukocytes resume the immune response functions, while platelets maintain hemostasis (2).

For decades, these two systems were thought to act independently, but this concept has recently been challenged. Emerging evidence suggests that the boundaries between coagulation and immune defense are actually not as clear-cut as originally supposed. A new concept of involvement of immune cells in hemostasis, termed immunothrombosis, has been recently proposed, which assumes a function of innate immune cells in thrombosis (3). On the other hand, an important role of platelets in immune response becomes more and more apparent (4–7).

In 1882, Bizzozero discovered platelets as the third morphological element in blood and elucidated the function of these cells in hemostasis and thrombosis (8). For a long time, platelets and their granule content were thought to mainly mediate the activation of the coagulation system and the recruitment of further platelets to stop blood loss upon injury. Unwanted platelet activation was noted to occur as a response to internal injuries, for example, denudation and erosion of the endothelial surface or rupture of an atherosclerotic plaque (9). Consequently, platelets were assumed to be responsible for the lethal steps of cardiovascular diseases, where unstable thrombi occlude small vessels, thereby compromising oxygen supply of target organs. Nevertheless, platelets are involved in far more processes and they respond to and interact with far more triggers than initially thought. Besides their central role in hemostasis, platelets assist and modulate inflammatory reactions and immune responses by direct interaction with leukocytes as well as endothelial cells and via release of soluble inflammatory mediators that enhance recruitment of leukocytes and trigger their activation (2, 4, 6, 10). Platelets also express surface receptors, such as lectins, integrins, and toll-like receptors (TLR), allowing them to directly interact with several pathogens. Moreover, they express Fc receptors by which they can recognize immunocomplexes.

Thus, platelet activation does not only occur in response to injury and is not only limited to hemostatic processes but also modulates host response and virus survival (3–5, 7, 10–12). Therefore, potential immune-modulatory side effects should be considered during anti-platelet therapy.

To date, the role of platelets in response to invading pathogens is not fully understood. Platelet–microbe interactions seem to be beneficial for the host due to up-regulation of immune responses. However, platelet interaction with invading pathogens is also speculated to benefit virus or bacteria, as platelets provide shelter against leukocytes, antiviral agents, and antibiotics. Moreover, the stickiness of platelets might attenuate microbe–endothelial interactions and facilitate infection by similar mechanisms to those recently described for platelet interactions with circulating tumor cells (13).

This review aims to summarize the current knowledge on platelet interaction with different types of viruses, the viral impact on platelet activation, and platelet-mediated modulations of innate and adaptive immune responses.

MECHANISMS OF VIRUS-INDUCED THROMBOCYTOPENIA

Platelets are, after erythrocytes, the second most abundant cell population in the blood. Normal platelet counts range from 150,000 to 450,000 platelets per microliter. Thrombocytopenia, which represents a drop in platelet count caused by either decreased platelet production or increased platelet destruction, is associated with an increased bleeding risk. Thrombocytopenia is frequent following viral infections and viruses use a variety of distinct strategies to decrease the levels of circulating platelets. The different mechanisms of how virus infection can interfere with platelet production and might trigger platelet destruction are summarized in the first chapter and **Figure 1**. As virus-mediated thrombocytopenia is often multifactorial and differs between virus infections, the mechanisms by which viruses trigger

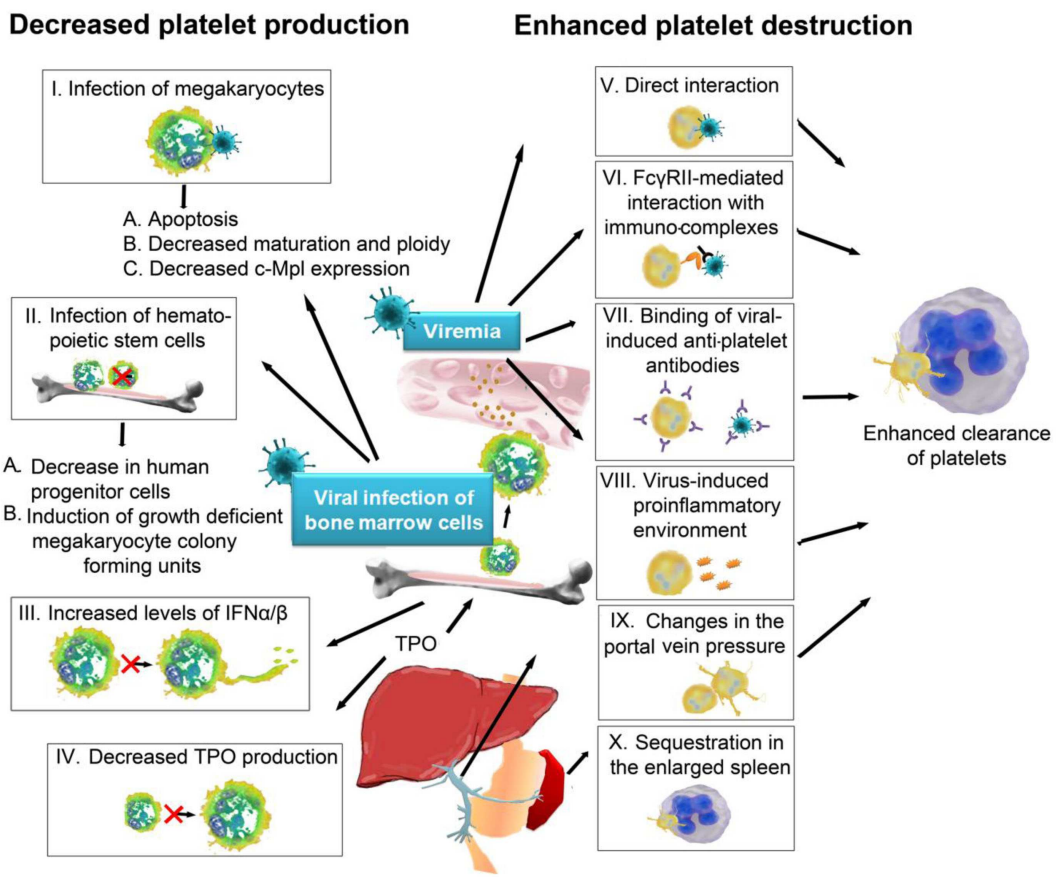


FIGURE 1 | Mechanisms of virus-induced thrombocytopenia: thrombocytopenia occurs either via decreased platelet production or increased platelet destruction. Viruses can trigger a decrease in platelet production by (I) infection of megakaryocytes, which can lead to (A) apoptosis of megakaryocytes, (B) decreased maturation and ploidy of megakaryocytes, or (C) decreased expression of thrombopoietin receptor c-Mpl. Viruses can also infect hematopoietic stem cells (II), which results (A) in a decrease of progenitor cells and (B) the induction of growth deficient megakaryocyte colony forming units, due to disturbed cytokine production by the infected cells in the bone marrow. Viruses can further indirectly influence platelet production (III) via induction of IFNα/β, which suppresses proplatelet formation or (IV) by targeting and modulating liver functions, which are important for the production of megakaryocyte growth and development factor thrombopoietin. Another mechanism for how viruses cause thrombocytopenia is by favoring platelet destruction, which frequently occurs during viremia. Viruses can either (V) directly interact with platelets or (VI) platelets recognize immunocomplexes of IgGs and viral antigens. Antiviral antigens often show cross-reactivity with platelet surface integrins (VII), which provides another mechanism of virus-induced destruction of platelets. The virus-induced pro-inflammatory environment itself often leads to further platelet activation in viremic patients (VIII). Additionally, changes in the portal vein pressure (IX) and an enlarged spleen (X) can serve as trigger for platelet activation. Once activated, platelets are recognized by circulating leukocytes or by cells in spleen and liver and rapidly cleared from the circulation. IFN, interferon, TPO, thrombopoietin; FcγRII, Fc receptor γ R II.

thrombocytopenia are separately discussed for the most prominent viruses and an overview on the receptors involved in direct platelet–virus interactions is depicted in **Figure 2**.

DECREASED PLATELET PRODUCTION

Platelets are produced in the bone marrow by megakaryocytes, which develop from hematopoietic stem cells. Megakaryocytes first undergo linage commitment, followed by endomitosis resulting in polyploidy (14). Polyploidy is a state where one cell comprises multiple sets of chromosomes and this represents an important step in megakaryopoiesis. After endoplasmatic maturation, megakaryocytes form proplatelets, which bud off thousands of platelets and microparticles into the blood stream (15). Megakaryopoiesis is triggered by a variety of cytokines (e.g., GM-CSF, IL-3, IL-6, IL-11, FGF4, and SDF-1), with thrombopoietin (TPO) being the most important. Nevertheless, TPO does not only trigger megakaryocyte development but also fulfils an important role in maintaining stem cells (16).

Viruses can modulate platelet production at various steps of development. They are able to influence the cytokine profile of the host, resulting in altered TPO production in the liver. Examples of this include: simian immunodeficiency virus (SIV), which triggers TPO production via up-regulation of tumor growth factor (TGF) β (17); human herpes virus 6, which can interfere with TPO-inducible megakaryocytic colony formation (18); human herpes virus 7, which impairs the survival and differentiation of megakaryocytes (19).

Some viruses also directly interfere with TPO production by destruction of liver tissue as shown for hepatitis C virus (HCV) (20). The resulting drop in TPO production results in delayed megakaryocyte development and a decrease in platelet production. Other viruses infect bone marrow stromal cells and hematopoietic stem cells, resulting in altered cytokine production and decreased number of progenitor cells, thereby disturbing hematopoiesis (21).

Human immunodeficiency virus (HIV), cytomegalovirus (CMV), and HCV are known to replicate in megakaryocytes (22–24) and many more viruses can interact with megakaryocytes modulating their proliferation and function (23, 25–29). Viral infection of megakaryocytes can increase apoptosis and decreases the maturation and ploidy of megakaryocytes. Moreover, virus-infected megakaryocytes have been shown to express less surface c-Mpl, which is the receptor for TPO (30). An overview on the different mechanisms on how viruses can interfere with platelet production is depicted in **Figure 1**.

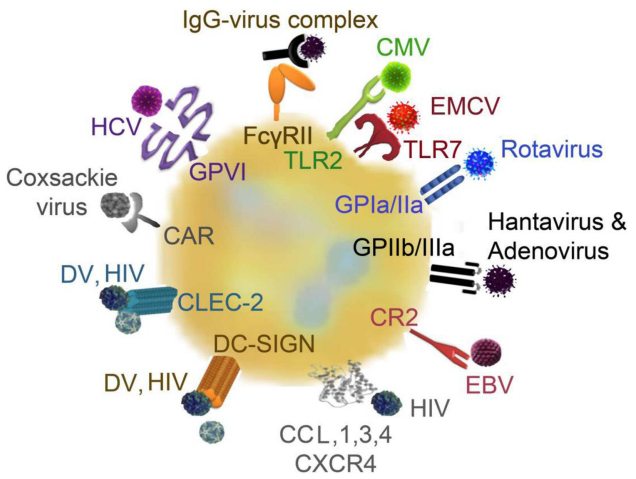


FIGURE 2 | Platelet receptors for viruses: platelets and viruses can directly interact via a plethora of surface receptors. CMV binds to platelets via TLR2, EMCV interacts via TLR7, rotavirus utilizes GPIa/IIa to bind to platelets and hantavirus and adenovirus interact with platelets via GPIIb/IIIa. EBV–platelet interaction occurs via CR2. HIV and DV bind to lectin receptors such as CLEC-2 and DC-SIGN. HIV further interacts with CXCR4 and CCL3 and CCL5. Platelets express the Coxsackie virus-specific receptor, CAR, and HCV interacts with platelets via GPVI. CAR, Coxsackie-adenovirus receptor; CLEC-2, C-type lectin domain family 2; CCL, chemokine (C–C motif) ligand; CMV, cytomegalovirus; CR, complement receptor; CXCR4, C–X–C chemokine receptor type 4; EBV, Epstein–Barr virus; EMCV, encephalomyocarditis virus; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grapping non-integrin; DV, Dengue virus; FcγRII, Fc receptor γ II; GP, glycoprotein; HCV, hepatitis virus C; HIV, human immunodeficient virus; IgG, immunoglobulin G; TLR, toll-like receptor;

ENHANCED PLATELET DESTRUCTION

While thrombocytopenia induced by decreased platelet production is observed at later stages of infection, rapidly induced thrombocytopenia in response to viral infections is mediated via enhanced platelet destruction.

The most rapid way of platelet destruction occurs via direct interaction between platelets and viruses. Platelet–virus interaction can occur via a variety of receptors and are mainly mediated by integrins, surface lectins, and TLR (25, 31, 32) (see **Figure 2**). Platelets can bind CMV via TLR2, which triggers platelet activation and degranulation and results in enhanced platelet interaction with neutrophils (32). Encephalomyocarditis virus (EMCV) was recently shown to interact with platelet TLR7, which also leads to platelet degranulation and direct platelet–neutrophil interactions (33). Platelet fragments subsequently get phagocytized by neutrophils, thereby contributing to the drop in platelet count (33).

Rotavirus utilizes the collagen receptor GPIa/IIa to bind to platelets (25, 34), while hantavirus and adenoviruses interact with platelets via the fibrinogen receptor GPIIb/IIIa (35). However, GPIIb/IIIa does not seem to be the unique receptor for platelet–adenovirus interaction, as inhibition of GPIIb/IIIa does not alter platelet internalization of adenoviruses (36). In addition, hantavirus-infected endothelial cells can bind to quiescent platelets via GPIIb/IIIa (37), which results in platelet activation and clearance but might also influence vascular permeability (38).

GPIIb/IIIa is the most abundant platelet integrin and displays bi-directional signaling functions. Inside-out signaling, which is positively regulated by various platelet agonists, is mediated by intracellular protein–protein interactions and biochemical reactions that regulate GPIIb/IIIa affinity (39). These intracellular processes triggering GPIIb/IIIa activation are complex and include recruitment of talin, which separates the GPIIb and the GPIIIa subunits, and kindlins, which are involved in GPIIb/IIIa activation (40) independent of talin recruitment (41). Further, G protein subunit $G\alpha_{13}$ directly binds to the cytoplasmic domain of GPIIIa and promotes ligand binding to GPIIb/IIIa (42).

Outside-in signaling via receptor binding promotes actin polymerization and platelet spreading (39) and can thereby enhance virus attachment to endothelial cells but also promote platelet clearance.

Epstein–Barr virus (EBV) interaction with platelets occurs via complement receptor 2 (CR2) (43). HIV and dengue virus activate platelets by binding to lectin receptors such as C-type lectin domain family 2 (CLEC-2) and cell-specific intercellular adhesion molecule-3-grapping non-integrin (DC-SIGN) (44). Platelets and/or megakaryocytes can further interact with HIV envelope proteins via C–X–C chemokine receptor type 4 (CXCR4) or via chemokine (C–C motif) ligand (CCL) 3 (MIP-1α) and 5 (RANTES) (25). However, HIV-1 changes its co-receptor usage from CCR5 to CXCR4 only after many years of infection and this receptor change represents a switch to non-CD4-dependent platelet activation at late stages of disease.

In addition, platelets express the Coxsackie virus-specific Coxsackie-adenovirus receptor (CAR) (45) and HCV interacts with platelets via collagen receptor GPVI (46).

These direct interactions often result in platelet activation and adhesion of activated platelets to leukocytes. Platelet binding to neutrophils triggers phagocytosis of platelets (33, 47) and platelet activation itself promotes platelet clearance in spleen and liver (48).

However, platelets are not only activated by direct interactions with viruses. Host defense mechanisms in response to viral infections can also lead to platelet activation. For example, many viral infections lead to systemic inflammation, which in turn triggers platelet activation and decreases platelet life span (49). Among others, influenza virus, rhinovirus, and CMV infection result in up-regulation of cytokines, such as interleukin 6 (IL-6), in target cells (50).

Platelets can be activated by these cytokines, leading to platelet–leukocyte interactions, which foster leukocyte and endothelial activation, further amplifying platelet activation and enhancing their clearance by splenic macrophages or Kupffer cells in the liver (45). Monocytes that encounter dengue virus, for example, start generating platelet activating factor (PAF) (51), which is a lipid mediator that triggers platelet activation. This leads to enhanced apoptosis of platelets and accelerates platelet clearance in secondary dengue infection (52).

Several virus infections activate the coagulation cascade via induction of tissue factor (TF) expression in target cells. Generation of thrombin by the activated coagulation cascade causes platelet activation and subsequent clearance via protease activating receptor (PAR) signaling (53). PARs on platelets, endothelial cells, and leukocytes are important modulators during viral infections, which modulate innate immune responses and exert positive and negative effects on TLR-dependent responses (53).

Platelets also recognize viral particles coated with immunoglobulins via their FcγRII receptor, which results in Fc receptor-mediated platelet activation, aggregation, and platelet clearance (54, 55). FcγRII-mediated platelet activation depends on IgG and GPIIb/IIIa engagement and involves ADP and thromboxane A_2 (TxA_2) feedback mechanisms to cause platelet aggregation, which is further enhanced by CXCL4 (56).

Furthermore, B-lymphocyte production of antibodies against some viruses has been shown to interfere with platelet survival. These antibodies, which usually target surface glycoproteins of viruses, show a cross-reactivity with platelet surface intergrins such as GPIIb/IIIa or GPIb-IX-V (38). This so called idiopathic thrombocytopenic purpura (ITP) or platelet autoantibody-induced thrombocytopenia has been described for HCV, HIV, CMV, EBV, hantavirus, varicella zoster virus, herpes viruses, and severe acute respiratory syndrome coronavirus (38).

Additionally, platelet destruction in response to viral infections can occur due to disturbed portal vein pressure and enhanced sequestration of platelets by the enlarged spleen as is the case in HCV infection (57).

MULTIFACTORIAL MECHANISMS LEADING TO THROMBOCYTOPENIA

Thrombocytopenia in response to viral infections is often multifactorial. In viral hepatitis, thrombocytopenia is caused by platelet-specific glycoprotein antibodies (58) as well as by immune complexes bound to the platelet surface (59). In the case of HCV, thrombocytopenia can be reversed using a selective thrombin receptor agonist (60), indicating that coagulation, inflammation, and platelet activation play a role in HCV-induced decrease of platelet count. HCV also indirectly affects megakaryopoiesis (25, 59). HCV-induced liver dysfunction results in a decrease in TPO production, which in turn results in impaired platelet production in the bone marrow (20). The recovery of TPO levels and increase in platelet count following successful liver transplantations in HCV-infected individuals (20) emphasizes the importance of this mechanism in HCV-induced thrombocytopenia. Moreover, portal hypertension leads to platelet activation and shortens platelet survival due to increased splenic platelet sequestration (57). However, treatment aiming at reversing portal hypertension did not always correct thrombocytopenia (20).

Human immunodeficiency virus-induced thrombocytopenia is caused by multiple mechanisms. The effects of HIV on megakaryocyte development were recently reviewed in Ref. (30). In brief, HIV results in impaired survival of bone marrow megakaryocytes and their precursors. HIV also decreases the number and activity of human progenitor cells and decreases megakaryocyte maturation and ploidy. HIV surface glycoprotein gp120 leads to increased megakaryocyte apoptosis *in vitro* due to increased TGFβ and down-regulation of the proliferation-inducing ligand tumor necrosis factor ligand superfamily member 13 (TNFSF13). Further, gp120 interacts with CD4, which is expressed by immature megakaryocytes, which also express CCR5, and leads to their infection (61). Furthermore, HIV infection of megakaryocytes can lead to reduced TPO receptor (c-Mpl) expression.

In dengue virus infection, platelet production is impaired by suppression of megakaryopoiesis via infection of hematopoietic progenitor cells or indirectly via altered cytokine levels in the bone marrow due to impaired stromal cell function (51). Platelets from patients with dengue infection present signs of activation, mitochondrial dysfunction, and enhanced apoptosis, which may contribute to the genesis of thrombocytopenia

(62, 63). Further, enhanced destruction of platelets occurs due to cross-reaction of platelets with anti-dengue virus antibodies. Dengue virus-induced anti-non-structural protein-1 (NS-1) induces complement-mediated lysis of platelets and thereby further accelerates thrombocytopenia (64). NS-1 can also activate endothelial cells and leads to increased vascular permeability and further platelet activation (65). Dengue virus-infected patients show increased levels of E-selectin on their endothelial cell surface, which promotes adhesion and clearance of platelets (65, 66) as well as enhanced activation of the coagulation cascade (67).

Arenaviruses infection by either lymphocytic choriomeningitis virus (LCMV) or Junin virus results in thrombocytopenia and decreased agonist-induced platelet responses in mice (68, 69). As a consequence, platelet depletion in LMCV-infected mice results in lethal hemorrhagic anemia (68). This effect is caused by diminished platelet responses, rather than solely a drop in platelet count. The underlying mechanism of altered platelet production and reduced platelet reactivity was found to rely on virus-induced production of interferon (IFN) α/β (68, 69). Junin virus mainly infects $CD34^+$ cells not megakaryocytes but impairs proplatelet formation and platelet release via IFNα/β receptor signaling (69). IFNα/β receptor signaling represents an important paracrine repressor of megakaryopoiesis, which directly inhibits TPO-induced signaling through induction of suppressor of cytokine signaling 1 (SOCS-1) (70), induction of 2′5′-oligoadenylate synthetase (OAS) (68), and decease of nuclear factor erythroid 2 (NF-E2) expression (69).

Hantavirus can directly interact with and activate platelets via GPIIb/IIIa (35) and infection of megakaryocytes with hantavirus induces the up-regulation of human leukocyte antigen (HLA) class 1 molecules on the megakaryocyte surface, which leads to cytotoxic T-lymphocyte-mediated destruction of megakaryocytes (27).

Viruses also possess enzymes, which can modulate platelet functions. For example, Influenza virus exhibits neuraminidase (sialidase), which hydrolyses the terminal sialic acid residues from host cell receptors and thereby decreases the life span of platelets by targeting platelets for rapid clearance in the liver and spleen (71). As another example, mycoviral neuraminidase has been shown to reduce platelet life span by cleaving sialic acid in the platelet membrane (72). Besides the effect on platelet live span, neuroaminidase further alters megakaryocyte ploidy as well as morphology and size of platelets (73). Newcastle virus can directly disrupt platelet cell membrane, resulting in platelet lysis (74). Human parvovirus 19 cannot reproduce in megakaryocytes, but triggers a drop in platelet count via platelet activation (75).

Despite its effects on TPO generation, direct interaction of the non-human SIV with platelets results in platelet–monocyte aggregate formation, which promotes monocyte differentiation into a more inflammatory phenotype. Furthermore, platelet activation triggers clearance of platelets (76, 77) as platelets become recognized by macrophages in the spleen, leading to a rapid drop in platelet count.

It is currently unclear if, and to what extent, viruses or the host could benefit from thrombocytopenia. It has been suggested that down-regulation of hematopoiesis, for example, in dengue virus infection might have a protective role for the microenvironment to limit injury during elimination of infected cells (78).

ANTIVIRAL EFFECTS OF PLATELETS

Direct interaction between platelets and viruses has been observed in various viral infections and could be proven by *in vitro* studies. The interactions leading to platelet activation do not only result in enhanced platelet clearance, the elimination of virus-laden platelets also contributes to the clearance of virus particles (25).

An overview on the different mechanisms by which platelets can interfere with virus infection is given in **Figure 3**. Platelet activation in response to direct and indirect interaction with viruses results in platelet activation and degranulation. Platelet α-granules provide platelet-mediated host defense mechanisms, as they contain kinocidins and microbicidal proteins important for antiviral host defense. Kinocidins have immune-modulatory functions including chemotaxis and activation of various immune cells (79). They can differentiate between host and pathogen cell membranes and distinct microbial spectra under different pH conditions. Thrombin, which is produced upon inflammation and injury, can proteolytically cleave kinocidins and platelet antimicrobial peptides resulting in cleavage products with a stronger and broader range of antimicrobial activity (49, 80). Pathogens can also cleave kinocidins on distinct sites, which potentially results in their inactivation.

The most abundant platelet kinocidin is platelet factor 4 (PF-4/CXCL4). CXCL4 is released into the blood stream upon tissue injury, inflammation, oxidative stress, or pathogen stimulation such as platelet–virus interactions. CXCL4 has been identified as a broad-spectrum HIV-1 inhibitor and suppresses HIV-1 infection of T-lymphocytes via steric inhibition by binding of CXCL4 proximal to the CD4 binding site on gb120 (81, 82).

Other important chemokine released during viral infections in host defense are CCL5 and CCL3 (83). CCL5 and CCL3 have been identified as major HIV-suppressive factors produced by cytotoxic T-lymphocytes (84) and also platelet-derived CCL5 and CCL3 could play a role in this process. It is currently unclear whether the effect of CCL5 on inhibiting HIV infection is due to perturbation of the viral envelope and/or due to competitive binding to its receptors (primarily CCR5) (79). HIV gp41 is able to decrease CCL5 release from platelets, thereby directly counteracting the antiviral responses of platelets (85). While CXCL4 is mainly platelet-derived, CCL5 can be released by various cells types and indeed, a variety of viruses has been shown to induce CCL5 release [reviewed in Ref. (86)].

CCL5 is further involved in viral lung diseases (87). It represents an important chemokine during the development and resolution of a pulmonary leukocyte response to influenza A virus infection in mice (88). CCL5 interaction with its receptor CCR5 provides an anti-apoptotic signal for macrophages during influenza virus infection (89) and is important for cell survival during viral infections. CCL5 has multifaceted roles in HCV infection, as it modulates inflammatory reactions and tissue injury and also induces the up-regulation of T-lymphocyte helper cells type 1 (Th1) (90).

Upon activation, platelets release β-defensins, which have been shown to neutralize a variety of viruses (91). Platelet antimicrobial peptides PD3 (KNGRKLCLDLQAALY) and PD4 (AALYKKKIIKKLLES), which are platelet α-granule-derived molecules, have also been demonstrated to potently reduce viral titers of vaccinia virus (92). Other platelet-derived antimicrobial peptides, for

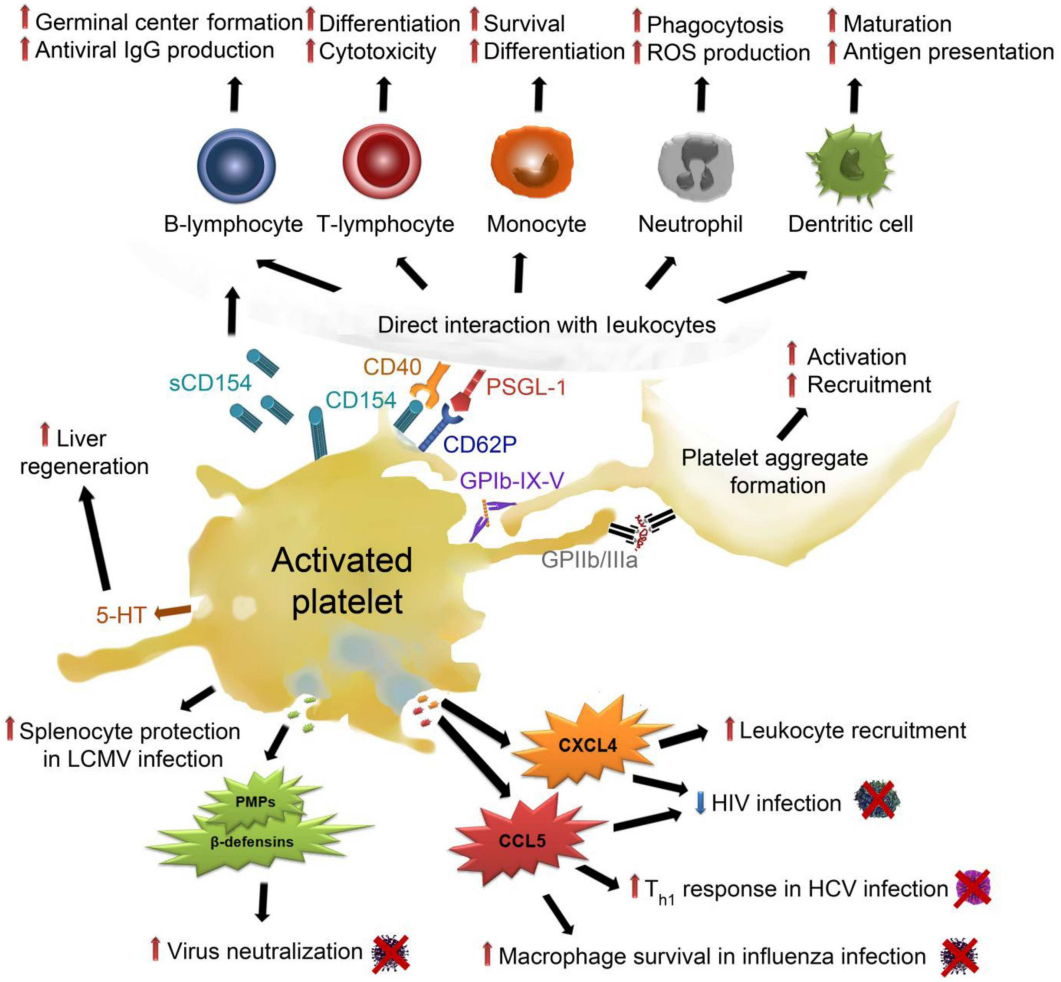


FIGURE 3 | Effects of activated platelets on virus infection: upon activation, platelets release their α-granules, containing high amounts of CXCL4, which up-regulates coagulation and leukocyte recruitment. Moreover, CXCL4 decreases HIV infection but also enhances liver fibrosis. CCL5, another α-granule-derived chemokine, also decreases HIV infection and enhances T_{h1} lymphocyte responses in HCV infection and serves as a survival signal for macrophages in influenza infection. Platelets release PMPs and β-defensins from their α-granules, which mediate virus neutralization. They protect splenocytes from necrosis in LCMV infection. Platelet dense granules contain 5-HT, which enables liver regeneration but also HBV and HCV infection. Platelet activation leads to the expression and release of CD154 and CD62P, which allow direct interaction between platelets and leukocytes. Platelet interaction with B-lymphocytes enhances germinal center formation and antiviral IgG production. Platelets trigger T-lymphocyte differentiation and cytotoxicity as well as survival and differentiation of monocytes. In neutrophils, platelet adhesion stimulates ROS production and boosts phagocytosis. Platelet interaction with dendritic cells promotes their maturation and facilitates antigen presentation. Finally, platelet activation results in interaction and activation of further platelets, which triggers platelet aggregation and amplifies the above described processes. CCL5, chemokine (C–C motif) ligand 5; CXCL4, C–X–C chemokine ligand 4, GP, glycoprotein; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficient virus; IgG, immunglobulin G; LCMV, lymphocytic choriomeningitis; PMPs, Platelet antimicrobial peptides; PSGL-1, P-selectin (CD62P) glycoprotein ligand-1; sCD154, soluble CD154/CD40 ligand; Th1, T-helper lymphocyte type 1; 5-HT, serotonin.

example, thymosin beta 4, CXCL7, or cleavage products of antimicrobial peptides (fibrinopeptide A and B, thrombocidins), do not directly act against viral infections but could prevent the host from bacterial super-infections (49).

The highest amounts of serotonin in blood are stored in platelet dense granules, which are also released upon platelet activation. Serotonin was found to be an important mediator of liver regeneration (93, 94) and also mediates early T-lymphocyte stimulation (95).

Platelet degranulation further results in surface expression of P-selectin, which is part of the inner α-granule membrane, which fuses with the outer platelet membrane upon granule exocytosis. P-selectin interacts with its counter-receptor P-selectin glycoprotein ligand-1 (PSGL-1), which is constitutively expressed on the surface of leukocytes (96). Platelet interactions with leukocytes mediate immune responses during viral infections (79). Direct interaction with platelets leads to activation of leukocytes, resulting in enhanced phagocytosis and reactive oxygen production of neutrophils, as well as neutrophil extracellular trap formation. The platelet–monocyte interaction results in an increased activation and differentiation and boosts monocyte surface expression of TF and formation of microparticles (96).

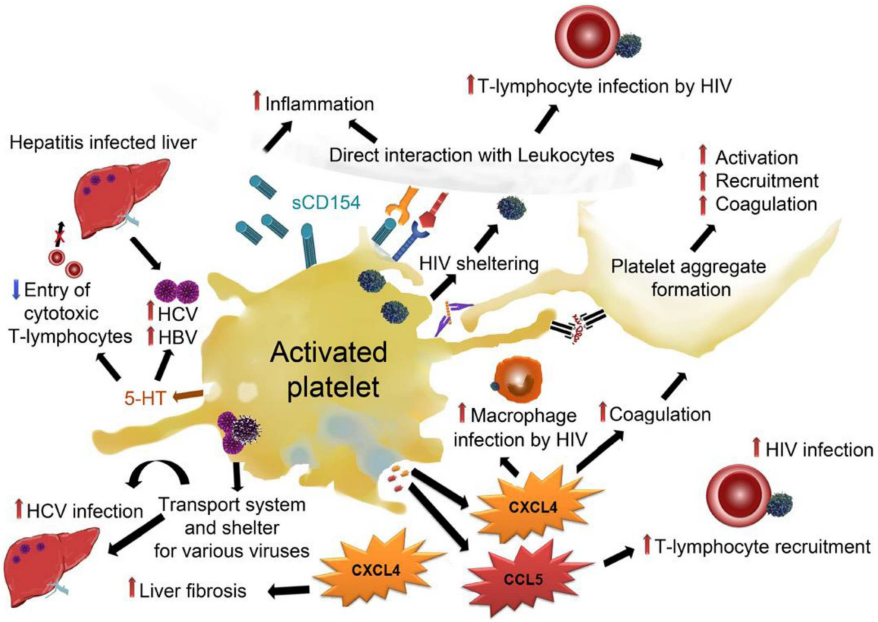


FIGURE 4 | Adverse effects of platelets in viral infections: platelets can shelter viruses like HIV, HVC, HBV, and influenza and facilitate their transportation throughout the circulation, thereby enabling *de novo* infections at distal sites. Platelet–virus interaction often results in platelet activation and subsequent release of α-granules and dense granules. CXCL4 from α-granules has been demonstrated to enhance HIV infection of macrophages and enhance liver fibrosis in hepatitis. CCL5 enhances T-lymphocyte recruitment and thereby promotes HIV infection of T-lymphocytes. Dense granule-derived 5-HT was shown to boost HBV and HCV infection by decreasing the entry of cytotoxic T-lymphocytes. Direct interaction of platelets with leukocytes facilitates infection of leukocytes and enhances both leukocyte activation and inflammation. Activation of platelets leads to activation and recruitment of further platelets and enhances pro-coagulatory processes.CCL5, chemokine (C–C motif) ligand 5; CXCL4, C–X–C chemokine ligand 4, GP, glycoprotein; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficient virus; sCD154, soluble CD154/CD40 ligand; 5-HT, serotonin.

In response to CMV and EMCV, platelet–virus interaction results in stimulation of TLRs, which trigger platelet degranulation and rapid direct interaction of platelets and neutrophils (32, 33). Of note, platelets were shown to decrease EMCV counts in a TLR7-dependent fashion and contribute to host survival while no pro-thrombotic consequences of platelet TLR7 and EMCV interaction could be observed (33).

Platelets recruit dendritic cells to sites of injury or infection (97) and products released from platelets promote maturation of dendritic cells and enhance their antigen presenting capacity (98, 99).

Platelet–leukocyte interaction and modulation of leukocyte functions can be further accelerated by CD40 ligand (CD40L/CD154) binding to CD40. Platelets express and secrete (soluble) CD154, thereby triggering host responses and boosting inflammation.

Platelets directly interact with T-lymphocytes and B-lymphocytes and modulate their function via direct cell–cell interaction as well as soluble mediators (100). Platelet-released CXCL4 and CCL5 enhance pro- and anti-inflammatory cytokine production of T-lymphocytes (101). CXCL4 represents an important mediator in T-cell differentiation, which leads to an increase in regulatory T-lymphocytes (102) and limits Th17 differentiation (103).

Platelets additionally enhance T-lymphocyte-mediated germinal center formation (104) and they can independently upregulate adenovirus specific IgGs via CD154 (105).

Binding of IgG-coated viruses causes platelet expression of CD154 and CCL5, which in turn primes protective T-cell-mediated immunity (79). Platelets in mice infected with lymphocytic choriomeningitis virus were found to be responsible for efficient cytotoxic T-cell responses, which depended on platelet GPIIIa (CD61) and CD154 (68). T-lymphocyte interaction with platelets also results in the activation of platelets, which amplifies the release of CCL5 (106).

In hepatitis virus B (HBV) infected mice, platelets were shown to be responsible for the intra-hepatic accumulation of virus-specific cytotoxic T-lymphocytes (107) and to mediate cytotoxic T-cell influx into liver tissue via P-selectin, which enhances viral clearance but also tissue damage (108). It was further shown an LCMV infection model that a drop in platelet count leads to necrotic destruction of splenocytes (109). This affects innate and adaptive immunity and leads to uncontrolled LCMV replication (109), indicating that platelets are necessary to maintain splenocyte survival.

ADVERSE EFFECTS OF PLATELET ACTIVATION IN VIRAL INFECTIONS

To date, it remains unclear if platelet–virus interactions are beneficial for the host or for the virus. Potential adverse effects of platelets in viral infections are summarized in **Figure 4**. HIV, for example, is internalized by platelets, which results in sheltering of virus particles from the host immune systems and allows dissemination

throughout the entire body [reviewed in Ref. (25)]. Moreover, in response to HIV interaction, platelets become activated and release CCL5, which results in the recruitment of highly susceptible target cells like T-lymphocytes and monocytes. It was demonstrated for influenza virus that platelets serve as a carrier for the virus in the circulation (72). Also HCV has been shown to take advantage of platelets as a safe transport system to reach the liver, where platelet activation further enhances the interaction of platelets and liver cells. This interaction prolongs the time for potential infection of liver tissue by the virus (46).

Virus-mediated activation of platelets and subsequent platelet-derived cytokine release not only protects the host but may also have unwanted implications for the host. While several reports indicate that CXCL4 is a broad-spectrum suppressor of HIV infection, one report indicates that platelet CXCL4 can also facilitate human macrophage infection with HIV-1 and potentiates virus replication (110). Furthermore, CXCL4 has been shown to mediate liver fibrosis in experimental mouse models (111), indicating that this chemokine could play a role in platelet-mediated acceleration of hepatitis-induced liver fibrosis.

Platelet-derived serotonin results in a delayed entry of activated cytotoxic T-lymphocytes into the liver, which slows down virus control and supports virus persistence in the liver (112). This results in aggravation of virus-induced immunopathology.

Megakaryocyte infection and subsequent modulation of platelet function by CMV have been speculated to be a reason for graft failure in allergenic bone marrow transplantation (25).

BLEEDING AND THROMBOTIC COMPLICATIONS IN RESPONSE TO VIRAL INFECTION

Primary and secondary hemostasis work tightly together. Imbalances of either system result in impaired function of the other. Activation of the coagulation cascade has been observed in various virus infections, including HIV, dengue, and Ebola virus infection, and might provide a host defense mechanism to limit pathogen dissemination (53). Changes in the activation of the coagulation cascade and modulation of platelet count and function, which are also observed during viral infections, lead to an increased risk of disseminated vascular coagulation (DIC), deep vein thrombosis (DVT) thrombosis, and hemorrhage in infected patients (38). Thrombocytopenia is a common result of viral infections and associated with an increased bleeding risk. Approximately 10% of HIV positive patients and up to 60% of patients with acquired immunodeficiency syndrome (AIDS) suffer from thrombocytopenia, which can lead to severe bleedings in these patients (30). In many viral infections, platelet function and aggregation in response to different agonists are diminished (25), causing bleeding complications in viral hemorrhagic fevers (VHF) (51). VHF outbreaks lead to the deaths of thousands of people every year and are caused by different enveloped RNA viruses, which include *Arenaviridae* (e.g., Lassa virus), *Bunyaviridea* (e.g., hantavirus), *Filioviridae* (e.g., Marburg and Ebola virus), and *Flaviviridae* (e.g., dengue virus).

Of note, it was recently shown that a reduction in platelet count of more than 85% is necessary for hemorrhages to occur, indicating that a very low percentage of platelets are sufficient to maintain vascular integrity (109). In an LCMV model, it was demonstrated that even severe thrombocytopenic mice develop only local hemorrhages at sites of inflammation and that LCMV-dependent bleedings are a result of IFNα/β signaling-induced platelet dysfunction (68). Mice lacking functional IFNα/β receptor show less severe anemia and hemorrhages due to restored platelet aggregation capacity during LCMV infection (68). This indicates that platelet dysfunction has more tremendous effects than thrombocytopenia in these pathologies. Of note, many VHF viruses inhibit platelet function. Junin virus, causative of Argentinean hemorrhagic fever, induces IFNα/β signaling-dependent decrease in platelet production and function (69). Ebola virus also induces an increase in IFNα, which correlated with increased fatality (113). Ebola infection further triggers TF expression, which is associated with Ebola hemorrhagic fever (114). Hantavirus and Lassavirus also abrogate platelet responses via plasma-mediated platelet inhibition and/or direct interaction (37, 115).

Although thrombocytopenia is frequently observed in patients with dengue infections, bleedings are rare. However, if bleedings do occur they are associated with high mortality risk. Of note, in dengue virus-infected patients, platelet count does not predict bleeding risk. However, systemic platelet activation might contribute to the pro-coagulatory state in these patients, which frequently develop DIC (67). Moreover, elevated platelet activation is associated with plasma leakage (116). H1N1 influenza infection enhances activation of circulating platelets (117) and results in increased events of thrombosis (118).

In line with the observation that platelet dysfunction increases the risk of hemorrhages and therefore mortality, it has been demonstrated that pharmacological inhibition of either platelets or the coagulation cascade increases the mortality of H1N1-infected mice (53). Thus, aspirin treatment, which inhibits platelet activation via inhibition of cyclooxygenase and subsequent TxA_2 production, has been hypothesized to have worsened the incidence and severity of the influenza pandemics in the 1910s (119).

Taken together, viruses can either enhance platelet activation resulting in pro-thrombotic events or diminish platelet responses thereby leading to bleeding complications. Platelet count alone does not seem to be sufficient to predict adverse platelet effects and parameters like platelet activation and reactivity might be more accurate to predict thrombotic or bleeding risks in patients.

PLATELET–VIRUS INTERPLAY IN CARDIOVASCULAR DISEASE

Platelets are responsible for the lethal consequences of cardiovascular disease but emerging evidence suggests that they also play a role in the initiation and progression of atherosclerosis. Several viruses have been associated with cardiovascular disease and herpes simplex virus (HSV), HIV, CMV, HCV, EBV as well as influenza DNA, and/or protein has been found in atherosclerotic plaques (120, 121). Large cohort studies indicate that seropositivity for CMV or HCV represent an independent risk marker for cardiovascular diseases (122, 123), while other studies could not confirm this observation (121). CMV and HCV are further associated with increased graft rejection rates, restenosis following coronary angioplasty and transplant vascular sclerosis, indicating that latent virus re-activates during immunosuppression and contributes to adverse effects (121).

In animal studies, CMV increased T-lymphocyte influx into plaques (124) and neutrophil extravasation, which is further supported in the presence of platelets (32). Influenza virus infection correlates with acute coronary syndromes and myocardial infarction (125), while influenza seropositivity and cardiovascular disease show no clear correlation (121). Animal experiments revealed that influenza triggers inflammatory and thrombotic responses in atherosclerotic plaques (126) and reverses the protective role of high-density lipoproteins (127). Both mechanisms are likely to modulate platelet function and reactivity.

There is some discrepancy regarding the strength of data regarding HSV infection and cardiovascular disease and despite its pro-inflammatory responses, a negative association between EBV infection and cardiovascular disease has been observed.

Moreover, the association between HIV infection and cardiovascular disease remains controversial and anti-retroviral therapy itself alters platelet function (128) and represents an independent risk factor for atherosclerosis (121). Further studies are warranted to evaluate the contribution of platelet–virus interactions in cardiovascular disease.

CONCLUSION

Platelets and their released products have been reported to directly and indirectly suppress infection but also to support virus persistence in response to certain viruses, making platelets a double-edged sword during viral infections. Platelets are involved in a variety of complications in response to viral infection but also fulfill a pivotal role in retaining adequate host responses. Thrombocytopenia is a common complication in several viral infections and viruses exert various strategies to mediate platelet decay. The question, if thrombocytopenia is a viral strategy to evade immune responses or if it also has host protecting functions, seems to depend on the virus variant and the underlying pathology. Further studies are warranted to fully understand the role of platelets in viral infections and to gain a clear understanding of the effects of anti-platelet therapies in viral infections. These studies will help us to predict the benefit or drawback of platelets and their inhibition during viral infections.

ACKNOWLEDGMENTS

The author would like to thank Dr. Mattias Forsell and Dr. Rona Strawbridge for critical comments on the manuscript and the Austrian Science Fund for financial support (FWF-P24978).

REFERENCES

1. Grigorian M, Hartenstein V. Hematopoiesis and hematopoietic organs in arthropods. *Dev Genes Evol* (2013) **223**:103–15. doi:10.1007/s00427-012-0428-2
2. Weyrich AS, Lindemann S, Zimmerman GA. The evolving role of platelets in inflammation. *J Thromb Haemost* (2003) **1**:1897–905. doi:10.1046/j.1538-7836.2003.00304.x
3. Engelmann B, Massberg S. Thrombosis as an intravascular effector of innate immunity. *Nat Rev Immunol* (2013) **13**:34–45. doi:10.1038/nri3345
4. von Hundelshausen P, Weber C. Platelets as immune cells: bridging inflammation and cardiovascular disease. *Circ Res* (2007) **100**:27–40. doi:10.1161/01.RES.0000252802.25497.b7
5. Morrell CN, Aggrey AA, Chapman LM, Modjeski KL. Emerging roles for platelets as immune and inflammatory cells. *Blood* (2014) **123**:2759–67. doi:10.1182/blood-2013-11-462432
6. Semple JW, Italiano JE Jr, Freedman J. Platelets and the immune continuum. *Nat Rev Immunol* (2011) **11**:264–74. doi:10.1038/nri2956
7. Weyrich AS, Zimmerman GA. Platelets: signaling cells in the immune continuum. *Trends Immunol* (2004) **25**:489–95. doi:10.1016/j.it.2004.07.003
8. Ribatti D, Crivellato E. Giulio Bizzozero and the discovery of platelets. *Leuk Res* (2007) **31**:1339–41. doi:10.1016/j.leukres.2007.02.008
9. Badimon L, Chesebro JH, Badimon JJ. Thrombus formation on ruptured atherosclerotic plaques and rethrombosis on evolving thrombi. *Circulation* (1992) **86**:III74–85.
10. Karshovska E, Weber C, von Hundelshausen P. Platelet chemokines in health and disease. *Thromb Haemost* (2013) **110**:894–902. doi:10.1160/TH13-04-0341
11. Rondina MT, Weyrich AS, Zimmerman GA. Platelets as cellular effectors of inflammation in vascular diseases. *Circ Res* (2013) **112**:1506–19. doi:10.1161/CIRCRESAHA.113.300512
12. Langer HF, Weber C, Gawaz M. The platelet – thrombosis and beyond. *Thromb Haemost* (2013) **110**:857–8. doi:10.1160/TH13-09-0805
13. Gay LJ, Felding-Habermann B. Contribution of platelets to tumour metastasis. *Nat Rev Cancer* (2011) **11**:123–34. doi:10.1038/nrc3004
14. Patel SR, Hartwig JH, Italiano JE Jr. The biogenesis of platelets from megakaryocyte proplatelets. *J Clin Invest* (2005) **115**:3348–54. doi:10.1172/JCI26891
15. Kuter DJ. Milestones in understanding platelet production: a historical overview. *Br J Haematol* (2014) **165**:248–58. doi:10.1111/bjh.12781
16. de Graaf CA, Metcalf D. Thrombopoietin and hematopoietic stem cells. *Cell Cycle* (2011) **10**:1582–9. doi:10.4161/cc.10.10.15619
17. Metcalf Pate KA, Lyons CE, Dorsey JL, Queen SE, Adams RJ, Morrell CN, et al. TGFbeta-mediated downregulation of thrombopoietin is associated with platelet decline in asymptomatic SIV infection. *J Acquir Immune Defic Syndr* (2014) **65**:510–6. doi:10.1097/QAI.0000000000000048
18. Isomura H, Yoshida M, Namba H, Fujiwara N, Ohuchi R, Uno F, et al. Suppressive effects of human herpesvirus-6 on thrombopoietin-inducible megakaryocytic colony formation in vitro. *J Gen Virol* (2000) **81**:663–73. doi:10.1002/(SICI)1096-9071(199708)52:4<406::AID-JMV11>3.0.CO;2-E
19. Gonelli A, Mirandola P, Grill V, Secchiero P, Zauli G. Human herpesvirus 7 infection impairs the survival/differentiation of megakaryocytic cells. *Haematologica* (2002) **87**:1223–5.
20. Afdhal N, McHutchison J, Brown R, Jacobson I, Manns M, Poordad F, et al. Thrombocytopenia associated with chronic liver disease. *J Hepatol* (2008) **48**:1000–7. doi:10.1016/j.jhep.2008.03.009
21. Kolb-Maurer A, Goebel W. Susceptibility of hematopoietic stem cells to pathogens: role in virus/bacteria tropism and pathogenesis. *FEMS Microbiol Lett* (2003) **226**:203–7. doi:10.1016/S0378-1097(03)00643-8
22. Chelucci C, Federico M, Guerriero R, Mattia G, Casella I, Pelosi E, et al. Productive human immunodeficiency virus-1 infection of purified megakaryocytic progenitors/precursors and maturing megakaryocytes. *Blood* (1998) **91**:1225–34.
23. Li X, Jeffers LJ, Garon C, Fischer ER, Scheffel J, Moore B, et al. Persistence of hepatitis C virus in a human megakaryoblastic leukaemia cell line. *J Viral Hepat* (1999) **6**:107–14. doi:10.1046/j.1365-2893.1999.00140.x
24. Crapnell K, Zanjani ED, Chaudhuri A, Ascensao JL, St Jeor S, Maciejewski JP. In vitro infection of megakaryocytes and their precursors by human cytomegalovirus. *Blood* (2000) **95**:487–93.
25. Flaujac C, Boukour S, Cramer-Borde E. Platelets and viruses: an ambivalent relationship. *Cell Mol Life Sci* (2010) **67**:545–56. doi:10.1007/s00018-009-0209-x
26. Yang M, Ng MH, Li CK. Thrombocytopenia in patients with severe acute respiratory syndrome (review). *Hematology* (2005) **10**:101–5. doi:10.1080/10245330400026170
27. Lutteke N, Raftery MJ, Lalwani P, Lee MH, Giese T, Voigt S, et al. Switch to high-level virus replication and HLA class I upregulation in differentiating megakaryocytic cells after infection with pathogenic hantavirus. *Virology* (2010) **405**:70–80. doi:10.1016/j.virol.2010.05.028
28. Passos AM, Treitinger A, Spada C. An overview of the mechanisms of HIV-related thrombocytopenia. *Acta Haematol* (2010) **124**:13–8. doi:10.1159/000313782
29. Carballal G, Cossio PM, Laguens RP, Ponzinibbio C, Oubina JR, Meckert PC, et al. Junin virus infection of guinea pigs: immunohistochemical and ultrastructural studies of hemopoietic tissue. *J Infect Dis* (1981) **143**:7–14. doi:10.1093/infdis/143.1.7

30. Gibellini D, Clo A, Morini S, Miserocchi A, Ponti C, Re MC. Effects of human immunodeficiency virus on the erythrocyte and megakaryocyte lineages. *World J Virol* (2013) **2**:91–101. doi:10.5501/wjv.v2.i2.91
31. Speth C, Loffler J, Krappmann S, Lass-Florl C, Rambach G. Platelets as immune cells in infectious diseases. *Future Microbiol* (2013) **8**:1431–51. doi:10.2217/fmb.13.104
32. Assinger A, Kral JB, Yaiw KC, Schrottmaier WC, Kurzejamska E, Wang Y, et al. Human cytomegalovirus-platelet interaction triggers toll-like receptor 2-dependent proinflammatory and proangiogenic responses. *Arterioscler Thromb Vasc Biol* (2014) **34**:801–9. doi:10.1161/ATVBAHA.114.303287
33. Koupenova M, Vitseva O, MacKay CR, Beaulieu LM, Benjamin EJ, Mick E, et al. Platelet-TLR7 mediates host survival and platelet count during viral infection in the absence of platelet-dependent thrombosis. *Blood* (2014) **124**:791–802. doi:10.1182/blood-2013-11-536003
34. Coulson BS, Londrigan SL, Lee DJ. Rotavirus contains integrin ligand sequences and a disintegrin-like domain that are implicated in virus entry into cells. *Proc Natl Acad Sci U S A* (1997) **94**:5389–94. doi:10.1073/pnas.94.10.5389
35. Mackow ER, Gavrilovskaya IN. Cellular receptors and hantavirus pathogenesis. *Curr Top Microbiol Immunol* (2001) **256**:91–115. doi:10.1007/978-3-642-56753-7_6
36. Gupalo E, Kuk C, Qadura M, Buriachkovskaia L, Othman M. Platelet-adenovirus vs. inert particles interaction: effect on aggregation and the role of platelet membrane receptors. *Platelets* (2013) **24**:383–91. doi:10.3109/09537104.2012.703792
37. Gavrilovskaya IN, Gorbunova EE, Mackow ER. Pathogenic hantaviruses direct the adherence of quiescent platelets to infected endothelial cells. *J Virol* (2010) **84**:4832–9. doi:10.1128/JVI.02405-09
38. Goeijenbier M, van Wissen M, van de Weg C, Jong E, Gerdes VE, Meijers JC, et al. Review: viral infections and mechanisms of thrombosis and bleeding. *J Med Virol* (2012) **84**:1680–96. doi:10.1002/jmv.23354
39. Coller BS, Shattil SJ. The GPIIb/IIIa (integrin alphaIIbbeta3) odyssey: a technology-driven saga of a receptor with twists, turns, and even a bend. *Blood* (2008) **112**:3011–25. doi:10.1182/blood-2008-06-077891
40. Moser M, Nieswandt B, Ussar S, Pozgajova M, Fassler R. Kindlin-3 is essential for integrin activation and platelet aggregation. *Nat Med* (2008) **14**:325–30. doi:10.1038/nm1722
41. Kahner BN, Kato H, Banno A, Ginsberg MH, Shattil SJ, Ye F. Kindlins, integrin activation and the regulation of talin recruitment to alphaIIbbeta3. *PLoS One* (2012) **7**:e34056. doi:10.1371/journal.pone.0034056
42. Gong H, Shen B, Flevaris P, Chow C, Lam SC, Voyno-Yasenetskaya TA, et al. G protein subunit Galpha13 binds to integrin alphaIIbbeta3 and mediates integrin "outside-in" signaling. *Science* (2010) **327**:340–3. doi:10.1126/science.1174779
43. Nunez D, Charriaut-Marlangue C, Barel M, Benveniste J, Frade R. Activation of human platelets through gp140, the C3d/EBV receptor (CR2). *Eur J Immunol* (1987) **17**:515–20. doi:10.1002/eji.1830170413
44. Chaipan C, Soilleux EJ, Simpson P, Hofmann H, Gramberg T, Marzi A, et al. DC-SIGN and CLEC-2 mediate human immunodeficiency virus type 1 capture by platelets. *J Virol* (2006) **80**:8951–60. doi:10.1128/JVI.00136-06
45. Othman M, Labelle A, Mazzetti I, Elbatarny HS, Lillicrap D. Adenovirus-induced thrombocytopenia: the role of von Willebrand factor and P-selectin in mediating accelerated platelet clearance. *Blood* (2007) **109**:2832–9. doi:10.1182/blood-2006-06-032524
46. Zahn A, Jennings N, Ouwehand WH, Allain JP. Hepatitis C virus interacts with human platelet glycoprotein VI. *J Gen Virol* (2006) **87**:2243–51. doi:10.1099/vir.0.81826-0
47. Maugeri N, Cattaneo M, Rovere-Querini P, Manfredi AA. Platelet clearance by circulating leukocytes: a rare event or a determinant of the "immune continuum"? *Platelets* (2014) **25**:224–5. doi:10.3109/09537104.2012.763116
48. Grozovsky R, Hoffmeister KM, Falet H. Novel clearance mechanisms of platelets. *Curr Opin Hematol* (2010) **17**:585–9. doi:10.1097/MOH.0b013e32833e7561
49. Yeaman MR. Platelets in defense against bacterial pathogens. *Cell Mol Life Sci* (2010) **67**:525–44. doi:10.1007/s00018-009-0210-4
50. Bouwman JJ, Visseren FL, Bosch MC, Bouter KP, Diepersloot RJ. Procoagulant and inflammatory response of virus-infected monocytes. *Eur J Clin Invest* (2002) **32**:759–66. doi:10.1046/j.1365-2362.2002.01041.x
51. Zapata JC, Cox D, Salvato MS. The role of platelets in the pathogenesis of viral hemorrhagic fevers. *PLoS Negl Trop Dis* (2014) **8**:e2858. doi:10.1371/journal.pntd.0002858
52. Alonzo MT, Lacuesta TL, Dimaano EM, Kurosu T, Suarez LA, Mapua CA, et al. Platelet apoptosis and apoptotic platelet clearance by macrophages in secondary dengue virus infections. *J Infect Dis* (2012) **205**:1321–9. doi:10.1093/infdis/jis180
53. Antoniak S, Mackman N. Multiple roles of the coagulation protease cascade during virus infection. *Blood* (2014) **123**:2605–13. doi:10.1182/blood-2013-09-526277
54. Anderson CL, Chacko GW, Osborne JM, Brandt JT. The Fc receptor for immunoglobulin G (Fc gamma RII) on human platelets. *Semin Thromb Hemost* (1995) **21**:1–9. doi:10.1055/s-2007-1000374
55. Cox D, Kerrigan SW, Watson SP. Platelets and the innate immune system: mechanisms of bacterial-induced platelet activation. *J Thromb Haemost* (2011) **9**:1097–107. doi:10.1111/j.1538-7836.2011.04264.x
56. Arman M, Krauel K, Tilley DO, Weber C, Cox D, Greinacher A, et al. Amplification of bacteria-induced platelet activation is triggered by FcgammaRIIA, integrin alphaIIbbeta3, and platelet factor 4. *Blood* (2014) **123**:3166–74. doi:10.1182/blood-2013-11-540526
57. Aster RH. Pooling of platelets in the spleen: role in the pathogenesis of "hypersplenic" thrombocytopenia. *J Clin Invest* (1966) **45**:645–57. doi:10.1172/JCI105380
58. Zhang W, Nardi MA, Borkowsky W, Li Z, Karpatkin S. Role of molecular mimicry of hepatitis C virus protein with platelet GPIIIa in hepatitis C-related immunologic thrombocytopenia. *Blood* (2009) **113**:4086–93. doi:10.1182/blood-2008-09-181073
59. Weksler BB. Review article: the pathophysiology of thrombocytopenia in hepatitis C virus infection and chronic liver disease. *Aliment Pharmacol Ther* (2007) **26**(Suppl 1):13–9. doi:10.1111/j.1365-2036.2007.03512.x
60. McHutchison JG, Dusheiko G, Shiffman ML, Rodriguez-Torres M, Sigal S, Bourliere M, et al. Eltrombopag for thrombocytopenia in patients with cirrhosis associated with hepatitis C. *N Engl J Med* (2007) **357**:2227–36. doi:10.1056/NEJMoa073255
61. Louache F, Bettaieb A, Henri A, Oksenhendler E, Farcet JP, Bierling P, et al. Infection of megakaryocytes by human immunodeficiency virus in seropositive patients with immune thrombocytopenic purpura. *Blood* (1991) **78**:1697–705.
62. Hottz ED, Oliveira MF, Nunes PC, Nogueira RM, Valls-de-Souza R, Da Poian AT, et al. Dengue induces platelet activation, mitochondrial dysfunction and cell death through mechanisms that involve DC-SIGN and caspases. *J Thromb Haemost* (2013) **11**:951–62. doi:10.1111/jth.12178
63. Hottz ED, Medeiros-de-Moraes IM, Vieira-de-Abreu A, de Assis EF, Valls-de-Souza R, Castro-Faria-Neto HC, et al. Platelet activation and apoptosis modulate monocyte inflammatory responses in dengue. *J Immunol* (2014) **193**:1864–72. doi:10.4049/jimmunol.1400091
64. Sun DS, King CC, Huang HS, Shih YL, Lee CC, Tsai WJ, et al. Antiplatelet autoantibodies elicited by dengue virus non-structural protein 1 cause thrombocytopenia and mortality in mice. *J Thromb Haemost* (2007) **5**:2291–9. doi:10.1111/j.1538-7836.2007.02754.x
65. Hottz ED, Lopes JF, Freitas C, Valls-de-Souza R, Oliveira MF, Bozza MT, et al. Platelets mediate increased endothelium permeability in dengue through NLRP3-inflammasome activation. *Blood* (2013) **122**:3405–14. doi:10.1182/blood-2013-05-504449
66. Chuansumrit A, Chaiyaratana W. Hemostatic derangement in dengue hemorrhagic fever. *Thromb Res* (2014) **133**:10–6. doi:10.1016/j.thromres.2013.09.028
67. Nascimento EJ, Hottz ED, Garcia-Bates TM, Bozza F, Marques ET Jr, Barratt-Boyes SM. Emerging concepts in dengue pathogenesis: interplay between plasmablasts, platelets, and complement in triggering vasculopathy. *Crit Rev Immunol* (2014) **34**:227–40. doi:10.1615/CritRevImmunol.2014010212
68. Iannacone M, Sitia G, Isogawa M, Whitmire JK, Marchese P, Chisari FV, et al. Platelets prevent IFN-alpha/beta-induced lethal hemorrhage promoting CTL-dependent clearance of lymphocytic choriomeningitis virus. *Proc Natl Acad Sci U S A* (2008) **105**:629–34. doi:10.1073/pnas.0711200105
69. Pozner RG, Ure AE, Jaquenod de Giusti C, D'Atri LP, Italiano JE, Torres O, et al. Junin virus infection of human hematopoietic progenitors impairs in vitro proplatelet formation and platelet release via a bystander effect involving type I IFN signaling. *PLoS Pathog* (2010) **6**:e1000847. doi:10.1371/journal.ppat.1000847

70. Wang Q, Miyakawa Y, Fox N, Kaushansky K. Interferon-alpha directly represses megakaryopoiesis by inhibiting thrombopoietin-induced signaling through induction of SOCS-1. *Blood* (2000) **96**:2093–9.
71. Sorensen AL, Rumjantseva V, Nayeb-Hashemi S, Clausen H, Hartwig JH, Wandall HH, et al. Role of sialic acid for platelet life span: exposure of beta-galactose results in the rapid clearance of platelets from the circulation by asialoglycoprotein receptor-expressing liver macrophages and hepatocytes. *Blood* (2009) **114**:1645–54. doi:10.1182/blood-2009-01-199414
72. Terada H, Baldini M, Ebbe S, Madoff MA. Interaction of influenza virus with blood platelets. *Blood* (1966) **28**:213–28.
73. Stenberg PE, Levin J, Baker G, Mok Y, Corash L. Neuraminidase-induced thrombocytopenia in mice: effects on thrombopoiesis. *J Cell Physiol* (1991) **147**:7–16. doi:10.1002/jcp.1041470103
74. Turpie AG, Chernesky MA, Larke RP, Packham MA, Mustard JF. Effect of Newcastle disease virus on human or rabbit platelets. Aggregation and loss of constituents. *Lab Invest* (1973) **28**:575–83.
75. Srivastava A, Bruno E, Briddell R, Cooper R, Srivastava C, van Besien K, et al. Parvovirus B19-induced perturbation of human megakaryocytopoiesis in vitro. *Blood* (1990) **76**:1997–2004.
76. Metcalf Pate KA, Lyons CE, Dorsey JL, Shirk EN, Queen SE, Adams RJ, et al. Platelet activation and platelet-monocyte aggregate formation contribute to decreased platelet count during acute simian immunodeficiency virus infection in pig-tailed macaques. *J Infect Dis* (2013) **208**:874–83. doi:10.1093/infdis/jit278
77. Singh MV, Davidson DC, Kiebala M, Maggirwar SB. Detection of circulating platelet-monocyte complexes in persons infected with human immunodeficiency virus type-1. *J Virol Methods* (2012) **181**:170–6. doi:10.1016/j.jviromet.2012.02.005
78. La Russa VF, Innis BL. Mechanisms of dengue virus-induced bone marrow suppression. *Baillieres Clin Haematol* (1995) **8**:249–70. doi:10.1016/S0950-3536(05)80240-9
79. Yeaman MR. Platelets: at the nexus of antimicrobial defence. *Nat Rev Microbiol* (2014) **12**:426–37. doi:10.1038/nrmicro3269
80. Yeaman MR. The role of platelets in antimicrobial host defense. *Clin Infect Dis* (1997) **25**:951–68. doi:10.1086/516120
81. Auerbach DJ, Lin Y, Miao H, Cimbro R, Difiore MJ, Gianolini ME, et al. Identification of the platelet-derived chemokine CXCL4/PF-4 as a broad-spectrum HIV-1 inhibitor. *Proc Natl Acad Sci U S A* (2012) **109**:9569–74. doi:10.1073/pnas.1207314109
82. Solomon Tsegaye T, Gnirss K, Rahe-Meyer N, Kiene M, Kramer-Kuhl A, Behrens G, et al. Platelet activation suppresses HIV-1 infection of T cells. *Retrovirology* (2013) **10**:48. doi:10.1186/1742-4690-10-48
83. Klinger MH, Wilhelm D, Bubel S, Sticherling M, Schroder JM, Kuhnel W. Immunocytochemical localization of the chemokines RANTES and MIP-1 alpha within human platelets and their release during storage. *Int Arch Allergy Immunol* (1995) **107**:541–6. doi:10.1159/000237097
84. Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC, Lusso P. Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. *Science* (1995) **270**:1811–5. doi:10.1126/science.270.5243.1811
85. Cognasse F, Hamzeh-Cognasse H, Berthet J, Damien P, Lucht F, Pozzetto B, et al. Altered release of regulated upon activation, normal T-cell expressed and secreted protein from human, normal platelets: contribution of distinct HIV-1MN gp41 peptides. *AIDS* (2009) **23**:2057–9. doi:10.1097/QAD.0b013e328330da65
86. Melchjorsen J, Sorensen LN, Paludan SR. Expression and function of chemokines during viral infections: from molecular mechanisms to in vivo function. *J Leukoc Biol* (2003) **74**:331–43. doi:10.1189/jlb.1102577
87. Culley FJ, Pennycook AM, Tregoning JS, Dodd JS, Walzl G, Wells TN, et al. Role of CCL5 (RANTES) in viral lung disease. *J Virol* (2006) **80**:8151–7. doi:10.1128/JVI.00496-06
88. Wareing MD, Lyon AB, Lu B, Gerard C, Sarawar SR. Chemokine expression during the development and resolution of a pulmonary leukocyte response to influenza A virus infection in mice. *J Leukoc Biol* (2004) **76**:886–95. doi:10.1189/jlb.1203644
89. Tyner JW, Uchida O, Kajiwara N, Kim EY, Patel AC, O'Sullivan MP, et al. CCL5-CCR5 interaction provides antiapoptotic signals for macrophage survival during viral infection. *Nat Med* (2005) **11**:1180–7. doi:10.1038/nm1303
90. Katsounas A, Schlaak JF, Lempicki RA. CCL5: a double-edged sword in host defense against the hepatitis C virus. *Int Rev Immunol* (2011) **30**:366–78. doi:10.3109/08830185.2011.593105
91. Wilson SS, Wiens ME, Smith JG. Antiviral mechanisms of human defensins. *J Mol Biol* (2013) **425**:4965–80. doi:10.1016/j.jmb.2013.09.038
92. Mohan KV, Rao SS, Atreya CD. Antiviral activity of selected antimicrobial peptides against vaccinia virus. *Antiviral Res* (2010) **86**:306–11. doi:10.1016/j.antiviral.2010.03.012
93. Lesurtel M, Graf R, Aleil B, Walther DJ, Tian Y, Jochum W, et al. Platelet-derived serotonin mediates liver regeneration. *Science* (2006) **312**:104–7. doi:10.1126/science.1123842
94. Starlinger P, Assinger A, Haegele S, Wanek D, Zikeli S, Schauer D, et al. Evidence for serotonin as a relevant inducer of liver regeneration after liver resection in humans. *Hepatology* (2014) **60**:257–66. doi:10.1002/hep.26950
95. Leon-Ponte M, Ahern GP, O'Connell PJ. Serotonin provides an accessory signal to enhance T-cell activation by signaling through the 5-HT7 receptor. *Blood* (2007) **109**:3139–46. doi:10.1182/blood-2006-10-052787
96. Polgar J, Matuskova J, Wagner DD. The P-selectin, tissue factor, coagulation triad. *J Thromb Haemost* (2005) **3**:1590–6. doi:10.1111/j.1538-7836.2005.01373.x
97. Langer HF, Daub K, Braun G, Schonberger T, May AE, Schaller M, et al. Platelets recruit human dendritic cells via Mac-1/JAM-C interaction and modulate dendritic cell function in vitro. *Arterioscler Thromb Vasc Biol* (2007) **27**:1463–70. doi:10.1161/ATVBAHA.107.141515
98. Hagihara M, Higuchi A, Tamura N, Ueda Y, Hirabayashi K, Ikeda Y, et al. Platelets, after exposure to a high shear stress, induce IL-10-producing, mature dendritic cells in vitro. *J Immunol* (2004) **172**:5297–303. doi:10.4049/jimmunol.172.9.5297
99. Elzey BD, Sprague DL, Ratliff TL. The emerging role of platelets in adaptive immunity. *Cell Immunol* (2005) **238**:1–9. doi:10.1016/j.cellimm.2005.12.005
100. Li N. Platelet-lymphocyte cross-talk. *J Leukoc Biol* (2008) **83**:1069–78. doi:10.1189/jlb.0907615
101. Gerdes N, Zhu L, Ersoy M, Hermansson A, Hjemdahl P, Hu H, et al. Platelets regulate CD4(+) T-cell differentiation via multiple chemokines in humans. *Thromb Haemost* (2011) **106**:353–62. doi:10.1160/TH11-01-0020
102. Liu CY, Battaglia M, Lee SH, Sun QH, Aster RH, Visentin GP. Platelet factor 4 differentially modulates CD4+CD25+ (regulatory) versus CD4+CD25- (non-regulatory) T cells. *J Immunol* (2005) **174**:2680–6. doi:10.4049/jimmunol.174.5.2680
103. Shi G, Field DJ, Ko KA, Ture S, Srivastava K, Levy S, et al. Platelet factor 4 limits Th17 differentiation and cardiac allograft rejection. *J Clin Invest* (2014) **124**:543–52. doi:10.1172/JCI71858
104. Elzey BD, Grant JF, Sinn HW, Nieswandt B, Waldschmidt TJ, Ratliff TL. Cooperation between platelet-derived CD154 and CD4+ T cells for enhanced germinal center formation. *J Leukoc Biol* (2005) **78**:80–4. doi:10.1189/jlb.1104669
105. Elzey BD, Tian J, Jensen RJ, Swanson AK, Lees JR, Lentz SR, et al. Platelet-mediated modulation of adaptive immunity. A communication link between innate and adaptive immune compartments. *Immunity* (2003) **19**:9–19. doi:10.1016/S1074-7613(03)00177-8
106. Danese S, de la Motte C, Reyes BM, Sans M, Levine AD, Fiocchi C. Cutting edge: T cells trigger CD40-dependent platelet activation and granular RANTES release: a novel pathway for immune response amplification. *J Immunol* (2004) **172**:2011–5. doi:10.4049/jimmunol.172.4.2011
107. Iannacone M, Sitia G, Isogawa M, Marchese P, Castro MG, Lowenstein PR, et al. Platelets mediate cytotoxic T lymphocyte-induced liver damage. *Nat Med* (2005) **11**:1167–9. doi:10.1038/nm1317
108. Iannacone M, Sitia G, Ruggeri ZM, Guidotti LG. HBV pathogenesis in animal models: recent advances on the role of platelets. *J Hepatol* (2007) **46**:719–26. doi:10.1016/j.jhep.2007.01.007
109. Loria GD, Romagnoli PA, Moseley NB, Rucavado A, Altman JD. Platelets support a protective immune response to LCMV by preventing splenic necrosis. *Blood* (2013) **121**:940–50. doi:10.1182/blood-2011-08-376822
110. Schwartzkopff F, Grimm TA, Lankford CS, Fields K, Wang J, Brandt E, et al. Platelet factor 4 (CXCL4) facilitates human macrophage infection with HIV-1 and potentiates virus replication. *Innate Immun* (2009) **15**:368–79. doi:10.1177/1753425909106171
111. Zaldivar MM, Pauels K, von Hundelshausen P, Berres ML, Schmitz P, Bornemann J, et al. CXC chemokine ligand 4 (Cxcl4) is a platelet-derived mediator

of experimental liver fibrosis. *Hepatology* (2010) **51**:1345–53. doi:10.1002/hep.23435
112. Lang PA, Contaldo C, Georgiev P, El-Badry AM, Recher M, Kurrer M, et al. Lang, aggravation of viral hepatitis by platelet-derived serotonin. *Nat Med* (2008) **14**:756–61. doi:10.1038/nm1780
113. Villinger F, Rollin PE, Brar SS, Chikkala NF, Winter J, Sundstrom JB, et al. Markedly elevated levels of interferon (IFN)-gamma, IFN-alpha, interleukin (IL)-2, IL-10, and tumor necrosis factor-alpha associated with fatal Ebola virus infection. *J Infect Dis* (1999) **179**(Suppl 1):S188–91. doi:10.1086/514283
114. Geisbert TW, Hensley LE, Jahrling PB, Larsen T, Geisbert JB, Paragas J, et al. Treatment of Ebola virus infection with a recombinant inhibitor of factor VIIa/tissue factor: a study in rhesus monkeys. *Lancet* (2003) **362**:1953–8. doi:10.1016/S0140-6736(03)15012-X
115. Cummins D, Fisher-Hoch SP, Walshe KJ, Mackie IJ, McCormick JB, Bennett D, et al. A plasma inhibitor of platelet aggregation in patients with Lassa fever. *Br J Haematol* (1989) **72**:543–8. doi:10.1111/j.1365-2141.1989.tb04321.x
116. Michels M, Alisjahbana B, De Groot PG, Indrati AR, Fijnheer R, Puspita M, et al. Platelet function alterations in dengue are associated with plasma leakage. *Thromb Haemost* (2014) **112**:352–62. doi:10.1160/TH14-01-0056
117. Rondina MT, Brewster B, Grissom CK, Zimmerman GA, Kastendieck DH, Harris ES, et al. In vivo platelet activation in critically ill patients with primary 2009 influenza A(H1N1). *Chest* (2012) **141**:1490–5. doi:10.1378/chest.11-2860
118. Bunce PE, High SM, Nadjafi M, Stanley K, Liles WC, Christian MD. Pandemic H1N1 influenza infection and vascular thrombosis. *Clin Infect Dis* (2011) **52**:e14–7. doi:10.1093/cid/ciq125
119. Starko KM. Salicylates and pandemic influenza mortality, 1918-1919 pharmacology, pathology, and historic evidence. *Clin Infect Dis* (2009) **49**:1405–10. doi:10.1086/606060
120. Tufano A, Di Capua M, Coppola A, Conca P, Cimino E, Cerbone AM, et al. The infectious burden in atherothrombosis. *Semin Thromb Hemost* (2012) **38**:515–23. doi:10.1055/s-0032-1315759
121. Rosenfeld ME, Campbell LA. Pathogens and atherosclerosis: update on the potential contribution of multiple infectious organisms to the pathogenesis of atherosclerosis. *Thromb Haemost* (2011) **106**:858–67. doi:10.1160/TH11-06-0392
122. Haji SA, Starling RC, Avery RK, Mawhorter S, Tuzcu EM, Schoenhagen P, et al. Donor hepatitis-C seropositivity is an independent risk factor for the development of accelerated coronary vasculopathy and predicts outcome after cardiac transplantation. *J Heart Lung Transplant* (2004) **23**:277–83. doi:10.1016/S1053-2498(03)00148-7
123. Simanek AM, Dowd JB, Pawelec G, Melzer D, Dutta A, Aiello AE. Seropositivity to cytomegalovirus, inflammation, all-cause and cardiovascular disease-related mortality in the United States. *PLoS One* (2011) **6**:e16103. doi:10.1371/journal.pone.0016103
124. Vliegen I, Stassen F, Grauls G, Blok R, Bruggeman C. MCMV infection increases early T-lymphocyte influx in atherosclerotic lesions in apoE knockout mice. *J Clin Virol* (2002) **25**(Suppl 2):S159–71. doi:10.1016/S1386-6532(02)00095-1
125. Warren-Gash C, Smeeth L, Hayward AC. Influenza as a trigger for acute myocardial infarction or death from cardiovascular disease: a systematic review. *Lancet Infect Dis* (2009) **9**:601–10. doi:10.1016/S1473-3099(09)70233-6
126. Naghavi M, Wyde P, Litovsky S, Madjid M, Akhtar A, Naguib S, et al. Influenza infection exerts prominent inflammatory and thrombotic effects on the atherosclerotic plaques of apolipoprotein E-deficient mice. *Circulation* (2003) **107**:762–8. doi:10.1161/01.CIR.0000048190.68071.2B
127. Van Lenten BJ, Wagner AC, Nayak DP, Hama S, Navab M, Fogelman AM. High-density lipoprotein loses its anti-inflammatory properties during acute influenza a infection. *Circulation* (2001) **103**:2283–8. doi:10.1161/01.CIR.103.18.2283
128. Damien P, Cognasse F, Lucht F, Suy F, Pozzetto B, Garraud O, et al. Highly active antiretroviral therapy alters inflammation linked to platelet cytokines in HIV-1-infected patients. *J Infect Dis* (2013) **208**:868–70. doi:10.1093/infdis/jit260

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 27 October 2014; paper pending published: 24 November 2014; accepted: 05 December 2014; published online: 18 December 2014.
Citation: Assinger A (2014) Platelets and infection – an emerging role of platelets in viral infection. Front. Immunol. 5:649. doi: 10.3389/fimmu.2014.00649
This article was submitted to Inflammation, a section of the journal Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 26 February 2015
doi: 10.3389/fimmu.2015.00082

Platelets and infections – complex interactions with bacteria

Hind Hamzeh-Cognasse[1], Pauline Damien[1], Adrien Chabert[1], Bruno Pozzetto[1], Fabrice Cognasse[1,2] and Olivier Garraud[1,3]*

[1] *GIMAP-EA3064, Université de Lyon, Saint-Etienne, France*
[2] *Etablissement Français du Sang Auvergne-Loire, Saint-Etienne, France*
[3] *Institut National de la Transfusion Sanguine, Paris, France*

Edited by:
Claudia Monaco, Catholic University of Rome, Italy; Imperial College London, UK

Reviewed by:
Kottarappat N. Dileepan, The University of Kansas Medical Center, USA
Kai Fang, University of California Los Angeles, USA

***Correspondence:**
Olivier Garraud, Faculty of Medicine, GIMAP-EA3064, 15 rue Ambroise Pare, Saint-Etienne Cedex 2 42023, France
e-mail: olivier.garraud@univ-st-etienne.fr

Platelets can be considered sentinels of vascular system due to their high number in the circulation and to the range of functional immunoreceptors they express. Platelets express a wide range of potential bacterial receptors, including complement receptors, FcγRII, Toll-like receptors but also integrins conventionally described in the hemostatic response, such as GPIIb–IIIa or GPIb. Bacteria bind these receptors either directly, or indirectly via fibrinogen, fibronectin, the first complement C1q, the von Willebrand Factor, etc. The fate of platelet-bound bacteria is questioned. Several studies reported the ability of activated platelets to internalize bacteria such as *Staphylococcus aureus* or *Porphyromonas gingivalis*, though there is no clue on what happens thereafter. Are they sheltered from the immune system in the cytoplasm of platelets or are they lysed? Indeed, while the presence of phagolysosome has not been demonstrated in platelets, they contain antimicrobial peptides that were shown to be efficient on *S. aureus*. Besides, the fact that bacteria can bind to platelets via receptors involved in hemostasis suggests that they may induce aggregation; this has indeed been described for *Streptococcus sanguinis*, *S. epidermidis*, or *C. pneumoniae*. On the other hand, platelets are able to display an inflammatory response to an infectious triggering. We, and others, have shown that platelet release soluble immunomodulatory factors upon stimulation by bacterial components. Moreover, interactions between bacteria and platelets are not limited to only these two partners. Indeed, platelets are also essential for the formation of neutrophil extracellular traps by neutrophils, resulting in bacterial clearance by trapping bacteria and concentrating antibacterial factors but in enhancing thrombosis. In conclusion, the platelet–bacteria interplay is a complex game; its fine analysis is complicated by the fact that the inflammatory component adds to the aggregation response.

Keywords: platelet, inflammation, adhesion, infection, cytokine, chemokine, sepsis

INTRODUCTION

The molecular make-up of platelets, which are specialized in repair and in innate immunity (1–4), makes these "cells" unique blood elements. The recognition of platelets as cells is still controversial, primarily due to their lack of a nucleus. However, the multitude of functions that have recently been attributed to them and that are presented in this manuscript support our decision to consider them as such throughout these studies. The platelet response, which was once believed to only be involved in hemostasis, is in fact extremely complex and probably adapts when required. In this review, we will address the inflammatory potential of platelets when confronted with pathogenic invasion, and more specifically when it involves bacteria or viruses. We will focus on their ability to directly trigger an immune response, ranging from recognition of the pathogen to the orchestration of its elimination.

Abbreviations: Bcl, B-cell lymphoma; C1q, first complement component; CD40L, CD40Ligand; Clf, clumping factor; CTAP3, connective tissue activating protein-3; DIC, disseminated intravascular coagulation; *E. coli*, *Escherichia coli*; Fas, apoptosis stimulating fragment; Fnbp, fibronectin-binding protein; gC1q-R, gC1q receptor; Gsp, glycosylated streptococcal protein; hBD-1, human beta-defensin-1; IE, infectious endocarditis; Ig, immunoglobulin; IL, interleukin; Isd, iron-regulated surface determinant; ITAM, immunoreceptor tyrosine-based activation motif; LPS, lipopolysaccharide; Mac-1, macrophage-1 antigen; mRNA, messenger RNA; MSCRAMM, microbial surface components recognizing adhesive matrix molecules; NET, neutrophil extracellular trap; NO, nitric oxide; NOS, nitric oxide synthase; OCS, open canalicular system; *P. gingivalis*, *Porphyromonas gingivalis*; PAF, platelet-activating factor; PAFR, platelet-activating factor receptor; PAR, protease-activated receptor; PF4, platelet factor-4; PMP, platelet microparticles; PmP, platelet microbicidal proteins; PS, phosphatidylserines; RANTES, regulated on activation, normal T cell expressed and secreted; ROS, reactive oxygen species; *S. aureus*, *Staphylococcus aureus*; *S. epidermidis*, *Staphylococcus epidermidis*; *S. gordonii*, *Streptococcus gordonii*; *S. pyogenes*, *Streptococcus pyogenes*; *S. sanguinis*, *Streptococcus sanguinis*; sCD40L, soluble CD40L; Sdr, serine–aspartate dipeptide repeat; SEB, staphylococcal enterotoxin B; SrpA, serine-rich protein A; SSL, staphylococcal superantigen-like; TLR, Toll-like receptor; TNF, tumor necrosis factor; Tx, thromboxane; vWF, von Willebrand factor; $\Delta\Psi m$, mitochondrial membrane potential.

PLATELETS AND BACTERIAL INFECTIONS

PLATELETS AT THE INTERFACE BETWEEN BACTERIAL INFECTION AND THROMBOSIS

Example of infectious endocarditis

Cardiovascular diseases, although varied, may have infectious origins, as was described by Beynon et al. concerning infectious endocarditis (IE) (5). The main bacterial agents involved in IE are *Staphylococcus aureus*, *Streptococcus sanguinis*, and *Streptococcus gordonii (S. gordonii)*. According to epidemiological studies, bacteremia that leads to the development of this disease may be the consequence of a local intervention, but there may be a more distant origin such as the recurrent administration of a drug or a surgical dental procedure.

By creating an inflammatory environment, bacteria adhere to the valvular endothelium and increase its permeability, thus leading to the exposure of subendothelial tissue factors. The circulating platelets then adhere to the subendothelium, and their hemostatic activation causes the formation of a thrombus, which can then lead to arterial ischemia, and even pulmonary embolism (6). IE is therefore a disease that links inflammation and hemostasis, though it was long accepted that platelet activation occurred indirectly.

In IE, analysis of a newly formed thrombus in the myocardium showed the presence of bacteria inside the platelet clot (7). The first hypotheses suggested that this bacterial presence stabilized the platelet clot due to the activity of bacterial enzymes specialized in coagulation [coagulase for *S. aureus* (8) or the "clumping factor" (Clf) for the other staphylococci (9)], although without direct participation in platelet activation.

In sepsis, microthrombi form in the blood capillaries (10). As with IE, this phenomenon was attributed to the inflammatory environment that promoted platelet aggregation. The study by Osterud et al. also supports this hypothesis, since the authors show that in severe sepsis, the circulating monocytes show an increased expression of tissue factors, which thus support platelet aggregation (11).

Beginning in 2005, publications describing the role of platelets in immunity put prior observations related to the direct interaction of platelets and bacteria back in the spotlight (12). They then suggested that the binding of bacteria to platelets should even be considered a factor in the immune response. Today, the growing number of studies based on the inflammatory potential of platelets show that these cells express a variety of receptors, soluble molecules, and signaling factors (both hemostatic and inflammatory), enabling them to secure their position as direct effectors of antibacterial defense. This function is presented in the following sections.

Inflammatory and thrombotic role of platelet microparticles

Platelets also form the link between thrombosis and inflammation through the production of microparticles. Platelet microparticles (PMP) are phospholipid vesicles (100–1000 nm) that are released after budding from the platelet plasma membrane. As a result, PMP express the same antigens as their parent cells, i.e., GPIIb–IIIa, GPIb, CD31, CD61, and CD62P. This distinguishes them from microparticles derived from other cell types (red blood cells, leukocytes, monocytes, endothelial cells). PMP thus make up between 70 and 90% of the circulating vesicles. PMP differ from exosomes by their size, but also due to the fact that they are not derived from exocytosis of multivesicular bodies (13).

Platelet microparticles are released by the activated platelets in apoptosis or senescence. A central factor in the induction of this event is the detachment of the actin cytoskeleton from the plasma membrane, which occurs primarily through the increase in intracellular calcium concentration. The calcium then interacts directly with the proteins involved in proteolysis of the cytoskeleton, such as calpain (14, 15). The formation of PMP may also occur independent of calcium, in which case it involves the C5B–9 complement factor and activation of protein kinases, such as calmodulin (16).

Bacterial infection also appears to be a source of PMP formation. This has already been well described during the involvement of platelet Toll-like receptor (TLR) 4 (17–19) but also in response to the Shiga toxin (20).

Platelet microparticle formation results in an asymmetrical distribution of the membrane phospholipids. The circulating PMP thus express phosphatidylserines (PS), which are highly procoagulant phospholipids, on their surface (21). PMP also express tissue factor, the major initiator of the coagulation cascade (22–24). PMP have also been described as a surface that enables the *in vitro* generation of plasmin; this ability was not found in microparticles isolated from endothelial cells (25).

Platelet microparticle are also capable of issuing immunomodulatory factors, such as regulated on activation, normal T cell expressed and secreted (RANTES) (26), interleukin (IL)-1β (18), and CD40Ligand (CD40L) (27), and can also modulate the activation of inflammatory cells such as neutrophils (28, 29). PMP even seem to be able to exert their pro-inflammatory activity outside of the blood compartment (30). The pro-inflammatory function of PMP is referred to in greater detail throughout this manuscript.

Finally, the proportion of PMP in the circulation is increased in some illnesses, such as cardiovascular disease (22), sepsis (31), or HIV infection (32), which suggests they may be involved in the pathophysiology of these diseases.

MECHANISMS OF INTERACTION BETWEEN PLATELETS AND BACTERIA

Three mechanisms of interactions between bacteria and platelets have been described to date: (1) the indirect binding of bacteria to a plasma protein, which itself is a ligand of a platelet receptor; (2) the direct binding of bacteria to platelet receptors; and (3) the binding of secreted bacterial products, particularly toxins, to platelets. The mechanisms of interaction are made more complex by the diversity of platelet receptors involved in bacterial recognition.

Role of glycoprotein IIb–IIIa

In addition to ensuring their usual function in hemostasis, the platelet glycoproteins play a role in the adhesion to bacteria. The first platelet receptor identified as such was GPIIb–IIIa. This integrin, specifically from the megakaryocyte cell line, is the receptor for fibrinogen. Its involvement results in adhesion and platelet aggregation (33, 34).

Staphylococci express surface receptors that are specific for fibrinogen and fibronectin (**Figure 1**). These are surface proteins characterized by regions rich with serine–aspartate repeats,

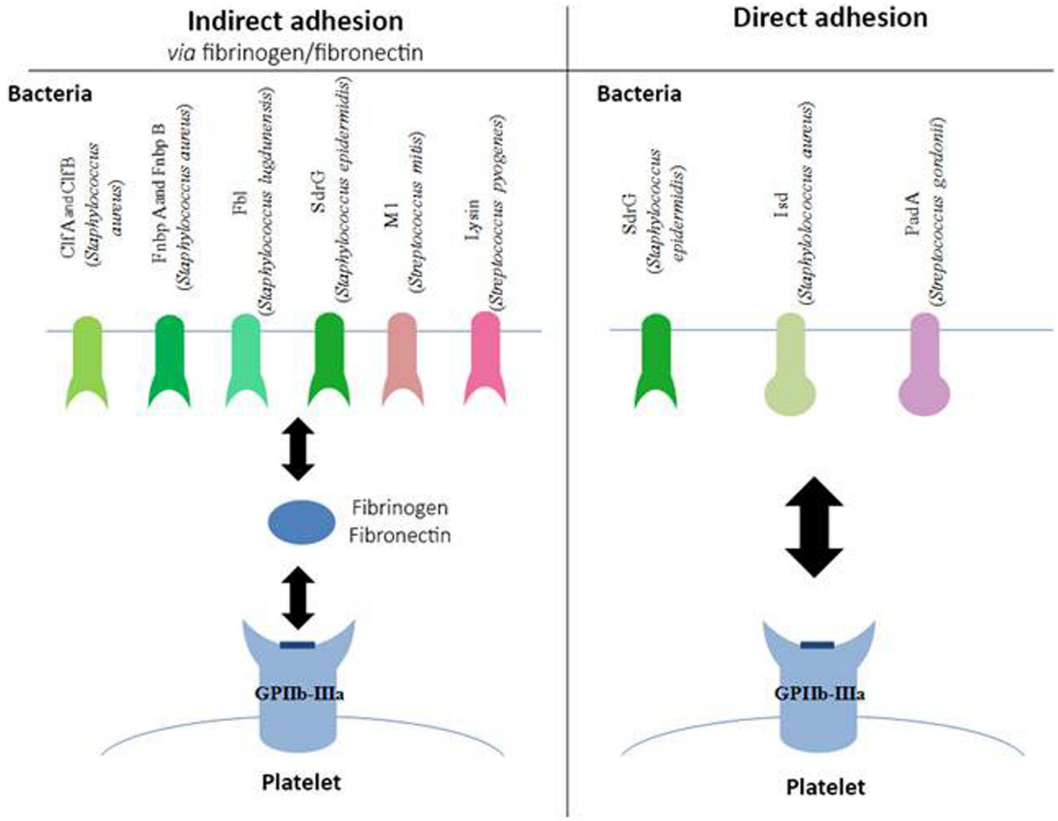


FIGURE 1 | Involvement of GPIIb–IIIa in the adhesion of bacteria to platelets. Schematic representation of different bacterial components, which bind to the platelet GPIIb–IIIa either indirectly via fibrinogen or fibronectin (left side of the diagram) or directly (right side of the diagram). Clf, clumping factor; Fnbp, fibronectin-binding protein; SdrG, serine–aspartate repeat protein; Isd, iron-regulated surface determinant; Pad, platelet adherence protein.

belonging to the microbial surface components recognizing adhesive matrix molecules (MSCRAMM) family (35). These molecules enable bacteria to adhere to tissues, a critical step in the establishment of infection. Some of the most common examples of MSCRAMM are found in *S. aureus*: ClfA (36), ClfB (37), fibronectin-binding protein (Fnbp) A, and FnbpB (38). *Staphylococcus lugdunensis* binds to fibrinogen via its Fbl protein, which is 58% identical to ClfA (39).

The binding of *Staphylococcus epidermidis* was long unknown, even though it had already been shown that trypsin treatment in a culture of *S. epidermidis* prevented its adhesion to platelets (40), suggesting the involvement of a membrane factor. In 2009, Brennan et al. showed that serine–aspartate dipeptide repeat (Sdr) G proteins expressed on the surface of *S. epidermidis* are necessary for the adhesion of bacteria to platelets via fibrinogen (41).

Even though these various MSCRAM are highly similar proteins, they bind to fibrinogen via different binding sites. ClfA and Fbl, as well as FnbpA and B, bind to the C-terminal region of the fibrinogen γ-chain. ClfB has its binding site on the C-terminal region of the fibrinogen α-chain, and SdrG on the β-chain. Other types of bacteria can also bind to fibrinogen, particularly *Streptococcus pyogenes (S. pyogenes)* via the M1 protein, and *Streptococcus mitis* via the enzyme, lysine (42).

More recently, bacteria have been described that also express surface proteins, enabling them to bind directly to GPIIb–IIIa, independent of fibrinogen (**Figure 1**). Such is the case for SdrG from *S. epidermidis*, which in addition to binding fibrinogen, can also directly target the platelet glycoprotein (41).

In vivo, *S. aureus* must find a source of iron that will enable it to grow and ensure its pathogenicity. To do so, it expresses iron-regulated surface determinant (Isd) proteins that are capable of binding the heme from hemoglobin and internalizing it. Yet, it has been shown that IsdB in particular can bind to GPIIb–IIIa in the absence of plasma protein. This adhesion is inhibited in the presence of platelets that have been pre-incubated with anti-GPIIb–IIIa antibodies, and in bacterial strains mutated for IsdB, confirming the specificity of the binding (43). *S. gordonii* also expresses a platelet adherence factor that has been recently described, platelet adherence protein A (PadA), and for which no other known function has been found to date (44).

The binding site(s) involved with GPIIb–IIIa have still not been mapped; however the use of peptides mimicking the arginine–glycine–aspartic acid chain, the ligand usually described for the involvement of the glycoprotein in hemostatic conditions, prevents the direct attachment of bacteria on platelets (42). This

observation suggests that the bond may be the same type as with fibrinogen.

Role of glycoprotein Ibα

GPIbα is a membrane glycoprotein and is also only found in the megakaryocyte cell line. It belongs to the family of leucine-rich repeat proteins. It is capable of binding several ligands and is essential in primary hemostasis through its high affinity with von Willebrand factor (vWF). It is important to remember that GPIbα is found as a complex with GPIb β, GPIX, and GPV at a ratio of 2:2:2:1 (34).

It has been shown that several species of *Streptococcus* are able to bind directly to GPIbα (**Figure 2**). This interaction involves a family of highly glycosylated, serine-rich bacterial proteins. This family includes serine-rich protein A (SrpA) from *S. sanguinis* (45), as well as glycosylated streptococcal protein B (GspB) and hemagglutinin salivary antigen (Hsa) from *S. gordonii* (46). These bacterial proteins, which are highly similar, bind to the sialic acids of the host's receptors. The staphylococcal accessory regulator (Sar) P protein expressed by *S. aureus* also allows adhesion to platelets (47). The fact that SrpA and GspB are molecularly very close has led to the hypothesis that the SrpA–platelet bond could involve GPIbα.

Bacterial proteins are capable of binding to vWF, although they are fewer than those binding to fibrinogen (**Figure 2**). It has been shown that protein A from *S. aureus* is capable of binding to vWF (48–50), which in turn interacts with GPIb β. The same applies to a surface protein of *Helicobacter pylori*, though it has still not been completely characterized. This study on the platelet–bacteria interaction involving vWF shows that if vWF is already bound to bacteria, it does not require shear forces to adhere to GPIb β (51).

Complement receptors

The literature describes platelets' ability to interact with the complement system. This is mainly observed in activated platelets, thereby allowing their clearance, but also in platelets in a pathological environment (**Figure 3**). For example, C5b–9 is found at detectable levels on the surface of platelets in 14% of patients with coronary artery disease. From a molecular perspective, complement is capable of activating platelets by inducing the expression of pro-coagulant factors, such as prothrombinase complex, on the surface of the cells (52).

Complement proteins also interact with bacteria both through the conventional pathway and the alternative pathway (53, 54). *S. sanguinis* for example induces platelet aggregation involving complement (55). ClfA and ClfB from *S. aureus* also induce aggregation that is dependent on complement (56).

Platelets express gC1q-R, the receptor of C1q, and could thus serve as a receptor for bacteria coated with these complement factors. Following platelet activation, the expression of gC1q-R on the

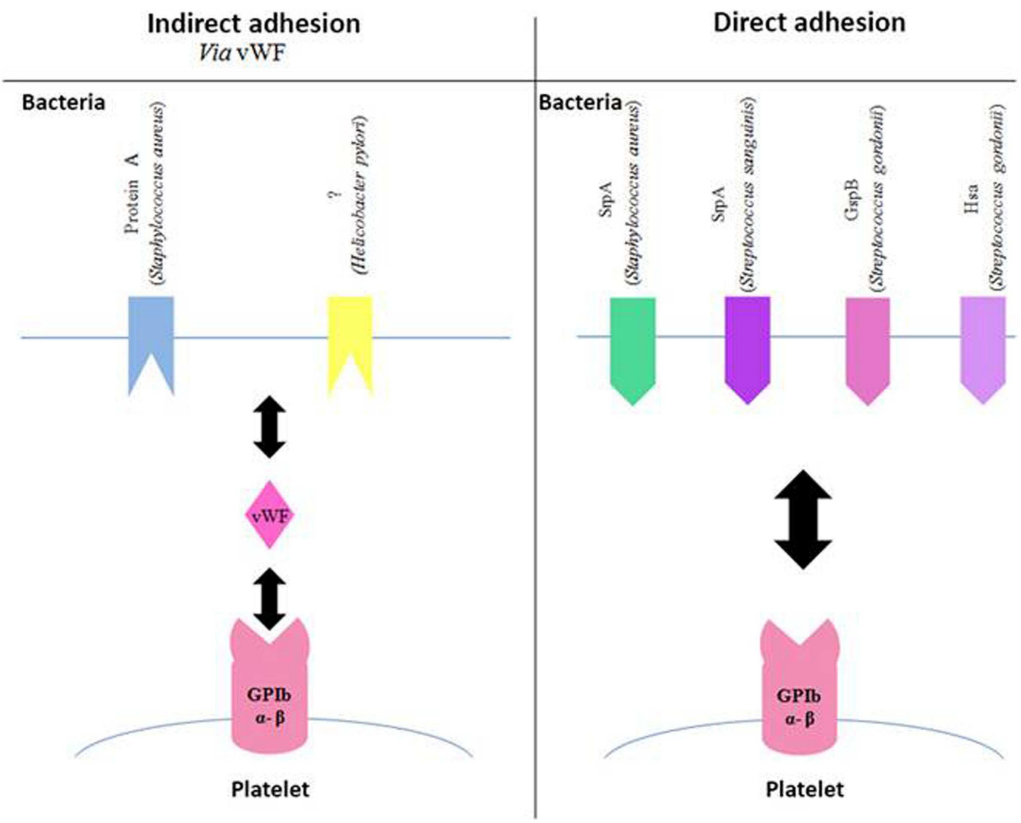


FIGURE 2 | Involvement of GPIb in the adhesion of bacteria to platelets. Schematic representation of different bacterial components, which bind to the platelet GPIb either indirectly via the von Willebrand Factor (vWF, left side of the diagram) or directly (right side of the diagram). Gsp, glycosylated streptococcal protein; Srp, serine-rich protein; Hsa, sialic acid-binding hemagglutinin.

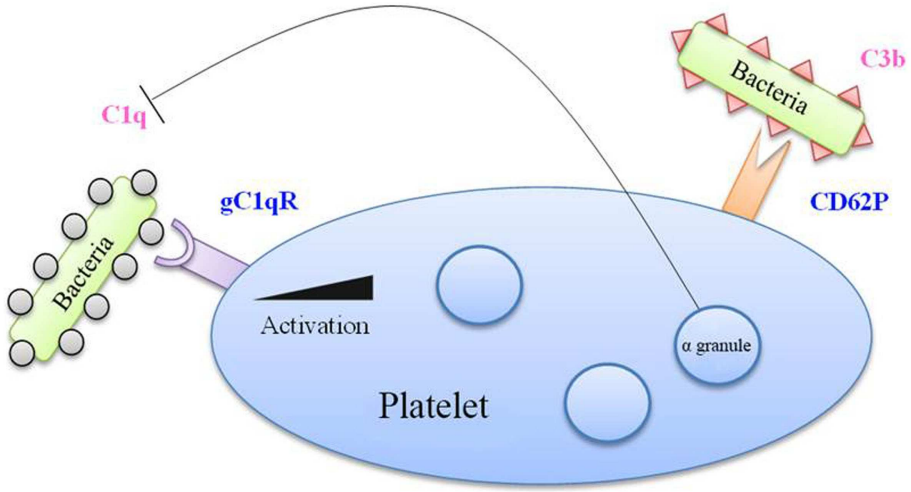


FIGURE 3 | Complement factors in bacterial binding to platelets. Bacteria coated with C1q factor recognized by the gC1q receptor (gC1q-R) on platelet membrane, which is overexpressed upon platelet activation. Moreover, the CD62P marker, expressed by activated platelets, is able to recognize the C3b factor. Besides, alpha-granules contain a C1q inhibitor, which limits the interaction between C1q and gC1q-R.

platelet surface is significantly increased (57). Platelet activation also leads to the increase of CD62P at the membrane, which has been reported to bind C3b, another complement protein (58).

This interaction of platelets with complement can be bivalent. On the one hand, platelets can assist in the destruction of bacteria by increasing the activity of complement, but since they bind complement proteins, they themselves can become the target of complement's lytic activity. This is notably what occurs in the case of thrombocytopenic purpura (52). Platelets however possess a C1 inhibitor in their α-granule, which, during platelet stimulation, would enable complement activation to be modulated (59).

This concept of platelet–bacteria binding by complement molecules involves a mechanism that is more immunologic than hemostatic, which therefore highlights the dual function of platelets.

FcγRIIa receptor

The expression of this immune receptor, which recognizes the Fc domain of immunoglobulin (Ig)G, is typically described for phagocytes, such as neutrophils and monocytes. FcγRIIa enables the binding and internalization of immune complexes involving IgG, whether soluble or cellular. This mechanism is regulated by the fact that complexed IgG have a strong affinity for the receptor, while it is very weak for monomeric IgG (60).

Blood platelets also express FcγRIIa (61), and it is the only type of Fcγ receptor that has been described on platelets to date. One of the first functions associated with platelet FcγRIIa involves its role in the pathophysiology of autoimmune disorders. In cases of heparin-induced thrombocytopenia, Reilly et al. described autoantibodies that recognized the platelet factor-4 (PF4)–heparin complexes binding to the platelet FcγRIIa. The involvement of the receptor then results in strong hemostatic activation, followed by clearance of the activated platelets (62).

Immunoglobulin G bound to bacteria are also capable of being taken by this platelet receptor. As with leukocytes, the immune complexes that bind FcγRIIa can even be internalized by the platelets (63).

The stimulation of other platelet receptors by bacteria very often requires the simultaneous involvement of FcγRIIa in order to obtain an effective platelet response. This suggests a link between the involvement of FcγRIIa and the mechanisms of aggregation.

On average, platelets express approximately 5,000 copies of FcγRIIa (42). Considering the large number of circulating platelets, these are thus the richest reservoir of FcγRIIa, and indeed, they are therefore a significant cell in the antibacterial platelet response.

BACTERIAL TOXINS

Bacteria may also secrete toxins that are capable of activating platelets. *Porphyromonas gingivalis* secretes a family of cysteine proteases called gingipains. These toxins are capable of recognizing the platelet protease-activated receptor (PAR) 1 and cleave it in a manner similar to that of thrombin, thereby making it functional (64).

Alpha-toxin, expressed by strains of *S. aureus*, binds to the lipid bilayer membrane of platelets to form a pore, followed by a flow of calcium, similar to that induced by calcium ionophore (65). Other toxins capable of forming pores on the platelet surface have also been described. These include streptolysin O from *S. pyogenes* (66) and pneumolysin from *Streptococcus pneumoniae* (67).

Staphylococcus aureus and *S. pyogenes* produce a superfamily of toxins called staphylococcal superantigen-like (SSL) toxins, which have a known superantigenic effect. Of them, SSL5 interacts directly with GPIbα via the sialyl lactosamine residues that terminate its glycan chain. This toxin also has a direct affinity for GPIV (42).

EFFECTS OF BACTERIA ON PLATELET FUNCTION

Most studies focusing on the adhesion of bacteria to platelets show that it is a result of aggregation. The scope of this manuscript does

not include coagulation; only the characteristics of aggregation and inflammation after bacterial contact are addressed.

INTERNALIZATION OF BACTERIA

During systemic bacterial infection, pathogens are generally captured by phagocytes. When Clawson studied the interaction between platelets and bacteria in the 1970s, he occasionally observed the internalization of *S. aureus* in some platelets (12, 68, 69). Youssefian et al. (70) confirmed these observations, which concerned the internalization of *S. aureus* in particular. Electron microscopy photographs show the internalization of *S. aureus* in platelets, within vacuoles that are independent of the open canalicular system (OCS), suggesting active internalization.

Platelets have a better capacity for internalization when they are activated by a conventional agonist [adenosine diphosphate (ADP) or thrombin], which underscores a common mechanism between activation and internalization. Immunohistochemical labeling of vacuoles containing *S. aureus* shows the presence of CD62P and GPIIb–IIIa, but not GPIb, the phenotype that corresponds to that of an activated platelet membrane. The vacuole may therefore be formed through invagination of the plasma membrane (endocytosis) after activation.

A Japanese team confirmed the internalization of *S. aureus* in platelets but only after activation of the latter by ADP. The same study shows that *P. gingivalis* can also be internalized in platelets. There appears however to be a different mechanism of internalization at work in both bacteria. Indeed, *P. gingivalis* is capable of inducing it alone, without the addition of another platelet agonist, as the platelet aggregates are adequate for internalization of the bacteria (71).

Staphylococcus aureus and *P. gingivalis* share a common element with regard to internalization, as both types of bacteria are internalized in the vacuoles independent of the OCS (71). Although the final result is the same, it is thus possible that Gram-positive and Gram-negative bacteria may have different internalization mechanisms, suggesting that one of them has an additional molecule promoting its internalization. It must be emphasized that on the pictures from the study by Li et al., the presence of some *P. gingivalis* cells at the OCS may be explained by passive trapping of bacteria during platelet aggregation (71).

Platelet FcγRII may also initiate the internalization of IgG–pathogen complexes (63). One study indeed shows that platelets are capable, after involvement of FcγRII, of internalizing polystyrene beads (0.5–1.5 μm diameter) covered with IgG. This internalization is inhibited by cytochalasin D, suggesting the need to remodel platelet actin for bacteria to be internalized (72).

There remains a question as to the outcome of the internalized bacteria. White et al. reviewed arguments favoring the inability of platelets to degrade/kill bacteria. Their main argument is the absence of phagolysosomes in platelets (73). The internalization of bacteria in platelets would enable them to escape the immune system. Platelets could however use another pathway for destroying bacteria. Indeed, the endosome containing the pathogens has the ability to merge with the alpha-granules containing many bactericidal molecules (70). Finally, it is possible for *Escherichia coli* (*E. coli*) to be destroyed by platelets via internalization by FcγRII, provided that the bacteria have first been opsonized by IgG (72).

The fate of the bacteria internalized in the platelets thus remains a subject of discussion, being either a means of defending the host or an escape mechanism for the bacteria. Without progressing to internalization however, adhesion of the bacteria or bacterial products on the platelet surface is sufficient for inducing a defense response from the platelets. The main reactions are described in the following section.

PLATELET ACTIVATION BY BACTERIA

Effect on aggregation

Since bacterial binding to platelets includes receptors that are also involved in hemostasis, some data show aggregation to be dependent on bacteria. Bacteria that indirectly use GPIIb–IIIa (via fibrinogen or fibronectin) bring about aggregation similar to that observed with other fibrinogen-coated surfaces (42). However, when there is direct adhesion between the bacteria and GPIIb–IIIa, a different mechanism is used, and aggregation induction is often controversial in the literature. *S. gordonii*, for example, which binds directly to GPIIb–IIIa through its PadA protein, does not induce aggregation (44), potentially due to a weaker affinity for the receptor.

Bacteria that bind to GPIb via vWF can (contrary to soluble or immobilized vWF) cause aggregation in the absence of shear force. This is the case for *S. sanguinis* and *S. gordonii*, which bind to platelets through their SrpA and GspB proteins, respectively. Deletion of these two proteins completely eliminates this aggregation (42). These bacterial components are capable of substituting for the shear forces, particularly by themselves ensuring platelet rolling.

For other bacteria, *S. pyogenes* and *S. aureus* in particular, shear forces are not necessary to induce the thrombus formation, but the observed aggregation may involve other platelet proteins. The hypothesis issued by Cox et al. is that the binding of bacteria to GPIb might bring them close to functional platelet receptors such as FcγRIIa or GPIIb–IIIa (42).

After stimulation by *S. sanguinis*, which involves GPIb, the platelets release the contents of their dense granules that contain vasoactive substances, including the adenosine nucleotides, adenosine triphosphate (ATP) and ADP. Once released, ATP is taken by the ecto-ATPases present on the surface of *S. sanguinis* and hydrolyzed to ADP. The newly formed ADP, as well as that released previously, will bind to their platelet receptor. The P2Y pathway is therefore involved (42).

The use of aspirin during the adhesion of *S. sanguinis* to platelets totally inhibits aggregation, also suggesting the role of cyclooxygenase and the production of thromboxane (Tx)A_2. The platelets exposed to *S. sanguinis* produce TxA_2, and the TPα receptor then amplifies platelet activation by binding the newly released TxA_2.

Platelet activation induced by *S. sanguinis* may also involve the MAP kinase pathways. McNicol et al. recently showed that MAP kinases Erk2 and p38 underwent the triphasic stages of phosphorylation/dephosphorylation observed in other phosphoproteins. Aspirin has no effect on phosphorylation and dephosphorylation of Erk2 but is able to inhibit its rephosphorylation stage (74).

There is little data available concerning platelet signaling related to the adhesion of *S. aureus*. Cox et al. showed that during the

interaction of *S. aureus* with platelets, the induced aggregation is dependent on the cyclooxygenase and Tx pathways (75). This study focused mainly on the mechanisms of interaction, and therefore further details on the intracellular mechanisms were not provided.

Several studies on platelet aggregation after bacterial adhesion have also highlighted the need for FcγRIIa involvement if there is to be an effective response (42). However, FcγRIIa functioning could differ from that which is usually described, since, although the observed aggregation requires FcγRIIa, IgG does not seem to be essential (76). The colocalization of FcγRIIa with GPIbα during bacterial stimulation might be the first step in signal transduction (76, 77).

One of the most accepted hypotheses concerning the alternative role of FcγRIIa is based on platelet remodeling, since the GPIb sequence that binds to FcγRIIa (R542G543R544) is the same that binds to actin during platelet activation (77).

A similar type of study was conducted on FcγRIIa and GPIIb–IIIa. Newman et al. showed that Src residue from GPIIb–IIIa, capable of ensuring a role of tyrosine kinase, phosphorylates the immunoreceptor tyrosine-based activation motif (ITAM) residue of FcγRIIa and thus amplifies the platelet activation signals (78). Phosphorylation of the ITAM motif may take place within 30 s following contact, which shows that secondary signaling pathways can be established very quickly.

Aggregation induced by bacteria is different from that observed with the conventional ADP, ATP, and thrombin platelet agonists. It is "binary" aggregation, meaning that aggregation is not observed below a certain bacterial density, and aggregation is already at a maximum when above that density (79).

The lag time required before the appearance of aggregation is another parameter that differs according to whether the platelet stimulation is bacterial or not. This lag time is generally longer with bacterial stimulation than with hemostatic activation. Although about 10 s are needed with a hemostatic agonist, some bacteria can have a very quick lag time of 90–120 s, while others may need more than 20 min before inducing platelet aggregation. Increased bacterial density can decrease this time to a limited extent (79). Several hypotheses have been proposed to explain the lag time variations according to the bacteria: (1) the time required for platelets to bind bacteria, particularly if it done indirectly; and (2) activation of the receptor, which may not be as strong.

Bacteria-induced platelet aggregation nevertheless remains controversial, and several studies show that bacterial stimulation may not result in aggregation but a more targeted inflammatory response [chemokines release, leukocyte activation, neutrophil extracellular trap (NET) formation]. Based on current knowledge, particularly on platelet TLR, other platelet responses to bacteria, in addition to aggregation, may include the release of adapted molecules.

Effect on the release of immunomodulatory factors

Platelets possess many bioactive molecules in their alpha-granules, including cytokines/chemokines, which are released during their activation and enable them to act during the immune response (80, 81). The Canadian team that partners with our laboratory and studies intraplatelet signaling after *S. sanguinis* stimulation also performed a study on the secretion of platelet cytokines. Four strains of *S. sanguinis* and one of *S. gordonii* were used for the study.

The observation of aggregation and phosphorylation of signaling molecules such as PCg2 and Erk confirms the pro-thrombotic role of streptococci and also shows the release of RANTES, PF4, soluble (s)CD40L, and platelet-derived growth factor (PDGF-AB). CD62P is only released in the presence of one strain of *S. sanguinis* (82). Another study had also shown that platelets were capable of releasing soluble CD40L (sCD40L) and RANTES after stimulation with IgG-bead complexes, with the absence of aggregation and a very weak expression of CD62P (72).

Epinephrine, known to stimulate fibrinogen binding and aggregation (83), causes the opposite effect on the release of platelet cytokines. The mechanism of inhibition used is unknown but could be linked to the activation of type 3 nitric oxide synthase (NOS) after involvement of the β_2 adrenoreceptors; the resultant generation of nitric oxide (NO) and cGMP has already been described in platelet activation inhibition (82). This clearly demonstrates that platelet aggregation and exocytosis of immunomodulating molecules are two independent functions.

One other platelet cytokine that is important in the antibacterial response is PF4. In heparin-induced thrombopenia syndrome (HIT syndrome), PF4 links to heparin through its positive charge, and together they form a neoantigen that is recognized by IgG. Likewise, soluble PF4 can bind to bacteria and thus form a new recognition site for IgG and the effector immune cells (84). This is particularly seen in Gram-negative bacteria, since PF4 presents an affinity for bisphosphorylated lipid A of lipopolysaccharide (LPS) bacteria. The newly formed complex is taken up the phagocytic cells. Platelet PF4 might thus facilitate the clearance of certain bacteria.

Finally, CD40L is the lead immunoregulatory molecule of platelets. It has been found that over 95% of plasma sCD40L originate from platelets (85). By comprising the main source of this molecule, platelets become an indisputable immunoregulatory factor; they participate both in the activation of the effector cells of innate immunity and that of adaptive immunity, since CD40L is involved in immunological synapses, as well as in class switching of B lymphocytes.

Following platelet activation, CD40L is first exposed to the membrane in trimeric form (the biologically most active form) and is then cleaved by proteolytic activity. Matrix metalloprotease (MMP)-9 is currently thought to be the most likely candidate. It should also be pointed out that the soluble form of platelet CD40L may also have an autocrine effect due to the presence of CD40 on the platelet surface (4, 86).

The involvement of CD40L in the full range of platelet functions is substantiated throughout this manuscript. This molecule is particularly implicated in the platelet response to bacteria, since the involvement of platelet TLR-2 (87–91) and TLR-4 (87, 88, 90–99) by means of their bacterial ligands, can specifically modulate the release of sCD40L. As with leukocytes, the following triad is thus observed: bacterial stimulation–platelet–detected release of immunoregulatory molecules. Hence, the role of platelet sCD40L in the pathophysiology of sepsis is an expanding field of study.

The platelet immunoregulatory molecules presented above are of great interest in the context of the immune system activation,

leading to bacterial elimination. The growing number of studies on the interaction of bacteria and platelets shows that platelets can also directly influence the elimination of microorganisms through the release of bactericidal molecules.

Release of antibacterial platelet molecules

Kraemer et al. recently showed that platelets incubated with *S. aureus* limit the growth of this microorganism (100). The smallest platelet concentration for obtaining a bacteriostatic effect may even be possible to determine, though this would vary depending on the bacterial species. A 2013 study conducted on 17 volunteers was able to determine a critical platelet concentration for pathogens from the human oral cavity: *E. faecalis* (resistant or non-resistant to vancomycin), *C. albicans*, *S. agalactiae*, *S. oralis*, and *P. aeruginosa* (101). It should be noted that the growth of *P. aeruginosa* was not inhibited by platelet-rich plasma (PRP).

Despite these very recent studies, the first demonstration of the antibacterial role of platelets occurred very long ago. As Yeaman recounts in his literature review on the subject, Fodor already reported in 1887, the bactericidal effect of heated sera (102). The thermostable molecule involved was then identified and named β-lysine. Its platelet origin is based on the fact that it is released in coagulated plasma and is not found in the other blood cells.

Yeaman et al. were particularly interested in this platelet function and established terminology that classified these antimicrobial platelet molecules as platelet microbicidal proteins (PmP) (102), also known as thrombocidins. PmP, of which there are two (PmP1 and PmP2), are released under the induction of thrombin or bacteria, and differ from classically described defensins by their molecular mass, their sequence, and the chaining of lysine and arginine residues, which gives them a cationic charge. To become functional, these molecules must be cleaved by thrombin; the two sub-units then act in an autonomous but complementary manner by alternating the permeability of the bacterial wall (102).

The platelet signaling pathways that lead to the release of PmP depend primarily on the ATP/ADP pair and the P2 receptors. The signal is amplified through the release of ADP, and autocrine activation of the platelets is produced, which can even extend to neighboring platelets (103).

The PMP family was enlarged through the integration of kinocidins, which includes the platelet cytokines that have a direct bactericidal effect (104). They are divided into two subgroups according to the nomenclature of the cytokines. Les α-kinocidins include the CXC-cytokines [PF4, platelet basic protein (PBP), connective tissue activating peptide (CTAP3), and neutrophil activating peptide (NAP2)], while the β-kinocidins are the CC-type (RANTES). These molecules even have a synergistic effect among themselves. For example, CTAP3 does not have an effect on the viability of *E. coli*, but the presence of PF4 potentiates its activity and thereby reduces the bacterial density by 2 logs. This result is not obtained for PF4 alone (105). Structural biochemical analyses identified the 60–74 structural domain in PF4 as being responsible for the bactericidal activity (106).

Kinocidins are integrated in the mechanisms of innate immunity, to the degree that they conserve their primary role, which is the chemoattraction of leukocytes, enabling cooperation between platelet and leukocyte factors in bacterial clearance (107, 108).

In addition, Kraemer et al. showed the presence of human β-defensins 1 (hBD-1) in megakaryocytes and platelets at the level messenger RNA (mRNA) and peptides (100). Platelet hBD-1 is thus released in response to the alpha-toxin of *S. aureus*; it is not released however in response to thrombin, thrombin receptor activating peptide, or platelet-activating factor (PAF), suggesting that hBD-1 release is independent of degranulation. hBD-1 may therefore not be found in the alpha-granules, especially since the authors did not observe colocalization of the markers of these granules and of hBD-1 (100).

While Kraemer et al. were unable to observe the expression of mRNA coding for hBD-2 and -3 in the platelets, other studies have demonstrated their presence through ELISA, Western Blot, and immunohistochemistry, as well as their microbicidal activity (109, 110). As a result, it appears that platelets are involved in the infectious immune response, both directly through the release of antimicrobial factors, and indirectly through the release of cytokines, enabling them to modulate the cell-mediated immune response.

PLATELETS–BACTERIA: FOCUS ON *STAPHYLOCOCCUS AUREUS* INFECTION

There are many studies on the interaction of platelets with *Streptococcus*, particularly oral streptococci, while comparatively few concern *S. aureus*. In 2005, a prospective study was conducted in 39 medical centers throughout 16 countries that included 1779 patients with IE. The final analysis showed that *S. aureus* was the most common pathogen implicated, with 31.6% of cases versus 18% of *Streptococcus viridans* (111). Invasive infections by methicillin-resistant *S. aureus* generally tend to spread in health care centers and result in a high level of mortality (112). It is therefore of interest to consider the role of *S. aureus* on platelets.

Most of the studies focus on molecules involved on both sides during the adhesion of *S. aureus* to platelets (**Figure 4**). We first looked at the interactions between the molecules present in the membrane of *S. aureus* and platelets.

Protein A, which is a surface protein of *S. aureus*, can be identified by anti-*S. aureus* antibodies. The immune complexes formed can attach to the FcγRII of the platelets, resulting in serotonin release and platelet aggregation. This reaction is dependent on the stimulation time and the quantity of immune complexes formed. The activation was found to be optimal at 5 min, and from two bacteria per platelet (75). In a more recent study, protein A was found to be incapable of inducing aggregation by itself, but it was able to maintain it (113).

Protein A from *S. aureus* can also attach to vWF, which binds to GPIbα. The use of an antibody to block vWF partially inhibits the platelet activation by *S. aureus* (79) providing evidence of the involvement of several adhesion pathways.

Clumping factor A from *S. aureus* is also involved in its attachment to platelets via fibrinogen (114). An alternative receptor exists however, since *S. aureus* adheres to platelets through the intermediary of fibrinogen/fibronectin, which does not necessarily involve GPIIb–IIIa (115). This could be shown by the persistence of aggregation, even if the two fibrinogen-binding sites on GPIIb–IIIa are blocked beforehand. It is not impossible for bacteria to transform fibrinogen so that it can be recognized by another receptor.

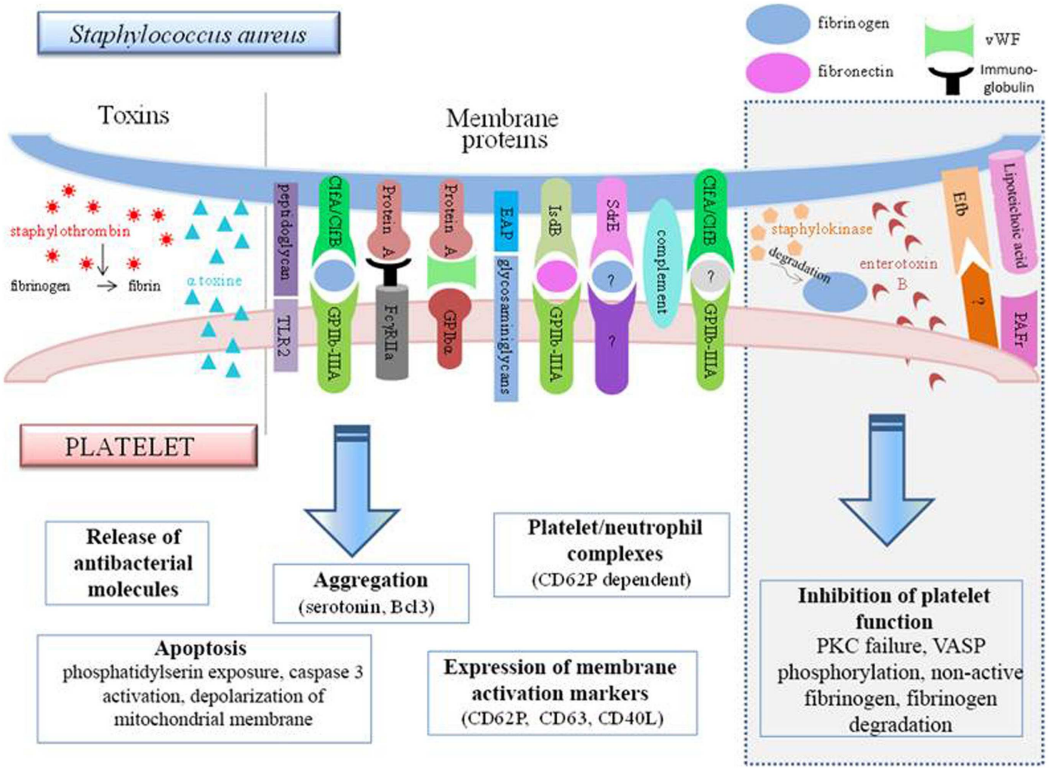


FIGURE 4 | Interconnections between *S. aureus* and platelets. *S. aureus* can induce platelet activation by several ways, e.g., through toxin release or by using membrane protein that bind platelet receptors either directly or indirectly. However, some bacterial factors induce the inhibition of platelet function (gray frame on the right side of the diagram). TLR, Toll-like receptor; Clf, clumping factor; FcγR, Fcγ receptor; EAP, extracellular adherence protein; Isd, iron-regulated surface determinant; Sdr, serine–aspartate repeat protein; Efb, extracellular fibrinogen-binding protein; PAFR, platelet-activating factor receptor.

This might thus be a method for the bacteria to increase their pathogenicity.

FcγRII and GPIIb–IIIa both have a functional role in the adhesion of *S. aureus*. The interconnection between these two receptors explains why the aggregation induced by *S. aureus* is dependent on FcγRII. This hypothesis also reflects the fact that the involvement of FcγRII alone is not sufficient for inducing aggregation, since its presence is meant to optimize the functionality of GPIIb–IIIa (79).

The identification of genetic mutations of *S. aureus* and the expression of candidate proteins in *L. lactis* (non-aggregative bacteria) showed that ClfB and the SdrE protein are also involved in platelet aggregation. The results also confirm the involvement of ClfA, which is moreover the first factor, before ClfB, that leads to aggregation, since it is the protein inducing the shortest lag time. Aggregation induced by both these factors can be inhibited by GPIIb–IIIa antagonists, aspirin, or prostaglandin E_1 (75). Aggregation is also seen with filtered platelets, suggesting a direct link between *S. aureus* and platelets, independent of fibrinogen. Different results have been observed for the SdrE protein. In order to attach to platelets, the bacterial protein requires the presence of a plasma protein other than fibrinogen, which has yet to be identified.

An important element to take into consideration is that *S. aureus* does not express the same factors depending on its stage of growth, which could be a bias in *in vitro* studies. ClfA is the dominant pro-aggregant protein in the stationary phase of growth, whereas in the exponential phase, FnBP expression dominates (79). As a result of these indications, it becomes difficult, for example, to evaluate the results of Fitzgerald, which confirm his modeling of the interaction between FnBP and platelets on bacteria in the stationary phase (116).

The complement system is important in platelet aggregation induced by *S. aureus*. It is capable of substituting for ClfA. In this instance, the lag time will be longer (between 8 and 20 min), but simultaneous involvement of the Fcγ receptor remains necessary (56).

The *Staphylococcus* protein, IsdB, could promote the adhesion and internalization of bacteria within platelets in the presence of fibronectin (117). In addition, this protein, unlike the IsdA and IsdH proteins, might induce platelet aggregation (117).

Studies on the interaction between platelets and *S. aureus* began in the 1970s and still continue today. The extracellular adherence protein, EAP, in the form of an oligomer, can bind directly to the glycosaminoglycans of platelets (118). This leads to the stimulation of thiol isomerase in the platelet and resultant platelet activation, ranging from stabilization of the fibrinogen binding, to the membrane expression of platelet activation molecules, such as CD62P, CD63, and CD40L.

In addition to having to integrate the alternative role of hemostasis receptors when confronted with *S. aureus*, there are also newly described platelet receptors that increase the range of functions. This particularly applies to TLR-2, which, by responding to the peptidoglycan of *S. aureus*, results in platelet activation after 30 min in association with a process of apoptosis characterized by depolarization of the mitochondrial membrane, exposure of PS to the plasma membrane, and caspase 3 activation.

In addition to the membrane components and the bacterial wall, *S. aureus* toxins can also modulate the platelet response (**Figure 4**). It has been shown *in vitro* that the alpha-toxin can interact with the platelet membrane and induce the production of microbicidal proteins and lysis of bacteria (102). This toxin might also be capable of generating dose-dependent platelet aggregation (119). It can also lead to the *de novo* synthesis in the platelet of B-cell lymphoma-3 (Bcl_3) (119), a protein involved in the withdrawal of the platelet plug. This exotoxin is also the source of the formation of many platelet–neutrophil complexes via CD62P, an activation marker expressed on the platelet surface. Formation of the complexes increases the activation of neutrophils, which can be measured through the increase of CD11b. These aggregates could thus participate in the destruction of alveolar capillaries and be the cause of *S. aureus* hemorrhagic pneumonia (120).

Staphylococcus aureus releases staphylocoagulase and vWF-binding protein. Both of these molecules bind to prothrombin and form the enzymatic complex known as staphylothrombin (121). Staphylothrombin has no direct action on platelet activation, but by transforming fibrinogen to fibrin, it plays a role in stabilization of the aggregation, as well as in the initiation of secondary activation.

It has been noted, however, that several *S. aureus* molecules seem to actually inhibit the hemostatic function of platelets. In this respect, staphylococcal enterotoxin B (SEB) has been observed to cause platelet overactivation of protein kinase C (PKC). This enzyme, essential for platelet response, is therefore no longer found in physiological conditions and therefore cannot ensure its function. This explains why platelets incubated with SEB are incapable of ensuring correct aggregation in response to thrombin (122).

Lipoteichoic acid uses the PAF receptor to increase the level of cAMP within platelets. This latter then increases its phosphorylation activity on vasodilator-stimulated phosphoprotein (VASP) and inhibits aggregation and thrombus formation (123). The antithrombotic role of *S. aureus* can also be attributed to the extracellular fibrinogen-binding protein (Efb). *In vitro*, this protein has been described as adhering to platelets (on a non-characterized receptor or on fibrinogen). Once attached, it recruits fibrinogen but in a non-conventional form that is rather inclined to inhibit platelet activation. The inhibitory action of this molecule was confirmed *in vivo*, in which it is able to prevent thrombosis following treatment with platelet agonists (124).

Finally, staphylokinase also exerts inhibitory activity on the platelets although indirectly. This enzyme degrades plasmin and fibrinogen, thereby preventing aggregation (125). Several groups are also studying the interaction of *S. aureus* and platelets under conditions similar to those seen *in vivo*, particularly with regard to preservation of the necessary shear forces. Mice infected by *S. aureus* develop thrombi through a ClfA-dependent mechanism (126). The use of a molecule occupying the binding site of ClfA on fibrinogen completely prevents aggregation, which demonstrates the predominance of ClfA, despite the multitude of other factors present in the microenvironment.

Dogs with *S. aureus* infection quickly develop sepsis, accompanied by platelet dysfunction (reduction in the capacity of growth in response to a PAR-4 agonist). This latter result suggests that beyond their role in IE due to *S. aureus*, platelets participate in the pathophysiology of *S. aureus*-induced sepsis (127).

In addition, a duality can be seen in the effect of *S. aureus* bacteria on platelets, indicating the complexity of the interaction. All of the studies are based on whether the bacteria have pro-aggregant or non-aggregant abilities. There is no data however on the release of platelet cytokines, which is nevertheless a very important component of bacterial infection.

ROLE OF PLATELETS IN THE PATHOPHYSIOLOGY OF SEPSIS

SEPSIS AND COAGULOPATHY

Data from the early 2000s show that between 30 and 50% of patients with severe sepsis have disseminated intravascular coagulation (DIC), resulting in organ hypoxia (128). During the inflammatory response, the neutrophils release tissue factors that trigger the coagulation cascade, leading to platelet activation. IL-1 and IL-6 are also strong inductors of coagulation (129, 130). This phenomenon is amplified by deregulation of the anticoagulant balance. Patients with sepsis have a strong release of PAI-1, a natural plasmin inhibitor. There is also a reduction in protein C, the active form of which is an inhibitor of coagulation factors Va and VIIIa (10, 129–133). In addition, these natural anticoagulants have their role in thrombin generation, with anti-inflammatory properties influencing nuclear factor κB (NFκB) (134).

Reactive oxygen species (ROS) released in massive quantities during the acute phase of sepsis are also responsible for coagulopathies. In mice with induced sepsis that are knocked-out for NOS (the enzyme that produces ROS), vasoconstriction is reduced compared to wild mice. By favoring vasoconstriction, ROS participates in circulatory alteration in the blood capillaries. ROS also have a direct effect on the hemostatic activation of platelets (132).

The fourth element favoring excessive coagulation is the increase of adhesion factors. In endotoxemia models, the expression of adhesion molecules is increased in both the platelet and endothelial membranes. The adhesion of platelets to the endothelium promotes mutual activation and an accumulation of platelets, resulting in vessel occlusion (118, 123). Furthermore, in endotoxemia induced in a mouse model, it was seen that overexpression of the endothelial PAI-1 molecule in the lungs limits the *in situ* recruitment of regulatory T lymphocytes but promotes that of neutrophils (135).

Sepsis-related coagulation disorders have highlighted the role of platelets in the pathophysiology of sepsis, particularly through their hemostatic function. However, it is now clear that platelets also possess an inflammatory function that may enable them to directly participate in the amplification of inflammation associated with the early phases of sepsis. In addition to their sensitivity to thrombin, adhesion molecules, and cytokines/chemokines, the hypothesis of their direct involvement in sepsis is also supported by the TLR expression on their surface and the broad range of

inflammatory molecules that they can release during bacterial stimulation.

SEPSIS AND INFLAMMATORY PLATELET MOLECULES

The first elements supporting the participation of platelets in sepsis-associated inflammation come from studies showing that the level of circulating sCD40L is greater in patients with sepsis than in individual controls (age- and gender-matched) but independent of the severity of the sepsis (136–141). This sCD40L released during sepsis comes from the platelets, since mice that have undergone platelet depletion do not present this increased plasma level (142). One recent study suggests that matrix metalloproteinase-9, which is also increased during sepsis, might be the source of platelet CD40L cleavage (143). This molecule has important inflammatory properties affecting many cells, both immune and non-immune, and may significantly amplify inflammation (144, 145).

In sepsis, sCD40L participates in the recruitment of neutrophils. CD40L gene-deficient C57BL/6 mice that had sepsis induced through cecal ligation puncture do not show neutrophil activation, edema formation, or neutrophil infiltration in the lungs, and they maintain their alveolar microarchitecture (142, 146).

Soluble CD40L may enable the expression of the macrophage-1 antigen (Mac-1) adhesion protein by neutrophils, promoting their recruitment at the mucosa. The mechanism of action of sCD40L may involve macrophage inflammatory protein 2 (MIP-2) and its receptor, CXCR2. This is supported by the fact that *in vitro* recombinant sCD40L does not increase the expression of Mac-1 on the neutrophils in culture (142).

Platelet-activating factor is a molecule synthesized by various cells and that possesses a cytokine function. Its receptor, platelet-activating factor receptor (PAFR), is attached to G proteins and is expressed by the cells that participate in immune defense and coagulation, including platelets. Platelet PAFR activation results in the release of inflammatory factors, degranulation, and the initiation of coagulation cascades. In physiological conditions, signaling associated with PAFR is finely regulated in order to avoid an excessive thrombo-inflammatory response. During sepsis however, this regulatory balance is disrupted, and PAF is then involved in the activation of neutrophils, monocytes, platelets, and in the formation of leukocyte–endothelium, leukocyte–platelet, and platelet–endothelium complexes (147).

A recent study suggested an association between the duration of storage of apheresis platelets before transfusion and the occurrence of complications in patients from a trauma center. This study was conducted on 381 patients who had been admitted to a trauma center and received apheresis platelet concentrates that had been stored for 3 days or less, 4 days, or 5 days. The results show that the transfusion of platelets stored for over 3 days may increase the risk of complications for the patient, sepsis in particular (148). It was shown that during the storage of platelet concentrates, the platelets are activated, and the platelet inflammatory factors, including sCD40L, are released and then accumulate (149–152). This suggests that the inflammatory molecules that accumulate during the storage of platelet concentrates could promote the onset of sepsis.

It has also recently been shown that IL-27 could be a predictive molecule of sepsis in children (153), and that the activated platelets could be a significant source of this cytokine (154). Thrombin formed during sepsis could lead to this release of platelet IL-27.

In addition, an increase in the level of circulating microparticles has been reported in septic patients. These vesicles (granulocytes, monocytes, endothelial cells, and platelets) may arise from several cell types (22). In sepsis, the release of PMP is accompanied by an increase of CD62P at the platelet membrane and an increase of platelet–monocyte aggregates (31). The role of microparticles in sepsis requires further exploration. It has been described however that they possess strong pro-coagulant pathogenicity through the expression of tissue factors. Some studies conducted in other contexts have shown that PMP are rich in CD40L (155) and IL-1 β (18, 30), which are two pro-inflammatory molecules that are strongly associated with the pathophysiology of sepsis. It therefore becomes important to consider PMP in the development of inflammation during sepsis.

The role of platelets in the inflammatory phase of sepsis may not be limited to the production of inflammatory molecules. Indeed, in patients with uncomplicated sepsis, the level of circulating platelet–leukocyte complexes is higher than in controls. However, in sepsis complicated by organ failure, the number of platelet–leukocyte complexes is decreased. This can be explained by sequestration of the complexes in the damaged organs, for example the lungs (156). The same type of observation occurred *in vitro*, in which strains of *S. aureus* isolated from bacteremia result in aggregation, the formation of platelet–neutrophil complexes, and activation of these neutrophils (157).

Moreover, since platelets are capable of binding to bacteria, and even keeping them alive intracellularly, they could promote their dissemination within the body. This mechanism was proposed in a mouse model infected by *S. pyogenes* via the intraperitoneal route. Platelet-depleted animals are unable to ensure the transport of bacteria in the blood, lungs, and spleen, which is characterized by a reduction in the bacterial load (through a CFU count) in these organs after sacrifice of the animals (158).

PLATELET APOPTOSIS IN THE MICROENVIRONMENT OF SEPSIS

The involvement of platelets in sepsis is also characterized by persistent thrombocytopenia in patients (159, 160). There have been various studies done that consider thrombocytopenia a predictive factor of the mortality rate of patients admitted to intensive care (160, 161). Several hypotheses have been made with regard to the decrease in circulating platelets and are presented below.

During sepsis, platelets express activation factors that promote their sequestration in the spleen and then their destruction (162). Platelet depletion can be accelerated if the platelet–bacteria contact involves the complement system (52) or Fc-γ receptor (163). Furthermore, sepsis is a pathological state that might promote hemophagocytosis of platelets by macrophages which is partly dependent on macrophage colony-stimulating factor (164).

The failure of thrombopoiesis is an unlikely hypothesis to the degree that the plasma levels of IL-6, tumor necrosis factor (TNF)-α, and thrombopoietin are increased in sepsis (129, 130, 165). On the contrary, the involvement of megakaryocyte TLRs might rather promote an overproduction of platelets.

Another hypothesis points at sepsis-induced coagulopathy leading to DIC, in which disseminated thrombi may immobilize the platelets (166). The results of Tyml et al. confirmed this in a mouse model of sepsis (132).

More recently, the scientific community has been interested in platelet apoptosis. This mechanism of cellular death involves an important step of nuclear transformation involving chromatin condensation, followed by DNA fragmentation (167–169).

Apoptosis, which is known as "programed cell death," can normally be triggered by two types of stimuli. These are referred to as the intrinsic pathway and the extrinsic pathway.

The extrinsic pathway involves cell death receptors, such as the apoptosis stimulating fragment (Fas) receptor, the ligands of which are the proteins from the TNF family. The involvement of Fas causes trimerization of the receptor, which then becomes active, enabling it to recruit an adaptor molecule, Fas-associated protein with death domain (FADD), which also contains a binding domain for procaspase-8. The formation of this complex leads to cleaving of caspase-8, which is then produced in its active dimeric form. Caspase-8 will then either activate the sequential cascade of the different caspases, or the previously described mitochondrial pathway.

The intrinsic pathway is triggered following cellular stress, such as oxidative stress. Under physiological conditions, Bcl_2 protein and other similar proteins maintain the integrity of the mitochondrial membrane. Under stress conditions, these proteins are degraded. Transition pores then form on the mitochondrial surface, causing them to swell and then burst. Cytochrome C is then released in the cytoplasm and can activate the caspase cascade (170).

In both cases, the pathways converge to activate caspase-3, which has many substrates. The effects of caspase-3 are seen both on the nuclear proteins involved in DNA repair and on cytoplasm proteins such as gelsolin, which is a cytoskeletal regulator. Cleaving of the molecules involved in cellular structure and repair causes DNA and cytoskeletal fragmentation, but the plasma membrane remains intact. During the formation of apoptotic bodies, a change in the distribution of phospholipids is produced, termed "membrane flip flop," which exposes the PS on the membrane surface. The PS constitute an "eat me" signal for the phagocytic cells, which enables the elimination of the apoptotic bodies (170).

Platelets have also been found to possess apoptotic machinery. The platelets express caspases 1, 3, 4, and 9. Caspases 2, 6, and 8A have a more reduced expression, and no expression for caspases 5, 7, and 10 has been demonstrated. Fas are not expressed in the platelets, but they have other cell death receptors, such as DR3, DR5, TNF-receptor p55, and RIP. Platelets also express proteins from the Bcl-2 family: Bcl-X, Bfl1, Bad, Bak, Bax, and Mc1 (171).

Activation of these apoptotic molecules has been demonstrated during storage of platelet concentrates. After 5 days of storage in standard blood bank conditions, platelet viability decreases. This platelet death is accompanied by an increase in PS at the cell surface and an increase in caspase-3 activity. Caspase activation occurs in a specific manner, since the use of an inhibitor (z-VAD-fmt) stops this process. Platelet death is thus well associated with a specific apoptosis (171).

This first study showed the independence of apoptosis and platelet activation, since PS exposure was independent of the increase in the expression of the CD63 activation marker, and thrombin activation had no effect on caspase-3 activity. The scientific community however is not in unanimous agreement. It has even been shown that thrombin stimulation induced depolarization of the mitochondrial membrane, the expression of pro-apoptotic molecules (Bax, Bak), activation of caspase-3, and exposure of PS to the membrane (172). The action of thrombin may not be direct and may require the release of platelet factors that can induce apoptosis. During storage, platelets release many soluble factors, including TNF-α, which is a strong inductor of apoptosis through the induction of caspases.

The life span of platelets could therefore be controlled through apoptosis. Caspase-9, for example, is required for platelet and megakaryocyte death but is not involved in their production or their functionality (159).

The Bcl-X family is also at the center of platelet survival. These molecules may even determine their life span. Two missense mutations of Bcl-X are capable of accelerating platelet death resulting in severe thrombocytopenia (173).

It has also been shown that a strain of isolated *E. coli* in a patient with sepsis was able to induce apoptotic manifestations of platelets, such as actin condensation, a decrease of the mitochondrial potential, and degradation of Bcl-X. The use of mutant and non-pathogenic strains showed that only pathogenic strains that release toxins forming pores have the ability to degrade Bcl-X. This applies to the alpha-toxin of *E. coli* and *S. aureus*. These toxins probably act on calpain, since this protein is the source of Bcl-X degradation. However, proteasome inhibition is ineffective in preventing degradation of the pro-apoptotic protein (174). This study is the first demonstration that pathogenic bacteria can influence the intrinsic initiation of platelet apoptosis. The degradation of Bcl-X suggests a new mechanism by which bacteria may be able to cause the thrombocytopenia observed in patients with bacteremia. This mechanism could then explain why depolarization of the mitochondrial membrane potential ($\Delta\Psi m$) is increased in the platelets of patients with SIRS (175). A decrease in $\Delta\Psi m$ could even be associated with SRIS progression.

INVOLVEMENT OF PLATELETS IN SEPSIS-RELATED NETose

Another phenomenon, the formation of NETs involving platelets, neutrophils, and bacteria, has been demonstrated and could play an important role in sepsis. It was first described in 2004. Neutrophils that have been activated, particularly by IL-8, release their granular (peptides and enzymes) and nuclear (chromatin and histones) components in the microvessels where they combine to form a network called NET (176). The use of high-resolution electron microscopy has confirmed the structure of NET, which is characterized by extracellular chromatin stretches that are associated with globular proteins. It has also been shown that a bacterial environment, or that mimicked through the injection of LPS, results in the *in vivo* release of NET that are able to trap bacteria and thus reduce their spread during sepsis (177).

It was noted that bacterial trapping at NET is greater under flow conditions, as in the blood circulation (94). NET can reach a

diameter of 25 nm, and once combined, they form a structure that can be over 100 nm both in diameter as well as length (94).

Aligned with DNA, NET contain the histones H1, H2A, H2B, and H4, as well as granular proteins such as elastase, myeloperoxidase, and bactericidal permeability increasing protein, which enable bacterial degradation (176). NET are able to stop both Gram-positive and Gram-negative bacteria. The histones and BPI have a proteolytic action on the alpha-toxin of *S. aureus*, as well as on the IpaB of *S. flexneri* (177). *C. albicans*, although not a bacteria, is also sensitive to NET, but its destruction may only depend on granular proteins and not histones (177).

Neutrophil extracellular trap release occurs within 5–10 min after stimulation of the neutrophils. This time period is too short for the implementation of an apoptosis or necrosis mechanism. It is therefore an active mechanism and not the consequence of disintegration of the neutrophilic plasma membrane (176).

From a molecular viewpoint, the formation of NET involves (178, 179):

- peptidylarginine deiminase type 4, for chromatin decondensation;
- ROS formation, which is NADPH oxidase-dependent for disintegration of the nuclear membrane;
- actin cytoskeleton and microtubules for NET release.

Neutrophil extracellular trap can be released according to three mechanisms (180): (1) a rapid mechanism (30–60 min) involving vesicles; in this case, the neutrophils remain viable; (2) a slower mechanism (3–4 h), resulting in rupture of the neutrophilic plasma membrane; or (3) directly from the mitochondria. At present, the third mechanism and the existence of NET composed from mitochondrial DNA remains controversial, although one study shows that NET might be majorly composed of mitochondrial DNA rather than nuclear DNA (181).

Lipopolysaccharide, which has traditionally been described as a neutrophil activator, is surprisingly incapable of inducing *in vitro* NET release by neutrophils. In contrast, in 2007, Clark et al. showed in a mouse model that the intravenous injection of LPS leads to NET formation within the first 5 min (94). A more detailed investigation showed that LPS-induced NET formation is not direct and requires platelet participation. Indeed, platelet stimulation by LPS in the presence of neutrophils may not cause a standard platelet response but might promote their adhesion; the neutrophils would then be activated and form NET (94).

Kraemer et al. then showed that type 1 beta-defensins released by platelets after bacterial stimulation were responsible for NET formation (100).

Kubes presents platelets as a barometer that can detect a substantial level of bacteria. The platelets are activated by an LPS concentration 100 times greater than that inducing neutrophil activation. The platelets may therefore come to the assistance of the neutrophils by enabling them to form NET when the bacterial load is too high and their normal functions are insufficient for correctly eliminating the bacteria (177). NET could be the innate "last chance" defense.

Neutrophil extracellular traps have been shown to have a positive impact on the destruction of pathogens during bacteremia. Conversely, they may alter the microvascular circulation by promoting the formation of microthrombi, thereby also preventing the immune cells from reaching the bacteria. In addition, their components may have a toxic effect on the host cells. This was confirmed *in vitro* on human umbilical vein endothelial cell (HUVEC) (94). Hepatotoxicity has been observed *in vivo* following the release of NET, which was measured by the release of alanine aminotransferase and an occlusion of the liver sinusoids (94).

The histones released in the extracellular medium possess prothrombotic activity and are capable of activating the platelets via their TLR-2 and -4 (90). This phenomenon may be extrapolated to the NET histones. In this case, NET would be responsible for platelet overactivation, which may lead to thrombi formation. The potential detrimental effect of NET is another illustration of the alteration of platelet function during sepsis.

CONCLUSION

The implication of platelets in the inflammatory response marks a veritable turning point in the understanding of platelet physiology and opens new fields of investigation that have been up to now somewhat neglected. The identification of platelets in the various inflammatory mechanisms places them at the center of innate immunity, whether in the recognition of pathogens, signal transduction, or the release of cytokines/chemokines. This functional similarity with leukocytes shows that both of these cells types are not so different.

Anucleated platelets are only found in mammals. In the lower vertebrates, such as birds, reptiles, amphibians, or fish, hemostatic function is ensured by nucleated thrombocytes. With regard to invertebrate species, they do not possess platelets *per se*, but the hemolymph contains a type of nucleated cell called a hemocyte that expresses TLR, which is capable of phagocytizing the foreign body or secreting antimicrobial proteins. It is this same cell however that regulates coagulation and healing. By compiling all of these characteristics, it is possible to discuss a possible common evolution between platelets and leukocytes, followed by a dissociation, proportional to the evolution of the species (1, 3, 4, 182), despite this is debated.

All of the studies presented in this thesis generally show that platelets are able to cover the majority of the steps of inflammation and thus confirm their total involvement in the orchestration of this pathophysiological state. By modulating both the acute effector phase of inflammation and the maintenance of this process, platelets may become a therapeutic target.

AUTHOR CONTRIBUTIONS

All authors contributed substantially to the conception of this review. HC, PD, and AC drafted it while BP, FC, and OG revised it critically for important intellectual content. All authors approved the final version and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

ACKNOWLEDGMENTS

Authors would like to thank Mr. Charles Antoine Arthaud, Mrs. Marie Ange Eyraud, and Jocelyne Fagand for their contribution to the team's work cited in the review. They also acknowledge

the staff of Etablissement Français du Sang Auvergne-Loire, Saint-Etienne, France and the healthy volunteer blood donors for their participation. The studies of the team that are cited were supported by grants from the French National Blood Service – EFS (Grant APR), France; the Association for Research in Transfusion (ART), Paris, France; the Agence Nationale de la Sécurité et du Médicament et des produits de santé (ANSM – AAP-2012-011, Reference 2012S055); the "Agence Nationale de la Recherche" (ANR), reference ANR-12-JSV1-0012-01; and the Association "Les Amis de Rémi," Savigneux, France. The authors declare they have no conflict of interest.

REFERENCES

1. Elzey BD, Tian J, Jensen RJ, Swanson AK, Lees JR, Lentz SR, et al. Platelet-mediated modulation of adaptive immunity. A communication link between innate and adaptive immune compartments. *Immunity* (2003) **19**:9–19. doi: 10.1016/S1074-7613(03)00177-8
2. Weyrich AS, Zimmerman GA. Platelets: signaling cells in the immune continuum. *Trends Immunol* (2004) **25**:489–95. doi:10.1016/j.it.2004.07.003
3. Semple JW, Italiano JE Jr, Freedman J. Platelets and the immune continuum. *Nat Rev Immunol* (2011) **11**:264–74. doi:10.1038/nri2956
4. Garraud O, Hamzeh-Cognasse H, Pozzetto B, Cavaillon JM, Cognasse F. Bench-to-bedside review: platelets and active immune functions – new clues for immunopathology? *Crit Care* (2013) **17**:236. doi:10.1186/cc12716
5. Beynon RP, Bahl VK, Prendergast BD. Infective endocarditis. *BMJ* (2006) **333**:334–9. doi:10.1136/bmj.333.7563.334
6. Leask RL, Jain N, Butany J. Endothelium and valvular diseases of the heart. *Microsc Res Tech* (2003) **60**:129–37. doi:10.1002/jemt.10251
7. Pessi T, Karhunen V, Karjalainen PP, Ylitalo A, Airaksinen JK, Niemi M, et al. Bacterial signatures in thrombus aspirates of patients with myocardial infarction. *Circulation* (2013) **127**(1219–1228):e1211–6. doi:10.1161/CIRCULATIONAHA.112.001254
8. McAdow M, Missiakas DM, Schneewind O. *Staphylococcus aureus* secretes coagulase and von Willebrand factor binding protein to modify the coagulation cascade and establish host infections. *J Innate Immun* (2012) **4**:141–8. doi:10.1159/000333447
9. Moreillon P, Entenza JM, Francioli P, Mcdevitt D, Foster TJ, Francois P, et al. Role of *Staphylococcus aureus* coagulase and clumping factor in pathogenesis of experimental endocarditis. *Infect Immun* (1995) **63**:4738–43.
10. Levi M, Schultz M, Van Der Poll T. Sepsis and thrombosis. *Semin Thromb Hemost* (2013) **39**:559–66. doi:10.1055/s-0033-1343894
11. Osterud B, Bjorklid E. Sources of tissue factor. *Semin Thromb Hemost* (2006) **32**:11–23. doi:10.1055/s-2006-933336
12. Clawson CC, Rao GH, White JG. Platelet interaction with bacteria. IV. Stimulation of the release reaction. *Am J Pathol* (1975) **81**:411–20.
13. Burnier L, Fontana P, Kwak BR, Angelillo-Scherrer A. Cell-derived microparticles in haemostasis and vascular medicine. *Thromb Haemost* (2009) **101**:439–51. doi:10.1160/TH08-08-0521
14. Nomura S, Fukuhara S. Platelet microparticles. *Methods Mol Biol* (2004) **272**:269–77. doi:10.1385/1-59259-782-3:269
15. Flaumenhaft R. Formation and fate of platelet microparticles. *Blood Cells Mol Dis* (2006) **36**:182–7. doi:10.1016/j.bcmd.2005.12.019
16. Wiedmer T, Sims PJ. Participation of protein kinases in complement C5b-9-induced shedding of platelet plasma membrane vesicles. *Blood* (1991) **78**:2880–6.
17. Hashimoto K, Jayachandran M, Owen WG, Miller VM. Aggregation and microparticle production through toll-like receptor 4 activation in platelets from recently menopausal women. *J Cardiovasc Pharmacol* (2009) **54**:57–62. doi:10.1097/FJC.0b013e3181ab373d
18. Brown GT, McIntyre TM. Lipopolysaccharide signaling without a nucleus: kinase cascades stimulate platelet shedding of proinflammatory IL-1beta-rich microparticles. *J Immunol* (2011) **186**:5489–96. doi:10.4049/jimmunol.1001623
19. Kappelmayer J, Beke Debreceni I, Vida A, Antal-Szalmas P, Clemetson KJ, Nagy B Jr. Distinct effects of Re- and S-forms of LPS on modulating platelet activation. *J Thromb Haemost* (2013) **11**(4):775–8. doi:10.1111/jth.12151
20. Ge S, Hertel B, Emden SH, Beneke J, Menne J, Haller H, et al. Microparticle generation and leucocyte death in Shiga toxin-mediated HUS. *Nephrol Dial Transplant* (2012) **27**:2768–75. doi:10.1093/ndt/gfr748
21. Italiano JE Jr, Mairuhu AT, Flaumenhaft R. Clinical relevance of microparticles from platelets and megakaryocytes. *Curr Opin Hematol* (2010) **17**:578–84. doi:10.1097/MOH.0b013e32833e77ee
22. George FD. Microparticles in vascular diseases. *Thromb Res* (2008) **122**(Suppl 1):S55–9. doi:10.1016/S0049-3848(08)70020-3
23. Vasina E, Heemskerk JW, Weber C, Koenen RR. Platelets and platelet-derived microparticles in vascular inflammatory disease. *Inflamm Allergy Drug Targets* (2010) **9**:346–54. doi:10.2174/187152810793938008
24. Markiewicz M, Richard E, Marks N, Ludwicka-Bradley A. Impact of endothelial microparticles on coagulation, inflammation, and angiogenesis in age-related vascular diseases. *J Aging Res* (2013) **2013**:734509. doi:10.1155/2013/734509
25. Lacroix R, Sabatier F, Mialhe A, Basire A, Pannell R, Borghi H, et al. Activation of plasminogen into plasmin at the surface of endothelial microparticles: a mechanism that modulates angiogenic properties of endothelial progenitor cells in vitro. *Blood* (2007) **110**:2432–9. doi:10.1182/blood-2007-02-069997
26. Mause SF, Von Hundelshausen P, Zernecke A, Koenen RR, Weber C. Platelet microparticles: a transcellular delivery system for RANTES promoting monocyte recruitment on endothelium. *Arterioscler Thromb Vasc Biol* (2005) **25**:1512–8. doi:10.1161/01.ATV.0000170133.43608.37
27. Sowa JM, Crist SA, Ratliff TL, Elzey BD. Platelet influence on T- and B-cell responses. *Arch Immunol Ther Exp* (2009) **57**:235–41. doi:10.1007/s00005-009-0032-y
28. Jy W, Mao WW, Horstman L, Tao J, Ahn YS. Platelet microparticles bind, activate and aggregate neutrophils in vitro. *Blood Cells Mol Dis* (1995) **21**:217–31. doi:10.1006/bcmd.1995.0025
29. Lo SC, Hung CY, Lin DT, Peng HC, Huang TF. Involvement of platelet glycoprotein Ib in platelet microparticle mediated neutrophil activation. *J Biomed Sci* (2006) **13**:787–96. doi:10.1007/s11373-006-9107-5
30. Boilard E, Nigrovic PA, Larabee K, Watts GF, Coblyn JS, Weinblatt ME, et al. Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science* (2010) **327**:580–3. doi:10.1126/science.1181928
31. Ogura H, Kawasaki T, Tanaka H, Koh T, Tanaka R, Ozeki Y, et al. Activated platelets enhance microparticle formation and platelet-leukocyte interaction in severe trauma and sepsis. *J Trauma* (2001) **50**:801–9. doi:10.1097/00005373-200105000-00005
32. Wang J, Zhang W, Nardi MA, Li Z. HIV-1 Tat induced-platelet activation and release of CD154 contribute to HIV-1 associated autoimmune thrombocytopenia. *J Thromb Haemost* (2011) **9**(3):562–73. doi:10.1111/j.1538-7836.2010.04168.x
33. Calvete JJ. Platelet integrin GPIIb/IIIa: structure-function correlations. An update and lessons from other integrins. *Proc Soc Exp Biol Med* (1999) **222**:29–38. doi:10.1111/j.1525-1373.1999.09993.pp.x
34. Bennett JS, Berger BW, Billings PC. The structure and function of platelet integrins. *J Thromb Haemost* (2009) **7**(Suppl 1):200–5. doi:10.1111/j.1538-7836.2009.03378.x
35. Josefsson E, Mccrea KW, Ni Eidhin D, O'connell D, Cox J, Hook M, et al. Three new members of the serine-aspartate repeat protein multigene family of *Staphylococcus aureus*. *Microbiology* (1998) **144**(Pt 12):3387–95. doi:10.1099/00221287-144-12-3387
36. McDevitt D, Francois P, Vaudaux P, Foster TJ. Molecular characterization of the clumping factor (fibrinogen receptor) of *Staphylococcus aureus*. *Mol Microbiol* (1994) **11**:237–48. doi:10.1111/j.1365-2958.1994.tb00304.x
37. Ni Eidhin D, Perkins S, Francois P, Vaudaux P, Hook M, Foster TJ. Clumping factor B (ClfB), a new surface-located fibrinogen-binding adhesin of *Staphylococcus aureus*. *Mol Microbiol* (1998) **30**:245–57. doi:10.1046/j.1365-2958.1998.01050.x
38. Flock JI, Froman G, Jonsson K, Guss B, Signas C, Nilsson B, et al. Cloning and expression of the gene for a fibronectin-binding protein from *Staphylococcus aureus*. *EMBO J* (1987) **6**:2351–7.
39. Mitchell J, Tristan A, Foster TJ. Characterization of the fibrinogen-binding surface protein Fbl of *Staphylococcus lugdunensis*. *Microbiology* (2004) **150**:3831–41. doi:10.1099/mic.0.27337-0
40. Usui Y, Ohshima Y, Ichiman Y, Ohtomo T, Suganuma M, Yoshida K. Platelet aggregation induced by strains of various species of coagulase-negative staphylococci. *Microbiol Immunol* (1991) **35**:15–26. doi:10.1111/j.1348-0421.1991.tb01529.x

41. Brennan MP, Loughman A, Devocelle M, Arasu S, Chubb AJ, Foster TJ, et al. Elucidating the role of *Staphylococcus epidermidis* serine-aspartate repeat protein G in platelet activation. *J Thromb Haemost* (2009) **7**:1364–72. doi:10.1111/j.1538-7836.2009.03495.x
42. Cox D, Kerrigan SW, Watson SP. Platelets and the innate immune system: mechanisms of bacterial-induced platelet activation. *J Thromb Haemost* (2011) **9**:1097–107. doi:10.1111/j.1538-7836.2011.04264.x
43. Miajlovic H, Zapotoczna M, Geoghegan JA, Kerrigan SW, Speziale P, Foster TJ. Direct interaction of iron-regulated surface determinant IsdB of *Staphylococcus aureus* with the GPIIb/IIIa receptor on platelets. *Microbiology* (2010) **156**:920–8. doi:10.1099/mic.0.036673-0
44. Petersen HJ, Keane C, Jenkinson HF, Vickerman MM, Jesionowski A, Waterhouse JC, et al. Human platelets recognize a novel surface protein, PadA, on *Streptococcus gordonii* through a unique interaction involving fibrinogen receptor GPIIbIIIa. *Infect Immun* (2010) **78**:413–22. doi:10.1128/IAI.00664-09
45. Plummer C, Wu H, Kerrigan SW, Meade G, Cox D, Ian Douglas CW. A serine-rich glycoprotein of *Streptococcus sanguinis* mediates adhesion to platelets via GPIb. *Br J Haematol* (2005) **129**:101–9. doi:10.1111/j.1365-2141.2005.05421.x
46. Bensing BA, Lopez JA, Sullam PM. The *Streptococcus gordonii* surface proteins GspB and Hsa mediate binding to sialylated carbohydrate epitopes on the platelet membrane glycoprotein Ibalpha. *Infect Immun* (2004) **72**:6528–37. doi:10.1128/IAI.72.11.6528-6537.2004
47. Siboo IR, Chambers HF, Sullam PM. Role of SraP, a serine-rich surface protein of *Staphylococcus aureus*, in binding to human platelets. *Infect Immun* (2005) **73**:2273–80. doi:10.1128/IAI.73.4.2273-2280.2005
48. O'Seaghdha M, Van Schooten CJ, Kerrigan SW, Emsley J, Silverman GJ, Cox D, et al. *Staphylococcus aureus* protein A binding to von Willebrand factor A1 domain is mediated by conserved IgG binding regions. *FEBS J* (2006) **273**:4831–41. doi:10.1111/j.1742-4658.2006.05482.x
49. Thomer L, Schneewind O, Missiakas D. Multiple ligands of von Willebrand factor-binding protein (vWbp) promote *Staphylococcus aureus* clot formation in human plasma. *J Biol Chem* (2013) **288**:28283–92. doi:10.1074/jbc.M113.493122
50. Claes J, Vanassche T, Peetermans M, Liesenborghs L, Vandenbriele C, Vanhoorelbeke K, et al. Adhesion of *Staphylococcus aureus* to the vessel wall under flow is mediated by von Willebrand factor-binding protein. *Blood* (2014) **124**:1669–76. doi:10.1182/blood-2014-02-558890
51. Byrne MF, Kerrigan SW, Corcoran PA, Atherton JC, Murray FE, Fitzgerald DJ, et al. Helicobacter pylori binds von Willebrand factor and interacts with GPIb to induce platelet aggregation. *Gastroenterology* (2003) **124**:1846–54. doi:10.1016/S0016-5085(03)00397-4
52. Peerschke EI, Yin W, Ghebrehiwet B. Complement activation on platelets: implications for vascular inflammation and thrombosis. *Mol Immunol* (2010) **47**:2170–5. doi:10.1016/j.molimm.2010.05.009
53. Verschoor A, Langer HF. Crosstalk between platelets and the complement system in immune protection and disease. *Thromb Haemost* (2013) **110**:910–9. doi:10.1160/TH13-02-0102
54. Gadjeva M. The complement system. Overview. *Methods Mol Biol* (2014) **1100**:1–9. doi:10.1007/978-1-62703-724-2_1
55. Ford I, Douglas CW, Heath J, Rees C, Preston FE. Evidence for the involvement of complement proteins in platelet aggregation by *Streptococcus sanguinis* NCTC 7863. *Br J Haematol* (1996) **94**:729–39. doi:10.1046/j.1365-2141.1996.d01-1857.x
56. Loughman A, Fitzgerald JR, Brennan MP, Higgins J, Downer R, Cox D, et al. Roles for fibrinogen, immunoglobulin and complement in platelet activation promoted by *Staphylococcus aureus* clumping factor A. *Mol Microbiol* (2005) **57**:804–18. doi:10.1111/j.1365-2958.2005.04731.x
57. Peerschke EI, Murphy TK, Ghebrehiwet B. Activation-dependent surface expression of gC1qR/p33 on human blood platelets. *Thromb Haemost* (2003) **89**:331–9. doi:10.1267/THRO03020331
58. Hamad OA, Nilsson PH, Wouters D, Lambris JD, Ekdahl KN, Nilsson B. Complement component C3 binds to activated normal platelets without preceding proteolytic activation and promotes binding to complement receptor 1. *J Immunol* (2010) **184**:2686–92. doi:10.4049/jimmunol.0902810
59. Schmaier AH, Smith PM, Colman RW. Platelet C1-inhibitor. A secreted alpha-granule protein. *J Clin Invest* (1985) **75**:242–50. doi:10.1172/JCI111680
60. Capel PJ, Van De Winkel JG, Van Den Herik-Oudijk IE, Verbeek JS. Heterogeneity of human IgG Fc receptors. *Immunomethods* (1994) **4**:25–34. doi:10.1006/immu.1994.1004
61. Cassel DL, Keller MA, Surrey S, Schwartz E, Schreiber AD, Rappaport EF, et al. Differential expression of Fc gamma RIIA, Fc gamma RIIB and Fc gamma RIIC in hematopoietic cells: analysis of transcripts. *Mol Immunol* (1993) **30**:451–60. doi:10.1016/0161-5890(93)90113-P
62. Reilly RF. The pathophysiology of immune-mediated heparin-induced thrombocytopenia. *Semin Dial* (2003) **16**:54–60. doi:10.1046/j.1525-139X.2003.03013.x
63. Worth RG, Chien CD, Chien P, Reilly MP, Mckenzie SE, Schreiber AD. Platelet FcgammaRIIA binds and internalizes IgG-containing complexes. *Exp Hematol* (2006) **34**:1490–5. doi:10.1016/j.exphem.2006.06.015
64. Fitzpatrick RE, Wijeyewickrema LC, Pike RN. The gingipains: scissors and glue of the periodontal pathogen, *Porphyromonas gingivalis*. *Future Microbiol* (2009) **4**:471–87. doi:10.2217/fmb.09.18
65. Arvand M, Bhakdi S, Dahlback B, Preissner KT. *Staphylococcus aureus* alpha-toxin attack on human platelets promotes assembly of the prothrombinase complex. *J Biol Chem* (1990) **265**:14377–81.
66. Bryant AE, Bayer CR, Chen RY, Guth PH, Wallace RJ, Stevens DL. Vascular dysfunction and ischemic destruction of tissue in *Streptococcus pyogenes* infection: the role of streptolysin O-induced platelet/neutrophil complexes. *J Infect Dis* (2005) **192**:1014–22. doi:10.1086/432729
67. Johnson MK, Boese-Marrazzo D, Pierce WA Jr. Effects of pneumolysin on human polymorphonuclear leukocytes and platelets. *Infect Immun* (1981) **34**:171–6.
68. Clawson CC. Platelet interaction with bacteria. 3. Ultrastructure. *Am J Pathol* (1973) **70**:449–71.
69. Clawson CC, White JG. Platelet interaction with bacteria. V. Ultrastructure of congenital afibrinogenemic platelets. *Am J Pathol* (1980) **98**:197–211.
70. Youssefian T, Drouin A, Masse JM, Guichard J, Cramer EM. Host defense role of platelets: engulfment of HIV and *Staphylococcus aureus* occurs in a specific subcellular compartment and is enhanced by platelet activation. *Blood* (2002) **99**:4021–9. doi:10.1182/blood-2001-12-0191
71. Li X, Iwai T, Nakamura H, Inoue Y, Chen Y, Umeda M, et al. An ultrastructural study of *Porphyromonas gingivalis*-induced platelet aggregation. *Thromb Res* (2008) **122**:810–9. doi:10.1016/j.thromres.2008.03.011
72. Antczak AJ, Vieth JA, Singh N, Worth RG. Internalization of IgG-coated targets results in activation and secretion of soluble CD40 ligand and RANTES by human platelets. *Clin Vaccine Immunol* (2011) **18**:210–6. doi:10.1128/CVI.00296-10
73. White JG. Why human platelets fail to kill bacteria. *Platelets* (2006) **17**:191–200. doi:10.1080/09537100500441234
74. Abdulrehman AY, Jackson EC, Mcnicol A. Platelet activation by *Streptococcus sanguinis* is accompanied by MAP kinase phosphorylation. *Platelets* (2013) **24**:6–14. doi:10.3109/09537104.2012.661105
75. O'Brien L, Kerrigan SW, Kaw G, Hogan M, Penades J, Litt D, et al. Multiple mechanisms for the activation of human platelet aggregation by *Staphylococcus aureus*: roles for the clumping factors ClfA and ClfB, the serine-aspartate repeat protein SdrE and protein A. *Mol Microbiol* (2002) **44**:1033–44. doi:10.1046/j.1365-2958.2002.02935.x
76. Sullam PM, Hyun WC, Szollosi J, Dong J, Foss WM, Lopez JA. Physical proximity and functional interplay of the glycoprotein Ib-IX-V complex and the Fc receptor FcgammaRIIA on the platelet plasma membrane. *J Biol Chem* (1998) **273**:5331–6. doi:10.1074/jbc.273.9.5331
77. Sun B, Li J, Kambayashi J. Interaction between GPIbalpha and FcgammaIIA receptor in human platelets. *Biochem Biophys Res Commun* (1999) **266**:24–7. doi:10.1006/bbrc.1999.1761
78. Boylan B, Gao C, Rathore V, Gill JC, Newman DK, Newman PJ. Identification of FcgammaRIIa as the ITAM-bearing receptor mediating alphaIIbbeta3 outside-in integrin signaling in human platelets. *Blood* (2008) **112**:2780–6. doi:10.1182/blood-2008-02-142125
79. Kerrigan SW, Cox D. Platelet-bacterial interactions. *Cell Mol Life Sci* (2010) **67**:513–23. doi:10.1007/s00018-009-0207-z
80. Fong KP, Barry C, Tran AN, Traxler EA, Wannemacher KM, Tang HY, et al. Deciphering the human platelet sheddome. *Blood* (2011) **117**:e15–26. doi:10.1182/blood-2010-05-283838

81. Karshovska E, Weber C, Von Hundelshausen P. Platelet chemokines in health and disease. *Thromb Haemost* (2013) **110**:894–902. doi:10.1160/TH13-04-0341
82. McNicol A, Agpalza A, Jackson EC, Hamzeh-Cognasse H, Garraud O, Cognasse F. *Streptococcus sanguinis*-induced cytokine release from platelets. *J Thromb Haemost* (2011) **9**:2038–49. doi:10.1111/j.1538-7836.2011.04462.x
83. Haaland HD, Holmsen H. Potentiation by adrenaline of agonist-induced responses in normal human platelets in vitro. *Platelets* (2011) **22**:328–37. doi:10.3109/09537104.2011.551949
84. Krauel K, Weber C, Brandt S, Zahringer U, Mamat U, Greinacher A, et al. Platelet factor 4 binding to lipid A of gram-negative bacteria exposes PF4/heparin-like epitopes. *Blood* (2012) **120**:3345–52. doi:10.1182/blood-2012-06-434985
85. Andre P, Nannizzi-Alaimo L, Prasad SK, Phillips DR. Platelet-derived CD40L: the switch-hitting player of cardiovascular disease. *Circulation* (2002) **106**:896–9. doi:10.1161/01.CIR.0000028962.04520.01
86. Garraud O, Hamzeh-Cognasse H, Cognasse F. Platelets and cytokines: how and why? *Transfus Clin Biol* (2012) **19**:104–8. doi:10.1016/j.tracli.2012.02.004
87. Cognasse F, Lafarge S, Chavarin P, Acquart S, Garraud O. Lipopolysaccharide induces sCD40L release through human platelets TLR4, but not TLR2 and TLR9. *Intensive Care Med* (2007) **33**:382–4. doi:10.1007/s00134-006-0488-8
88. Knapp S, Von Aulock S, Leendertse M, Haslinger I, Draing C, Golenbock DT, et al. Lipoteichoic acid-induced lung inflammation depends on TLR2 and the concerted action of TLR4 and the platelet-activating factor receptor. *J Immunol* (2008) **180**:3478–84. doi:10.4049/jimmunol.180.5.3478
89. Assinger A, Laky M, Schabbauer G, Hirschl AM, Buchberger E, Binder BR, et al. Efficient phagocytosis of periodontopathogens by neutrophils requires plasma factors, platelets and TLR2. *J Thromb Haemost* (2011) **9**:799–809. doi:10.1111/j.1538-7836.2011.04193.x
90. Semeraro F, Ammollo CT, Morrissey JH, Dale GL, Friese P, Esmon NL, et al. Extracellular histones promote thrombin generation through platelet-dependent mechanisms: involvement of platelet TLR2 and TLR4. *Blood* (2011) **118**:1952–61. doi:10.1182/blood-2011-03-343061
91. Assinger A, Laky M, Badrnya S, Esfandeyari A, Volf I. Periodontopathogens induce expression of CD40L on human platelets via TLR2 and TLR4. *Thromb Res* (2012) **130**:e73–8. doi:10.1016/j.thromres.2012.04.017
92. Ward JR, Bingle L, Judge HM, Brown SB, Storey RF, Whyte MK, et al. Agonists of toll-like receptor (TLR)2 and TLR4 are unable to modulate platelet activation by adenosine diphosphate and platelet activating factor. *Thromb Haemost* (2005) **94**:831–8. doi:10.1160/TH05-01-0009
93. Stahl AL, Svensson M, Morgelin M, Svanborg C, Tarr PI, Mooney JC, et al. Lipopolysaccharide from enterohemorrhagic *Escherichia coli* binds to platelets via TLR4 and CD62 and is detected on circulating platelets in patients with hemolytic uremic syndrome. *Blood* (2006) **108**:167–76. doi:10.1182/blood-2005-08-3219
94. Clark SR, Ma AC, Tavener SA, Mcdonald B, Goodarzi Z, Kelly MM, et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat Med* (2007) **13**:463–9. doi:10.1038/nm1565
95. Jayachandran M, Brunn GJ, Karnicki K, Miller RS, Owen WG, Miller VM. In vivo effects of lipopolysaccharide and TLR4 on platelet production and activity: implications for thrombotic risk. *J Appl Physiol* (2007) **102**:429–33. doi:10.1152/japplphysiol.01576.2005
96. Zhang G, Han J, Welch EJ, Ye RD, Voyno-Yasenetskaya TA, Malik AB, et al. Lipopolysaccharide stimulates platelet secretion and potentiates platelet aggregation via TLR4/MyD88 and the cGMP-dependent protein kinase pathway. *J Immunol* (2009) **182**:7997–8004. doi:10.4049/jimmunol.0802884
97. Soliman A, Michelsen KS, Karahashi H, Lu J, Meng FJ, Qu X, et al. Platelet-activating factor induces TLR4 expression in intestinal epithelial cells: implication for the pathogenesis of necrotizing enterocolitis. *PLoS One* (2010) **5**:e15044. doi:10.1371/journal.pone.0015044
98. Wang B, Wang YQ, Cao SH, Liang Y. [Platelet parameters and platelet toll-like receptor 4 (TLR4) expression in patients with sepsis, and the effect of a joint treatment-plan integrating traditional Chinese and western medicine: a clinical study]. *Zhongguo Wei Zhong Bing Ji Jiu Yi Xue* (2011) **23**:616–20.
99. Berthet J, Damien P, Hamzeh-Cognasse H, Arthaud CA, Eyraud MA, Zeni F, et al. Human platelets can discriminate between various bacterial LPS isoforms via TLR4 signaling and differential cytokine secretion. *Clin Immunol* (2012) **145**:189–200. doi:10.1016/j.clim.2012.09.004
100. Kraemer BF, Campbell RA, Schwertz H, Cody MJ, Franks Z, Tolley ND, et al. Novel anti-bacterial activities of beta-defensin 1 in human platelets: suppression of pathogen growth and signaling of neutrophil extracellular trap formation. *PLoS Pathog* (2011) **7**:e1002355. doi:10.1371/journal.ppat.1002355
101. Drago L, Bortolin M, Vassena C, Taschieri S, Del Fabbro M. Antimicrobial activity of pure platelet-rich plasma against microorganisms isolated from oral cavity. *BMC Microbiol* (2013) **13**:47. doi:10.1186/1471-2180-13-47
102. Yeaman MR. Platelets in defense against bacterial pathogens. *Cell Mol Life Sci* (2010) **67**:525–44. doi:10.1007/s00018-009-0210-4
103. Trier DA, Gank KD, Kupferwasser D, Yount NY, French WJ, Michelson AD, et al. Platelet antistaphylococcal responses occur through P2X1 and P2Y12 receptor-induced activation and kinocidin release. *Infect Immun* (2008) **76**:5706–13. doi:10.1128/IAI.00935-08
104. Yang D, Chen Q, Hoover DM, Staley P, Tucker KD, Lubkowski J, et al. Many chemokines including CCL20/MIP-3alpha display antimicrobial activity. *J Leukoc Biol* (2003) **74**:448–55. doi:10.1189/jlb.0103024
105. Tang YQ, Yeaman MR, Selsted ME. Antimicrobial peptides from human platelets. *Infect Immun* (2002) **70**:6524–33. doi:10.1128/IAI.70.12.6524-6533.2002
106. Yeaman MR, Yount NY, Waring AJ, Gank KD, Kupferwasser D, Wiese R, et al. Modular determinants of antimicrobial activity in platelet factor-4 family kinocidins. *Biochim Biophys Acta* (2007) **1768**:609–19. doi:10.1016/j.bbamem.2006.11.010
107. Agerberth B, Gudmundsson GH. Host antimicrobial defence peptides in human disease. *Curr Top Microbiol Immunol* (2006) **306**:67–90. doi:10.1007/3-540-29916-5_3
108. Guani-Guerra E, Santos-Mendoza T, Lugo-Reyes SO, Teran LM. Antimicrobial peptides: general overview and clinical implications in human health and disease. *Clin Immunol* (2010) **135**:1–11. doi:10.1016/j.clim.2009.12.004
109. Tohidnezhad M, Varoga D, Podschun R, Wruck CJ, Seekamp A, Brandenburg LO, et al. Thrombocytes are effectors of the innate immune system releasing human beta defensin-3. *Injury* (2011) **42**:682–6. doi:10.1016/j.injury.2010.12.010
110. Tohidnezhad M, Varoga D, Wruck CJ, Podschun R, Sachweh BH, Bornemann J, et al. Platelets display potent antimicrobial activity and release human beta-defensin 2. *Platelets* (2012) **23**:217–23. doi:10.3109/09537104.2011.610908
111. Fowler VG Jr, Miro JM, Hoen B, Cabell CH, Abrutyn E, Rubinstein E, et al. *Staphylococcus aureus* endocarditis: a consequence of medical progress. *JAMA* (2005) **293**:3012–21. doi:10.1001/jama.293.24.3012
112. Klevens RM, Morrison MA, Nadle J, Petit S, Gershman K, Ray S, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA* (2007) **298**:1763–71. doi:10.1001/jama.298.15.1763
113. Fitzgerald JR, Foster TJ, Cox D. The interaction of bacterial pathogens with platelets. *Nat Rev Microbiol* (2006) **4**:445–57. doi:10.1038/nrmicro1425
114. Sullam PM, Bayer AS, Foss WM, Cheung AL. Diminished platelet binding in vitro by *Staphylococcus aureus* is associated with reduced virulence in a rabbit model of infective endocarditis. *Infect Immun* (1996) **64**:4915–21.
115. Bayer AS, Sullam PM, Ramos M, Li C, Cheung AL, Yeaman MR. *Staphylococcus aureus* induces platelet aggregation via a fibrinogen-dependent mechanism which is independent of principal platelet glycoprotein IIb/IIIa fibrinogen-binding domains. *Infect Immun* (1995) **63**:3634–41.
116. Fitzgerald JR, Loughman A, Keane F, Brennan M, Knobel M, Higgins J, et al. Fibronectin-binding proteins of *Staphylococcus aureus* mediate activation of human platelets via fibrinogen and fibronectin bridges to integrin GPIIb/IIIa and IgG binding to the FcgammaRIIa receptor. *Mol Microbiol* (2006) **59**:212–30. doi:10.1111/j.1365-2958.2005.04922.x
117. Zapotoczna M, Jevnikar Z, Miajlovic H, Kos J, Foster TJ. Iron-regulated surface determinant B (IsdB) promotes *Staphylococcus aureus* adherence to and internalization by non-phagocytic human cells. *Cell Microbiol* (2013) **15**:1026–41. doi:10.1111/cmi.12097
118. Heptinstall S. Platelet activation by an extracellular adherence protein from *Staphylococcus aureus* acting via modulation of sulfhydryl groups on platelets. *Arterioscler Thromb Vasc Biol* (2012) **32**:1751–2. doi:10.1161/ATVBAHA.112.252460
119. Schubert S, Schwertz H, Weyrich AS, Franks ZG, Lindemann S, Otto M, et al. *Staphylococcus aureus* alpha-toxin triggers the synthesis of B-cell lymphoma 3 by human platelets. *Toxins (Basel)* (2011) **3**:120–33. doi:10.3390/toxins3020120

120. Parimon T, Li Z, Bolz DD, Mcindoo ER, Bayer CR, Stevens DL, et al. *Staphylococcus aureus* alpha-hemolysin promotes platelet-neutrophil aggregate formation. *J Infect Dis* (2013) **208**:761–70. doi:10.1093/infdis/jit235
121. Vanassche T, Kauskot A, Verhaegen J, Peetermans WE, Van Ryn J, Schneewind O, et al. Fibrin formation by staphylothrombin facilitates *Staphylococcus aureus*-induced platelet aggregation. *Thromb Haemost* (2012) **107**:1107–21. doi:10.1160/TH11-12-0891
122. Tran U, Boyle T, Shupp JW, Hammamieh R, Jett M. Staphylococcal enterotoxin B initiates protein kinase C translocation and eicosanoid metabolism while inhibiting thrombin-induced aggregation in human platelets. *Mol Cell Biochem* (2006) **288**:171–8. doi:10.1007/s11010-006-9134-6
123. Waller AK, Sage T, Kumar C, Carr T, Gibbins JM, Clarke SR. *Staphylococcus aureus* lipoteichoic acid inhibits platelet activation and thrombus formation via the Paf receptor. *J Infect Dis* (2013) **208**:2046–57. doi:10.1093/infdis/jit398
124. Shannon O, Uekotter A, Flock JI. Extracellular fibrinogen binding protein, Efb, from *Staphylococcus aureus* as an antiplatelet agent in vivo. *Thromb Haemost* (2005) **93**:927–31. doi:10.1267/THRO05050927
125. Suehiro A, Oura Y, Ueda M, Kakishita E. Inhibitory effect of staphylokinase on platelet aggregation. *Thromb Haemost* (1993) **70**:834–7.
126. Liu CZ, Huang TF, Tsai PJ, Chang LY, Chang MC. A segment of *Staphylococcus aureus* clumping factor A with fibrinogen-binding activity (ClfA221-550) inhibits platelet-plug formation in mice. *Thromb Res* (2007) **121**:183–91. doi:10.1016/j.thromres.2007.03.019
127. Ferkau A, Gillmann HJ, Mischke R, Calmer S, Ecklebe S, Abid M, et al. Infection-associated platelet dysfunction of canine platelets detected in a flow chamber model. *BMC Vet Res* (2013) **9**:112. doi:10.1186/1746-6148-9-112
128. Levi M. The coagulant response in sepsis and inflammation. *Hamostaseologie* (2010) **30**(10–12):14–6. doi:10.1016/j.ccm.2008.06.006
129. Cohen J. The immunopathogenesis of sepsis. *Nature* (2002) **420**:885–91. doi:10.1038/nature01326
130. Perl M, Chung CS, Swan R, Ayala A. Role of programmed cell death in the immunopathogenesis of sepsis. *Drug Discov Today Dis Mech* (2007) **4**:223–30. doi:10.1016/j.ddmec.2008.02.010
131. Cavaillon JM, Adib-Conquy M, Fitting C, Adrie C, Payen D. Cytokine cascade in sepsis. *Scand J Infect Dis* (2003) **35**:535–44. doi:10.1080/00365540310015935
132. Tyml K. Critical role for oxidative stress, platelets, and coagulation in capillary blood flow impairment in sepsis. *Microcirculation* (2011) **18**:152–62. doi:10.1111/j.1549-8719.2010.00080.x
133. Iskander KN, Osuchowski MF, Stearns-Kurosawa DJ, Kurosawa S, Stepien D, Valentine C, et al. Sepsis: multiple abnormalities, heterogeneous responses, and evolving understanding. *Physiol Rev* (2013) **93**:1247–88. doi:10.1152/physrev.00037.2012
134. Okajima K. Regulation of inflammatory responses by natural anticoagulants. *Immunol Rev* (2001) **184**:258–74. doi:10.1034/j.1600-065x.2001.1840123.x
135. Poggi M, Paulmyer-Lacroix O, Verdier M, Peiretti F, Bastelica D, Boucraut J, et al. Chronic plasminogen activator inhibitor-1 (PAI-1) overexpression dampens CD25+ lymphocyte recruitment after lipopolysaccharide endotoxemia in mouse lung. *J Thromb Haemost* (2007) **5**:2467–75. doi:10.1111/j.1538-7836.2007.02757.x
136. Inwald DP, Faust SN, Lister P, Peters MJ, Levin M, Heyderman R, et al. Platelet and soluble CD40L in meningococcal sepsis. *Intensive Care Med* (2006) **32**:1432–7. doi:10.1007/s00134-006-0250-2
137. Chew M, Rahman M, Ihrman L, Erson A, Zhang S, Thorlacius H. Soluble CD40L (CD154) is increased in patients with shock. *Inflamm Res* (2010) **59**:979–82. doi:10.1007/s00011-010-0213-5
138. Lorente L, Martin MM, Varo N, Borreguero-Leon JM, Sole-Violan J, Blanquer J, et al. Association between serum soluble CD40 ligand levels and mortality in patients with severe sepsis. *Crit Care* (2011) **15**:R97. doi:10.1186/cc10104
139. Zhang S, Rahman M, Qi Z, Thorlacius H. Simvastatin antagonizes CD40L secretion, CXC chemokine formation, and pulmonary infiltration of neutrophils in abdominal sepsis. *J Leukoc Biol* (2011) **89**:735–42. doi:10.1189/jlb.0510279
140. Rahman M, Zhang S, Chew M, Syk I, Jeppsson B, Thorlacius H. Platelet shedding of CD40L is regulated by matrix metalloproteinase-9 in abdominal sepsis. *J Thromb Haemost* (2013) **11**:1385–98. doi:10.1111/jth.12273
141. Luo L, Zhang S, Wang Y, Rahman M, Syk I, Zhang E, et al. Proinflammatory role of neutrophil extracellular traps in abdominal sepsis. *Am J Physiol Lung Cell Mol Physiol* (2014) **307**:L586–96. doi:10.1152/ajplung.00365.2013
142. Rahman M, Zhang S, Chew M, Ersson A, Jeppsson B, Thorlacius H. Platelet-derived CD40L (CD154) mediates neutrophil upregulation of Mac-1 and recruitment in septic lung injury. *Ann Surg* (2009) **250**:783–90. doi:10.1097/SLA.0b013e3181bd95b7
143. Rahman M, Roller J, Zhang S, Syk I, Menger MD, Jeppsson B, et al. Metalloproteinases regulate CD40L shedding from platelets and pulmonary recruitment of neutrophils in abdominal sepsis. *Inflamm Res* (2012) **61**:571–9. doi:10.1007/s00011-012-0446-6
144. Banchereau J, Bazan F, Blanchard D, Briere F, Galizzi JP, Van Kooten C, et al. The CD40 antigen and its ligand. *Annu Rev Immunol* (1994) **12**:881–922. doi:10.1146/annurev.iy.12.040194.004313
145. van Kooten C, Banchereau J. CD40-CD40 ligand. *J Leukoc Biol* (2000) **67**:2–17. doi:10.1189/jlb.0805480
146. Asaduzzaman M, Lavasani S, Rahman M, Zhang S, Braun OO, Jeppsson B, et al. Platelets support pulmonary recruitment of neutrophils in abdominal sepsis. *Crit Care Med* (2009) **37**:1389–96. doi:10.1097/CCM.0b013e31819ceb71
147. Yost CC, Weyrich AS, Zimmerman GA. The platelet activating factor (PAF) signaling cascade in systemic inflammatory responses. *Biochimie* (2010) **92**:692–7. doi:10.1016/j.biochi.2010.02.011
148. Inaba K, Branco BC, Rhee P, Blackbourne LH, Holcomb JB, Spinella PC, et al. Impact of the duration of platelet storage in critically ill trauma patients. *J Trauma* (2011) **71**:1766–73. doi:10.1097/TA.0b013e31823bdbf9
149. Cognasse F, Boussoulade F, Chavarin P, Acquart S, Fabrigli P, Lamy B, et al. Release of potential immunomodulatory factors during platelet storage. *Transfusion* (2006) **46**:1184–9. doi:10.1111/j.1537-2995.2006.00869.x
150. Cognasse F, Osselaer JC, Payrat JM, Chavarin P, Corash L, Garraud O. Release of immune modulation factors from platelet concentrates during storage after photochemical pathogen inactivation treatment. *Transfusion* (2008) **48**:809–13. doi:10.1111/j.1537-2995.2008.01655.x
151. Nguyen KA, Hamzeh-Cognasse H, Sebban M, Fromont E, Chavarin P, Absi L, et al. A computerized prediction model of hazardous inflammatory platelet transfusion outcomes. *PLoS One* (2014) **9**:e97082. doi:10.1371/journal.pone.0097082
152. Hamzeh-Cognasse H, Damien P, Nguyen KA, Arthaud CA, Eyraud MA, Chavarin P, et al. Immune-reactive soluble OX40 ligand, soluble CD40 ligand, and interleukin-27 are simultaneously oversecreted in platelet components associated with acute transfusion reactions. *Transfusion* (2014) **54**:613–25. doi:10.1111/trf.12378
153. Wong HR, Cvijanovich NZ, Hall M, Allen GL, Thomas NJ, Freishtat RJ, et al. Interleukin-27 is a novel candidate diagnostic biomarker for bacterial infection in critically ill children. *Crit Care* (2012) **16**:R213. doi:10.1186/cc11847
154. Hamzeh-Cognasse H, Damien P, Nguyen KA, Zeni F, Pozzetto B, Cognasse F, et al. Contribution of activated platelets to plasma IL-27 levels. *Crit Care* (2013) **17**:411. doi:10.1186/cc11925
155. Nomura S, Fujita S, Nakanishi T, Yokoi T, Shimamoto K, Miyamoto R, et al. Platelet-derived microparticles cause CD154-dependent activation of dendritic cells. *Platelets* (2012) **23**:81–2. doi:10.3109/09537104.2011.595850
156. Gawaz M, Fateh-Moghadam S, Pilz G, Gurland HJ, Werdan K. Platelet activation and interaction with leucocytes in patients with sepsis or multiple organ failure. *Eur J Clin Invest* (1995) **25**:843–51. doi:10.1111/j.1365-2362.1995.tb01694.x
157. Johansson D, Shannon O, Rasmussen M. Platelet and neutrophil responses to Gram positive pathogens in patients with bacteremic infection. *PLoS One* (2011) **6**:e26928. doi:10.1371/journal.pone.0026928
158. Kahn F, Hurley S, Shannon O. Platelets promote bacterial dissemination in a mouse model of streptococcal sepsis. *Microbes Infect* (2013) **15**:669–76. doi:10.1016/j.micinf.2013.05.003
159. Vandijck DM, Blot SI, De Waele JJ, Hoste EA, Vandewoude KH, Decruyenaere JM. Thrombocytopenia and outcome in critically ill patients with bloodstream infection. *Heart Lung* (2010) **39**:21–6. doi:10.1016/j.hrtlng.2009.07.005
160. Gafter-Gvili A, Mansur N, Bivas A, Zemer-Wassercug N, Bishara J, Leibovici L, et al. Thrombocytopenia in *Staphylococcus aureus* bacteremia: risk factors and prognostic importance. *Mayo Clin Proc* (2011) **86**:389–96. doi:10.4065/mcp.2010.0705
161. Moreau D, Timsit JF, Vesin A, Garrouste-Orgeas M, De Lassence A, Zahar JR, et al. Platelet count decline: an early prognostic marker in critically ill patients with prolonged ICU stays. *Chest* (2007) **131**:1735–41. doi:10.1378/chest.06-2233

162. Dormehl IC, Kilian JG, Maree M, Jacobs L. Investigation by scintigraphic methods of platelet kinetics under normal and septic shock conditions in the experimental baboon model. *Am J Physiol Imaging* (1990) **5**:75–9.
163. McKenzie SE, Taylor SM, Malladi P, Yuhan H, Cassel DL, Chien P, et al. The role of the human Fc receptor Fc gamma RIIA in the immune clearance of platelets: a transgenic mouse model. *J Immunol* (1999) **162**:4311–8.
164. Francois B, Trimoreau F, Vignon P, Fixe P, Praloran V, Gastinne H. Thrombocytopenia in the sepsis syndrome: role of hemophagocytosis and macrophage colony-stimulating factor. *Am J Med* (1997) **103**:114–20. doi:10.1016/S0002-9343(97)00136-8
165. Zakynthinos SG, Papanikolaou S, Theodoridis T, Zakynthinos EG, Christopoulou-Kokkinou V, Katsaris G, et al. Sepsis severity is the major determinant of circulating thrombopoietin levels in septic patients. *Crit Care Med* (2004) **32**:1004–10. doi:10.1097/01.CCM.0000121433.61546.A0
166. Secor D, Li F, Ellis CG, Sharpe MD, Gross PL, Wilson JX, et al. Impaired microvascular perfusion in sepsis requires activated coagulation and P-selectin-mediated platelet adhesion in capillaries. *Intensive Care Med* (2010) **36**:1928–34. doi:10.1007/s00134-010-1969-3
167. Josefsson EC, White MJ, Dowling MR, Kile BT. Platelet life span and apoptosis. *Methods Mol Biol* (2012) **788**:59–71. doi:10.1007/978-1-61779-307-3_5
168. Leytin V. Apoptosis in the anucleate platelet. *Blood Rev* (2012) **26**:51–63. doi:10.1016/j.blre.2011.10.002
169. Nguyen KA, Hamzeh-Cognasse H, Palle S, Anselme-Bertrand I, Arthaud CA, Chavarin P, et al. Role of siglec-7 in apoptosis in human platelets. *PLoS One* (2014) **9**:e106239. doi:10.1371/journal.pone.0106239
170. Elmore S. Apoptosis: a review of programmed cell death. *Toxicol Pathol* (2007) **35**:495–516. doi:10.1080/01926230701320337
171. Li J, Xia Y, Bertino AM, Coburn JP, Kuter DJ. The mechanism of apoptosis in human platelets during storage. *Transfusion* (2000) **40**:1320–9. doi:10.1046/j.1537-2995.2000.40111320.x
172. Leytin V, Allen DJ, Mykhaylov S, Lyubimov E, Freedman J. Thrombin-triggered platelet apoptosis. *J Thromb Haemost* (2006) **4**:2656–63. doi:10.1111/j.1538-7836.2006.02200.x
173. Qi B, Hardwick JM. A Bcl-xL timer sets platelet life span. *Cell* (2007) **128**:1035–6. doi:10.1016/j.cell.2007.03.002
174. Kraemer BF, Campbell RA, Schwertz H, Franks ZG, Vieira De Abreu A, Grundler K, et al. Bacteria differentially induce degradation of Bcl-xL, a survival protein, by human platelets. *Blood* (2012) **120**:5014–20. doi:10.1182/blood-2012-04-420661
175. Yamakawa K, Ogura H, Koh T, Ogawa Y, Matsumoto N, Kuwagata Y, et al. Platelet mitochondrial membrane potential correlates with severity in patients with systemic inflammatory response syndrome. *J Trauma Acute Care Surg* (2013) **74**:411–7. doi:10.1097/TA.0b013e31827a34cf
176. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. *Science* (2004) **303**:1532–5. doi:10.1126/science.1092385
177. Ma AC, Kubes P. Platelets, neutrophils, and neutrophil extracellular traps (NETs) in sepsis. *J Thromb Haemost* (2008) **6**:415–20. doi:10.1111/j.1538-7836.2007.02865.x
178. von Kockritz-Blickwede M, Nizet V. Innate immunity turned inside-out: antimicrobial defense by phagocyte extracellular traps. *J Mol Med* (2009) **87**:775–83. doi:10.1007/s00109-009-0481-0
179. Chow OA, Von Kockritz-Blickwede M, Bright AT, Hensler ME, Zinkernagel AS, Cogen AL, et al. Statins enhance formation of phagocyte extracellular traps. *Cell Host Microbe* (2010) **8**:445–54. doi:10.1016/j.chom.2010.10.005
180. Phillipson M, Kubes P. The neutrophil in vascular inflammation. *Nat Med* (2011) **17**:1381–90. doi:10.1038/nm.2514
181. Yousefi S, Mihalache C, Kozlowski E, Schmid I, Simon HU. Viable neutrophils release mitochondrial DNA to form neutrophil extracellular traps. *Cell Death Differ* (2009) **16**:1438–44. doi:10.1038/cdd.2009.96
182. Wong CH, Jenne CN, Petri B, Chrobok NL, Kubes P. Nucleation of platelets with blood-borne pathogens on Kupffer cells precedes other innate immunity and contributes to bacterial clearance. *Nat Immunol* (2013) **14**:785–92. doi:10.1038/ni.2631

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 14 November 2014; accepted: 11 February 2015; published online: 26 February 2015.

*Citation: Hamzeh-Cognasse H, Damien P, Chabert A, Pozzetto B, Cognasse F and Garraud O (2015) Platelets and infections – complex interactions with bacteria. Front. Immunol. **6**:82. doi: 10.3389/fimmu.2015.00082*

This article was submitted to Inflammation, a section of the journal Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 13 February 2015
doi: 10.3389/fimmu.2015.00048

Breaking the mold: transcription factors in the anucleate platelet and platelet-derived microparticles

Katie L. Lannan[1], Julie Sahler[1,2], Nina Kim[1], Sherry L. Spinelli[3], Sanjay B. Maggirwar[1], Olivier Garraud[4], Fabrice Cognasse[4,5], Neil Blumberg[3] and Richard P. Phipps[1,3,6]*

[1] Department of Microbiology and Immunology, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA
[2] Department of Biological and Environmental Engineering, Cornell University, Ithaca, NY, USA
[3] Department of Pathology and Laboratory Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA
[4] Faculté de Médecine, Université de Lyon, Saint-Etienne, France
[5] Etablissement Français du Sang Auvergne-Loire, Saint-Etienne, France
[6] Department of Environmental Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA

Edited by:
Masaaki Murakami, Hokkaido University, Japan

Reviewed by:
Daisuke Kamimura, Hokkaido University, Japan
Hideki Ogura, Yale University, USA

Correspondence:
Richard P. Phipps, Department of Environmental Medicine, University of Rochester, 601 Elmwood Avenue, Rochester, NY 14642, USA
e-mail: richard_phipps@urmc.rochester.edu

Platelets are small anucleate blood cells derived from megakaryocytes. In addition to their pivotal roles in hemostasis, platelets are the smallest, yet most abundant, immune cells and regulate inflammation, immunity, and disease progression. Although platelets lack DNA, and thus no functional transcriptional activities, they are nonetheless rich sources of RNAs, possess an intact spliceosome, and are thus capable of synthesizing proteins. Previously, it was thought that platelet RNAs and translational machinery were remnants from the megakaryocyte. We now know that the initial description of platelets as "cellular fragments" is an antiquated notion, as mounting evidence suggests otherwise. Therefore, it is reasonable to hypothesize that platelet transcription factors are not vestigial remnants from megakaryocytes, but have important, if only partly understood functions. Proteins play multiple cellular roles to minimize energy expenditure for maximum cellular function; thus, the same can be expected for transcription factors. In fact, numerous transcription factors have non-genomic roles, both in platelets and in nucleated cells. Our lab and others have discovered the presence and non-genomic roles of transcription factors in platelets, such as the nuclear factor kappa β (NFκB) family of proteins and peroxisome proliferator-activated receptor gamma (PPARγ). In addition to numerous roles in regulating platelet activation, functional transcription factors can be transferred to vascular and immune cells through platelet microparticles. This method of transcellular delivery of key immune molecules may be a vital mechanism by which platelet transcription factors regulate inflammation and immunity. At the very least, platelets are an ideal model cell to dissect out the non-genomic roles of transcription factors in nucleated cells. There is abundant evidence to suggest that transcription factors in platelets play key roles in regulating inflammatory and hemostatic functions.

Keywords: platelets, microparticles, transcription factors, NF-kappa B, PPAR gamma, non-genomic, PPAR alpha, steroid receptors

INTRODUCTION

Platelets are central players in hemostasis and inflammation, contributing to numerous pathophysiologic conditions (1). They are unique from the majority of mammalian cells apart from red blood cells in that they lack a nucleus, and thus have previously been discounted as "cellular fragments." This antiquated notion has been refuted many times over as platelets are now emerging as cellular mediators of cancer cell metastasis, atherosclerosis, type II diabetes, and even mediate adaptive immune responses (2, 3). Platelets are metabolically active cells that contain numerous functional organelles, such as endoplasmic reticulum, Golgi apparatus, mitochondria, and granules that can be released upon activation. Although their lack of a nucleus prevents *de novo* transcription, they can be activated very rapidly to release copious amounts of biological mediators within seconds to minutes of stimulation. The idea that platelets contain transcription factors is a relatively new concept and has led to the discovery of a large number of transcription factors in platelets (**Table 1**). This review will discuss the newly described roles of transcription factors in platelets, in addition to proposing uninvestigated potential roles of transcription factors in platelets, as extrapolated from findings in nucleated cells (**Table 2**).

NUCLEAR FACTOR KAPPA β

In the Immunology field, nuclear factor kappa β (NFκB) is the most widely recognized transcription factor for its quintessential roles in regulating inflammation and immune responses. Almost any immunologist could rattle off key parts of its signaling pathways in response to toll-like receptor (TLR) signaling, influenza infection, or in cytokine production. Although we are quick to

identify its essential roles in regulating transcription of inflammatory genes, the non-genomic roles of NFκB are often overlooked. In all fairness, the concept that NFκB has non-genomic roles in nucleated and non-nucleated cells is a relatively new area of study that is still in its early stages (4). NFκB signaling molecules regulate several different stages of the inflammatory response, without ever entering the nucleus. For example, the NFκB regulatory protein, IkappaB kinase β (IKKβ), can alter the function of numerous proteins via phosphorylation in addition to regulating signaling through direct interactions with cellular effector molecules (5).

Table 1 | Identified transcription factors in platelets.

Transcription factor	Activation of transcription factor	Agonist-induced activation
p65	Phosphorylation	↑ Aggregation, spreading, clot retraction, GPIBα shedding
PPARγ	Ligand binding (15d-PGJ_2, TZDs)	↓ Aggregation, CD40L, TXB_2, P-selectin
	Phosphorylation	↑ Collagen-induced activation and granule secretion
PPARβ/δ	Ligand binding (GW501516, PGI_2)	↓ Aggregation
PPARα	Ligand binding (fenofibrate)	↓ Aggregation
	Phosphorylation	↑ Activation
LXRβ	Ligand binding	↓ Collagen-induced aggregation
RXRα	Ligand binding (9cRA)	↓ Activation
GR	Ligand binding (prednisolone)	↓ Activation
	Ligand binding (dexamethasone)	Unknown
AHR	Ligand binding	↑ Activation
STAT3	Phosphorylation	↑ Aggregation, P-selectin, thrombosis

IKAPPAB KINASE IN PLATELETS

Specifically in platelets, the presence and non-genomic functions of NFκB family members have been demonstrated by several groups, including our own (6–9). Our group discovered the presence of the majority of NFκB family members in human platelets, including the canonical p50/p65 subunits, RelB and c-Rel. Additionally, we identified the presence of IκB proteins and IKK members, which regulate NFκB activation (6). Importantly, these findings suggest that platelets contain an intact, functional, and complete NFκB pathway. The use of the irreversible inhibitor of IKKβ phosphorylation, BAY 11-7082 (BAY), has elucidated complex roles for NFκB in platelet signaling. In human platelets, BAY inhibits platelet spreading and clot retraction, and may alter aggregation at higher doses (6, 10, 11). Malaver et al. (7) and Chen et al. (12) show that high concentrations of BAY (10–25 μM) inhibit platelet aggregation, while our group saw no effect when platelets were treated with 0.5–5 μM BAY (6). As the IC_{50} of BAY for inhibition of IKK-mediated phosphorylation of IκBα is 10 μM, <5 μM BAY may be too low to inhibit IKK sufficiently to affect aggregation (13). Another possibility is that IKK inhibition exerts a threshold response in affecting platelet aggregation, rather than a dose–response. Using genetic approaches, Karim et al. demonstrated that IKKβ knockout mice have variable, but generally attenuated, aggregation responses to platelet agonists, which may explain some of the variations observed in human studies as well (14).

Interestingly, the data reported by Chen et al. and Malavar et al. suggest that the second wave of aggregation was most affected by IKKβ inhibition, thus affecting the maximum aggregation and potentiation of the response in human platelets (7, 12). In accordance with this finding, dense granule release was consistently and potently reduced by IKKβ inhibition in these and other studies. This finding is likely explained by the ability of IKKβ to phosphorylate synaptosomal-associated protein-23 (SNAP-23), an integral regulator of granule secretion (14). SNAP-23 is a member of the target sensitive factor attachment protein receptors (SNARE) complex, which interacts with the vesicle-associated membrane protein (VAMP) to facilitate granule release. IKKβ appears to be central in enhancing soluble *N*-ethylmaleimide–SNARE complex formation

Table 2 | Identified and possible interactions of transcription factors in platelets with other proteins.

Transcription factor	Known protein interaction		Possible protein interactions	
	Binding partners	Outcome	Binding partners	Outcome
PPARγ	Syk, LAT	Platelet activation	NFκB (p65)	Inhibit NFκB activity → anti-platelet effect
	p-ERK, p-p38	Granule secretion	MEK/ERK	Increased activation
	PKCα	Reduce PKCα activation		
PPARβ/δ	PKCα	Dampen platelet adhesion	–	–
PPARα	NFkB	Inhibit NFκB activity → anti-platelet effect	PKCα	Increased activation
LXRβ	Syk, LAT	Dampen platelet activation	PPARγ	Unknown
RXRα	PPARγ	Unknown	G_q	Decreased activation
GR	HSP90	Unknown	–	–
	Mineralocorticoid Receptor	Unknown	–	–
AHR	–	–	NFκB (p65)	Unknown

though the phosphorylation of SNAP-23. Consequently, platelets from IKKβ knockout mice have a reduced ability to release alpha, dense, and lysosomal granules upon thrombin stimulation by approximately 30%. Likewise, treatment of mouse or human platelets with an inhibitor of IKKβ similarly reduced granule secretion upon stimulation. These data suggest that the NFκB signaling molecule, IKKβ, plays an important regulatory role in platelet activation by transducing critical stimulatory signals.

In general, genetic ablation or pharmacological inhibition of IKKβ in mice results in a hyporesponsive phenotype to agonist-induced platelet activation. Inhibition of IKKβ in mice prolonged thrombus formation and increased bleeding times (14). Additionally, a second IKKβ inhibitor, IKK inhibitor VII, recapitulated several of the aforementioned findings in both human and mouse platelets, including preventing P-selectin expression and dampening aggregation (15). Of note, Gambaryan et al. found that IKK inhibitor VII potentiated collagen and thrombin-induced aggregation, rather than having an inhibitory effect (8). They proposed a model in which thrombin and collagen induce a negative feedback loop in platelets that inhibits platelet function through NFκB. Although the work delves into the complex agonist-induced signaling pathways of NFκB in platelets, their data showing potentiation of platelet activation by IKK inhibitor VII is less clear-cut. Treatment of human platelets with IKK inhibitor VII enhanced very low dose (0.001 U/mL) thrombin-induced PAC1 binding by approximately 15%. Additionally, IKK inhibitor VII treatment only mildly potentiated collagen (10 μg/mL) and thrombin (0.01 U/mL)-induced platelet aggregation, with a slight left shift in the aggregation traces compared to control. However, the maximum amplitude of aggregation was indistinguishable between IKK inhibitor VII treatment and control. Although the data demonstrating NFκB signaling post-activation are intriguing, the current consensus is that NFκB primarily plays an important role in positively regulating platelet activation.

As much of the investigations into the non-genomic roles of the transcription factor, NFκB, has involved manipulation of its upstream regulatory kinase, IKK, careful consideration must be taken into account when interpreting the findings of these studies. IKK acts as a kinase that plays a crucial role in regulating NFκB activation, but can also phosphorylate other proteins that may play regulatory roles in platelet activation (5). Thus, inhibition of IKK in platelets may dampen platelet function in a non-canonical fashion, independent of NFκB. On the other hand, many studies have observed changes in p65 phosphorylation in platelets, although the effects of direct inhibition or deletion of p65 has not been investigated to date. It is likely that IKK activation in platelets results in NFκB-dependent and -independent regulation of platelet function. This is supported by the data showing that IKK phosphorylates SNAP-23 (14), leading to granule secretion, but can also activate p65, which can regulate protein kinase A (PKA) (8).

NFκB IN PLATELETS DURING INFLAMMATION

Nuclear factor kappa β in nucleated cells is known to play a crucial role in inflammatory diseases, although its functions in platelets during inflammation are still under active investigation. In one study, IKKβ deficiency increased neointimal formation in low-density lipoprotein receptor (LDLR) knockout mice and exhibited increased leukocyte adherence to the vessel walls after injury (16). Upon further investigation, IKKβ-deficient platelets were unable to shed GPIbα in response to thrombin stimulation. Interestingly, GPIbα shedding in response to ADP or collagen was not affected, suggesting that IKKβ is uniquely involved in thrombin-induced GPIbα shedding. These data are intriguing in light of the fact that many studies evaluating the role of IKKβ in platelets focused on thrombin-induced activation and signaling. However, collagen and ADP-induced aggregation and granule secretion were also dampened by IKKβ deficiency or pharmacological inhibition. Furthermore, no differences in GPVI, GPIX, or αIIbβ3 shedding were found by loss of IKKβ in mouse platelets. These data suggest that although IKKβ plays an important role in the activation of platelets, it may also induce an inhibitory feedback loop, as proposed by Gambaryan et al., perhaps through shedding of GPIbα (8). Sustained levels of GPIbα on the platelet surface can enhance platelet–leukocyte interactions, and thus, may exacerbate certain conditions.

Platelets are also known to response to various immunologic stimuli, such as TLR ligands (17). In nucleated cells, bacterial lipopolysaccharide (LPS) signaling through TLR4 is largely through NFκB, leading to the production of proinflammatory cytokines and chemokines. We have recently shown that platelets can discriminate between different isoforms of LPS (18). This suggests that platelets are capable of specifically sensing and responding to various bacterial products. Thus, it is possible that LPS signaling in platelets involves differential NFkB activation, although this has not been investigated in platelets to date.

SIGNALING MECHANISM OF NFκB IN PLATELETS

Thrombin activates the NFκB signaling cascade in platelets, although the complete pathway has not yet been elucidated. Several lines of evidence suggest that p38 mitogen-activated protein kinase (MAPK) signaling is upstream of NFκB in platelets, while the extracellular signal-regulated kinase (ERK) pathway is downstream of NFκB activation (19, 20). In human platelets, inhibition of MAPK prevented collagen-induced IKKβ and p65 phosphorylation, while treatment with an ERK inhibitor had no effect. Furthermore, collagen-induced ERK phosphorylation was prevented by pretreatment with an MAPK inhibitor or the NFκB inhibitor BAY, suggesting that NFκB signaling regulates ERK activation. ERK activates phospholipase A_2 (PLA_2), which releases arachidonic acid, leading to platelet aggregation and mediator release. This explains why arachidonic acid-induced platelet aggregation is unaffected by NFκB inhibitors, while aggregation in response to other agonists is dampened. Interestingly, one study suggests that activation of NFκB by CD40L signaling may be independent of p38 MAPK, but may instead involve TRAF2 activation of IKKβ (15). Additionally, protease-activated receptor 4 (PAR4) stimulation led to ceramide production by sphingomyelin phosphodiesterase (Smase), which in turn activated MAPK, while PAR1 signaling was independent of ceramide (12). This is consistent with the finding that exogenous treatment of platelets with ceramide leads to *in vitro* activation and enhances thrombosis *in vivo*. PAR1

and PAR4 are the thrombin receptors on human platelets, with PAR1 having a lower threshold for activation by thrombin than PAR4 (21). PAR1 activation typically induces a rapid, but transient spike in calcium, while PAR4 activation involves a more sustained response, suggesting overlapping, but distinct roles for these receptors (22). These data reveal a novel and distinct signaling pathway for PAR1 and PAR4 receptors, although both converge on NFκB signaling.

Taken together, these data present compelling evidence that NFκB plays an important, albeit complex, role in platelet activation (**Figure 1**). The data support a model whereby platelet activation through various receptors leads to phosphorylation and activation of IKKβ, release of p65, and subsequent platelet aggregation and granule release. This model, however, is not mutually exclusive of the idea that NFκB may induce a negative inhibitory feedback loop in platelets (8). After dissociation of p65 from its inhibitory complex, PKA is free to induce vasodilator-stimulated phosphoprotein (VASP) phosphorylation, which mediates platelet inhibitory signaling. This may represent a mechanism to fine tune platelet activation after thrombin stimulation. In fact, PIP_3 can induce VASP phosphorylation, leading to inhibitory signaling, in addition to activating IKK through protein kinase B, also known as Akt. Thrombin signaling also appears to be unique in that it stimulates GPIb shedding through NFκB and ADAM17, unlike ADP or collagen-induced activation (16). ADAM17 is a sheddase that is critical for platelet surface receptor shedding and can be activated by p38 MAPK (23). Unlike pharmacological inhibition of NFκB in human platelets, IKK-deficient mouse platelets are unable to phosphorylate p38 MAPK after thrombin stimulation (16). This raises the question as to whether defective GPIb shedding in IKKβ-deficient mouse platelets is an artifact of interspecies variability, differences in inhibition versus complete lack of IKKβ, or merely a technical timing issue. Regardless, it will be necessary to investigate the role of ADAM17 and GPIb shedding in human platelet NFκB signaling.

NON-GENOMIC FUNCTIONS OF NFκB: LESSONS FROM NUCLEATED CELLS

Nuclear factor kappa β is a versatile family of proteins capable of performing multiple functions in nucleated cells apart

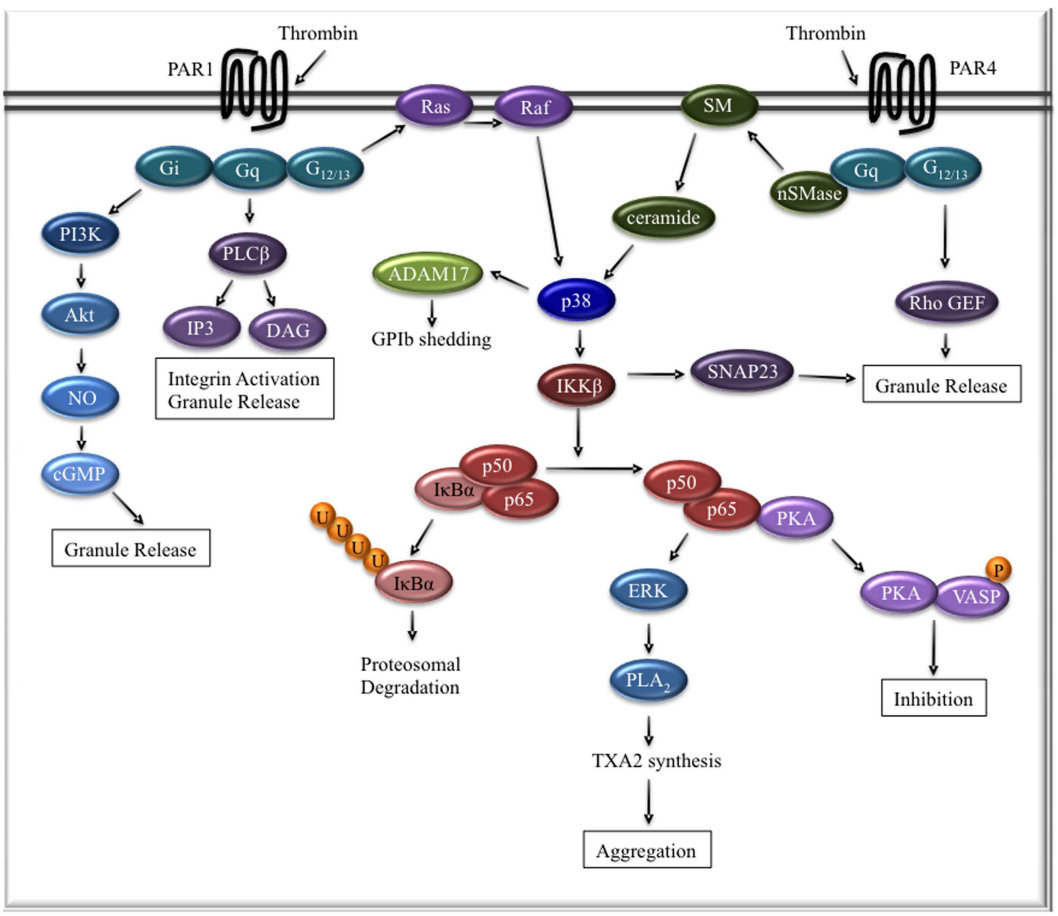


FIGURE 1 | The role of NFκB in platelet thrombin signaling. Thrombin signaling through PAR1 in human platelets is mostly independent of NFκB. PAR4 signaling in human platelets involves activation of p38 MAPK through ceramide signaling. Activated p38 MAPK phosphorylates IKKβ, leading to the subsequent ubiquitination and proteosomal degradation of the inhibitory IκBα protein. Active p50/p65 dimers activate the ERK signaling cascade, which is involved in thromboxane TXA_2 synthesis and aggregation. The p65 subunit can also bind PKA, inducing a negative feedback loop through VASP phosphorylation.

from acting as a transcription factor (5). Evaluating these identified non-genomic roles in nucleated cells is likely to translate to important regulatory functions in platelets. Although roles for IKKβ in platelets have already been identified, studies from nucleated cells suggest that there may be more. A strong contender is the role of IKKβ in platelet spreading, as inhibition of IKKβ in human platelets leads to a spreading defect. In epithelial cells and B-lymphocytes, IKKβ-mediated phosphorylation of Dok1 inhibits ERK activation, leading to increased cell motility (24). Platelets are known to transiently activate ERK, which is important for alpha granule release. However, outside-in integrin signaling through the fibrinogen receptor, as is likely to occur during platelet spreading, inhibits ERK signaling (25). Thus, inhibiting IKKβ may impair platelet spreading through a similar Dok1-mediated mechanism. Proteomic data have identified the presence of Dok1 in human platelets, although its function has yet to be studied in this context (26).

The NFκB protein p65 and IκBα, are found in the mitochondria and appear to be differentially regulated compared to their cytoplasmic counterparts (27). Interestingly, canonical NFκB stimulatory signals had no effect on the expression or phosphorylation of mitochondria-localized p65 (28). Conversely, stimulation of liver or Jurkat cells with tumor necrosis factor α (TNFα) or FAS ligand, respectively, led to non-proteasomal degradation of IκBα and induction of apoptosis. IKKβ, on the other hand, was not localized to the mitochondria. To date, most studies of NFκB in platelets have involved the use of IKKβ inhibitors and IKKβ knockout mice. Investigations in platelets centering on the role of IκBα and p65, in lieu of IKKβ, may reveal novel apoptotic regulatory elements in platelets.

A second intriguing possibility is the role of 14–3–3β in regulating mRNA stability in platelets. 14–3–3β is a highly conserved protein that binds AU-rich elements (ARE) on mRNA, leading to destabilization of mRNA (29). Phosphorylation of 14–3–3β by IKK results in the release of a 14–3–3β-tritetraprolin complex from the mRNA, thus preventing its destabilizing effects (30). This is interesting in light of the fact that platelets contain mRNA, which can be translated upon stimulation (31) Furthermore, 14–3–3 proteins can interact with p65 and IκBα to facilitate the export of p65 from the nucleus. As proteomic data show that human platelets express 14–3–3β (32), it is possible that it plays an important role in regulating mRNA stability or NFκB signaling in the anucleate platelet.

PPARγ IN PLATELETS

Peroxisome proliferator-activated receptor γ (PPARγ) is a ligand-activated transcription factor that is important in the regulation of lipid and glucose metabolism and is essential for fat production. Inactive PPARγ is localized to the cytoplasm of nucleated cells complexed with co-repressors. Upon ligand binding, the co-repressors dissociate, leading to a conformational change in PPARγ, allowing dimerization with retinoid X receptor α (RXRα) (33). This heterodimeric complex then acts as a scaffold for the recruitment of coactivator proteins, and PPARγ-dependent gene transcription commences. Our group discovered that human and mouse platelets express functional PPARγ protein that is regulated by activation or treatment with PPARγ ligands (34).

PPARγ LIGANDS ARE CARDIOPROTECTIVE

Thiazolidines (TZDs) are a class of oral antidiabetic drugs that exert their insulin-sensitizing actions through activation of PPARγ. In addition to regulating glucose homeostasis, TZDs, such as rosiglitazone, pioglitazone, and troglitazone, have potent anti-inflammatory properties (35). Type II diabetics taking TZDs have improved glucose metabolism, decreased markers of inflammation, and improved cardiovascular health. Much of this can be attributed to the beneficial actions of TZDs on vascular cells and both direct and indirect actions on circulating platelets. Platelets from type II diabetics exhibit a more activated phenotype and are hyper-responsive to agonist (36, 37). These changes include increased platelet numbers and mean platelet volume (MPV), which may indicate alterations in megakaryocyte function or increased platelet turnover (38). Additionally, platelets from type II diabetics have enhanced surface expression of the collagen receptor (GPVI) and the fibrinogen receptor (αIIbβ3), leading to increased adhesiveness and aggregation (39). Plasma from type II diabetics contains higher levels of the platelet-derived inflammatory mediators, soluble CD40L (sCD40L), soluble P-selectin (sP-selectin), and C-reactive protein (40, 41). Dsyregulated platelet function in type II diabetics is likely due to both inherent changes in the platelet and decreased prostaglandin I_2 (PGI_2) and nitric oxide (NO) production from endothelial cells, which exert potent anti-platelet effects.

Numerous studies have demonstrated that TZDs beneficially regulate cardiovascular function in type II diabetics. They have consistently been shown to reduce the elevated levels of plasminogen activator inhibitor 1 (PAI-1) (42–44). PAI-1 rapidly binds and inactivates tissue plasminogen activator, thus preventing fibrinolysis and increasing thrombotic risk. Additionally, TZDs reduce markers of cardiovascular disease, which are typically elevated in type II diabetics, including C-reactive protein, serum amyloid A, fibrinogen, and matrix metalloproteinase 9 (45–49). Many of these beneficial changes can be attributed to improved glucose metabolism and restored vascular and endothelial homeostasis, although similar results can be seen in non-diabetic patients (50).

PPARγ LIGANDS IMPROVE PLATELET FUNCTION IN TYPE II DIABETIC

Consistent with their ability to reduce thrombotic risk, TZDs reduce markers of platelet activation. Rosiglitazone monotherapy decreased plasma sCD40L and P-selectin levels (51, 52). These data suggest that TZDs can directly or indirectly reduce platelet activation in type II diabetics. Supporting these findings, Khanolkar et al. demonstrated that patients receiving rosiglitazone and metformin had a significantly greater reduction in platelet aggregation compared to patients receiving gliclazide and metformin (53). Similarly, a second study found that pioglitazone in combination with metformin improved platelet function to a greater degree than glimepiride and metformin (49). A major unanswered question from these studies is whether TZDs improve cardiovascular health secondary to improved lipid metabolism or due to the anti-inflammatory actions of PPARγ stimulation. For example, macrophages, which play a key role in atherosclerosis, efflux cholesterol and are less inflammatory in response to PPARγ activation. In an attempt to investigate the insulin-sensitizing independent

effects of TZDs, non-diabetic patients with coronary artery disease were treated with TZDs for 12 weeks. In this setting, TZDs reduced the inflammatory markers, C-reactive protein, tumor necrosis factor α (TNFα), and interleukin-6 (IL-6) (50, 54). Additionally, circulating platelet activity was dampened, as evidenced by fewer P-selectin positive platelets, suggesting an important role for the anti-inflammatory actions of PPARγ.

PPARγ LIGANDS DAMPEN PLATELET FUNCTION FROM HEALTHY DONORS

Due to the global effects of TZDs, it is difficult to tease out indirect actions from potential direct effects of TZDs on platelet function. However, many recent studies have aimed to elucidate the direct effects of TZDs on platelet function. Our lab was the first to show that human platelets and megakaryocytes express functional PPARγ (55). The PPARγ ligands 15d-PGJ_2 and rosiglitazone potently inhibit thrombin-induced CD40L surface expression and thomboxane B_2 (TXB_2) production and dampen ADP-induced aggregation (56). Other studies have confirmed these findings and further demonstrated that rosiglitazone and 15d-PGJ_2 inhibited collagen-induced aggregation and prevented P-selectin exposure *in vitro* and *in vivo* (54, 57). Utilizing the specific PPARγ antagonist, GW9662, these effects were partially mediated through PPARγ in platelets from healthy donors (58). Moreover, pioglitazone was protective in a mouse model of thrombosis (57, 59). Similarly, using platelets from type II diabetics, which are hyperresponsive to agonist, rosiglitazone reduced aggregation and mediator release (36, 60). These data support the hypothesis that TZDs can regulate platelet function by directly acting on platelet PPARγ.

Interestingly, PPAR-independent pathways are evident upon treatment with some ligands. 15d-PGJ_2 is an electrophilic compound that is known to form adducts with other cellular proteins, and could explain some of the PPAR-independent effects (61). Most interestingly, the mechanism of troglitazone differs from that of the structurally similar TZD, pioglitazone, in platelets. Although troglitazone and pioglitazone decreased platelet activation *in vivo*, only troglitazone directly inhibited platelet aggregation *in vitro* (62). However, in this study, only 1 μM of each TZD was examined for their effects on platelet function. In some cell systems, troglitazone is more potent than pioglitazone, despite having a higher EC_{50} for binding PPARγ and this may also be the case in platelets (63). It is possible that higher concentrations of pioglitazone would exhibit similar effects as troglitazone. Another possibility is that there may be PPAR-independent effects or differential signaling of PPARγ in human platelets. Clinical data points to some PPAR-independent actions of TZDs as pioglitazone has been shown to decrease the risk of myocardial infarction and stroke in type II diabetics, while rosiglitazone had no effect and may actually increase the relative risk (64).

SIGNALING MECHANISM OF PPARγ IN PLATELETS

Differential signaling of PPARγ is not an unprecedented finding, as PPARγ is known to recruit various co-activators after stimulation with different ligands. Although most TZDs bind identical binding pockets in PPARγ, their biological profiles are distinct (65, 66). This is in part due to differential recruitment of co-activators, but also possibly due to variations in availability of cofactors. In cell-based systems, PPARγ ligands can act as partial agonists in some cell types and full agonists in others (67). Additionally, different PPARγ ligands can recruit different co-activators in the same cell type, leading to different outcomes (68–70). These differences likely explain many of the adverse effects observed with some TZDs in clinical trials. Although still poorly understood, differential binding and recruitment of cofactors may explain the complex and sometimes contradictory actions of PPARγ in platelets, although no studies have evaluated this role of PPARγ in platelets to date.

Although a transcription factor, PPARγ has been shown to serve many other important non-genomic roles in platelets and nucleated cells (**Figure 2**). Specifically in platelets, collagen stimulation results in PPARγ recruitment to the GPVI signalosome and interacts with the adapter molecule, Syk (71). As a result, linker of activated T cells (LAT) is recruited and forms a complex with Syk and PPARγ. In this setting, PPARγ appears to be necessary for enhancement of GPVI-mediated activation as treatment with PPARγ antagonists only partially blocks phosphorylation of LAT and the downstream targets phospholipase Cγ (PLCγ), PI3K, and Akt. Interestingly, several other groups have demonstrated that cytosolic platelet PPARγ is released into the supernatants and in platelet-derived microparticles rapidly upon activation, leaving very low levels of PPARγ in the activated platelets (34, 72). This may suggest that early signaling

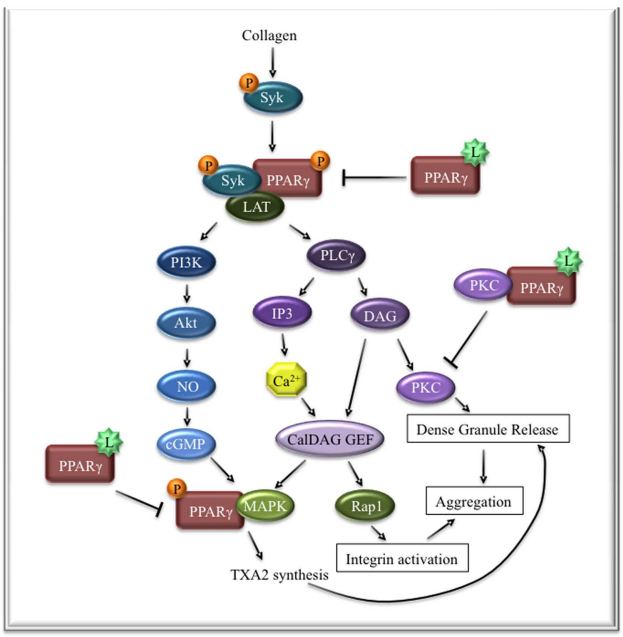


FIGURE 2 | The role of PPARγ in collagen signaling in platelets. GPVI stimulation with collagen leads to the recruitment and activation of Syk. Syk recruits and phosphorylates PPARγ, which in turn leads to the recruitment of LAT, resulting in enhanced signal transduction downstream of GPVI. PPARγ ligands prevent the interaction of PPARγ with the Syk signaling complex, which is necessary for enhanced signaling of Syk and LAT. Ligand-bound PPARγ can also interact with PKCα, preventing it from mediating dense granule release. Lastly, ligand-activated PPARγ prevents PPARγ phosphorylation and interaction with MAPK, an essential downstream mediator of collagen signaling.

roles of PPARγ include recruitment to the LAT/Syk signaling complex, which results in its packaging and export into microparticles, possibly due to its proximity to the cell membrane. Alternatively, there may be different pools of PPARγ that could have different subcellular localization patterns. It is not known whether platelets contain an unknown endogenous PPARγ ligand that plays a role in this signaling pathway. However, exogenous treatment of activated platelets with PPARγ ligands inhibit its interaction with the LAT/Syk signaling complex and reduces the amplitude of the signal, thus reducing aggregation and mediator release. Similarly, PPARγ ligands also blunt release of PPARγ into the supernatants and platelet-derived microparticles (34).

Separate studies have suggested that PPARγ interacts with ERK and p38 MAPK, which are common downstream mediators of platelet activation, leading to granule secretion (73). Stimulation of human platelets with collagen led to phosphorylation of PPARγ and its subsequent association with p-ERK and p38 MAPK. Treatment with PPARγ ligands prevented these interactions, reducing granule release. Similarly, PPARγ has been shown to interact with PKCα in platelets in response to PPAR agonists, consequently reducing the activation of PKCα (74). Although complex, there is sufficient evidence to suggest that PPARγ plays an important role in platelet signaling. PPARγ knockout mice are embryonic lethal; thus, a megakaryocyte and platelet-specific PPARγ knockout mouse model would be invaluable in determining the biologic role of PPARγ in platelets. As platelets do not contain a nucleus, any genomic deletion of PPARγ in platelets must also be deleted in the parent megakaryocyte. Therefore, changes in platelet function in these mice could be due to the loss of PPARγ in the platelet or megakaryocyte. Our group has recently shown that overexpression of PPARγ in a megakaryocyte cell line resulted in enhanced platelet production upon stimulation with a PPARγ ligand (72). These data suggest that PPARγ may play an important role in platelet production from megakaryocytes and could potentially convolute findings from megakaryocyte and platelet-specific PPARγ knockout mice.

PPARβ/δ

PPARβ/δ is broadly expressed and plays an important role in skeletal muscle where it regulates cellular proliferation, differentiation, and fatty acid catabolism (75). Similar to PPARγ, PPARβ/δ is found in human and mouse platelets and PPARβ/δ ligands inhibit platelet function (76). Treatment with the specific PPARβ/δ ligand, GW501516, significantly increased cAMP levels in mouse platelets and prevented aggregation (77). Utilizing platelets from PPARβ/δ knockout mice, the increase in cAMP was shown to be specific to PPARβ/δ signaling, as cAMP levels in knockout platelets were drastically lower in response to ligand. Interestingly, no difference in aggregation was observed between knockout and control platelets treated with GW501516, suggesting that the inhibition of platelet aggregation by GW501516 occurs independent of PPARβ/δ. Furthermore, no differences were observed between knockout and control platelets' ability to produce cAMP or aggregate in response to ADP in the absence of PPARβ/δ ligand. This indicates that PPARβ/δ in platelets may require ligand binding to exert its inhibitory effects on platelet function. Nevertheless, these data demonstrate that PPARβ/δ is capable of signaling in platelets, although the physiologic relevance remains unclear at this point.

One possible mechanism by which PPARβ/δ dampens platelet activation may involve the endogenous antithrombotic molecule, prostacyclin I_2 (PGI_2). PGI_2 is produced by endothelial cells and is reported to be a ligand for PPARβ/δ (78), although this has not been proven in platelets. Previous studies have shown that PGI_2 can synergize with endothelial-derived NO to inhibit platelet aggregation in response to variety of agonists through a mechanism involving increased cAMP (76). Another possible mechanism of action of PPARβ/δ in platelets may involve modulation of PKCα function. Treatment of human platelets with GW501516 induced association of PPARβ/δ with PKCα in a dose-dependent manner (77). PKCα has been shown to positively regulate platelet adhesion, a function that was dampened with PPARβ/δ activation. Overall, very little is known about the biological significance and mechanism of PPARβ/δ in platelets, although the available data suggest a role in inhibiting platelet activation.

PPARα

PPARα is a major regulator of fatty acid homeostasis and inflammation. It is highly expressed in brown adipose tissue, liver, kidney, heart, and skeletal muscle (75). PPARα agonists are used as a treatment for elevated plasma lipid levels and for their ability to increase the uptake of fatty acids and improve the high-density lipoprotein (HDL) to low-density lipoprotein (LDL) ratio. Little is known about the function of PPARα in regulating hemostasis, but its presence was recently discovered in platelets. Statins and fibrates are PPARα agonists and are widely prescribed for the prevention of coronary artery disease and atherosclerosis, thus reducing the risk of thrombotic events (74). In addition to their effects on lipid metabolism, statins and fibrates have been shown to activate PPARα (79). Specifically, fenofibrate decreased agonist-induced platelet activation and increased bleeding times in mice (74). Fenofibrate's ability to inhibit platelet aggregation was abolished in the presence of a specific PPARα antagonist. Additionally, bleeding times in PPARα knockout mice were not affected by treatment with fenofibrate, unlike control mice. Interestingly, the baseline bleeding times in PPARα knockout mice were longer than control mice, suggesting an additional role for PPARα in maintaining hemostasis. Due to the usage of PPARα global knockout mice, it is unclear whether this difference was due to platelet-specific or multivariate effects. Although the mechanisms of action of PPARα in platelets have not been thoroughly investigated, some evidence suggests that it may regulate PKCα activity, similar to PPARγ and PPARβ/δ (80).

NON-GENOMIC FUNCTIONS OF PPARs: LESSONS FROM NUCLEATED CELLS

The wide use of PPAR ligands in the clinic has led to the discovery of numerous pleotropic effects of these compounds. These include PPAR-dependent, non-genomic actions in addition to PPAR-independent signaling of ligands (81, 82). The PPAR-independent signaling mechanisms of PPAR ligands will not be discussed here, but these effects must be kept in mind when interpreting data investigating the effects of PPAR ligands on platelet function. In fact, these likely play an important role in regulating

platelet function, perhaps through affecting mitochondrial activity, as many effects of PPAR ligands in platelets cannot be reversed using PPAR antagonists. Additionally, many PPAR ligands, such as unsaturated fatty acids, can cross-react with all PPAR isoforms at higher concentrations, further complicating the interpretation of these findings (83). It may be possible to generate useful hypotheses about how PPARs signal in platelets based upon similar studies in nucleated cells.

PPARγ–PKCα CROSSTALK

In human epithelial colorectal adenocarcinoma cells, PPARγ has been shown to regulate NFκB activity in a non-genomic fashion involving their direct association (84). In this model, PPARγ bound to p65 in the nucleus, resulting in export of both proteins to the cytoplasm. Consequently, cytoplasmic localization of NFκB prevented its proinflammatory transcription-dependent effects. It is possible that PPARγ can also physically interact with p65 in platelets. This interaction could inhibit NFκB activity in platelets, which would exert anti-inflammatory and anti-platelet effects. PPARγ has also been shown to form a direct interaction with PKCα upon stimulation with various PPARγ ligands in macrophages (85). The physical association of PKCα with PPARγ prevented PKCα translocation to the membrane and subsequent degradation. However, in this system PPARγ was only able to negatively regulate PKCα activation in response to low-dose PMA stimulation. This suggests that the inhibitory effect of PPARγ can be overridden with stronger stimulation.

PPARα–PKCα CROSSTALK

Interestingly, PPARα has also been shown to directly interact with PKCα in macrophages (86). In response to LPS stimulation, PKCα bound and phosphorylated PPARα. However, in the presence of the PPARα ligand, simvastatin, PPARα no longer bound PKCα and consequently was able to transrepress NFκB activation. In this model, PKCα likely acts to deactivate PPARα's inhibitory actions on the proinflammatory NFκB signaling. This may represent a mechanism by which PPARα ligands, such as statins, exert potent and rapid anti-inflammatory actions. Although the interaction of PPARα with PKCα was postulated to negatively regulate PPARα's function in this system, it is possible that this complex serves additional and possibly proinflammatory functions. Similarly, these data suggest that in platelets, ligand-induced activation of PPARα may function differently than phosphorylation-induced activation, resulting in different or possibly contradictory actions. This may even explain the discrepancy between the bleeding phenotype observed in PPARα knockout mice, while control mice also had increased bleeding times with PPARα activation. Perhaps, PPARα, independent of ligand, acts as a positive regulator of platelet hemostatic function by enhancing activation of PKCα, while ligand-activated PPARα sequesters and inhibits NFκB thus, negatively regulating platelet activation (**Figure 3**).

PPARγ AND PPARα REGULATE MAP KINASES

Another possible role of PPARα in platelets involves regulation of MAP kinases. In T cells, PPARα was shown to inhibit p38 MAPK

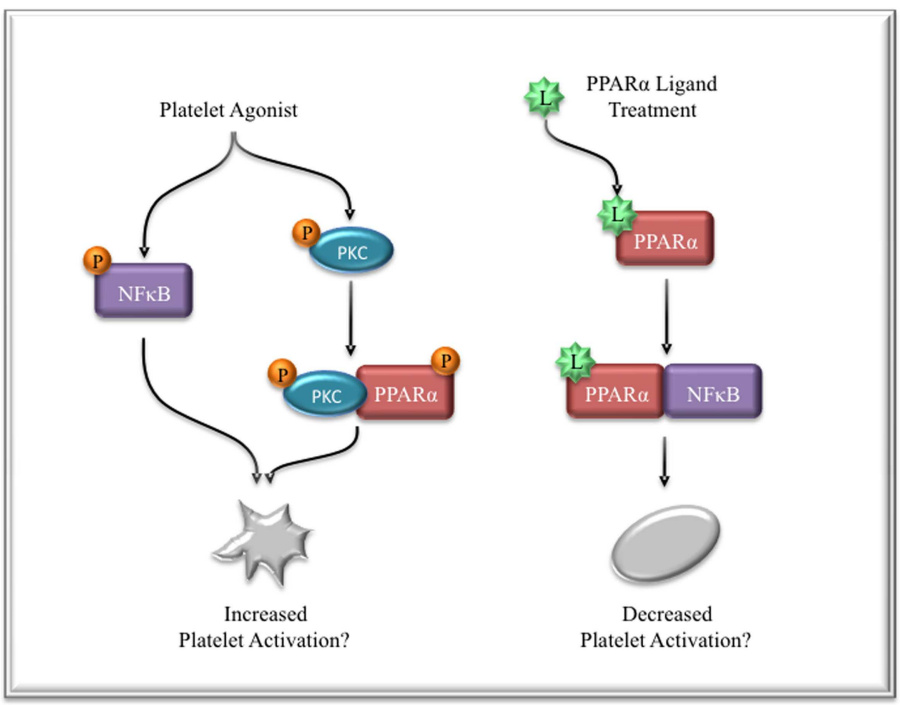


FIGURE 3 | Possible mechanism of PPARα and NFκB regulation in platelets. Platelet activation leads to NFκB phosphorylation and activation, which potentiates activation signals. PKCα is also phosphorylated and activated in response to stimulation. This may lead to an interaction between PPARα and PKCα, leading to increased activation. Treatment with PPARα ligands prevents PPARα binding to PKCα, but instead enhances its interaction with NFκB, thus functionally sequestering PPARα and NFκB from participating in activation signaling.

activation, but only in the absence of ligand (87). Additionally, this regulation of p38 MAPK appeared to be independent of direct interaction with PPARα, but likely required an unknown secondary mediator. Activation of p38 MAPK occurs early in platelet activation, albeit transiently, to induce granule release that stimulates the second wave of aggregation (88, 89). Subsequent ligand binding to αIIbβ3 downregulates active p38 MAPK (90). Perhaps, PPARα mediates the regulation of p38 MAPK in these conditions or possibly under shear stress, whereby p38 MAPK mediates adhesion in flow.

PPARγ has also been shown to regulate MAPK pathways in nucleated cells through non-genomic mechanisms (91). PPARγ reversibly interacts with MEK1 via its AF2 domain, resulting in export from the nucleus to the cytoplasm. This corroborates evidence suggesting that PPARγ ligands regulate downstream ERK signaling (92). Interestingly, PPARγ activation led to rapid ERK phosphorylation in human prostate cancer cells, vascular smooth muscle cells, and human microvascular endothelial cells, but inhibited ERK activation in adrenocortical cancer cells (93–95). Similarly, PPARγ enhanced ERK activation in rabbit renal cortex cells, but had no effect in mouse cells (96). These data suggest cell type-specific and species-specific effects of PPARγ, possibly through availability of different co-activators. The effects of PAPRγ activation on the MEK/ERK pathway in platelets has not been investigated, but represents a promising avenue of further research.

LIVER X RECEPTORS

Liver X receptors (LXRs) are transcription factors that play key roles in cholesterol homeostasis by regulating genes, such as apolipoprotein E and cytochrome P450 7α-hydroxylase 1 (Cyp7a1) (97, 98). Recent studies have demonstrated that platelets express LXRβ and its ligands inhibited collagen-induced aggregation (99). Thrombin and fibrinogen-induced activation was affected to a lesser degree, requiring high doses of LXR ligands to exert comparable inhibitory effects. Collagen-induced Syk phosphorylation was strongly inhibited by pretreatment with LXR ligands, while LAT and PLCγ phosphorylation were inhibited to a lesser degree, which could be explained by the high concentration of collagen used for activation (50 μg/mL). Interestingly, and opposite of the results seen with PPARγ (71), LXR ligands induced association of LXR with Syk and PLCγ. Moreover, stimulation of platelets with PPARγ or LXR ligands led to an association between the two transcription factors, which was diminished with higher concentrations of ligand. Taken together, these data suggest that LXR and PPARγ can physically interact in platelets and this may represent a novel regulatory mechanism of collagen-induced activation (**Figure 4**). In cell free systems, LXRβ was able to bind all three PPAR isoforms with different affinities (100). Moreover, different PPAR ligands altered the affinities of these interactions. For example, troglitazone inhibited PPARγ/LXRβ interactions, while GI262570, a PPARγ ligand with high binding affinity, had no effect on this interaction. This may help to explain why different

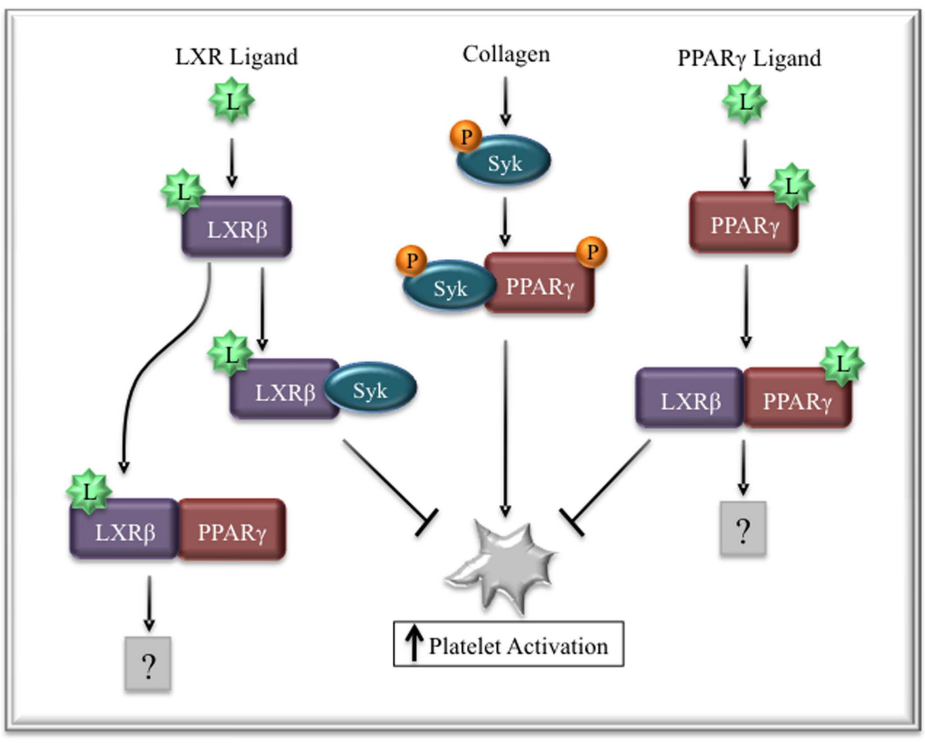


FIGURE 4 | Regulation of collagen signaling by LXRβ and PPARγ. Collagen signaling leads to the phosphorylation of Syk and PPARγ. Ligand-activated LXR binds Syk, preventing its interaction with PPARγ, thus decreasing platelet activation. Similarly, PPARγ ligands or LXR ligands can induce the interaction of PPARγ and LXR, reducing the availability of PAPRγ to participate in collagen signaling.

PPAR ligands exert different effects on platelet signaling and function.

RETINOID X RECEPTORS

Retinoid X receptors comprise a group of nuclear receptors that recognize vitamin A metabolites (101). The three different isoforms of RXRs (α, β, and γ) are expressed in different tissues, with RXRα and RXRβ being fairly widely expressed and RXRγ is mainly expressed in skeletal muscle and heart tissue (102). RXRα is the heterodimeric binding partner of PPARγ, which is essential for mediating the genomic effects of PPARγ. Platelets express the RXRα and β isoforms, but not RXRγ, and RXRs immunoprecipitate with PPARγ in platelets. (103) Similar to PPARγ ligands, the RXR ligand, 9-cis-retinoic acid (9cRA), inhibited agonist-induced platelet activation. Interestingly, however, 9cRA preferentially inhibited ADP and U46619 (a thromboxane mimetic)-induced aggregation, but did not alter collagen-induced activation. This is surprising in light of the fact that PPARγ ligands dampen signaling through the collagen receptor (**Figure 2**), suggesting that RXR in platelets can signal non-genomically independent of PPARγ. RXRα co-immunoprecipitated with Gq11 in resting platelets and this interaction was enhanced when platelets were stimulated with 9cRA. In this manner, ligand-bound RXRα may sequester Gq from its traditional role in propagating signals from G-protein coupled receptors, such as the ADP and thromboxane receptors.

GLUCOCORTICOID RECEPTORS

The glucocorticoid receptor (GR) is a nuclear hormone receptor that binds glucocorticoids, such as dexamethasone and prednisolone, resulting in an anti-inflammatory response (104). Human platelets express GR and differentially respond to its ligands (105). One study found that human platelets were less responsive to activation when treated with prednisolone, but not dexamethasone (106). Furthermore, the inhibitory effects of prednisolone on platelet function could be prevented by treatment with the GR antagonist, RU486. Interestingly, binding assays in human platelets revealed that both glucocorticoids could bind GR and increase its association with the chaperone protein, HSP90. In nucleated cells, ligand binding to GR causes HSP90 dissociation from the GR complex, which reveals its nuclear localization signal (107). However, the opposite effect was found in platelets, suggesting a different method of regulation. Moreover, a unique dimerization was identified in human platelets between GR and the mineralocorticoid receptor (MR), which can bind some of the same ligands as GR, such as dexamethasone. This may help to explain why dexamethasone affected platelet function differently from the more specific GR agonist, prednisolone. Additionally, mineralocorticoids activate PI3K, consistent with the finding that mineralocorticoid actions are associated with increased risk of vascular disease (108).

ARYL HYDROCARBON RECEPTOR

The aryl hydrocarbon receptor (AHR) is a well-known toxicant-sensing receptor that also plays essential roles in immune function and hematopoiesis (109). Common AHR ligands include polycyclic aromatic hydrocarbons, lipoxin A4, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Mouse platelets express AHR, which is known to play an important role in hematopoiesis and megakaryocyte development (110). Platelets from AHR knockout mice exhibit defective collagen signaling, with inhibited collagen-induced aggregation and spreading. Of note, platelets from AHR knockout mice had lower levels of the collagen receptor, GPVI, and undetectable levels of Vav proteins. Vav1 and Vav3 are activated downstream of many signaling pathways in platelets, and play a role in responding to collagen (111). Although the defects in platelet collagen signaling in AHR knockout mice are intriguing and may suggest an important role for this transcription factor in platelet biology, a more thorough investigation is needed. It is still unknown whether platelets respond to AHR ligands and whether they affect agonist-induced activation. Moreover, it is possible that the defects in collagen signaling in AHR knockout mice could be solely attributed to defective thrombopoiesis and altered platelet composition. Limited studies have evaluated the effects of AHR ligands in hemostasis. Polychlorinated biphenyls, which are non-specific AHR ligands, enhanced human platelet activation (112). Additionally, the specific AHR ligand, TCDD, was shown to induce thrombocyte aggregation in zebrafish (113). This can likely be attributed to its non-genomic actions in thrombocytes, as these effects were seen as early as 30 min post-treatment. Further research investigating the effects of AHR activation in platelets may reveal novel actions of environmental toxicants on hemostasis and cardiovascular risk.

The AHR has been shown to form a complex with the p65 subunit of NFκB in breast cancer cells (114). Furthermore, this interaction was found to be functionally relevant, resulting in proliferation and tumorigenesis. Additional roles for the AHR in regulation of NFκB signaling have been shown by our group in fibroblasts (115, 116). We found that absence of the AHR coincided with decreased expression of the non-canonical NFκB member, RelB. Signal-specific phosphorylation of RelB targets it for proteosomal degradation. It is possible that physical association between the AHR and RelB prevents its proteosomal degradation and allows it to exert anti-inflammatory effects, similar to its regulation in T cells (117).

SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTIONS

The signal transducer and activator of transcription (STAT) family of proteins were some of the first identified functional transcription factors in platelets (118). Human platelets express STATs 1, 3, and 5, which traditionally play a role in transducing signals from cytokine receptors to elicit an immune response (119). Early on, it was known that thrombopoietin could act on human platelets and this resulted in STAT3 phosphorylation, although the biological significance was unclear. Recently, the role of STAT3 in platelet signaling has been investigated more thoroughly, uncovering an important role for it in GPVI signaling (120). Pharmacologic inhibition of STAT3 signaling or dimerization resulted in a hyporesponsive phenotype to low-dose collagen activation. Additionally, platelets from platelet and megakaryocyte-specific STAT3 knockout mice had lower levels of collagen-induced aggregation, decreased P-selectin expression, and slowed thrombus formation. Interrogation of the signaling cascade revealed that STAT3 was activated by JAK2, in the same manner as in nucleated cells.

Traditionally, STAT3 homodimerizes upon phosphorylation by JAK2, then translocates to the nucleus where it acts as a traditional transcription factor. However, in platelets, phosphorylation of STAT3 by JAK2 resulted in dimerization and activation of PLCγ. Syk was found to be an upstream activator of this pathway and was complexed with STAT3 and PLCγ after activation with collagen (121). These data suggest that STAT3 plays an important regulatory role in platelet activation downstream of collagen and thrombopoietin signaling.

THE DIVERGENT ROLES OF TRANSCRIPTION FACTORS

The investigation of transcription factors in platelets has often led to convoluted and sometimes contradictory findings. This can most clearly been seen in the evaluation of the roles of PPARα in platelets. In this case, PPARα appears to play an important role in platelet activation, but has the opposite effect in the presence of a PPARα ligand (74). Additionally, phosphorylated PPARγ positively regulates collagen signaling, while ligand-bound PPARγ inhibits activation (71). These data support the hypothesis that ligand-induced activation of transcription factors can function differently than phosphorylation-induced activation (**Figure 3**).

MICROPARTICLES AS TRANSPORTS OF TRANSCRIPTION FACTORS

Microparticles are plasma membrane-derived vesicles ranging in diameter from 0.1 to 1 μm that are present at levels of approximately 5–50 μg/mL in blood plasma (122). Platelets and megakaryocytes are the primary source of microparticles in the blood circulation (about 80%), whereas other microparticles are derived from erythrocytes, endothelial cells, and granulocytes (122–124). Since their discovery, the role of platelet microparticles in coagulation was evident, and later was supported by identification of tissue factor expression (123) and a phosphatidylserine-rich outer membrane (125) that binds coagulation factors to aid in their assembly and enzymatic processing. Microparticles are produced from resting cells, during apoptosis or during cell activation, likely resulting in different internal and surface composition. Microparticles were shown to differ in composition between human samples and between microparticle size classes (126, 127). Packaging mechanisms for microparticles have not yet been identified and such studies are crucial for the understanding of microparticle influences on their environment. Whether microparticle packaging is a passive or active process, increased proximity of mediators to the cell plasma membrane would likely increase their chances of becoming encapsulated by the released microparticles.

MICROPARTICLE FUNCTIONS AND ROLES IN INFLAMMATION

Microparticles are postulated to have several means to influence their environment. Burger et al. described their ability to (1) promote coagulation, (2) scavenge NO, (3) generate reactive oxygen species, (4) cleave cellular surface proteins via metalloproteinases, (5) signal cells via surface proteins, and (6) deliver cargo via transfer of membrane and internal contents (128). Additionally, microparticles are postulated to contribute to, and sometimes exacerbate inflammation. It is likely that surface interactions and the less studied delivery of internal microparticle contents are both contributing to the influences of microparticles on their environment.

TRANSCELLULAR COMMUNICATION

Circulating microparticles can interact with other blood cells they encounter, such as leukocytes, lymphocytes, platelets, and with endothelial cells. Specific modes of cell–microparticle interactions include surface receptor signaling, plasma membrane fusion, or internalization of microparticles (129). The fusion of platelet microparticle membranes to target cell membranes was demonstrated to transfer the surface protein CXCR4 to cells causing recipient cell susceptibility to human immunodeficiency virus infection (130). Membrane fusion or internalization of microparticles could also cause microparticle internal composition to be transferred into the recipient cell cytoplasm. Lipids (131), RNA, and protein (129) have been seen to be delivered in this fashion, and multiple examples of each are reviewed by Mause and Weber (132). Arachidonic acid is a lipid mediator delivered by platelet microparticles that can be further processed into thromboxane A_2 by recipient platelets and contribute to their activation (131). Overall, platelet microparticles contain various mediators that can be delivered to surrounding cells to impact their function.

TRANSCRIPTION FACTORS IN MICROPARTICLES AND ROLES IN TRANSCELLULAR COMMUNICATION

The influence of microparticles on recipient cell function is based on microparticle composition. Not surprisingly, blood microparticle protein composition was found to be highly variable between healthy humans (127). Our work showed that platelet microparticles contain transcription factors, such as PPARγ, derived from parent cells (34). Furthermore, proteomic analysis has led to the discovery of three other transcription factors in platelet microparticles, RuvB-like 2, STAT3, and STAT5A (133).

Transcription factors are transported from cells through microparticles and retain function within the recipient cells (34, 72). Our lab was the first to show this ability with platelet-derived microparticles delivering functional PPARγ to THP-1 monocytes, detected through an electrophoresis mobility gel shift assay (134). We have since developed a novel platform technology to engineer microparticles through overexpression of PPARγ in platelet and megakaryocyte-derived microparticles obtained from the cultured megakaryoblastic cell line, Meg-01 cells. We showed that these engineered microparticles could be taken up by THP-1 monocytes, and that the transferred PPARγ was functional within recipients shown by induction fatty acid binding protein-4 (FABP4) expression, a unique PPARγ-specific target gene (72) (**Figure 5**).

To identify the significance of the transferred PPARγ to recipient cells, we compared recipient cell responses incubated with microparticles that did or did not contain PPARγ, to inflammatory stimuli (135). Monocytes that received PPARγ-containing microparticles had decreased inflammatory mediator production compared to the control microparticles. PPARγ activation has been shown to induce cell differentiation (136, 137). Our work also supported the influence of PPARγ on monocyte differentiation as the cells receiving PPARγ-containing microparticles became more adherent through increased integrin expression and fibronectin

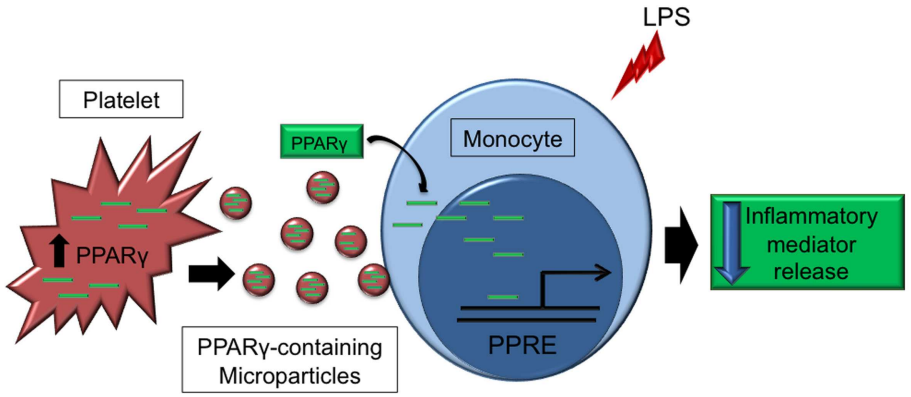


FIGURE 5 | Platelet microparticles as a method of transcellular delivery of transcription factors. Platelet-derived PPARγ is packaged into platelet microparticles, which can deliver intact PPARγ protein to target cells, such as monocytes. PPARγ can then bind to peroxisome proliferator response elements (PPRE) in the nucleus to affect transcription of target genes. Transfer of PPARγ to monocytes via microparticles has been shown to decrease LPS-induced inflammatory mediator release.

production (135). These data support the notion that circulating microparticles could have a profound impact on inflammatory cells during responses to insult and injury. Specifically, the anti-inflammatory PPARγ and proinflammatory NF-κB composition of microparticles could vastly dampen or heighten responses of recipient cells.

DIAGNOSTIC AND THERAPEUTIC POTENTIAL OF MICROPARTICLES

Measurements of microparticle abundance are becoming a more popular assessment of systemic inflammation. With current technology, it is easier and less expensive to measure microparticle number and surface composition rather than running entire microparticle proteomic studies with each patient sample. Microparticle number and surface phenotype have provided descriptive diagnostic tools indicative of disease severity. However, evaluation of the total protein composition of microparticles would tell an infinitely more detailed summary of patient health status. Particularly, looking at levels of the highly influential transcription factors within circulating microparticles would provide insight into the inflammatory state from the cells generating the microparticles as well as the potential inflammatory impact on recipient cells within the blood.

Delivery of platelet proteins such as PPARγ could have an anti-inflammatory and/or pro-differentiation influence on activated cells via microparticles and act as a physiological means of quenching inflammation. Transcription factors would be among the most influential mediators to deliver to a cell, as they are capable of initiating transcripts of multiple downstream products that could influence numerous networks and pathways. Certain transcription factors may require signal or ligand activation to fully function in a microparticle delivery setting. In the case of PPARγ, endogenous ligands such as 15d-PGJ_2 and others may be present or synthesized. Whereas the components delivered by microparticles may be short-lived, their effects on the recipient cells may sometimes be permanent, such as inducing irreversible differentiation or death of recipient cells.

Overall, platelet microparticles are abundant and influential transcellular vesicles. They contain proteins, lipids, and RNA derived from their parent cells. Therefore, these circulating biomarkers provide insight into the cumulative activation and inflammatory state of all blood cell populations. Importantly, microparticles have repeatedly been shown to not just be cell byproducts but rather influential delivery mechanisms. Transportation of transcription factors to cells could influence several ongoing or initiate new pathways causing profound impacts within recipient cells. Transcellular communication involving transcription factors via platelet microparticles substantiates another possible key role of transcription factors presence in platelets.

CONCLUSION

Transcription factors play numerous important and previously unrecognized roles in regulating platelet function in a non-genomic manner. Additionally, transfer of intact and functional transcription factors to other cells via microparticles may serve as a novel regulatory mechanism for inflammation. The initial discovery of transcriptional regulatory elements in a particular protein does not exclude the possibility of non-genomic roles for that protein as well. The anucleate platelet can serve as an important model system to study non-genomic roles of transcription factors. Further elucidation of the non-genomic functions of transcription factors will yield important discoveries that have the potential to illuminate platelet biology and also help to explain the pleotropic effects of pharmaceuticals that target these pathways.

ACKNOWLEDGMENTS

This work was supported in part by ES001247, HL095467, T32-AI007285, NS066801, T90 DE0 21985 and HL128129 a University of Rochester grant from Howard Hughes Medical Institute through the Med into Grad Initiative, and UL1RR024160 and UL1TR000042 from the National Center for Research Resources. The content is solely the responsibility of the authors and does not necessarily represent the views of NCRR or NIH.

REFERENCES

1. Rondina MT, Weyrich AS, Zimmerman GA. Platelets as cellular effectors of inflammation in vascular diseases. *Circ Res* (2013) **112**(11):1506–19. doi:10.1161/CIRCRESAHA.113.300512
2. Morrell CN, Aggrey AA, Chapman LM, Modjeski KL. Emerging roles for platelets as immune and inflammatory cells. *Blood* (2014) **123**(18):2759–67. doi:10.1182/blood-2013-11-462432
3. Garraud O, Hamzeh-Cognasse H, Pozzetto B, Cavaillon JM, Cognasse F. Bench-to-bedside review: platelets and active immune functions – new clues for immunopathology? *Crit Care* (2013) **17**(4):236. doi:10.1186/cc12716
4. Spinelli SL, Maggirwar SB, Blumberg N, Phipps RP. Nuclear emancipation: a platelet tour de force. *Sci Signal* (2010) **3**(144):e37. doi:10.1126/scisignal.3144pe37
5. Chariot A. The NF-kappa B-independent functions of IKK subunits in immunity and cancer. *Trends Cell Biol* (2009) **19**(8):404–13. doi:10.1016/jcb.2009.05.006
6. Spinelli SL, Casey AE, Pollock SJ, Gertz JM, McMillan DH, Narasipura SD, et al. Platelets and megakaryocytes contain functional nuclear factor-kappaB. *Arterioscler Thromb Vasc Biol* (2010) **30**(3):591–8. doi:10.1161/ATVBAHA.109.197343
7. Malaver E, Romaniuk MA, D'Atri LP, Pozner RG, Negrotto S, Benzadón R, et al. NF-kappa B inhibitors impair platelet activation responses. *J Thromb Haemost* (2009) **7**(8):1333–43. doi:10.1111/j.1538-7836.2009.03492.x
8. Gambaryan S, Kobsar A, Rukoyatkina N, Herterich S, Geiger J, Smolenski A, et al. Thrombin and collagen induce a feedback inhibitory signaling pathway in platelets involving dissociation of the catalytic subunit of protein kinase A from an NFkappaB-IkappaB complex. *J Biol Chem* (2010) **285**(24):18352–63. doi:10.1074/jbc.M109.077602
9. Liu F, Morris S, Epps J, Carroll R. Demonstration of an activation regulated NF-kappa B/I-kappaBalpha complex in human platelets. *Thromb Res* (2002) **106**(4–5):199–203. doi:10.1016/S0049-3848(02)00130-5
10. Chang CC, Lu WJ, Ong ET, Chiang CW, Lin SC, Huang SY, et al. A novel role of sesamol in inhibiting NF-κB-mediated signaling in platelet activation. *J Biomed Sci* (2011) **18**:93. doi:10.1186/1423-0127-18-93
11. Lee HS, Kim SD, Lee WM, Endale M, Kamruzzaman SM, Oh WJ, et al. A noble function of BAY 11-7082: inhibition of platelet aggregation mediated by an elevated cAMP-induced VASP, and decreased ERK2/JNK1 phosphorylations. *Eur J Pharmacol* (2010) **627**(1–3):85–91. doi:10.1016/j.ejphar.2009.11.005
12. Chen WF, Lee JJ, Chang CC, Lin KH, Wang SH, Sheu JR. Platelet protease-activated receptor (PAR)4, but not PAR1, associated with neutral sphingomyelinase responsible for thrombin-stimulated ceramide-NF-κB signaling in human platelets. *Haematologica* (2013) **98**(5):793–801. doi:10.3324/haematol.2012.072553
13. Pierce JW, Schoenleber R, Jesmok G, Best J, Moore SA, Collins T, et al. Novel inhibitors of cytokine-induced IkappaBalpha phosphorylation and endothelial cell adhesion molecule expression show anti-inflammatory effects in vivo. *J Biol Chem* (1997) **272**(34):21096–103. doi:10.1074/jbc.272.34.21096
14. Karim ZA, Zhang J, Banerjee M, Chicka MC, Al Hawas R, Hamilton TR, et al. IκB kinase phosphorylation of SNAP-23 controls platelet secretion. *Blood* (2013) **121**(22):4567–74. doi:10.1182/blood-2012-11-470468
15. Hachem A, Yacoub D, Zaid Y, Mourad W, Merhi Y. Involvement of nuclear factor κB in platelet CD40 signaling. *Biochem Biophys Res Commun* (2012) **425**(1):58–63. doi:10.1016/j.bbrc.2012.07.049
16. Wei S, Wang H, Zhang G, Lu Y, An X, Ren S, et al. Platelet IκB kinase-β deficiency increases mouse arterial neointima formation via delayed glycoprotein Ibα shedding. *Arterioscler Thromb Vasc Biol* (2013) **33**(2):241–8. doi:10.1161/ATVBAHA.112.300781
17. Cognasse F, Hamzeh-Cognasse H, Garraud O. [Platelets "toll-like receptor" engagement stimulates the release of immunomodulating molecules]. *Transfus Clin Biol* (2008) **15**(4):139–47. doi:10.1016/j.tracli.2008.07.010
18. Berthet J, Damien P, Hamzeh-Cognasse H, Arthaud CA, Eyraud MA, Zéni F, et al. Human platelets can discriminate between various bacterial LPS isoforms via TLR4 signaling and differential cytokine secretion. *Clin Immunol* (2012) **145**(3):189–200. doi:10.1016/j.clim.2012.09.004
19. Lu WJ, Lee JJ, Chou DS, Jayakumar T, Fong TH, Hsiao G, et al. A novel role of andrographolide, an NF-kappa B inhibitor, on inhibition of platelet activation: the pivotal mechanisms of endothelial nitric oxide synthase/cyclic GMP. *J Mol Med (Berl)* (2011) **89**(12):1261–73. doi:10.1007/s00109-011-0800-0
20. Lu WJ, Lin KH, Hsu MJ, Chou DS, Hsiao G, Sheu JR. Suppression of NF-κB signaling by andrographolide with a novel mechanism in human platelets: regulatory roles of the p38 MAPK-hydroxyl radical-ERK2 cascade. *Biochem Pharmacol* (2012) **84**(7):914–24. doi:10.1016/j.bcp.2012.06.030
21. Sambrano GR, Weiss EJ, Zheng YW, Huang W, Coughlin SR. Role of thrombin signalling in platelets in haemostasis and thrombosis. *Nature* (2001) **413**(6851):74–8. doi:10.1038/35092573
22. Mazharian A, Roger S, Berrou E, Adam F, Kauskot A, Nurden P, et al. Protease-activating receptor-4 induces full platelet spreading on a fibrinogen matrix: involvement of ERK2 and p38 and Ca2+ mobilization. *J Biol Chem* (2007) **282**(8):5478–87. doi:10.1074/jbc.M609881200
23. Brill A, Chauhan AK, Canault M, Walsh MT, Bergmeier W, Wagner DD. Oxidative stress activates ADAM17/TACE and induces its target receptor shedding in platelets in a p38-dependent fashion. *Cardiovasc Res* (2009) **84**(1):137–44. doi:10.1093/cvr/cvp176
24. Lee S, Andrieu C, Saltel F, Destaing O, Auclair J, Pouchkine V, et al. IkappaB kinase beta phosphorylates Dok1 serines in response to TNF, IL-1, or gamma radiation. *Proc Natl Acad Sci U S A* (2004) **101**(50):17416–21. doi:10.1073/pnas.0408061101
25. Flevaris P, Li Z, Zhang G, Zheng Y, Liu J, Du X. Two distinct roles of mitogen-activated protein kinases in platelets and a novel Rac1-MAPK-dependent integrin outside-in retractile signaling pathway. *Blood* (2009) **113**(4):893–901. doi:10.1182/blood-2008-05-155978
26. García A, Senis YA, Antrobus R, Hughes CE, Dwek RA, Watson SP, et al. A global proteomics approach identifies novel phosphorylated signaling proteins in GPVI-activated platelets: involvement of G6f, a novel platelet Grb2-binding membrane adapter. *Proteomics* (2006) **6**(19):5332–43. doi:10.1002/pmic.200600299
27. Cogswell PC, Kashatus DF, Keifer JA, Guttridge DC, Reuther JY, Bristow C, et al. NF-kappa B and I kappa B alpha are found in the mitochondria. Evidence for regulation of mitochondrial gene expression by NF-kappa B. *J Biol Chem* (2003) **278**(5):2963–8. doi:10.1074/jbc.M209995200
28. Bottero V, Rossi F, Samson M, Mari M, Hofman P, Peyron JF. Ikappa b-alpha, the NF-kappa B inhibitory subunit, interacts with ANT, the mitochondrial ATP/ADP translocator. *J Biol Chem* (2001) **276**(24):21317–24. doi:10.1074/jbc.M005850200
29. Gringhuis SI, García-Vallejo JJ, van Het Hof B, van Dijk W. Convergent actions of I kappa B kinase beta and protein kinase C delta modulate mRNA stability through phosphorylation of 14-3-3 beta complexed with tristetraprolin. *Mol Cell Biol* (2005) **25**(15):6454–63. doi:10.1128/MCB.25.15.6454-6463.2005
30. Aguilera C, Fernández-Majada V, Inglés-Esteve J, Rodilla V, Bigas A, Espinosa L. Efficient nuclear export of p65-IkappaBalpha complexes requires 14-3-3 proteins. *J Cell Sci* (2006) **119**(Pt 17):3695–704. doi:10.1242/jcs.03086
31. Denis MM, Tolley ND, Bunting M, Schwertz H, Jiang H, Lindemann S, et al. Escaping the nuclear confines: signal-dependent pre-mRNA splicing in anucleate platelets. *Cell* (2005) **122**(3):379–91. doi:10.1016/j.cell.2005.06.015
32. McRedmond JP, Park SD, Reilly DF, Coppinger JA, Maguire PB, Shields DC, et al. Integration of proteomics and genomics in platelets: a profile of platelet proteins and platelet-specific genes. *Mol Cell Proteomics* (2004) **3**(2):133–44. doi:10.1074/mcp.M300063-MCP200
33. Garcia-Bates TM, Lehmann GM, Simpson-Haidaris PJ, Bernstein SH, Sime PJ, Phipps RP. Role of peroxisome proliferator-activated receptor gamma and its ligands in the treatment of hematological malignancies. *PPAR Res* (2008) **2008**:834612. doi:10.1155/2008/834612
34. Ray DM, Spinelli SL, Pollock SJ, Murant TI, O'Brien JJ, Blumberg N, et al. Peroxisome proliferator-activated receptor gamma and retinoid X receptor transcription factors are released from activated human platelets and shed in microparticles. *Thromb Haemost* (2008) **99**(1):86–95. doi:10.1160/TH07-05-0328
35. Ghanim H, Dhindsa S, Aljada A, Chaudhuri A, Viswanathan P, Dandona P. Low-dose rosiglitazone exerts an antiinflammatory effect with an increase in adiponectin independently of free fatty acid fall and insulin sensitization in obese type 2 diabetics. *J Clin Endocrinol Metab* (2006) **91**(9):3553–8. doi:10.1210/jc.2005-2609
36. Randriamboavonjy V, Pistrosch F, Bölck B, Schwinger RH, Dixit M, Badenhoop K, et al. Platelet sarcoplasmic endoplasmic reticulum Ca2+-ATPase and mucalpain activity are altered in type 2 diabetes mellitus and restored by rosiglitazone. *Circulation* (2008) **117**(1):52–60. doi:10.1161/CIRCULATIONAHA.107.719807

37. Watala C, Boncler M, Gresner P. Blood platelet abnormalities and pharmacological modulation of platelet reactivity in patients with diabetes mellitus. *Pharmacol Rep* (2005) **57**(Suppl):42–58. doi:10.2174/1381612054367337
38. Gasparyan AY, Ayvazyan L, Mikhailidis DP, Kitas GD. Mean platelet volume: a link between thrombosis and inflammation? *Curr Pharm Des* (2011) **17**(1):47–58. doi:10.2174/138161211795049804
39. Trovati M, Anfossi G. Mechanisms involved in platelet hyperactivation and platelet-endothelium interrelationships in diabetes mellitus. *Curr Diab Rep* (2002) **2**(4):316–22. doi:10.1007/s11892-002-0020-7
40. Hetzel J, Balletshofer B, Rittig K, Walcher D, Kratzer W, Hombach V, et al. Rapid effects of rosiglitazone treatment on endothelial function and inflammatory biomarkers. *Arterioscler Thromb Vasc Biol* (2005) **25**(9):1804–9. doi:10.1161/01.ATV.0000176192.16951.9a
41. Pfützner A, Schöndorf T, Seidel D, Winkler K, Matthaei S, Hamann A, et al. Impact of rosiglitazone on beta-cell function, insulin resistance, and adiponectin concentrations: results from a double-blind oral combination study with glimepiride. *Metabolism* (2006) **55**(1):20–5. doi:10.1016/j.metabol.2005.06.021
42. Derosa G, Cicero AF, Gaddi A, Ragonesi PD, Piccinni MN, Fogari E, et al. A comparison of the effects of pioglitazone and rosiglitazone combined with glimepiride on prothrombotic state in type 2 diabetic patients with the metabolic syndrome. *Diabetes Res Clin Pract* (2005) **69**(1):5–13. doi:10.1016/j.diabres.2004.10.007
43. Fonseca VA, Reynolds T, Hemphill D, Randolph C, Wall J, Valiquet TR, et al. Effect of troglitazone on fibrinolysis and activated coagulation in patients with non-insulin-dependent diabetes mellitus. *J Diabetes Complications* (1998) **12**(4):181–6. doi:10.1016/S1056-8727(97)00109-8
44. Kato K, Yamada D, Midorikawa S, Sato W, Watanabe T. Improvement by the insulin-sensitizing agent, troglitazone, of abnormal fibrinolysis in type 2 diabetes mellitus. *Metabolism* (2000) **49**(5):662–5. doi:10.1016/S0026-0495(00)80045-1
45. Sidhu JS, Cowan D, Kaski JC. The effects of rosiglitazone, a peroxisome proliferator-activated receptor-gamma agonist, on markers of endothelial cell activation, C-reactive protein, and fibrinogen levels in non-diabetic coronary artery disease patients. *J Am Coll Cardiol* (2003) **42**(10):1757–63. doi:10.1016/j.jacc.2003.04.001
46. Haffner SM, Greenberg AS, Weston WM, Chen H, Williams K, Freed MI. Effect of rosiglitazone treatment on nontraditional markers of cardiovascular disease in patients with type 2 diabetes mellitus. *Circulation* (2002) **106**(6):679–84. doi:10.1161/01.CIR.0000025403.20953.23
47. Chu CS, Lee KT, Lee MY, Su HM, Voon WC, Sheu SH, et al. Effects of rosiglitazone alone and in combination with atorvastatin on nontraditional markers of cardiovascular disease in patients with type 2 diabetes mellitus. *Am J Cardiol* (2006) **97**(5):646–50. doi:10.1016/j.amjcard.2005.09.101
48. Yu J, Jin N, Wang G, Zhang F, Mao J, Wang X. Peroxisome proliferator-activated receptor gamma agonist improves arterial stiffness in patients with type 2 diabetes mellitus and coronary artery disease. *Metabolism* (2007) **56**(10):1396–401. doi:10.1016/j.metabol.2007.05.011
49. Schöndorf T, Musholt PB, Hohberg C, Forst T, Lehmann U, Fuchs W, et al. The fixed combination of pioglitazone and metformin improves biomarkers of platelet function and chronic inflammation in type 2 diabetes patients: results from the PIOfix study. *J Diabetes Sci Technol* (2011) **5**(2):426–32. doi:10.1177/193229681100500233
50. Samaha FF, Szapary PO, Iqbal N, Williams MM, Bloedon LT, Kochar A, et al. Effects of rosiglitazone on lipids, adipokines, and inflammatory markers in nondiabetic patients with low high-density lipoprotein cholesterol and metabolic syndrome. *Arterioscler Thromb Vasc Biol* (2006) **26**(3):624–30. doi:10.1161/01.ATV.0000200136.56716.30
51. Chu JW, Abbasi F, Lamendola C, McLaughlin T, Reaven GM, Tsao PS. Effect of rosiglitazone treatment on circulating vascular and inflammatory markers in insulin-resistant subjects. *Diab Vasc Dis Res* (2005) **2**(1):37–41. doi:10.3132/dvdr.2005.004
52. Marx N, Imhof A, Froehlich J, Siam L, Ittner J, Wierse G, et al. Effect of rosiglitazone treatment on soluble CD40L in patients with type 2 diabetes and coronary artery disease. *Circulation* (2003) **107**(15):1954–7. doi:10.1161/01.CIR.0000069272.06194.91
53. Khanolkar MP, Morris RH, Thomas AW, Bolusani H, Roberts AW, Geen J, et al. Rosiglitazone produces a greater reduction in circulating platelet activity compared with gliclazide in patients with type 2 diabetes mellitus – an effect probably mediated by direct platelet PPARgamma activation. *Atherosclerosis* (2008) **197**(2):718–24. doi:10.1016/j.atherosclerosis.2007.07.020
54. Sidhu JS, Cowan D, Tooze JA, Kaski JC. Peroxisome proliferator-activated receptor-gamma agonist rosiglitazone reduces circulating platelet activity in patients without diabetes mellitus who have coronary artery disease. *Am Heart J* (2004) **147**(6):e25. doi:10.1016/j.ahj.2003.12.035
55. Akbiyik F, Ray DM, Gettings KF, Blumberg N, Francis CW, Phipps RP. Human bone marrow megakaryocytes and platelets express PPARgamma, and PPARgamma agonists blunt platelet release of CD40 ligand and thromboxanes. *Blood* (2004) **104**(5):1361–8. doi:10.1182/blood-2004-03-0926
56. Hishinuma T, Yamazaki T, Mizugaki M. Troglitazone has a reducing effect on thromboxane production. *Prostaglandins Other Lipid Mediat* (2000) **62**(2):135–43. doi:10.1016/S0090-6980(00)00059-9
57. Bodary PF, Vargas FB, King SA, Jongeward KL, Wickenheiser KJ, Eitzman DT. Pioglitazone protects against thrombosis in a mouse model of obesity and insulin resistance. *J Thromb Haemost* (2005) **3**(10):2149–53. doi:10.1111/j.1538-7836.2005.01551.x
58. Rao F, Yang RQ, Chen XS, Xu JS, Fu HM, Su H, et al. PPARγ ligands decrease hydrostatic pressure-induced platelet aggregation and proinflammatory activity. *PLoS One* (2014) **9**(2):e89654. doi:10.1371/journal.pone.0089654
59. Li D, Chen K, Sinha N, Zhang X, Wang Y, Sinha AK, et al. The effects of PPAR-gamma ligand pioglitazone on platelet aggregation and arterial thrombus formation. *Cardiovasc Res* (2005) **65**(4):907–12. doi:10.1016/j.cardiores.2004.11.027
60. Varo N, Vicent D, Libby P, Nuzzo R, Calle-Pascual AL, Bernal MR, et al. Elevated plasma levels of the atherogenic mediator soluble CD40 ligand in diabetic patients: a novel target of thiazolidinediones. *Circulation* (2003) **107**(21):2664–9. doi:10.1161/01.CIR.0000074043.46437.44
61. Surh YJ, Na HK, Park JM, Lee HN, Kim W, Yoon IS, et al. 15-Deoxy-$\delta^{12,14}$-prostaglandin J_2, an electrophilic lipid mediator of anti-inflammatory and pro-resolving signaling. *Biochem Pharmacol* (2011) **82**(10):1335–51. doi:10.1016/j.bcp.2011.07.100
62. Ishizuka T, Itaya S, Wada H, Ishizawa M, Kimura M, Kajita K, et al. Differential effect of the antidiabetic thiazolidinediones troglitazone and pioglitazone on human platelet aggregation mechanism. *Diabetes* (1998) **47**(9):1494–500. doi:10.2337/diabetes.47.9.1494
63. Smith NJ, Stoddart LA, Devine NM, Jenkins L, Milligan G. The action and mode of binding of thiazolidinedione ligands at free fatty acid receptor 1. *J Biol Chem* (2009) **284**(26):17527–39. doi:10.1074/jbc.M109.012849
64. Simó R, Rodriguez A, Caveda E. Different effects of thiazolidinediones on cardiovascular risk in patients with type 2 diabetes mellitus: pioglitazone versus rosiglitazone. *Curr Drug Saf* (2010) **5**(3):234–44. doi:10.2174/157488610791698352
65. Pickavance L, Widdowson PS, King P, Ishii S, Tanaka H, Williams G. The development of overt diabetes in young Zucker diabetic fatty (ZDF) rats and the effects of chronic MCC-555 treatment. *Br J Pharmacol* (1998) **125**(4):767–70. doi:10.1038/sj.bjp.0702158
66. Choi J, Park Y, Lee HS, Yang Y, Yoon S. 1,3-diphenyl-1H-pyrazole derivatives as a new series of potent PPARγ partial agonists. *Bioorg Med Chem* (2010) **18**(23):8315–23. doi:10.1016/j.bmc.2010.09.068
67. Camp HS, Li O, Wise SC, Hong YH, Frankowski CL, Shen X, et al. Differential activation of peroxisome proliferator-activated receptor-gamma by troglitazone and rosiglitazone. *Diabetes* (2000) **49**(4):539–47. doi:10.2337/diabetes.49.4.539
68. Guan HP, Ishizuka T, Chui PC, Lehrke M, Lazar MA. Corepressors selectively control the transcriptional activity of PPARgamma in adipocytes. *Genes Dev* (2005) **19**(4):453–61. doi:10.1101/gad.1263305
69. Kodera Y, Takeyama K, Murayama A, Suzawa M, Masuhiro Y, Kato S. Ligand type-specific interactions of peroxisome proliferator-activated receptor gamma with transcriptional coactivators. *J Biol Chem* (2000) **275**(43):33201–4. doi:10.1074/jbc.C000517200
70. Lonard DM, O'Malley BW. Nuclear receptor coregulators: modulators of pathology and therapeutic targets. *Nat Rev Endocrinol* (2012) **8**(10):598–604. doi:10.1038/nrendo.2012.100
71. Moraes LA, Spyridon M, Kaiser WJ, Jones CI, Sage T, Atherton RE, et al. Non-genomic effects of PPARgamma ligands: inhibition of GPVI-stimulated platelet activation. *J Thromb Haemost* (2010) **8**(3):577–87. doi:10.1111/j.1538-7836.2009.03732.x

72. Sahler J, Woeller C, Spinelli S, Blumberg N, Phipps R. A novel method for over-expression of peroxisome proliferator-activated receptor-γ in megakaryocyte and platelet microparticles achieves transcellular signaling. *J Thromb Haemost* (2012) **10**(12):2563–72. doi:10.1111/jth.12017
73. Du H, Hu H, Zheng H, Hao J, Yang J, Cui W. Effects of peroxisome proliferator-activated receptor γ in simvastatin antiplatelet activity: influences on cAMP and mitogen-activated protein kinases. *Thromb Res* (2014) **134**(1):111–20. doi:10.1016/j.thromres.2014.05.005
74. Ali FY, Armstrong PC, Dhanji AR, Tucker AT, Paul-Clark MJ, Mitchell JA, et al. Antiplatelet actions of statins and fibrates are mediated by PPARs. *Arterioscler Thromb Vasc Biol* (2009) **29**(5):706–11. doi:10.1161/ATVBAHA.108.183160
75. Kliewer SA, Forman BM, Blumberg B, Ong ES, Borgmeyer U, Mangelsdorf DJ, et al. Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. *Proc Natl Acad Sci U S A* (1994) **91**(15):7355–9. doi:10.1073/pnas.91.15.7355
76. Ali FY, Davidson SJ, Moraes LA, Traves SL, Paul-Clark M, Bishop-Bailey D, et al. Role of nuclear receptor signaling in platelets: antithrombotic effects of PPARbeta. *FASEB J* (2006) **20**(2):326–8. doi:10.1096/fj.05-4395fje
77. Ali FY, Hall MG, Desvergne B, Warner TD, Mitchell JA. PPARbeta/delta agonists modulate platelet function via a mechanism involving PPAR receptors and specific association/repression of PKCalpha – brief report. *Arterioscler Thromb Vasc Biol* (2009) **29**(11):1871–3. doi:10.1161/ATVBAHA.109.193367
78. Lim H, Dey SK. PPAR delta functions as a prostacyclin receptor in blastocyst implantation. *Trends Endocrinol Metab* (2000) **11**(4):137–42. doi:10.1016/S1043-2760(00)00243-5
79. Fuentes E, Palomo I. Mechanism of antiplatelet action of hypolipidemic, antidiabetic and antihypertensive drugs by PPAR activation: PPAR agonists: new antiplatelet agents. *Vascul Pharmacol* (2014) **62**(3):162–6. doi:10.1016/j.vph.2014.05.008
80. Chou TC, Shih CY, Chen YT. Inhibitory effect of α-lipoic acid on platelet aggregation is mediated by PPARs. *J Agric Food Chem* (2011) **59**(7):3050–9. doi:10.1021/jf103940u
81. Feinstein DL, Spagnolo A, Akar C, Weinberg G, Murphy P, Gavrilyuk V, et al. Receptor-independent actions of PPAR thiazolidinedione agonists: is mitochondrial function the key? *Biochem Pharmacol* (2005) **70**(2):177–88. doi:10.1016/j.bcp.2005.03.033
82. Luconi M, Cantini G, Serio M. Peroxisome proliferator-activated receptor gamma (PPARgamma): is the genomic activity the only answer? *Steroids* (2010) **75**(8–9):585–94. doi:10.1016/j.steroids.2009.10.012
83. Robinson E, Grieve DJ. Significance of peroxisome proliferator-activated receptors in the cardiovascular system in health and disease. *Pharmacol Ther* (2009) **122**(3):246–63. doi:10.1016/j.pharmthera.2009.03.003
84. Kelly D, Campbell JI, King TP, Grant G, Jansson EA, Coutts AG, et al. Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. *Nat Immunol* (2004) **5**(1):104–12. doi:10.1038/ni1018
85. von Knethen A, Soller M, Tzieply N, Weigert A, Johann AM, Jennewein C, et al. PPARgamma1 attenuates cytosol to membrane translocation of PKCalpha to desensitize monocytes/macrophages. *J Cell Biol* (2007) **176**(5):681–94. doi:10.1083/jcb.200605038
86. Paumelle R, Blanquart C, Briand O, Barbier O, Duhem C, Woerly G, et al. Acute antiinflammatory properties of statins involve peroxisome proliferator-activated receptor-alpha via inhibition of the protein kinase C signaling pathway. *Circ Res* (2006) **98**(3):361–9. doi:10.1161/01.RES.0000202706.70992.95
87. Jones DC, Ding X, Zhang TY, Daynes RA. Peroxisome proliferator-activated receptor alpha negatively regulates T-bet transcription through suppression of p38 mitogen-activated protein kinase activation. *J Immunol* (2003) **171**(1):196–203. doi:10.4049/jimmunol.171.1.196
88. Saklatvala J, Rawlinson L, Waller RJ, Sarsfield S, Lee JC, Morton LF, et al. Role for p38 mitogen-activated protein kinase in platelet aggregation caused by collagen or a thromboxane analogue. *J Biol Chem* (1996) **271**(12):6586–9. doi:10.1074/jbc.271.12.6586
89. Li Z, Xi X, Du X. A mitogen-activated protein kinase-dependent signaling pathway in the activation of platelet integrin alpha IIbbeta3. *J Biol Chem* (2001) **276**(45):42226–32. doi:10.1074/jbc.M106129200
90. Li Z, Zhang G, Feil R, Han J, Du X. Sequential activation of p38 and ERK pathways by cGMP-dependent protein kinase leading to activation of the platelet integrin alphaIIb beta3. *Blood* (2006) **107**(3):965–72. doi:10.1182/blood-2005-03-1308
91. Burgermeister E, Seger R. PPARgamma and MEK interactions in cancer. *PPAR Res* (2008) **2008**:309469. doi:10.1155/2008/309469
92. Cantini G, Lombardi A, Borgogni E, Francalanci M, Ceni E, Degl'Innocenti S, et al. Peroxisome-proliferator-activated receptor gamma (PPARgamma) is required for modulating endothelial inflammatory response through a nongenomic mechanism. *Eur J Cell Biol* (2010) **89**(9):645–53. doi:10.1016/j.ejcb.2010.04.002
93. Cantini G, Lombardi A, Piscitelli E, Poli G, Ceni E, Marchiani S, et al. Rosiglitazone inhibits adrenocortical cancer cell proliferation by interfering with the IGF-IR intracellular signaling. *PPAR Res* (2008) **2008**:904041. doi:10.1155/2008/904041
94. Papageorgiou E, Pitulis N, Manoussakis M, Lembessis P, Koutsilieris M. Rosiglitazone attenuates insulin-like growth factor 1 receptor survival signaling in PC-3 cells. *Mol Med* (2008) **14**(7–8):403–11. doi:10.2119/2008-00021.Papageorgiou
95. Ichiki T, Tokunou T, Fukuyama K, Iino N, Masuda S, Takeshita A. 15-deoxy-delta12,14-prostaglandin J2 and thiazolidinediones transactivate epidermal growth factor and platelet-derived growth factor receptors in vascular smooth muscle cells. *Biochem Biophys Res Commun* (2004) **323**(2):402–8. doi:10.1016/j.bbrc.2004.08.101
96. Endo Y, Suzuki M, Yamada H, Horita S, Kunimi M, Yamazaki O, et al. Thiazolidinediones enhance sodium-coupled bicarbonate absorption from renal proximal tubules via PPARγ-dependent nongenomic signaling. *Cell Metab* (2011) **13**(5):550–61. doi:10.1016/j.cmet.2011.02.015
97. Lehmann JM, Kliewer SA, Moore LB, Smith-Oliver TA, Oliver BB, Su JL, et al. Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway. *J Biol Chem* (1997) **272**(6):3137–40. doi:10.1074/jbc.272.6.3137
98. Peet DJ, Turley SD, Ma W, Janowski BA, Lobaccaro JM, Hammer RE, et al. Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR alpha. *Cell* (1998) **93**(5):693–704. doi:10.1016/S0092-8674(00)81432-4
99. Spyridon M, Moraes LA, Jones CI, Sage T, Sasikumar P, Bucci G, et al. LXR as a novel antithrombotic target. *Blood* (2011) **117**(21):5751–61. doi:10.1182/blood-2010-09-306142
100. Yue L, Ye F, Gui C, Luo H, Cai J, Shen J, et al. Ligand-binding regulation of LXR/RXR and LXR/PPAR heterodimerizations: SPR technology-based kinetic analysis correlated with molecular dynamics simulation. *Protein Sci* (2005) **14**(3):812–22. doi:10.1110/ps.04951405
101. Mangelsdorf DJ, Ong ES, Dyck JA, Evans RM. Nuclear receptor that identifies a novel retinoic acid response pathway. *Nature* (1990) **345**(6272):224–9. doi:10.1038/345224a0
102. Chambon P. A decade of molecular biology of retinoic acid receptors. *FASEB J* (1996) **10**(9):940–54.
103. Moraes LA, Swales KE, Wray JA, Damazo A, Gibbins JM, Warner TD, et al. Nongenomic signaling of the retinoid X receptor through binding and inhibiting Gq in human platelets. *Blood* (2007) **109**(9):3741–4. doi:10.1182/blood-2006-05-022566
104. Schaaf MJ, Cidlowski JA. Molecular mechanisms of glucocorticoid action and resistance. *J Steroid Biochem Mol Biol* (2002) **83**(1–5):37–48. doi:10.1016/S0960-0760(02)00263-7
105. Sanner BM, Meder U, Zidek W, Tepel M. Effects of glucocorticoids on generation of reactive oxygen species in platelets. *Steroids* (2002) **67**(8):715–9. doi:10.1016/S0039-128X(02)00024-7
106. Moraes LA, Paul-Clark MJ, Rickman A, Flower RJ, Goulding NJ, Perretti M. Ligand-specific glucocorticoid receptor activation in human platelets. *Blood* (2005) **106**(13):4167–75. doi:10.1182/blood-2005-04-1723
107. Hutchison KA, Czar MJ, Pratt WB. Evidence that the hormone-binding domain of the mouse glucocorticoid receptor directly represses DNA binding activity in a major portion of receptors that are "misfolded" after removal of hsp90. *J Biol Chem* (1992) **267**(5):3190–5.
108. Jaffe IZ, Tintut Y, Newfell BG, Demer LL, Mendelsohn ME. Mineralocorticoid receptor activation promotes vascular cell calcification. *Arterioscler Thromb Vasc Biol* (2007) **27**(4):799–805. doi:10.1161/01.ATV.0000258414.59393.89

109. Lindsey S, Papoutsakis ET. The evolving role of the aryl hydrocarbon receptor (AHR) in the normophysiology of hematopoiesis. *Stem Cell Rev* (2012) **8**(4):1223–35. doi:10.1007/s12015-012-9384-5
110. Lindsey S, Jiang J, Woulfe D, Papoutsakis ET. Platelets from mice lacking the aryl hydrocarbon receptor exhibit defective collagen-dependent signaling. *J Thromb Haemost* (2014) **12**(3):383–94. doi:10.1111/jth.12490
111. Pearce AC, Senis YA, Billadeau DD, Turner M, Watson SP, Vigorito E. Vav1 and vav3 have critical but redundant roles in mediating platelet activation by collagen. *J Biol Chem* (2004) **279**(52):53955–62. doi:10.1074/jbc.M410355200
112. Raulf M, König W. In vitro effects of polychlorinated biphenyls on human platelets. *Immunology* (1991) **72**(2):287–91.
113. Kim S, Sundaramoorthi H, Jagadeeswaran P. Dioxin-induced thrombocyte aggregation in zebrafish. *Blood Cells Mol Dis* (2014) **54**(1):116–22. doi:10.1016/j.bcmd.2014.07.010
114. Kim DW, Gazourian L, Quadri SA, Romieu-Mourez R, Sherr DH, Sonenshein GE. The RelA NF-kappa B subunit and the aryl hydrocarbon receptor (AhR) cooperate to transactivate the c-myc promoter in mammary cells. *Oncogene* (2000) **19**(48):5498–506. doi:10.1038/sj.onc.1203945
115. Thatcher TH, Maggirwar SB, Baglole CJ, Lakatos HF, Gasiewicz TA, Phipps RP, et al. Aryl hydrocarbon receptor-deficient mice develop heightened inflammatory responses to cigarette smoke and endotoxin associated with rapid loss of the nuclear factor-kappaB component RelB. *Am J Pathol* (2007) **170**(3):855–64. doi:10.2353/ajpath.2007.060391
116. Baglole CJ, Maggirwar SB, Gasiewicz TA, Thatcher TH, Phipps RP, Sime PJ. The aryl hydrocarbon receptor attenuates tobacco smoke-induced cyclooxygenase-2 and prostaglandin production in lung fibroblasts through regulation of the NF-kappa B family member RelB. *J Biol Chem* (2008) **283**(43):28944–57. doi:10.1074/jbc.M800685200
117. Marienfeld R, Berberich-Siebelt F, Berberich I, Denk A, Serfling E, Neumann M. Signal-specific and phosphorylation-dependent RelB degradation: a potential mechanism of NF-kappa B control. *Oncogene* (2001) **20**(56):8142–7. doi:10.1038/sj.onc.1204884
118. Ezumi Y, Takayama H, Okuma M. Thrombopoietin, c-Mpl ligand, induces tyrosine phosphorylation of Tyk2, JAK2, and STAT3, and enhances agonists-induced aggregation in platelets in vitro. *FEBS Lett* (1995) **374**(1):48–52. doi:10.1016/0014-5793(95)01072-M
119. Miyakawa Y, Oda A, Druker BJ, Ozaki K, Handa M, Ohashi H, et al. Thrombopoietin and thrombin induce tyrosine phosphorylation of Vav in human blood platelets. *Blood* (1997) **89**(8):2789–98.
120. Zhou Z, Gushiken FC, Bolgiano D, Salsbery BJ, Aghakasiri N, Jing N, et al. Signal transducer and activator of transcription 3 (STAT3) regulates collagen-induced platelet aggregation independently of its transcription factor activity. *Circulation* (2013) **127**(4):476–85. doi:10.1161/CIRCULATIONAHA.112.132126
121. Lu WJ, Lin KC, Huang SY, Thomas PA, Wu YH, Wu HC, et al. Role of a Janus kinase 2-dependent signaling pathway in platelet activation. *Thromb Res* (2014) **133**(6):1088–96. doi:10.1016/j.thromres.2014.03.042
122. Ratajczak J, Wysoczynski M, Hayek F, Janowska-Wieczorek A, Ratajczak MZ. Membrane-derived microvesicles: important and underappreciated mediators of cell-to-cell communication. *Leukemia* (2006) **20**(9):1487–95. doi:10.1038/sj.leu.2404296
123. Del Conde I, Shrimpton CN, Thiagarajan P, López JA. Tissue-factor-bearing microvesicles arise from lipid rafts and fuse with activated platelets to initiate coagulation. *Blood* (2005) **106**(5):1604–11. doi:10.1182/blood-2004-03-1095
124. Horstman LL, Ahn YS. Platelet microparticles: a wide-angle perspective. *Crit Rev Oncol Hematol* (1999) **30**(2):111–42. doi:10.1016/S1040-8428(98)00044-4
125. Dachary-Prigent J, Freyssinet JM, Pasquet JM, Carron JC, Nurden AT. Annexin V as a probe of aminophospholipid exposure and platelet membrane vesiculation: a flow cytometry study showing a role for free sulfhydryl groups. *Blood* (1993) **81**(10):2554–65.
126. Dean WL, Lee MJ, Cummins TD, Schultz DJ, Powell DW. Proteomic and functional characterisation of platelet microparticle size classes. *Thromb Haemost* (2009) **102**(4):711–8. doi:10.1160/TH09-04-243
127. Bastos-Amador P, Royo F, Gonzalez E, Conde-Vancells J, Palomo-Diez L, Borras FE, et al. Proteomic analysis of microvesicles from plasma of healthy donors reveals high individual variability. *J Proteomics* (2012) **75**(12):3574–84. doi:10.1016/j.jprot.2012.03.054
128. Burger D, Schock S, Thompson CS, Montezano AC, Hakim AM, Touyz RM. Microparticles: biomarkers and beyond. *Clin Sci (Lond)* (2013) **124**(7):423–41. doi:10.1042/CS20120309
129. Camussi G, Deregibus MC, Bruno S, Cantaluppi V, Biancone L. Exosomes/microvesicles as a mechanism of cell-to-cell communication. *Kidney Int* (2010) **78**(9):838–48. doi:10.1038/ki.2010.278
130. Rozmyslowicz T, Majka M, Kijowski J, Murphy SL, Conover DO, Poncz M, et al. Platelet- and megakaryocyte-derived microparticles transfer CXCR4 receptor to CXCR4-null cells and make them susceptible to infection by X4-HIV. *AIDS* (2003) **17**(1):33–42. doi:10.1097/00002030-200301030-00006
131. Barry OP, Pratico D, Lawson JA, FitzGerald GA. Transcellular activation of platelets and endothelial cells by bioactive lipids in platelet microparticles. *J Clin Invest* (1997) **99**(9):2118–27. doi:10.1172/JCI119385
132. Mause SF, Weber C. Microparticles: protagonists of a novel communication network for intercellular information exchange. *Circ Res* (2010) **107**(9):1047–57. doi:10.1161/CIRCRESAHA.110.226456
133. Garcia BA, Smalley DM, Cho H, Shabanowitz J, Ley K, Hunt DF. The platelet microparticle proteome. *J Proteome Res* (2005) **4**(5):1516–21. doi:10.1021/pr0500760
134. Feldon SE, O'loughlin CW, Ray DM, Landskroner-Eiger S, Seweryniak KE, Phipps RP. Activated human T lymphocytes express cyclooxygenase-2 and produce proadipogenic prostaglandins that drive human orbital fibroblast differentiation to adipocytes. *Am J Pathol* (2006) **169**(4):1183–93. doi:10.2353/ajpath.2006.060434
135. Sahler J, Woeller CF, Phipps RP. Microparticles engineered to highly express peroxisome proliferator-activated receptor-γ decreased inflammatory mediator production and increased adhesion of recipient monocytes. *PLoS One* (2014) **9**(11):e113189. doi:10.1371/journal.pone.0113189
136. Liu H, Shi B, Huang CC, Eksarko P, Pope RM. Transcriptional diversity during monocyte to macrophage differentiation. *Immunol Lett* (2008) **117**(1):70–80. doi:10.1016/j.imlet.2007.12.012
137. Tontonoz P, Nagy L, Alvarez JG, Thomazy VA, Evans RM. PPARgamma promotes monocyte/macrophage differentiation and uptake of oxidized LDL. *Cell* (1998) **93**(2):241–52. doi:10.1016/S0092-8674(00)81575-5

Conflict of Interest Statement: Neil Blumberg has received lecture honoraria and consulting fees from Antek, Inc., Fenwal, Pall BioMedical, and Caridian (Terumo), manufacturers of leukoreduction filters, blood component equipment, and cell washing devices. The other authors have nothing to disclose.

Received: 09 December 2014; paper pending published: 06 January 2015; accepted: 26 January 2015; published online: 13 February 2015.
*Citation: Lannan KL, Sahler J, Kim N, Spinelli SL, Maggirwar SB, Garraud O, Cognasse F, Blumberg N and Phipps RP (2015) Breaking the mold: transcription factors in the anucleate platelet and platelet-derived microparticles. Front. Immunol. **6**:48. doi: 10.3389/fimmu.2015.00048*
This article was submitted to Inflammation, a section of the journal Frontiers in Immunology.

frontiers in
IMMUNOLOGY

MINI REVIEW ARTICLE
published: 06 January 2015
doi: 10.3389/fimmu.2014.00678

Platelets in inflammation: regulation of leukocyte activities and vascular repair

Angèle Gros[1,2†], Véronique Ollivier[1,2†] and Benoît Ho-Tin-Noé[1,2]*

[1] *Université Paris Diderot, Sorbonne Paris Cité, Paris, France*
[2] *Unit 1148, Laboratory for Vascular Translational Science, INSERM, Paris, France*

Edited by:
Olivier Garraud, Institut National de la Transfusion Sanguine, France

Reviewed by:
Daniel Duerschmied, University of Freiburg, Germany
Steve P. Watson, University of Birmingham, UK

***Correspondence:**
Benoît Ho-Tin-Noé, Unit 1148, INSERM, Hôpital Bichat, 46 rue Henri Huchard, Paris Cedex 18 75877, France
e-mail: benoit.ho-tin-noe@inserm.fr

†Angèle Gros and Véronique Ollivier have contributed equally to this work.

There is now a large body of evidence that platelets are central actors of inflammatory reactions. Indeed, platelets play a significant role in a variety of inflammatory diseases. These diseases include conditions as varied as atherosclerosis, arthritis, dermatitis, glomerulonephritis, or acute lung injury. In this context, one can note that inflammation is a convenient but imprecise catch-all term that is used to cover a wide range of situations. Therefore, when discussing the role of platelets in inflammation, it is important to clearly define the pathophysiological context and the exact stage of the reaction. Inflammatory reactions are indeed multistep processes that can be either acute or chronic, and their sequence can vary greatly depending on the situation and organ concerned. Here, we focus on how platelets contribute to inflammatory reactions involving recruitment of neutrophils and/or macrophages. Specifically, we review past and recent data showing that platelets intervene at various stages of these reactions to regulate parameters such as endothelial permeability, the recruitment of neutrophils and macrophages and their effector functions, as well as inflammatory bleeding. The mechanisms underlying these various modulating effect of platelets are also discussed.

Keywords: neutrophils, platelets, inflammation, monocytes, vascular permeability, bleeding, thrombocytopenia, ITAM

PLATELETS CONTROL ENDOTHELIAL PERMEABILITY AND LEUKOCYTE INFILTRATION

While platelets have long been known to promote the semi-permeable barrier function of the resting endothelium (1), it has become equally clear that under inflammatory conditions, platelets can promote vascular permeability. Indeed, both edema formation and leukocyte infiltration have been shown to be markedly reduced by thrombocytopenia in multiple models of acute or chronic inflammation. For instance, immunodepletion of platelets was shown to inhibit neutrophil extravasation and the efflux of Evans blue from mesenteric venules in a mouse model of thioglycollate-induced peritonitis (2). Thrombocytopenia also resulted in reduced skin edema and infiltration of neutrophils, eosinophils, and mast cells in models of cutaneous leukocytoclastic vasculitis (3) and of chronic contact hypersensitivity (4). These pro-inflammatory effects of platelets were also reported in the inflamed lungs (5–7), the very same organ where the anti-permeability effect of platelets was demonstrated in unchallenged thrombocytopenic animals (8).

Notably, while in the above-cited examples the efflux of leukocytes and plasma were both promoted by platelets, situations where only one of these parameters was impacted by platelet depletion have also been reported. In a mouse model of irritant contact dermatitis, experimental thrombocytopenia led to reduced edema formation without affecting neutrophil infiltration (9). Platelet depletion also resulted in complete abrogation of protein leakage without affecting neutrophil recruitment to the lungs in a model of transfusion-related acute lung injury (7). Concordantly, thrombocytopenia fully prevented endothelial gap formation and vascular leakage during experimental arthritis but only partially reduced the overall arthritis severity (10). Therefore, although promotion of plasma and leukocyte extravasation by platelets may share common mechanistic aspects, they are clearly distinct parameters with their own distinct regulatory mechanisms. Interestingly, it was recently shown that leukocyte extravasation and vascular permeability can be controlled separately by different tyrosine residues of VE-cadherin (11). Whether such a mechanism contributes to differential regulation of vascular permeability and leukocyte infiltration by platelets remains to be demonstrated but these results suggest that distinct and selective regulation of these two parameters is possible at the endothelial junction level.

Recruitment of platelets to the inflamed vasculature and local release of soluble compounds from activated platelets likely constitute the predominant basic mechanisms common to regulation of both vascular permeability and leukocyte extravasation by platelets. Interactions between platelets and inflamed vessels have been shown to be an early event in various models of acute and chronic inflammation (7, 9, 10, 12–19). Various adhesion receptors can support the early recruitment of platelets to inflamed microvessels and the subsequent actions of platelets on vascular permeability and leukocyte extravasation. The nature of these receptors is highly dependent on the inflammatory context. While platelet P-selectin was crucial for platelet recruitment and leukocyte extravasation in experimental colitis (20), acute lung injury (5), chronic cutaneous hypersensitivity (4), allergic asthma (21), glomerulonephritis (17), and cerebral and intestinal

ischemia/reperfusion (13, 14), it played only a minor role in the recruitment of neutrophils in thioglycollate-induced peritonitis (2). Likewise, GPIb was critical for platelet recruitment and leukocyte invasion at atheromatous lesion-prone sites in hypercholesterolemic mice (12) and during peritonitis (2) but dispensable for early recruitment of platelets to the inflamed glomerulus (19). In addition to P-selectin and GPIb, GPVI, GPIIb–IIIa, CD40L are among the other platelet receptors that have been reported to be involved in platelet recruitment and action on vascular permeability and/or leukocyte recruitment (**Table 1**).

Remarkably, immunohistological and intravital analyses have revealed that platelet recruitment during the early phase of the inflammatory reaction mostly occurs through transient and firm adhesion of individual platelets to endothelial cells and gaps in the endothelial lining, and/or to adherent leukocytes rather than through platelet aggregation and thrombus formation (7, 9, 10, 12–14, 16, 18–20). Binding of platelets to adherent leukocytes illustrates the fact that while platelets support the recruitment of leukocytes during inflammatory reactions, the opposite is also true. Inhibition of neutrophil recruitment by immunodepletion of neutrophils or by pharmacological or genetic targeting of their adhesion receptors was indeed shown to induce a reduction in platelet recruitment in various inflammation models (7, 13, 14, 19, 27). It is also noteworthy that interactions between activated platelets and leukocytes do not only occur at the inflammatory site but also in the circulation. The signals triggered by these interactions notably induce integrin activation, thus priming leukocytes for adhesion to inflamed vessels (24, 28). Increased formation of neutrophil/platelet and/or monocyte/platelet complexes has been reported in patients with various inflammatory diseases including rheumatoid and psoriatic arthritis (29–31), allergic asthma (32), perionditis (33), ulcerative colitis (34), ischemic stroke (35), dengue infection (36), and atherosclerosis (37). Considering the contribution of platelet/leukocyte interactions to the pathogenesis of various inflammatory diseases in experimental models, in part, via promotion of neutrophil infiltration, increased platelet/leukocyte complexes in human diseases may not only reflect platelet activation but also participate in disease regulation.

Table 1 | Regulation of vascular permeability and leukocyte recruitment by platelets.

	Inflammatory model	Receptors and/or soluble factors involved	References
Vascular permeability	Arthritis	GPVI/Serotonin	Cloutier et al. (10)
	Peritonitis	GPIb/vWF	Petri et al. (2)
	Leukocytoclastic vasculitis	P-selectin/PSGL-I	Hara et al. (3)
	Chronic cutaneous hypersensitivity	ND	Tamagawa-Mineoka et al. (4)
	Acute lung inflammation	P-selectin	Hidalgo et al. (7), Zarbock et al. (5)
Leukocyte recruitment	Glomerulonephritis	P-selectin/PSGL-1	Kuligowski et al. (17)
		P-selectin	Devi et al. (19)
	Atherosclerosis	GPVI/EMMPRIN	Schulz et al. (22)
		CD40L and MCP-I	Lievens et al. (23)
		GPIb, GPIIbIIIa	Massberg et al. (12)
		P-selectin	Huo et al. (24)
	Cerebral I/R	GPIIbIIIa	Massberg et al. (15)
		CD40/CD40L/sCD40L	Ishikawa et al. (14)
	Intestinal I/R	P-selectin	Ishikawa et al. (14)
	Abdominal sepsis	Serotonin	Duerschmied et al. (25)
		ND/Mac-1	Asaduzzaman et al. (6)
	Peritonitis	GPIb/vWF	Petri et al. (2)
		Serotonin	Duerschmied et al. (25)
	Experimental colitis	CD40L	Vowinkel et al. (20)
		P-selectin/PSGL-1	Vowinkel et al. (20)
	Aseptic wound	Serotonin	Duerschmied et al. (25)
	Acute Lung inflammation	Serotonin, TXA_2, P-selectin	Duerschmied et al. (25), Hidalgo et al. (7), Zarbock et al. (5)
	Leukocytoclastic vasculitis	P-selectin/PSGL-1	Hara et al. (3)
	Chronic cutaneous hypersensitivity	P-selectin	Tamagawa-Mineoka et al. (4)
	Allergic asthma	P-selectin, Serotonin	Pitchford et al. (21), Durk et al. (26)

Examples of various inflammatory situations in which platelets were shown to regulate vascular permeability and/or leukocyte recruitment. If identified, the adhesion receptors and/or soluble factors involved are indicated. Platelet-derived factors are underlined.

CD40, cluster of differentiation 40, CD40L, cluster of differentiation 40 ligand, EMMPRIN, extracellular matrix metalloproteinase inducer, Mac-1, macrophage-1 antigen, MCP-1, monocyte chemoattractant protein-1, ND, non-determined, PAF, platelet-activating factor, PSGL-1, P-selectin glycoprotein ligand 1, sCD40L, soluble cluster of differentiation 40 ligand, TXA2, thromboxane A2, VEGF, vascular endothelial growth factor, vWF, von Willebrand factor.

The release of soluble factors by platelets is also central to their ability to modulate endothelial permeability and leukocyte infiltration. In the same way, platelets contain endothelial barrier-enhancing factors [e.g., angiopoietin-1 and sphingosine-1-phosphate (S1P)] (1), they also contain a large range of chemokines, cytokines, and other soluble mediators capable of loosening endothelial junctions and/or of activating or attracting leukocytes. Among the platelet factors that can open interendothelial junctions are factors that are not specific to platelets such as vascular endothelial growth factor (38) or platelet-activating factor (PAF) (39, 40), and serotonin, for which platelets are the major peripheral source (41). In particular, platelet serotonin was recently shown to mediate the GPVI-dependent pro-permeability effect of platelets in inflamed joints during arthritis (10) and the recruitment of neutrophils and/or eosinophils in various models of acute inflammation (25, 26).

The panel of chemokines found in platelets is extensive and includes platelet factor 4, stromal cell-derived factor-1, macrophage inflammatory protein-1α (MIP-1α), regulated upon activation, normal T cell expressed and secreted (RANTES), and thymus and activation-regulated chemokine (TARC), to name but a few (4, 24, 42). Like P-selectin, these chemokines are stored in platelet granules and their contribution to recruitment of leukocytes by platelets highlights the need for platelet activation in this process. In agreement, ADP-dependent platelet activation was required for glomerular recruitment of leukocytes by platelets (19). Moreover, while some platelet-derived soluble factors directly target endothelial cells and/or leukocytes, others like PAF, S1P, and serotonin also target platelets themselves and could therefore amplify their regulatory effects on vascular permeability and/or leukocyte recruitment. Finally, another mechanism reported for the stimulation of neutrophil recruitment by activated platelets is the up-regulation of the expression of adhesion molecules on the endothelium. Activated platelets were shown to increase the expression of ICAM-1 and αvβ3 on endothelial cells through the secretion of IL-1β (43), and to induce the secretion of Weibel–Palade bodies (44). Importantly, together, these observations that platelets can engage their adhesion receptors and release bioactive factors independently of aggregation substantiate the concept that platelet activation is a finely tuned and graduated process that allows context-dependent responses (27, 45–48).

PLATELETS REGULATE NEUTROPHIL AND MACROPHAGE EFFECTOR FUNCTIONS

Many studies have demonstrated the ability of platelets to regulate most of the effector functions of neutrophils and macrophages such as the production of reactive oxygen species (ROS), the secretion of neutrophil granule content, phagocytosis, or the formation of neutrophil extracellular traps (NETs). Thrombin-activated platelets were shown to stimulate the respiratory burst in unstimulated monocytes and neutrophils *in vitro* (49–51). Zalavary et al. showed that resting platelets enhanced the respiratory burst of neutrophils stimulated with IgG-opsonized yeast particles (52). However, the opposite effect was found when the chemoattractant formyl-methionyl-leucyl-phenylalanine was used as an agonist (53–56). The fact that the pro- or anti-ROS effect of platelets depends on experimental conditions such as the platelet and neutrophil agonists used again highlights how the action of platelets may ultimately be context-dependent. Several non-mutually exclusive mechanisms have been proposed to support the modulation of the oxidative burst in innate immune cells by platelets. The release of adenine nucleotide derivatives by platelets has been consistently shown to inhibit ROS generation in neutrophils (49, 53, 54). Evidence that direct cell–cell contact between platelets and monocytes/neutrophils is also important for regulation of the oxidative burst in these cells has also been provided. Prevention of platelet adhesion to neutrophils was indeed shown to suppress the modulatory effect exerted by platelets on ROS generation by neutrophils, whether it was stimulatory (50, 51, 54, 57, 58) or inhibitory (58). *In vivo* data on the actual contribution of platelets to modulation of ROS generation by innate immune cells during inflammatory reactions are, however, scarce. Nevertheless, binding of platelets to adherent neutrophils induced the generation of ROS by neutrophils in a mouse model of transfusion-related acute lung injury (7), a mechanism that might contribute to vascular damage and lung injury. Stimulation of the neutrophil respiratory burst by platelets was also proposed to contribute to proteinuria during immune-complex-induced experimental glomerulonephritis (51), as ROS were previously shown to be one of the main causes of glomerular damage in this model (59).

Several *in vitro* studies have shown that platelets can inhibit the release of myeloperoxidase and neutrophil elastase (57, 60). In contrast, platelets amplified lysozyme secretion by neutrophils stimulated with opsonized zymosan (61, 62). It is likely that as for ROS generation and endothelial permeability, platelets regulate neutrophil degranulation in a stimulus-specific manner. Some groups have demonstrated that in addition to regulating neutrophil degranulation and ROS production, platelets can also stimulate neutrophil phagocytosis by both contact-dependent and contact-independent mechanisms (52, 57). More recently, it was shown that the uptake of oxidized low-density lipoproteins (OxLDL) by monocytes was increased by direct platelet–monocyte interactions *in vitro* and *in vivo* (63). These findings partly echoed those of previous studies with the difference that the earlier studies focused on the ability of platelet secretion products to enhance macrophage uptake of OxLDL (64–66).

The production and release of cytokines by monocytes can also be regulated by platelets. Thrombin-activated platelets were shown to induce expression and secretion of MCP-1 and IL-8 by monocytes in a P-selectin-dependent manner (67). The contribution of P-selectin-mediated platelet–monocyte interactions to cytokine responses was recently investigated in patients with dengue infection. Whereas P-selectin-dependent binding of platelets from patients with dengue to monocytes from healthy volunteers induced the secretion of the IL-1β, IL-8, IL-10, and MCP-1, platelets from healthy volunteers only induced the secretion of MCP-1 (36). Modulation of cytokine levels by platelets was also reported in the cecal ligation and puncture mouse model of sepsis, a model in which deficiency in platelet GPIbα led to reduced platelet–monocyte and platelet–neutrophil interactions, and to increased circulating levels of TNF-α, MCP-1, IL-6, and MIP-1β (68). A substantial contribution of platelets to the regulation of systemic inflammatory responses was also shown in

a model of pneumonia-derived sepsis (69) and in a model of thermal injury (70). In this latter model, plasma levels of TNF-α, IL-6, and MCP-1 were increased in thrombocytopenic mice, which also displayed reduced levels of TGF-β compared to control mice. In line with these studies, it was shown that secretion of PDGF by activated platelets was required for the release of MCP-1 and subsequent monocyte infiltration and killing in tissues infected with *Leishmania* parasites (71). It should be noted that thrombocytopenia led to a higher mortality rate in sepsis and thermal injury, and to reduced elimination of *Leishmania* parasites, thus demonstrating an overall beneficial role of platelets in these models. Thrombocytopenia also increased the mortality of mice infected with encephalomyocarditis virus, a situation in which platelet-TLR7-dependent formation of platelet–neutrophil aggregates played a protective role (72). These various examples where platelets exerted a beneficial effect under inflammatory conditions in some way balance those where platelets were shown to play a pathogenic role, as in glomerulonephritis (51), acute lung injury (5), atherosclerosis (12, 15), and rheumatoid arthritis (10, 73).

Neutrophil extracellular traps are extracellular webs made of DNA fibers decorated with neutrophil granule proteins that form according to a specific cell death process (74, 75). The contribution of platelets to NET formation was first demonstrated by a series of experiments showing that TLR4- and integrin-dependent platelet–neutrophil interactions led to the production of NETs capable of trapping bacteria and also causing tissue damage in models of endotoxemia and sepsis (27, 76). Stimulation of NET formation by platelets has since been shown in models of sterile acute lung injury where NETs contribute to lung endothelial damage (77, 78). Importantly, while platelets stimulate NET formation, in return, NETs cause platelet activation and aggregation, thus linking inflammation and thrombosis (79). This form of thrombosis triggered by neutrophil–platelet interactions has been called immunothrombosis and helps fight bacterial infection (80, 81). The downside of immunothrombosis is that it can also initiate deep vein thrombosis (82, 83) and contribute to cancer-associated thrombosis (84). Finally, immunothrombosis illustrates how interactions between platelets and leukocytes lead to reciprocal regulation, a notion previously introduced by earlier *in vitro* studies showing the modulation of platelet reactivity and adhesion properties by neutrophils and/or macrophages (48, 85–89).

PLATELETS PREVENT INFLAMMATORY BLEEDING

Inflammatory reactions have been identified as a cause of spontaneous bleeding during thrombocytopenia, thus demonstrating that platelets also intervene to prevent bleeding during inflammation. This protective role of platelets was notably established in situations where platelets were also shown to promote vascular permeability and/or leukocyte infiltration. For example, induction of thrombocytopenia resulted in immediate and severe skin bleeding during cutaneous leukocytoclastic vasculitis (90), the same model in which it caused reduced skin edema and neutrophil infiltration (3). Again, this highlights the duality of platelets that can exert apparently opposed effects during the course of a given inflammatory reaction. Nonetheless, though regulation of plasma leakage and prevention of bleeding can both be referred to as maintenance of vascular integrity, the mechanisms underlying edema formation and bleeding are essentially different. Opening of intercellular junctions favors edema formation by allowing plasma fluid and small molecules to filter out through endothelial gaps and the basement membrane. Larger elements like platelets and red blood cells are, however, retained by the vascular wall (91). For this reason, injection of the pro-permeability factor vascular endothelial growth factor (VEGF) did not cause any skin bleeding when injected in thrombocytopenic mice (92). In a similar manner, mice lacking the anti-permeability factor, S1P, in plasma exhibited basal vascular leakage of proteins and fluid in the lungs, but no bleeding (93). Therefore, while vascular permeability is tightly regulated and controlled through modulation of endothelial junctions bleeding implies vascular damage with rupture or distortion of the basement membrane mesh. There is strong evidence suggesting that neutrophils may cause this damage: in various inflammatory models, bleeding in inflamed organs of thrombocytopenic mice was prevented by depletion of neutrophils (18, 92). Prevention of inflammatory bleeding by platelets most likely relies on the inhibition or repair of neutrophil-dependent injury. Although this is unlikely to provide the full explanation, this vasculoprotective action of platelets has been shown to be independent of their ability to form thrombi. Indeed, it has been reported that platelet interactions with inflamed microvessels occur before any sign of thrombosis, and that prevention of this early initial platelet deposition results in accelerated and exacerbated local hemorrhage (18, 90). Moreover, mice lacking platelet adhesion receptors required for thrombus formation or with impaired G-protein coupled receptor (GPCR) signaling in platelets have shown no bleeding at sites of inflammation despite their compromised ability to form platelet plugs and to ensure classical hemostasis (90, 94). A recent study indicates that instead, the immunoreceptor tyrosine-based activation motif (ITAM)-associated GPVI and C-type lectin-like receptor-2 (CLEC-2) play a predominant role in the prevention of inflammatory bleeding by platelets (94, 95). Given that GPVI is the main platelet receptor for collagen, this suggests that platelets could stop bleeding by covering small areas where the basement membrane gets exposed and disrupted by recruited neutrophils. Whereas non-aggregated adherent platelets could exert a purely mechanical action by plugging small holes in the endothelial lining, recent results suggest that the release of soluble factors by platelets could further help seal vessel lesions induced by neutrophils. We have shown that even when not causing aggregation, engagement of GPVI by collagen leads to the release of soluble platelet factors capable of tightening interendothelial junctions such as angiopoietin-1 (48). Furthermore, platelets lacking the adapter protein SLP-76, which mediates GPVI-dependent platelet responses to collagen including degranulation and spreading, were shown to be unable to prevent inflammatory bleeding (94). Also, although absence of S1P is not in itself a cause of bleeding, it was shown to sensitize peripheral lymph nodes to bleeding in a mouse model of immunization characterized by high lymphocyte trafficking (95). Whether this protective role of S1P and, more specifically, of platelet S1P also applies to other inflammatory models remains to be verified but in any case, results from this study indicate that factors stimulating closure of interendothelial junctions can help stop bleeding associated with leukocyte trafficking.

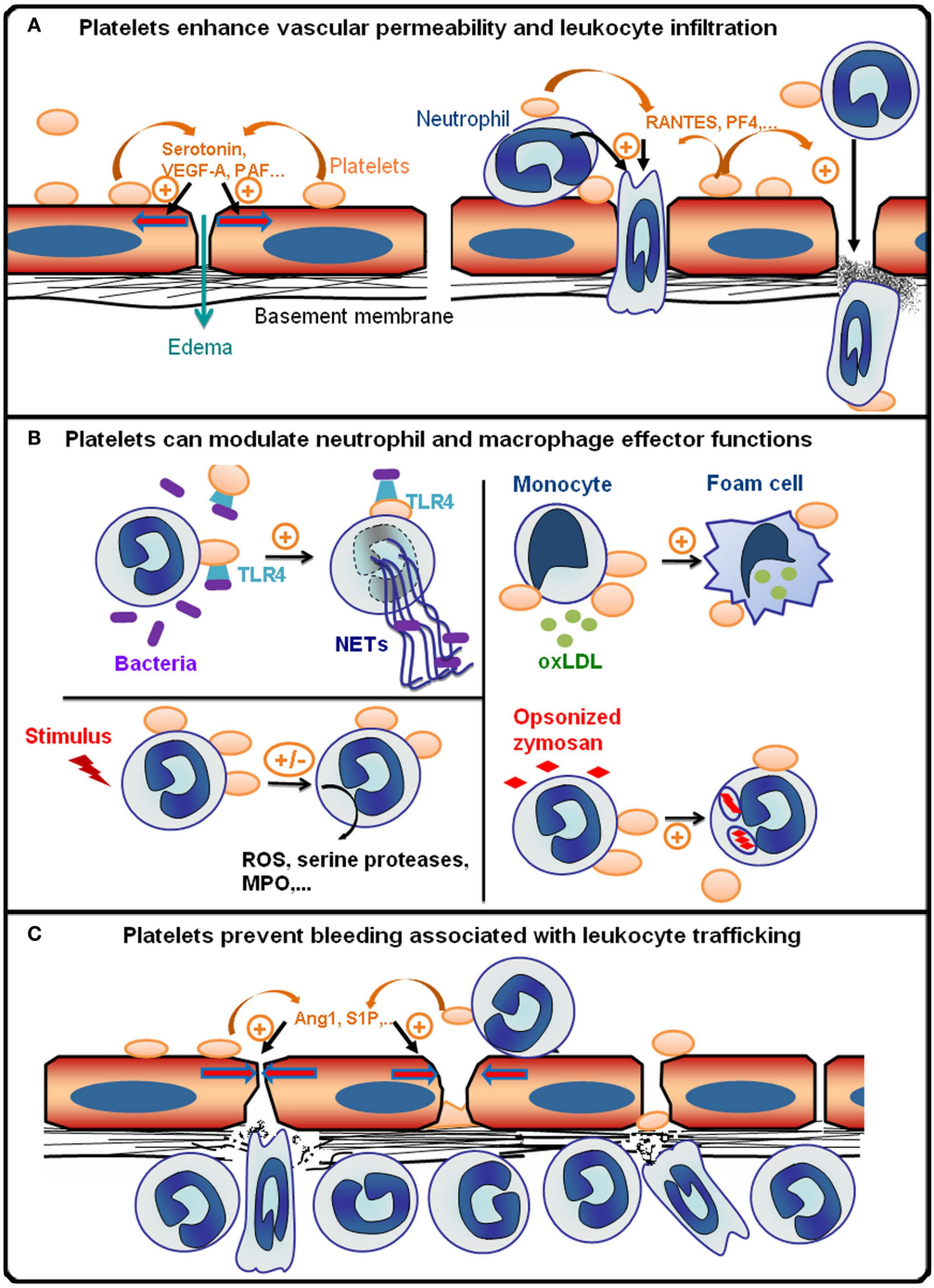


FIGURE 1 | Platelets are integral players of inflammatory reactions. **(A)** During the early phase of inflammatory reactions, platelets are recruited to inflamed vessels and enhance vascular permeability by secreting pro-permeability factors such as serotonin, vascular endothelial growth factor A (VEGF-A), or platelet-activating factor (PAF). Platelets further enhance the recruitment and infiltration of leukocytes by secreting chemokines [e.g., activation or normal T cell expressed and secreted (RANTES) or platelet factor 4 (PF-4)], and by upregulating the expression and/or activation of adhesion molecules on leukocytes and endothelial cells via direct cell–cell contacts and secretion of pro-inflammatory cytokines. The platelet receptors (e.g., GPVI, GPIb, GPIIbIIIa, or P-selectin) supporting the interactions of platelets with inflamed vessels and/or leukocytes vary with the organ and inflammatory reactions concerned. **(B)** Platelets can modulate various leukocyte effector functions: toll-like receptor 4 (TLR4)-dependent activation of platelets promotes the formation of antibacterial neutrophil extracellular traps (NETs); platelets can also either stimulate or inhibit the production of reactive oxygen species (ROS) and/or secretion of cytokines and cytotoxic enzymes [e.g., myeloperoxidase (MPO), serine proteases] by neutrophils and macrophages depending on the inflammatory situation; platelets can stimulate phagocytosis and thus enhance foam cell formation by promoting the uptake of oxidized low-density lipoprotein by monocytes/macrophages (oxLDL). **(C)** Platelets prevent bleeding associated with leukocyte trafficking. This protective action of platelets has been shown to be independent of thrombus formation and to rely on the engagement of platelet ITAM receptors, which might cause secretion of anti-permeability factors such as angiopoietin-1 (Ang1) and sphingosine-1-phosphate (S1P) by platelets.

The ITAM-dependent vasculoprotective action of platelets could also depend on their interactions with leukocytes. As mentioned above, platelets recruited to inflamed vessels bind to adherent leukocytes. The extracellular matrix metalloproteinase inducer (EMMPRIN) was recently identified as a counter-receptor for GPVI and is present on neutrophils and macrophages (22, 96). Additionally, podoplanin, the ligand for CLEC-2, is absent on blood endothelial cells but is expressed by inflammatory macrophages (97). ITAM-mediated interactions between platelets and innate immune cells thus represent another potential trigger for the anti-permeability and/or "neutrophil-dampening" effect of platelets.

In conclusion, all these data showing that in a given situation, platelets can intervene to regulate vascular permeability, leukocyte infiltration, and effector functions, as well as bleeding associated with leukocyte trafficking, demonstrate that platelets are integral players of inflammatory reactions (**Figure 1**). Also, it appears that the pro- or anti-inflammatory character of platelets is highly dependent on parameters such as the cause of inflammation, which defines the pathways and extent of leukocyte, platelet, and endothelial cell activation, and the stage and/or endpoint readouts of the inflammatory reaction considered. In a similar manner, the overall impact of platelets on inflammatory diseases can either be beneficial or deleterious depending on the pathophysiological situation. Finally, as illustrated by the increasing interest in platelets as potential targets for the treatment of allergic inflammation (26, 98, 99), the availability of numerous anti-platelet drugs could open up new therapeutic perspectives for the many inflammatory diseases where platelets have been shown to play a pathogenic role (100).

ACKNOWLEDGMENTS

We thank Dr. Mary J. Osborne-Pellegrin and Dr. Richard Bayles for their help in editing this manuscript. This work was supported by the Institut National de la Santé et de la Recherche Médicale and by grants from La Fondation de France, DHU FIRE, and CORDDIM Région Ile de France to Benoît Ho-Tin-Noé.

REFERENCES

1. Ho-Tin-Noe B, Demers M, Wagner DD. How platelets safeguard vascular integrity. *J Thromb Haemost* (2011) **9**(Suppl 1):56–65. doi:10.1111/j.1538-7836.2011.04317.x
2. Petri B, Broermann A, Li H, Khandoga AG, Zarbock A, Krombach F, et al. von Willebrand factor promotes leukocyte extravasation. *Blood* (2010) **116**:4712–9. doi:10.1182/blood-2010-03-276311
3. Hara T, Shimizu K, Ogawa F, Yanaba K, Iwata Y, Muroi E, et al. Platelets control leukocyte recruitment in a murine model of cutaneous arthus reaction. *Am J Pathol* (2010) **176**:259–69. doi:10.2353/ajpath.2010.081117
4. Tamagawa-Mineoka R, Katoh N, Ueda E, Takenaka H, Kita M, Kishimoto S. The role of platelets in leukocyte recruitment in chronic contact hypersensitivity induced by repeated elicitation. *Am J Pathol* (2007) **170**:2019–29. doi:10.2353/ajpath.2007.060881
5. Zarbock A, Singbartl K, Ley K. Complete reversal of acid-induced acute lung injury by blocking of platelet-neutrophil aggregation. *J Clin Invest* (2006) **116**:3211–9. doi:10.1172/JCI29499
6. Asaduzzaman M, Lavasani S, Rahman M, Zhang S, Braun OO, Jeppsson B, et al. Platelets support pulmonary recruitment of neutrophils in abdominal sepsis. *Crit Care Med* (2009) **37**:1389–96. doi:10.1097/CCM.0b013e31819ceb71
7. Hidalgo A, Chang J, Jang JE, Peired AJ, Chiang EY, Frenette PS. Heterotypic interactions enabled by polarized neutrophil microdomains mediate thromboinflammatory injury. *Nat Med* (2009) **15**:384–91. doi:10.1038/nm.1939
8. Lo SK, Burhop KE, Kaplan JE, Malik AB. Role of platelets in maintenance of pulmonary vascular permeability to protein. *Am J Physiol* (1988) **254**:H763–71.
9. Senaldi G, Piguet PF. Platelets play a role in the pathogenesis of the irritant reaction in mice. *J Invest Dermatol* (1997) **108**:248–52. doi:10.1111/1523-1747.ep12286444
10. Cloutier N, Pare A, Farndale RW, Schumacher HR, Nigrovic PA, Lacroix S, et al. Platelets can enhance vascular permeability. *Blood* (2012) **120**:1334–43. doi:10.1182/blood-2012-02-413047
11. Wessel F, Winderlich M, Holm M, Frye M, Rivera-Galdos R, Vockel M, et al. Leukocyte extravasation and vascular permeability are each controlled in vivo by different tyrosine residues of VE-cadherin. *Nat Immunol* (2014) **15**:223–30. doi:10.1038/ni.2824
12. Massberg S, Brand K, Gruner S, Page S, Muller E, Muller I, et al. A critical role of platelet adhesion in the initiation of atherosclerotic lesion formation. *J Exp Med* (2002) **196**:887–96. doi:10.1084/jem.20012044
13. Cooper D, Chitman KD, Williams MC, Granger DN. Time-dependent platelet-vessel wall interactions induced by intestinal ischemia-reperfusion. *Am J Physiol Gastrointest Liver Physiol* (2003) **284**:G1027–33. doi:10.1152/ajpgi.00457.2002
14. Ishikawa M, Cooper D, Arumugam TV, Zhang JH, Nanda A, Granger DN. Platelet-leukocyte-endothelial cell interactions after middle cerebral artery occlusion and reperfusion. *J Cereb Blood Flow Metab* (2004) **24**:907–15. doi:10.1097/01.WCB.0000132690.96836.7F
15. Massberg S, Schurzinger K, Lorenz M, Konrad I, Schulz C, Plesnila N, et al. Platelet adhesion via glycoprotein IIb integrin is critical for atheroprogression and focal cerebral ischemia: an in vivo study in mice lacking glycoprotein IIb. *Circulation* (2005) **112**:1180–8. doi:10.1161/CIRCULATIONAHA.105.539221
16. Schmitt-Sody M, Klose A, Gottschalk O, Metz P, Gebhard H, Zysk S, et al. Platelet-endothelial cell interactions in murine antigen-induced arthritis. *Rheumatology (Oxford)* (2005) **44**:885–9. doi:10.1093/rheumatology/keh638
17. Kuligowski MP, Kitching AR, Hickey MJ. Leukocyte recruitment to the inflamed glomerulus: a critical role for platelet-derived P-selectin in the absence of rolling. *J Immunol* (2006) **176**:6991–9. doi:10.4049/jimmunol.176.11.6991
18. Hirahashi J, Hishikawa K, Kaname S, Tsuboi N, Wang Y, Simon DI, et al. Mac-1 (CD11b/CD18) links inflammation and thrombosis after glomerular injury. *Circulation* (2009) **120**:1255–65. doi:10.1161/CIRCULATIONAHA.109.873695
19. Devi S, Kuligowski MP, Kwan RY, Westein E, Jackson SP, Kitching AR, et al. Platelet recruitment to the inflamed glomerulus occurs via an alphaIIbbeta3/GPVI-dependent pathway. *Am J Pathol* (2010) **177**:1131–42. doi:10.2353/ajpath.2010.091143
20. Vowinkel T, Wood KC, Stokes KY, Russell J, Tailor A, Anthoni C, et al. Mechanisms of platelet and leukocyte recruitment in experimental colitis. *Am J Physiol Gastrointest Liver Physiol* (2007) **293**:G1054–60. doi:10.1152/ajpgi.00350.2007
21. Pitchford SC, Momi S, Giannini S, Casali L, Spina D, Page CP, et al. Platelet P-selectin is required for pulmonary eosinophil and lymphocyte recruitment in a murine model of allergic inflammation. *Blood* (2005) **105**:2074–81. doi:10.1182/blood-2004-06-2282
22. Schulz C, Von Bruhl ML, Barocke V, Cullen P, Mayer K, Okrojek R, et al. EMMPRIN (CD147/basigin) mediates platelet-monocyte interactions in vivo and augments monocyte recruitment to the vascular wall. *J Thromb Haemost* (2011) **9**:1007–19. doi:10.1111/j.1538-7836.2011.04235.x
23. Lievens D, Zernecke A, Seijkens T, Soehnlein O, Beckers L, Munnix IC, et al. Platelet CD40L mediates thrombotic and inflammatory processes in atherosclerosis. *Blood* (2010) **116**(20):4317–27. doi:10.1182/blood-2010-01-261206
24. Huo Y, Schober A, Forlow SB, Smith DF, Hyman MC, Jung S, et al. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. *Nat Med* (2003) **9**:61–7. doi:10.1038/nm810
25. Duerschmied D, Suidan GL, Demers M, Herr N, Carbo C, Brill A, et al. Platelet serotonin promotes the recruitment of neutrophils to sites of acute inflammation in mice. *Blood* (2013) **121**:1008–15. doi:10.1182/blood-2012-06-437392
26. Durk T, Duerschmied D, Muller T, Grimm M, Reuter S, Vieira RP, et al. Production of serotonin by tryptophan hydroxylase 1 and release via platelets contribute to allergic airway inflammation. *Am J Respir Crit Care Med* (2013) **187**:476–85. doi:10.1164/rccm.201208-1440OC
27. Clark SR, Ma AC, Tavener SA, McDonald B, Goodarzi Z, Kelly MM, et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat Med* (2007) **13**:463–9. doi:10.1038/nm1565

28. Totani L, Evangelista V. Platelet-leukocyte interactions in cardiovascular disease and beyond. *Arterioscler Thromb Vasc Biol* (2010) **30**:2357–61. doi:10.1161/ATVBAHA.110.207480
29. Joseph JE, Harrison P, Mackie IJ, Isenberg DA, Machin SJ. Increased circulating platelet-leucocyte complexes and platelet activation in patients with antiphospholipid syndrome, systemic lupus erythematosus and rheumatoid arthritis. *Br J Haematol* (2001) **115**:451–9. doi:10.1046/j.1365-2141.2001.03101.x
30. Bunescu A, Seideman P, Lenkei R, Levin K, Egberg N. Enhanced Fcgamma receptor I, alphaMbeta2 integrin receptor expression by monocytes and neutrophils in rheumatoid arthritis: interaction with platelets. *J Rheumatol* (2004) **31**:2347–55.
31. Pamuk GE, Nuri Pamuk O, Orum H, Arican O, Turgut B, Demir M. Elevated platelet-monocyte complexes in patients with psoriatic arthritis. *Platelets* (2009) **20**:493–7. doi:10.3109/09537100903165174
32. Pitchford SC, Yano H, Lever R, Riffo-Vasquez Y, Ciferri S, Rose MJ, et al. Platelets are essential for leukocyte recruitment in allergic inflammation. *J Allergy Clin Immunol* (2003) **112**:109–18. doi:10.1067/mai.2003.1514
33. Nicu EA, Van Der Velden U, Nieuwland R, Everts V, Loos BG. Elevated platelet and leukocyte response to oral bacteria in periodontitis. *J Thromb Haemost* (2009) **7**:162–70. doi:10.1111/j.1538-7836.2008.03219.x
34. Pamuk GE, Vural O, Turgut B, Demir M, Umit H, Tezel A. Increased circulating platelet-neutrophil, platelet-monocyte complexes, and platelet activation in patients with ulcerative colitis: a comparative study. *Am J Hematol* (2006) **81**:753–9. doi:10.1002/ajh.20655
35. McCabe DJ, Harrison P, Mackie IJ, Sidhu PS, Purdy G, Lawrie AS, et al. Platelet degranulation and monocyte-platelet complex formation are increased in the acute and convalescent phases after ischaemic stroke or transient ischaemic attack. *Br J Haematol* (2004) **125**:777–87. doi:10.1111/j.1365-2141.2004.04983.x
36. Hottz ED, Medeiros-De-Moraes IM, Vieira-De-Abreu A, De Assis EF, Vals-De-Souza R, Castro-Faria-Neto HC, et al. Platelet activation and apoptosis modulate monocyte inflammatory responses in dengue. *J Immunol* (2014) **193**:1864–72. doi:10.4049/jimmunol.1400091
37. Furman MI, Benoit SE, Barnard MR, Valeri CR, Borbone ML, Becker RC, et al. Increased platelet reactivity and circulating monocyte-platelet aggregates in patients with stable coronary artery disease. *J Am Coll Cardiol* (1998) **31**:352–8. doi:10.1016/S0735-1097(97)00510-X
38. Nachman RL, Rafii S. Platelets, petechiae, and preservation of the vascular wall. *N Engl J Med* (2008) **359**:1261–70. doi:10.1056/NEJMra0800887
39. Vargaftig BB, Chignard M, Lefort J, Benveniste J. Platelet-tissue interaction: role of platelet-activating factor (PAF-acether). *Agents Actions* (1980) **10**:502–6. doi:10.1007/BF02024151
40. Knezevic II, Predescu SA, Neamu RF, Gorovoy MS, Knezevic NM, Easington C, et al. Tiam1 and Rac1 are required for platelet-activating factor-induced endothelial junctional disassembly and increase in vascular permeability. *J Biol Chem* (2009) **284**:5381–94. doi:10.1074/jbc.M808958200
41. Vanhoutte PM, Luscher TF. Serotonin and the blood vessel wall. *J Hypertens Suppl* (1986) **4**:S29–35.
42. Strussmann T, Tillmann S, Wirtz T, Bucala R, Von Hundelshausen P, Bernhagen J. Platelets are a previously unrecognised source of MIF. *Thromb Haemost* (2013) **110**:1004–13. doi:10.1160/TH13-01-0049
43. Gawaz M, Brand K, Dickfeld T, Pogatsa-Murray G, Page S, Bogner C, et al. Platelets induce alterations of chemotactic and adhesive properties of endothelial cells mediated through an interleukin-1-dependent mechanism. Implications for atherogenesis. *Atherosclerosis* (2000) **148**:75–85. doi:10.1016/S0021-9150(99)00241-5
44. Dole VS, Bergmeier W, Mitchell HA, Eichenberger SC, Wagner DD. Activated platelets induce Weibel-Palade-body secretion and leukocyte rolling in vivo: role of P-selectin. *Blood* (2005) **106**:2334–9. doi:10.1182/blood-2005-04-1530
45. Cognasse F, Hamzeh-Cognasse H, Lafarge S, Delezay O, Pozzetto B, McNicol A, et al. Toll-like receptor 4 ligand can differentially modulate the release of cytokines by human platelets. *Br J Haematol* (2008) **141**:84–91. doi:10.1111/j.1365-2141.2008.06999.x
46. Italiano JE Jr, Richardson JL, Patel-Hett S, Battinelli E, Zaslavsky A, Short S, et al. Angiogenesis is regulated by a novel mechanism: pro- and antiangiogenic proteins are organized into separate platelet {alpha} granules and differentially released. *Blood* (2008) **111**:1227–33. doi:10.1182/blood-2007-09-113837
47. Stalker TJ, Traxler EA, Wu J, Wannemacher KM, Cermignano SL, Voronov R, et al. Hierarchical organization in the hemostatic response and its relationship to the platelet-signaling network. *Blood* (2013) **121**:1875–85. doi:10.1182/blood-2012-09-457739
48. Ollivier V, Syvannarath V, Gros A, Butt A, Loyau S, Jandrot-Perrus M, et al. Collagen can selectively trigger a platelet secretory phenotype via glycoprotein VI. *PLoS One* (2014) **9**:e104712. doi:10.1371/journal.pone.0104712
49. Moon DG, Van Der Zee H, Weston LK, Gudewicz PW, Fenton JW II, Kaplan JE. Platelet modulation of neutrophil superoxide anion production. *Thromb Haemost* (1990) **63**:91–6.
50. Tsuji T, Nagata K, Koike J, Todoroki N, Irimura T. Induction of superoxide anion production from monocytes an neutrophils by activated platelets through the P-selectin-sialyl Lewis X interaction. *J Leukoc Biol* (1994) **56**:583–7.
51. Wu X, Helfrich MH, Horton MA, Feigen LP, Lefkowith JB. Fibrinogen mediates platelet-polymorphonuclear leukocyte cooperation during immune-complex glomerulonephritis in rats. *J Clin Invest* (1994) **94**:928–36. doi:10.1172/JCI117459
52. Zalavary S, Grenegard M, Stendahl O, Bengtsson T. Platelets enhance Fc(gamma) receptor-mediated phagocytosis and respiratory burst in neutrophils: the role of purinergic modulation and actin polymerization. *J Leukoc Biol* (1996) **60**:58–68.
53. McGarrity ST, Stephenson AH, Hyers TM, Webster RO. Inhibition of neutrophil superoxide anion generation by platelet products: role of adenine nucleotides. *J Leukoc Biol* (1988) **44**:411–21.
54. Bengtsson T, Zalavary S, Stendahl O, Grenegard M. Release of oxygen metabolites from chemoattractant-stimulated neutrophils is inhibited by resting platelets: role of extracellular adenosine and actin polymerization. *Blood* (1996) **87**:4411–23.
55. Jancinova V, Drabikova K, Nosal R, Danihelova E. Platelet-dependent modulation of polymorphonuclear leukocyte chemiluminescence. *Platelets* (2000) **11**:278–85. doi:10.1080/09537100050129297
56. Herbertsson H, Bengtsson T. Role of platelets and the arachidonic acid pathway in the regulation of neutrophil oxidase activity. *Scand J Clin Lab Invest* (2001) **61**:641–9. doi:10.1080/003655101753268008
57. Losche W, Dressel M, Krause S, Redlich H, Spangenberg P, Heptinstall S. Contact-induced modulation of neutrophil elastase secretion and phagocytic activity by platelets. *Blood Coagul Fibrinolysis* (1996) **7**:210–3. doi:10.1097/00001721-199603000-00025
58. Losche W, Temmler U, Redlich H, Vickers J, Krause S, Spangenberg P. Inhibition of leukocyte chemiluminescence by platelets: role of platelet-bound fibrinogen. *Platelets* (2001) **12**:15–9. doi:10.1080/09537100020031171
59. Shah SV. Role of reactive oxygen metabolites in experimental glomerular disease. *Kidney Int* (1989) **35**:1093–106. doi:10.1038/ki.1989.96
60. Del Principe D, Menichelli A, Di Giulio S, De Matteis W, Giordani M, Pentassuglio AM, et al. Stimulated platelets release factor(s) affecting the in vitro response of human polymorphonuclear cells. *J Leukoc Biol* (1990) **48**:7–14.
61. Del Maschio A, Maclouf J, Corvazier E, Grange MJ, Borgeat P. Activated platelets stimulate human neutrophils functions. *Nouv Rev Fr Hematol* (1985) **27**:275–8.
62. Del Maschio A, Corvazier E, Maillet F, Kazatchkine MD, Maclouf J. Platelet-dependent induction and amplification of polymorphonuclear leucocyte lysosomal enzyme release. *Br J Haematol* (1989) **72**:329–35. doi:10.1111/j.1365-2141.1989.tb07712.x
63. Badrnya S, Schrottmaier WC, Kral JB, Yaiw KC, Volf I, Schabbauer G, et al. Platelets mediate oxidized low-density lipoprotein-induced monocyte extravasation and foam cell formation. *Arterioscler Thromb Vasc Biol* (2014) **34**:571–80. doi:10.1161/ATVBAHA.113.302919
64. Hussein O, Brook GJ, Aviram M. Intraperitoneal injection of platelet secretory products into mice increases macrophage uptake of oxidized low density lipoprotein. *Isr J Med Sci* (1993) **29**:453–9.
65. Aviram M. LDL-platelet interaction under oxidative stress induces macrophage foam cell formation. *Thromb Haemost* (1995) **74**:560–4.
66. Aviram M, Hussein O, Rosenblat M, Schlezinger S, Hayek T, Keidar S. Interactions of platelets, macrophages, and lipoproteins in hypercholesterolemia: antiatherogenic effects of HMG-CoA reductase inhibitor therapy. *J Cardiovasc Pharmacol* (1998) **31**:39–45. doi:10.1097/00005344-199801000-00006

67. Weyrich AS, Elstad MR, McEver RP, McIntyre TM, Moore KL, Morrissey JH, et al. Activated platelets signal chemokine synthesis by human monocytes. *J Clin Invest* (1996) **97**:1525–34. doi:10.1172/jci118575
68. Corken A, Russell S, Dent J, Post SR, Ware J. Platelet glycoprotein Ib-IX as a regulator of systemic inflammation. *Arterioscler Thromb Vasc Biol* (2014) **34**:996–1001. doi:10.1161/ATVBAHA.113.303113
69. de Stoppelaar SF, van 't Veer C, Claushuis TA, Albersen BJ, Roelofs JJ, Van Der Poll T. Thrombocytopenia impairs host defense in Gram-negative pneumonia derived sepsis. *Blood* (2014) **124**(25):3781–90. doi:10.1182/blood-2014-05-573915
70. Fujimi S, Macconmara MP, Maung AA, Zang Y, Mannick JA, Lederer JA, et al. Platelet depletion in mice increases mortality after thermal injury. *Blood* (2006) **107**:4399–406. doi:10.1182/blood-2005-09-3776
71. Goncalves R, Zhang X, Cohen H, Debrabant A, Mosser DM. Platelet activation attracts a subpopulation of effector monocytes to sites of *Leishmania major* infection. *J Exp Med* (2011) **208**:1253–65. doi:10.1084/jem.20101751
72. Koupenova M, Vitseva O, Mackay CR, Beaulieu LM, Benjamin EJ, Mick E, et al. Platelet-TLR7 mediates host survival and platelet count during viral infection in the absence of platelet-dependent thrombosis. *Blood* (2014) **124**:791–802. doi:10.1182/blood-2013-11-536003
73. Boilard E, Nigrovic PA, Larabee K, Watts GF, Coblyn JS, Weinblatt ME, et al. Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science* (2010) **327**:580–3. doi:10.1126/science.1181928
74. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. *Science* (2004) **303**:1532–5. doi:10.1126/science.1092385
75. Fuchs TA, Abed U, Goosmann C, Hurwitz R, Schulze I, Wahn V, et al. Novel cell death program leads to neutrophil extracellular traps. *J Cell Biol* (2007) **176**:231–41. doi:10.1083/jcb.200606027
76. McDonald B, Urrutia R, Yipp BG, Jenne CN, Kubes P. Intravascular neutrophil extracellular traps capture bacteria from the bloodstream during sepsis. *Cell Host Microbe* (2012) **12**:324–33. doi:10.1016/j.chom.2012.06.011
77. Caudrillier A, Kessenbrock K, Gilliss BM, Nguyen JX, Marques MB, Monestier M, et al. Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury. *J Clin Invest* (2012) **122**:2661–71. doi:10.1172/jci61303
78. Rossaint J, Herter JM, Van Aken H, Napirei M, Doring Y, Weber C, et al. Synchronized integrin engagement and chemokine activation is crucial in neutrophil extracellular trap-mediated sterile inflammation. *Blood* (2014) **123**:2573–84. doi:10.1182/blood-2013-07-516484
79. Fuchs TA, Brill A, Duerschmied D, Schatzberg D, Monestier M, Myers DD Jr, et al. Extracellular DNA traps promote thrombosis. *Proc Natl Acad Sci U S A* (2010) **107**:15880–5. doi:10.1073/pnas.1005743107
80. Massberg S, Grahl L, Von Bruehl ML, Manukyan D, Pfeiler S, Goosmann C, et al. Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. *Nat Med* (2010) **16**:887–96. doi:10.1038/nm.2184
81. Engelmann B, Massberg S. Thrombosis as an intravascular effector of innate immunity. *Nat Rev Immunol* (2013) **13**:34–45. doi:10.1038/nri3345
82. Brill A, Fuchs TA, Savchenko AS, Thomas GM, Martinod K, De Meyer SF, et al. Neutrophil extracellular traps promote deep vein thrombosis in mice. *J Thromb Haemost* (2012) **10**:136–44. doi:10.1111/j.1538-7836.2011.04544.x
83. Von Bruhl ML, Stark K, Steinhart A, Chandraratne S, Konrad I, Lorenz M, et al. Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice in vivo. *J Exp Med* (2012) **209**:819–35. doi:10.1084/jem.20112322
84. Demers M, Krause DS, Schatzberg D, Martinod K, Voorhees JR, Fuchs TA, et al. Cancers predispose neutrophils to release extracellular DNA traps that contribute to cancer-associated thrombosis. *Proc Natl Acad Sci U S A* (2012) **109**:13076–81. doi:10.1073/pnas.1200419109
85. Evangelista V, Piccardoni P, White JG, De Gaetano G, Cerletti C. Cathepsin G-dependent platelet stimulation by activated polymorphonuclear leukocytes and its inhibition by antiproteinases: role of P-selectin-mediated cell-cell adhesion. *Blood* (1993) **81**:2947–57.
86. Valles J, Santos MT, Marcus AJ, Safier LB, Broekman MJ, Islam N, et al. Downregulation of human platelet reactivity by neutrophils. Participation of lipoxygenase derivatives and adhesive proteins. *J Clin Invest* (1993) **92**:1357–65. doi:10.1172/JCI116709
87. Pidard D, Renesto P, Rabhi S, Chignard M. Regulation of the structure and activity of platelet adhesion receptors by leukocyte proteinases. *Nouv Rev Fr Hematol* (1994) **36**(Suppl 1):S99–101.
88. Renesto P, Si-Tahar M, Moniatte M, Balloy V, Van Dorsselaer A, Pidard D, et al. Specific inhibition of thrombin-induced cell activation by the neutrophil proteinases elastase, cathepsin G, and proteinase 3: evidence for distinct cleavage sites within the aminoterminal domain of the thrombin receptor. *Blood* (1997) **89**:1944–53.
89. Ilton MK, Taylor ML, Misso NL, Thompson PJ, Hung J. Neutrophil cathepsin G modulates platelet P-selectin expression and inhibits P-selectin-mediated platelet-neutrophil adhesion. *Clin Sci (Lond)* (1998) **94**:437–45.
90. Goerge T, Ho-Tin-Noe B, Carbo C, Benarafa C, Remold-O'Donnell E, Zhao BQ, et al. Inflammation induces hemorrhage in thrombocytopenia. *Blood* (2008) **111**:4958–64. doi:10.1182/blood-2007-11-123620
91. Majno G, Palade GE. Studies on inflammation. 1. The effect of histamine and serotonin on vascular permeability: an electron microscopic study. *J Biophys Biochem Cytol* (1961) **11**:571–605. doi:10.1083/jcb.11.3.607
92. Ho-Tin-Noe B, Carbo C, Demers M, Cifuni SM, Goerge T, Wagner DD. Innate immune cells induce hemorrhage in tumors during thrombocytopenia. *Am J Pathol* (2009) **175**:1699–708. doi:10.2353/ajpath.2009.090460
93. Camerer E, Regard JB, Cornelissen I, Srinivasan Y, Duong DN, Palmer D, et al. Sphingosine-1-phosphate in the plasma compartment regulates basal and inflammation-induced vascular leak in mice. *J Clin Invest* (2009) **119**:1871–9. doi:10.1172/JCI38575
94. Boulaftali Y, Hess PR, Getz TM, Cholka A, Stolla M, Mackman N, et al. Platelet ITAM signaling is critical for vascular integrity in inflammation. *J Clin Invest* (2013) **123**(2):908–16. doi:10.1172/JCI65154
95. Herzog BH, Fu J, Wilson SJ, Hess PR, Sen A, McDaniel JM, et al. Podoplanin maintains high endothelial venule integrity by interacting with platelet CLEC-2. *Nature* (2013) **502**:105–9. doi:10.1038/nature12501
96. Wang CH, Dai JY, Wang L, Jia JF, Zheng ZH, Ding J, et al. Expression of CD147 (EMMPRIN) on neutrophils in rheumatoid arthritis enhances chemotaxis, matrix metalloproteinase production and invasiveness of synoviocytes. *J Cell Mol Med* (2011) **15**:850–60. doi:10.1111/j.1582-4934.2010.01084.x
97. Kerrigan AM, Navarro-Nunez L, Pyz E, Finney BA, Willment JA, Watson SP, et al. Podoplanin-expressing inflammatory macrophages activate murine platelets via CLEC-2. *J Thromb Haemost* (2012) **10**:484–6. doi:10.1111/j.1538-7836.2011.04614.x
98. Kornerup KN, Page CP. The role of platelets in the pathophysiology of asthma. *Platelets* (2007) **18**:319–28. doi:10.1080/09537100701230436
99. Pitchford SC. Defining a role for platelets in allergic inflammation. *Biochem Soc Trans* (2007) **35**:1104–8. doi:10.1042/BST0351104
100. Pitchford SC. Novel uses for anti-platelet agents as anti-inflammatory drugs. *Br J Pharmacol* (2007) **152**:987–1002. doi:10.1038/sj.bjp.0707364

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 03 November 2014; paper pending published: 24 November 2014; accepted: 16 December 2014; published online: 06 January 2015.

*Citation: Gros A, Ollivier V and Ho-Tin-Noé B (2015) Platelets in inflammation: regulation of leukocyte activities and vascular repair. Front. Immunol. **5**:678. doi: 10.3389/fimmu.2014.00678*

This article was submitted to Inflammation, a section of the journal Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 06 March 2015
doi: 10.3389/fimmu.2015.00098

Platelets in inflammation and atherogenesis

Henry M. Nording[1,2], Peter Seizer[1] and Harald F. Langer[1,2]*

[1] University Clinic for Cardiology and Cardiovascular Medicine, Eberhard Karls-University Tübingen, Tübingen, Germany
[2] Section for Cardioimmunology, Eberhard Karls-University Tübingen, Tübingen, Germany

Edited by:
Olivier Garraud, Institut National de la Transfusion Sanguine, France

Reviewed by:
Marco Emilio Bianchi, Universita' Vita Salute San Raffaele, Italy
Gaurav K. Gupta, Harvard Medical School, USA

***Correspondence:**
Harald F. Langer, Medizinische Klinik III, Universitätsklinikum Tübingen, Otfried-Müller Str. 10, Tübingen 72076, Germany
e-mail: harald.langer@med.uni-tuebingen.de

Platelets contribute to processes beyond thrombus formation and may play a so far underestimated role as an immune cell in various circumstances. This review outlines immune functions of platelets in host defense, but also how they may contribute to mechanisms of infectious diseases. A particular emphasis is placed on the interaction of platelets with other immune cells. Furthermore, this article outlines the features of atherosclerosis as an inflammatory vascular disease highlighting the role of platelet crosstalk with cellular and soluble factors involved in atheroprogression. Understanding, how platelets influence these processes of vascular remodeling will shed light on their role for tissue homeostasis beyond intravascular thrombosis. Finally, translational implications of platelet-mediated inflammation in atherosclerosis are discussed.

Keywords: platelets, inflammation, atherosclerosis

INTRODUCTION

The vasculature is one of the large networks of the human body and, thus, it needs to be well protected by immune mechanisms. When tissue is injured, the wound is paralleled by a severance of the vascular network, as well. Disruption of the endothelial monolayer lining all vessels from the inside triggers a process referred to as thrombus formation, a well regulated and complex cascade of events (1). During thrombus formation, other systems located within the vasculature can be activated, the most prominent of which is the immune system and inflammation being a part of its innate response. The inflammatory response to tissue injury triggers various events, which allow for defense against possible intruders and initiate the healing process (2). Simultaneously and in close proximity, platelets are recruited to the wound to restore endothelial integrity; they are activated and initiate thrombus formation. Given the close spatiotemporal relationship of these molecular processes, it is not surprising that growing evidence suggests that platelets are not only effectors of thrombus formation, but actively participate in inflammation and other processes related to tissue remodeling (3).

PLATELETS PRESERVE VASCULAR INTEGRITY

Repair of vascular damage while simultaneously preserving the patency of narrow capillaries is a complex task and requires a finely tuned machinery of pro- as well as anti-thrombotic mechanisms. Platelets are the key cells of primary hemostasis and thrombus formation. They mediate thrombus formation through cellular and soluble factors [recently also reviewed in Ref. (1, 4–7)]. GPIbα is a platelet transmembrane receptor associated with GPIX and GPV (8). GPIbα binding to von-Willebrand-factor (vWF) initiates primary hemostasis (8). In a shear-dependent fashion, GPIbα binding to vWF immobilized on collagen enables initial platelet rolling, which precedes all further steps of thrombus formation (9). Except at sites of high shear rates (10), stable platelet adhesion requires additional contribution of GPVI and integrins (11). GPVI is one of the platelet collagen receptors. It provides strong mechanic adhesion but also serves as the primary inducer of platelet activation mediated by its FcRγ-chain (12, 13). Amongst other signaling events, activation of GPVI causes an elevation of intracellular Ca^{2+} and subsequent platelet shape change (14). Platelet activation is paralleled by the secretion of soluble factors from platelet granules, the most important of which are ADP and TxA2 (15–17), as they activate platelets in an autocrine fashion (18). C-type lectin-like type II (CLEC-2) supports GPVI as it sustains a similar signaling pathway as the one induced by the GPVI/FcRγ complex (19, 20). In fact, CLEC-2 was found to be of particular importance for stable aggregate formation under flow conditions (21). The thrombotic activity of platelets is regulated by controlling the surface density of these major receptors by ectodomain shedding (22). A central process in platelet-mediated thrombus formation is integrin activation, as integrins connect the ECM to the platelets' cytoskeleton and enable platelet aggregate formation (11). The integrin $\alpha_{IIb}\beta_3$ has the ability to "crosslink" platelets via fibrinogen-bridging (23), thus stabilizing the forming thrombus. Due to its central importance, its inside-out activation is referred to as the "final common pathway of platelet activation" (24). The activation of $\alpha_{IIb}\beta_3$ is mediated by the classical platelet agonists ADP or TxA2, and interfering with these pathways was successfully transferred into patient treatment (25). Similar to other integrins, $\alpha_{IIb}\beta_3$ promotes "outside-in" signaling as well as platelet spreading and clot retraction (26). Finally, platelets also interact with the coagulation system in various ways stabilizing the developing thrombus by fibrin formation, which provides for provisional wound closure (27–29).

"NON-CLASSICAL" PLATELET FUNCTIONS

Although traditionally not conceived as immune cells, platelets hold important functions in the immune response, particularly in innate immunity (30–32). In both host defense and preservation of vascular functions, platelets are helpful in some and harmful in

other conditions (33). In the following, we will aim to exemplify how platelets mediate effects beyond thrombus formation.

PLATELETS IN HOST DEFENSE

Platelets contribute to pathogen recognition by interacting with immune cells, but also by interacting with the bacteria themselves (34–41). Recently, it was demonstrated that platelet-rich plasma (PRP) inhibited the growth of bacteria (42). The various receptor interactions involved in this platelet–bacteria crosstalk were already reviewed elsewhere (43, 44). For instance, platelets are able to recognize CpG islands upon thrombin activation and subsequent TLR9 expression (40). Furthermore, platelets react to fungal infections *in vitro* and *in vivo* (45). Finally, platelet "nuclear functions" are increasingly uncovered and recognized (2). Via transcription of mRNA and post-transcriptional modification (46–48), platelets seem to contribute to the inflammasome by producing IL-1β (49), they are involved in modulating NFκB (50) and may influence endothelial polarization by miRNA secretion (51).

PLATELETS CONTRIBUTING TO MECHANISMS OF INFECTIONS

On the other hand, there are a number of reports describing platelets as an important element in the progression of infections (52). The platelet receptor CLEC-2 has been shown to facilitate the entry of HI-viruses (53), and platelets contribute to disease progression via CD40L (54, 55). Furthermore, platelets are involved in the progression of HBV-infection and other viral diseases (56, 57) by the recruitment of cytotoxic T-cells (CTL) to the liver (58) or other organs in a serotonin-dependent manner (59). Verschoor et al. could recently show that platelets are a relevant factor in the process of immune evasion by intracellular bacteria such as *Listeria* (38). Furthermore, platelets play an important role in infections by *Leishmania* (60) and in the pathogenesis of Hantavirus infection (61). A fact, which complicates the picture even more, is that platelets can also modulate the function of further cells involved in the response to infections – the leukocyte.

PLATELET CROSSTALK WITH IMMUNE CELLS

One of the main immune mechanisms of platelets is their capability to recruit leukocytes to sites of infection and inflammation (32, 62). P-selectin–PSGL-1 binding (63, 64), ICAM1 (51), and GPIbα (65) play an important role in how platelets bring other immune cells to the scene (66), particularly under high shear conditions (67). Platelets have the ability to form aggregates with neutrophils (68). In periodontitis, aggregate formation of platelets and neutrophils (NPA) enhances neutrophil phagocytosis in a TLR-2-dependent manner (69). In acute lung injury, NPA formation mediates neutrophil extravasation (70) and platelet-derived platelet factor 4 (PF4) fostered neutrophil survival in a model of arterial occlusion (71). Furthermore, platelet–leukocyte aggregates can be used as a diagnostic tool, for example as a parameter to assess sepsis severity (72). Another recently discovered way, how platelets modulate neutrophil function is their involvement in neutrophil extracellular trap (NET) formation to ensnare intruders (73). Platelet TLR-4 (74) as well as platelet β-defensins have been implicated in NET formation (75, 76). Specifically, platelet-induced NET formation may play a role in viral infections (77) or transfusion-related lung injury (78). Rossaint et al. have recently proposed that simultaneous activation of neutrophils via Mac-1 outside-in signaling and Gαi engagement by platelet-derived RANTES–PF4 heterodimers is required for NET formation (79). Interestingly, platelets seem to form especially stable aggregates with monocytes (80), and activated platelets induce an inflammatory monocyte phenotype (81). As this process seems to be partially independent of P-selectin interaction with PSGL-1, paracrine mechanisms to strengthen platelet–monocyte aggregate formation have been proposed as an alternative mechanism (81). Platelet–monocyte interaction seems to be of functional relevance, as their formation increases the number of circulating monocytes with a higher affinity for adhesion to the endothelium (82). Furthermore, activated platelets are taken up by monocytes which induces enhancement of cytokine release from macrophages (83). Other authors, however, report on anti-inflammatory effects of platelet–monocyte interaction (84–86) via CXCR5 engagement of CXCL13 on monocytes (84) or, following experimental sepsis, by inhibition of macrophage tumor necrosis factor α (TNF-α) and IL-6 secretion (85, 86). Thus, the effect of platelets on monocytes appears to be context-dependent.

Via P-selectin PSGL-1 interaction, platelets can form aggregates with lymphocytes (PLA), as well. Platelet interaction with T-cells, B-cells, and NK-cells induces their homing, activation, and recruitment as recently reviewed (87). Platelets may even serve as a bridge directing T-cells to the endothelium (88). Furthermore, platelets modulate lymphocyte function via direct cell–cell interaction as well as soluble mediators (87). In rheumatoid arthritis patients, platelet binding to lymphocytes promoted activation-induced proliferation as well as IL-17 and interferon-γ production by GPVI positive CD4+ T-cells (89). Serotonin from platelet vesicles may also stimulate T-lymphocytes (90). Through release of PF4 and CCL5, platelets can enhance cytokine production in CD4+ T-cells (34). Furthermore, in HCV infection, platelet CCL5 causes upregulation of T-lymphocyte helper cells type 1 (Th1) (91). Finally, platelets may also enhance T-cell-mediated germinal center formation and release of specific IgGs from B-cells via CD40L signaling (35, 41). In fact, platelets may substitute CD40L when few CD40L-positive T-cells are present to stimulate B-cell maturation (92). However, platelets may also induce anti-inflammatory effects. PF4 released from platelets leads to an increase in regulatory T-lymphocytes (Tregs) (93) and limits Th17 differentiation (94). Interestingly, T-lymphocytes can activate platelets, which amplify the release of CCL5 (95). Addressing antigen-presenting cells, there are a number of ways in which platelets interact with dendritic cells (DCs). For instance, platelets can recruit DC via Mac-1 interaction with JAM-C and can activate them inducing platelet phagocytosis and subsequently apoptosis of DCs (96). High shear rates may be a trigger for platelets to recruit DCs and promote their maturation (97). In fact, direct contact of platelets and DCs seems to induce other effects than crosstalk via soluble factors suggesting that platelets have the ability to differentially regulate a DC response (98). This conclusion is supported by recent findings demonstrating that platelets can enhance DC-mediated Th-2 cell response in allergy by secreted RANKL (99). Platelets can, however, also impair DC differentiation or reduce DC production of proinflammatory cytokines IL-12p70 and TNF-α (100).

PLATELETS IN ATHEROSCLEROSIS

Atherosclerosis is a chronic inflammatory disease featuring various complex processes contributing to its pathophysiology and the development of the atherosclerotic plaque over decades. **Figure 1** summarizes the steps contributing to fatty streak formation, inflammation, progression of the plaque, and finally plaque rupture.

PLATELET CONTRIBUTION TO ATHEROSCLEROSIS

In atherosclerosis, platelets are known to contribute to early steps of this chronic vascular pathology (**Figure 2**) such as endothelial dysfunction (101, 102), but also to final events such as rupture of the vulnerable plaque [(103), see also **Figure 4**]. For instance, platelets participate in atherogenesis by chemokine release (79, 104, 105), surface association of oxLDL (106), direct cell–cell interaction (107, 108), release of microparticles (109), and provision of inflammatory mediators [(110), see also **Figure 3**]. Platelets within the atherosclerotic plaque may remain activated for a long time providing for proinflammatory IL-1β production [(111), see also **Figure 3**]. One of the most considered functions of platelets in atherosclerosis is the recruitment of leukocytes via direct receptor–ligand interactions or augmented by released factors such as chemokines [(112, 113), see also previous sections and **Figure 3**]. The role of a particular leukocyte subtype – DCs,

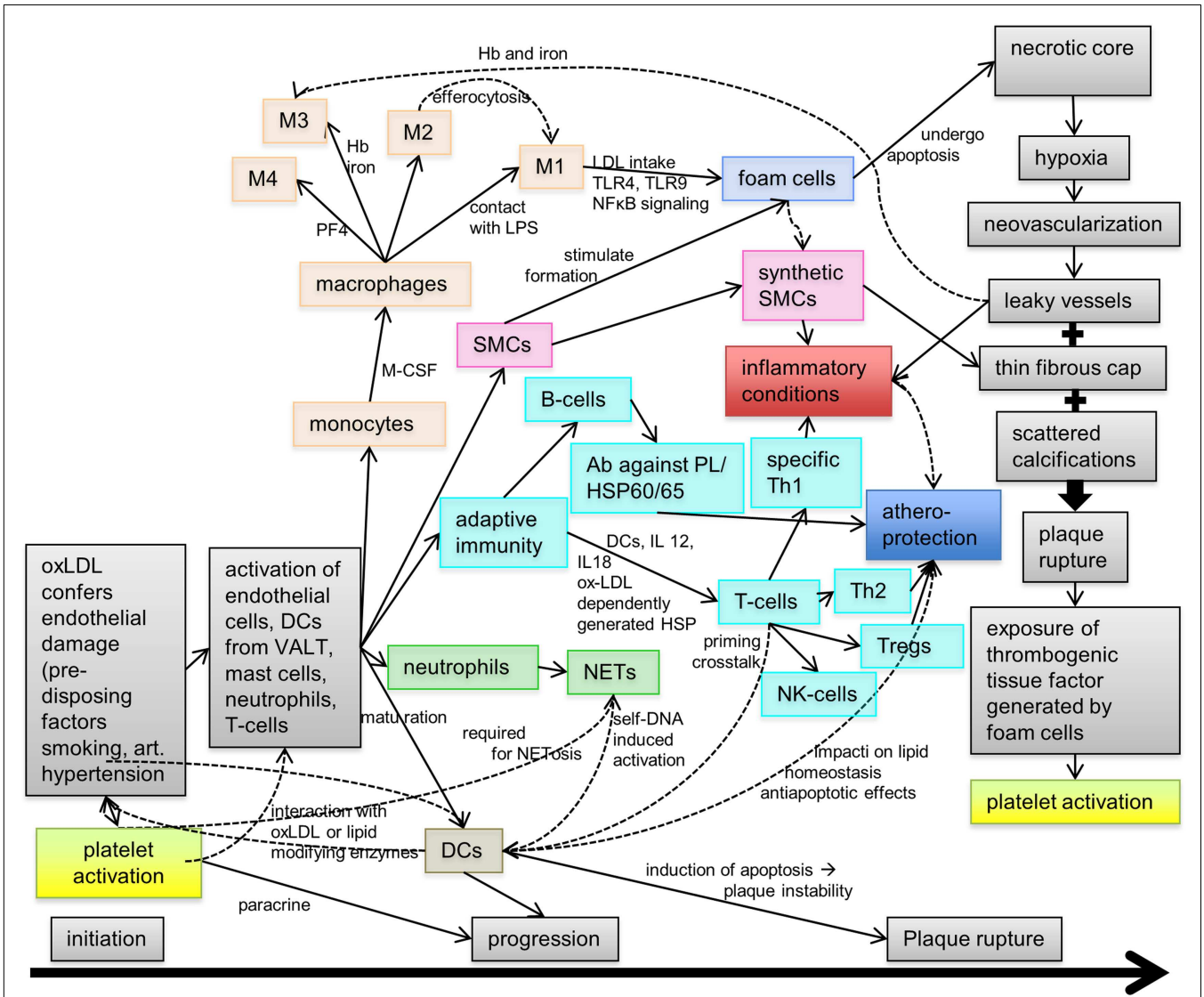


FIGURE 1 | Pathophysiology of atherosclerosis. During atherogenesis, a plaque forms on the luminal side of the arterial wall through a complex process involving lipid accumulation, cellular activation inducing the transformation and differentiation of monocytes into foam cells, and various immune reactions mediated by T- and B-cells, neutrophils, granulocytes (neutrophils), and dendritic cells (DCs). Hence, the progression of atherosclerosis is driven by inflammation, although some of these inflammatory cells/factors may also mediate atheroprotection under certain conditions. At later stages, the atherosclerotic plaque develops a necrotic core with areas of neovascularization, a thin fibrous cap, and scattered calcifications. Finally, plaque rupture exposes the thrombogenic atherosclerotic core inducing platelet activation and, subsequently, initiation of the coagulation cascade. Ab, antibody; Hb, hemoglobin; HSP, heat shock protein; NET, neutrophil extracellular trap; PF4, platelet factor 4; SMC, smooth muscle cell; Treg, regulatory T-cell; VALT, vascular-associated lymphatic tissue.

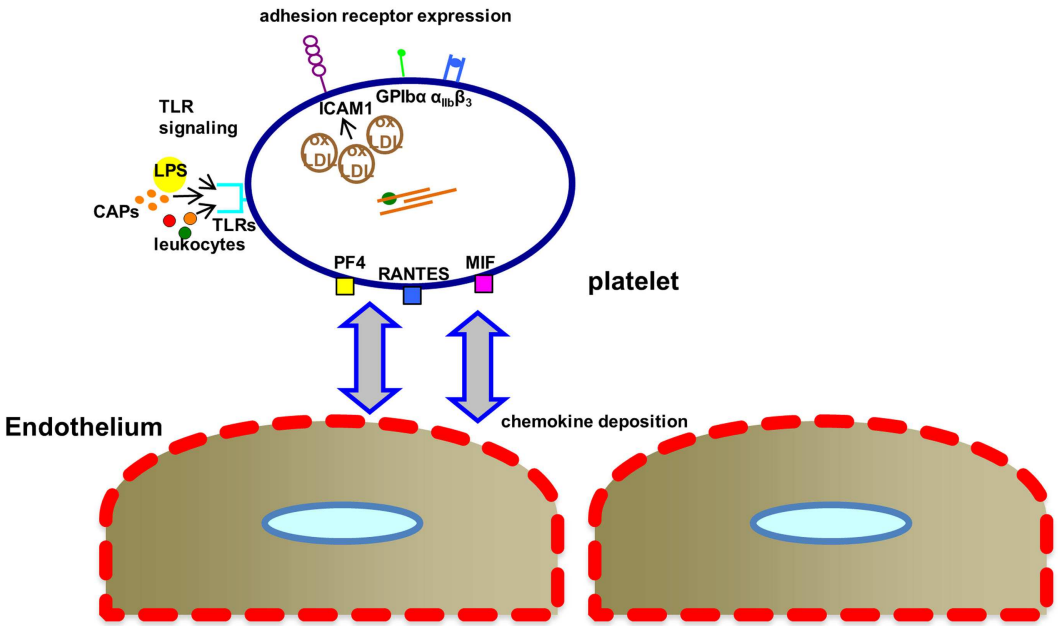


FIGURE 2 | Mechanisms, how platelets affect the initiation of atherosclerosis. Upon activation within the microenvironment of the atherosclerotic plaque or even at very early stages of atherogenesis in areas of high shear stress, platelets express increased levels of inflammatory adhesion receptors (ICAM1, intracellular adhesion molecule 1; GPIbα, glycoprotein Ibα; $\alpha_{IIb}\beta_3$, glycoprotein $\alpha_{IIb}\beta_3$) associated with oxLDL binding to platelets. TLR-mediated signaling may contribute via binding of lipopolysaccharides (LPS), leukocyte interaction, or binding of carboxyalkylpyrroles (CAPs). The complement system also contributes to atherogenesis. MIF, macrophage migratory inhibitory factor; PF4, platelet factor 4; RANTES, chemokine ligand 5 (CCL5); TLRs, Toll-like receptors; oxLDL, oxidized low-density lipoprotein.

the classical antigen presenting cells of our body – has been emphasized recently in the context of atherosclerosis and, interestingly, platelets interact with DCs (**Figure 3**). In fact, GPIb–Mac-1 interaction may be an interesting signaling mechanism in the context of platelet–DC crosstalk modulating atheroprogression (114, 115). This is of particular importance, as DCs have been proposed to play a significant role in the different steps of atherosclerosis (116).

PLATELETS IN THE COMPLEMENT SYSTEM AND ATHEROSCLEROSIS

A further part of our innate immune response, the complement system receives increasing attention in the context of atherosclerosis. This cascade of soluble plasma proteins constitutes a phylogenetically very old part of the inherited immune system (117). Complement activation is important for inflammatory conditions associated with vascular injury (118, 119). Most interestingly, platelets were reported to express a number of complement receptors relevant for platelet function and their crosstalk with the local microenvironment (120–122). Several complement components can be bound to the platelet surface (123, 124). We found that expression of complement anaphylatoxin receptors on platelets showed a strong and positive correlation with platelet activation markers such as P-selectin in patients with atherosclerosis (125). Further mechanistic studies will have to address the relevance of this association. In a recent review, the literature on platelets and potential intersection points with the complement system in diverse settings was summarized (126). Additional profound studies are needed to differentiate our understanding of the intersection points of platelet activation with the immunological elements of atherogenesis such as endothelial inflammation, leukocyte recruitment, antigen presentation, chemokine and cytokine production, or complement activation.

PLAQUE RUPTURE

At later stages, the atherosclerotic core becomes hypoxic inducing the outgrowth of vasa vasorum from the adventitia toward the intima [(127, 128), see also **Figure 4**]. As a consequence, fragile and leaky vessels form, which facilitate further invasion of immune cells and release of soluble factors into the surrounding atherosclerotic tissue (129, 130). Moreover, red blood cells get stuck in the plaque liberating hemoglobin and iron (131). These mechanisms ultimately result in plaque destabilization (132). Foam cells produce tissue factor (133), and as soon as the thrombogenic lipid core is exposed to the lumen, fibrin generation is initiated (134–136). In parallel, platelets as well as the coagulation cascade become activated (137, 138). Plaque rupture in the region of a thin fibrous cap is the final event, how atherosclerosis causes acute vascular complications such as myocardial infarction or stroke (139–143).

TRANSLATIONAL RELEVANCE

The fact that inflammation plays a key role in central steps of atherosclerosis (31, 144), is increasingly integrated into clinical

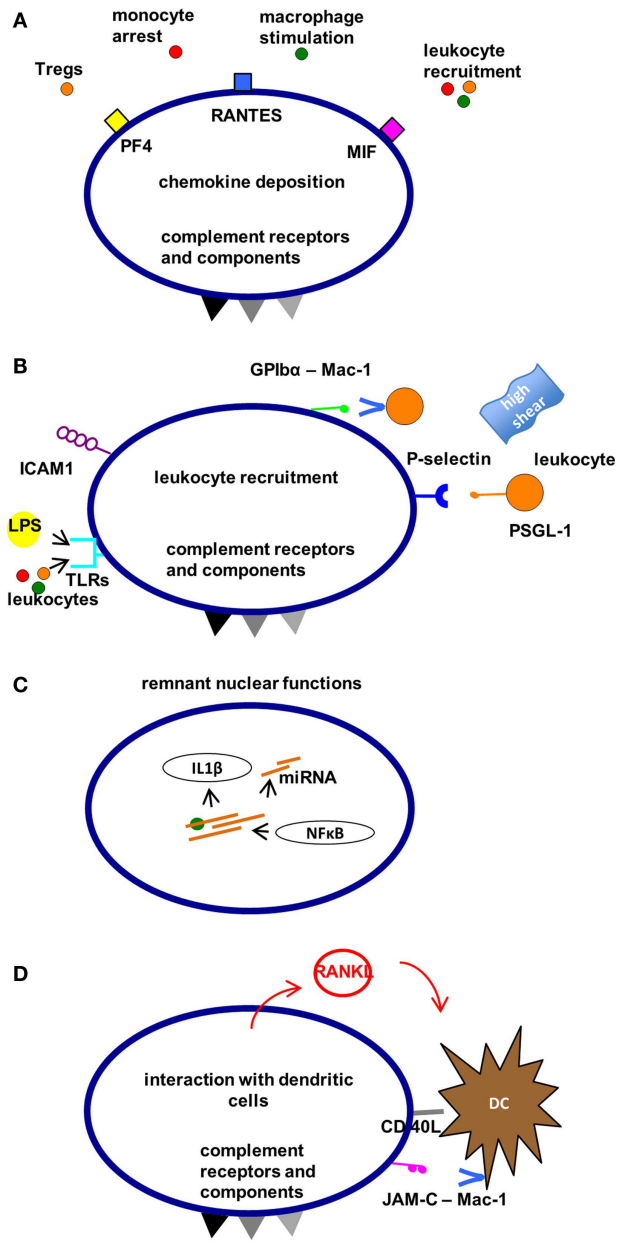


FIGURE 3 | Mechanisms, how platelets affect the progression of atherosclerosis. Platelets contribute to the progression of atherosclerosis by chemokine deposition **(A)** and leukocyte recruitment **(B)**. Remnant "nuclear" functions of platelets such as the ability to translate and modify mRNA promote inflammation and endothelial polarization **(C)**. Furthermore, the interaction with antigen-presenting dendritic cells can contribute to atheroprogression involving adhesion receptors and soluble mediators **(D)**. CXCR4, C-X-C chemokine receptor type 4 (CD184); SDF-1α, stromal cell-derived factor 1α.

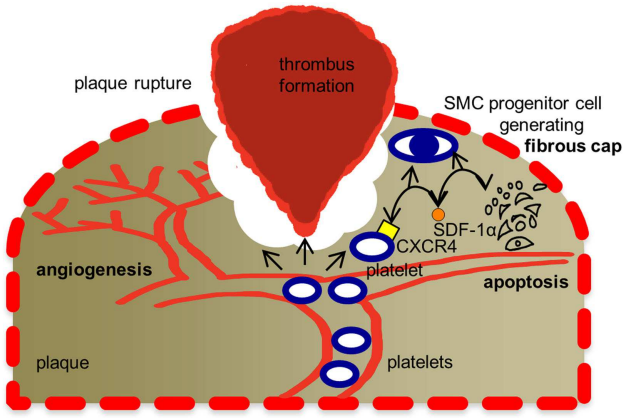


FIGURE 4 | Mechanisms, how platelets affect plaque rupture. Plaque rupture is tailored by platelets in several ways: platelets are known to modulate angiogenesis, which is a decisive factor for plaque stability. Platelets have also been found to promote smooth muscle cell (SMC) progenitor recruitment via CXCR4-SDF-1α signaling. SMCs are responsible for generating extracellular matrix stabilizing the fibrous cap of the plug. Thrombus formation in areas of the ruptured fibrous cap exposing components of the extracellular matrix is initiated by platelets. Future investigations will also have to test, whether platelets enter the plaque tissue and affect tissue remodeling. Ab, antibody; CD40L, CD40 ligand (CD154); DC, dendritic cell; IL-1α, interleukin 1α; JAM-C, junctional adhesion molecule type C; Mac-1, macrophage-1 antigen; MIF, macrophage migratory inhibitory factor; miRNA, microRNA; NFκB, nuclear factor kappa-light-chain-enhancer of activated B-cells; PF4, platelet factor 4; PSGL-1, P-selectin glycoprotein ligand-1; RANKL, receptor activator of nuclear factor kappa-B ligand; RANTES, chemokine ligand 5 (CCL5); TLRs, Toll-like receptors; Treg, regulatory T-cell.

considerations. Accordingly, this hypothesis is addressed by two current trials, the CANTOS trial launched in 2011 (145) and the CIRT trial (146) using immunosuppressants to treat atherosclerosis. Earlier in this article, we have aimed at depicting the importance of platelets for inflammation in atherosclerosis. Inhibition of platelet-mediated inflammation may already be everyday clinical practice considering the use of aspirin for the treatment of cardiovascular disease (147). As an irreversible inhibitor of the enzyme cyclooxygenase, aspirin is a mild inhibitor of platelet function (148). Despite its widespread use, the definite role of aspirin in the prevention of atherosclerosis and atherosclerosis-related diseases is still under discussion (147). There is evidence from preclinical studies that aspirin is able to inhibit the initiation (149) and even the progression of experimental atherosclerosis (150) via its effect on prostaglandin synthesis but also by other mechanisms such as the modulation of endothelial NO synthesis (151), NFκB signaling (152), CRP, or soluble CD40 ligand (sCD40L) (153). Clinical studies on the use of aspirin for primary prevention of atherosclerosis, however, have also yielded negative results [recently reviewed by Gaziano and Greenland (147)]. A large study in a Japanese population over 60 years of age could demonstrate no benefit of low-dose aspirin therapy (154, 155). In patients at low risk for cardiovascular events, the use of aspirin needs to be weighed very carefully against an elevated risk of bleeding events or even hemorrhagic stroke (147).

For the ADP-receptor antagonist clopidogrel, reports on an effect in the context of atherosclerosis exist, too. In animal models, clopidogrel has the ability to slow down the inflammatory progression of atherosclerosis (156, 157). Clopidogrel reduces platelet activation as measured by P-selectin expression and other inflammatory markers (158), while others stress that important inflammatory markers such as hsCRP are not affected (159, 160). On the other hand, platelet–leukocyte aggregate formation is inhibited more effectively by clopidogrel compared to aspirin (161, 162). In contrast, another group reported that under therapy with clopidogrel, the expression of some inflammatory chemokines may even be increased in peripheral blood mononuclear cells in patients with coronary artery disease (163). On the platelet surface, a number of inflammatory receptors may represent potential targets for new therapeutic approaches such as CXCL4, CCL5, CD40 ligand, PSGL-1 (164). Further targets may be platelet-activating factor (PAF) (165) or Annexin A5 (166). Finally, we have already described the evidence on complement receptor involvement in atherosclerosis (126, 167). This class of receptors are involved in a large number of inflammatory processes (117) and are also expressed on platelets (120–122). There are a number of substances targeting different parts of the complement system which are evaluated in different stages of clinical trials in conditions such as age-related macular degeneration or hereditary angioedema (168, 169). Some have even established themselves as first-line treatment such as eculizumab for paroxysmal nocturnal hemoglobinuria (170, 171). It is tempting to speculate, that these substances might be worth an evaluation in the context of atherosclerosis, as well.

Moreover, biomarkers of atherosclerosis are of great importance from a clinical point of view. Due to the high prevalence of cardiovascular disease, it is vital to identify which patient is at particular risk for adverse cardiovascular events and would benefit from preventive diagnostic or therapeutic interventions. A number of platelet surface receptors may be promising candidates to consider in this context. A prominent example is soluble CD40 ligand (sCD40L) released from platelets (172). In a number of settings such as on hospital admission of patients with acute coronary syndrome or in patients undergoing primary angioplasty, sCD40L levels appear to have predictive capacity (173–175). Some authors even discuss sCD40L as a therapeutic target (176). Apart from sCD40L, soluble P-selectin released from platelets is referred to as a further potential platelet-derived biomarker (177, 178).

Considering the achievements of platelet research over the last two decades with a bounty of platelet-targeted drugs, which found their way into everyday clinical practice, platelets and platelet-associated molecular mechanisms offer potential translational applications.

CONCLUSION

In conclusion, platelets – conceived as immune cells and mediators of vascular/tissue remodeling – have a strong impact on atherosclerosis through inflammatory mechanisms discussed here on the basis of selected cellular or soluble platelet-derived mediators. The net effect of platelet-mediated inflammation may be an atheropromoting one, although these anucleate cells may mediate distinct atheroprotective mechanisms, as well. Future investigations will have to identify these specific platelet aspects to enable us to develop better diagnostic markers and therapeutic approaches with fewer undesired side effects.

ACKNOWLEDGMENTS

This work was supported by the Volkswagen Foundation (Lichtenberg program), German Heart Foundation, Wilhelm Sander Foundation, the Juniorprofessorenprogramm of the county Baden-Wuerttemberg, and the Tuebingen Platelet Investigative Consortium (TuePIC) funded by the German Research Council [Deutsche Forschungsgemeinschaft (DFG), KFO 274 – Platelets – basic mechanisms and clinical implications].

REFERENCES

1. Jackson SP. Arterial thrombosis – insidious, unpredictable and deadly. *Nat Med* (2011) **17**(11):1423–36. doi:10.1038/nm.2515
2. Rondina MT, Weyrich AS, Zimmerman GA. Platelets as cellular effectors of inflammation in vascular diseases. *Circ Res* (2013) **112**(11):1506–19. doi:10.1161/CIRCRESAHA.113.300512
3. Langer HF, Weber C, Gawaz M. The platelet – thrombosis and beyond. *Thromb Haemost* (2013) **110**(5):857–8. doi:10.1160/TH13-09-0805
4. Hagedorn I, Vögtle T, Nieswandt B. Arterial thrombus formation. Novel mechanisms and targets. *Hämostaseologie* (2010) **30**(3):127–35.
5. Nieswandt B, Pleines I, Bender M. Platelet adhesion and activation mechanisms in arterial thrombosis and ischaemic stroke. *J Thromb Haemost* (2011) **9**(s1):92–104. doi:10.1111/j.1538-7836.2011.04361.x
6. Berndt M, Metharom P, Andrews R. Primary haemostasis: newer insights. *Haemophilia* (2014) **20**(s4):15–22. doi:10.1111/hae.12427
7. Etulain J, Schattner M. Glycobiology of platelet-endothelial cell interactions. *Glycobiology* (2014) **24**(12):1252–9. doi:10.1093/glycob/cwu056
8. Andrews RK, Berndt MC. The glycoprotein Ib-IX-V complex. In: Michelson A, editor. *Platelets*. 3rd ed. San Diego: Academic Press (2012). p. 195–212.
9. Bergmeier W, Piffath CL, Goerge T, Cifuni SM, Ruggeri ZM, Ware J, et al. The role of platelet adhesion receptor GPIbα far exceeds that of its main ligand, von Willebrand factor, in arterial thrombosis. *Proc Natl Acad Sci U S A* (2006) **103**(45):16900–5. doi:10.1073/pnas.0608207103
10. Nesbitt WS, Westein E, Tovar-Lopez FJ, Tolouei E, Mitchell A, Fu J, et al. A shear gradient-dependent platelet aggregation mechanism drives thrombus formation. *Nat Med* (2009) **15**(6):665–73. doi:10.1038/nm.1955
11. Nieswandt B, Varga-Szabo D, Elvers M. Integrins in platelet activation. *J Thromb Haemost* (2009) **7**(s1):206–9. doi:10.1111/j.1538-7836.2009.03370.x
12. Moroi M, Jung SM. Platelet glycoprotein VI: its structure and function. *Thromb Res* (2004) **114**(4):221–33. doi:10.1016/j.thromres.2004.06.046
13. Bergmeier W, Stefanini L. Platelet ITAM signaling. *Curr Opin Hematol* (2013) **20**(5):445–50. doi:10.1097/MOH.0b013e3283642267
14. Stefanini L, Boulaftali Y, Ouellette TD, Holinstat M, Désiré L, Leblond B, et al. Rap1-Rac1 circuits potentiate platelet activation. *Arterioscler Thromb Vasc Biol* (2012) **32**(2):434–41. doi:10.1161/ATVBAHA.111.239194
15. Moers A, Nieswandt B, Massberg S, Wettschureck N, Grüner S, Konrad I, et al. G13 is an essential mediator of platelet activation in hemostasis and thrombosis. *Nat Med* (2003) **9**(11):1418–22. doi:10.1038/nm943
16. Huang J-S, Ramamurthy SK, Lin X, Le Breton GC. Cell signalling through thromboxane A2 receptors. *Cell Signal* (2004) **16**(5):521–33. doi:10.1016/j.cellsig.2003.10.008
17. Jin J, Daniel JL, Kunapuli SP. Molecular basis for ADP-induced platelet activation II. The P2Y1 receptor mediates ADP-induced intracellular calcium mobilization and shape change in platelets. *J Biol Chem* (1998) **273**(4):2030–4. doi:10.1074/jbc.273.4.2030
18. Offermanns S. Activation of platelet function through G protein-coupled receptors. *Circ Res* (2006) **99**(12):1293–304. doi:10.1161/01.RES.0000251742.71301.16
19. Fuller GL, Williams JA, Tomlinson MG, Eble JA, Hanna SL, Pöhlmann S, et al. The C-type lectin receptors CLEC-2 and Dectin-1, but not DC-SIGN, signal via a novel YXXL-dependent signaling cascade. *J Biol Chem* (2007) **282**(17):12397–409. doi:10.1074/jbc.M609558200

20. Suzuki-Inoue K, Fuller GL, García Á, Eble JA, Pöhlmann S, Inoue O, et al. A novel Syk-dependent mechanism of platelet activation by the C-type lectin receptor CLEC-2. *Blood* (2006) **107**(2):542–9. doi:10.1182/blood-2005-05-1994
21. May F, Hagedorn I, Pleines I, Bender M, Vögtle T, Eble J, et al. CLEC-2 is an essential platelet-activating receptor in hemostasis and thrombosis. *Blood* (2009) **114**(16):3464–72. doi:10.1182/blood-2009-05-222273
22. Gardiner E, Karunakaran D, Shen Y, Arthur J, Andrews R, Berndt M. Controlled shedding of platelet glycoprotein (GP) VI and GPIb-IX-V by ADAM family metalloproteinases. *J Thromb Haemost* (2007) **5**(7):1530–7. doi:10.1111/j.1538-7836.2007.02590.x
23. Gawaz MP, Loftus J, Bajt M, Frojmovic M, Plow E, Ginsberg M. Ligand bridging mediates integrin alpha IIb beta 3 (platelet GPIIB-IIIA) dependent homotypic and heterotypic cell-cell interactions. *J Clin Invest* (1991) **88**(4):1128. doi:10.1172/JCI115412
24. Varga-Szabo D, Pleines I, Nieswandt B. Cell adhesion mechanisms in platelets. *Arterioscler Thromb Vasc Biol* (2008) **28**(3):403–12. doi:10.1161/ATVBAHA.107.150474
25. Ozaki Y, Asazuma N, Suzuki-Inoue K, Berndt MC. Platelet GPIb-IX-V-dependent signaling. *J Thromb Haemost* (2005) **3**(8):1745–51. doi:10.1111/j.1538-7836.2005.01379.x
26. Naik UP, Naik MU. Association of CIB with GPIIb/IIIa during outside-in signaling is required for platelet spreading on fibrinogen. *Blood* (2003) **102**(4):1355–62. doi:10.1182/blood-2003-02-0591
27. Munnix IC, Kuijpers MJ, Auger J, Thomassen CM, Panizzi P, van Zandvoort MA, et al. Segregation of platelet aggregatory and procoagulant microdomains in thrombus formation regulation by transient integrin activation. *Arterioscler Thromb Vasc Biol* (2007) **27**(11):2484–90. doi:10.1161/ATVBAHA.107.151100
28. Müller F, Mutch NJ, Schenk WA, Smith SA, Esterl L, Spronk HM, et al. Platelet polyphosphates are proinflammatory and procoagulant mediators in vivo. *Cell* (2009) **139**(6):1143–56. doi:10.1016/j.cell.2009.11.001
29. Kleinschnitz C, Stoll G, Bendszus M, Schuh K, Pauer H-U, Burfeind P, et al. Targeting coagulation factor XII provides protection from pathological thrombosis in cerebral ischemia without interfering with hemostasis. *J Exp Med* (2006) **203**(3):513–8. doi:10.1084/jem.20052458
30. Semple JW, Italiano JE, Freedman J. Platelets and the immune continuum. *Nat Rev Immunol* (2011) **11**(4):264–74. doi:10.1038/nri2956
31. Libby P, Ridker PM, Hansson GK. Inflammation in atherosclerosis: from pathophysiology to practice. *J Am Coll Cardiol* (2009) **54**(23):2129–38. doi:10.1016/j.jacc.2009.09.009
32. Herter JM, Rossaint J, Zarbock A. Platelets in inflammation and immunity. *J Thromb Haemost* (2014) **12**(11):1764–75. doi:10.1111/jth.12730
33. von Hundelshausen P, Weber C. Platelets as immune cells bridging inflammation and cardiovascular disease. *Circ Res* (2007) **100**(1):27–40. doi:10.1161/01.RES.0000252802.25497.b7
34. Gerdes N, Zhu L, Ersoy M, Hermansson A, Hjemdahl P, Hu H, et al. Platelets regulate CD4(+) T-cell differentiation via multiple chemokines in humans. *Thromb Haemost* (2011) **106**(2):353–62. doi:10.1160/TH11-01-0020
35. Elzey BD, Grant JF, Sinn HW, Nieswandt B, Waldschmidt TJ, Ratliff TL. Cooperation between platelet-derived CD154 and CD4+ T cells for enhanced germinal center formation. *J Leukoc Biol* (2005) **78**(1):80–4. doi:10.1189/jlb.1104669
36. Sprague DL, Elzey BD, Crist SA, Waldschmidt TJ, Jensen RJ, Ratliff TL. Platelet-mediated modulation of adaptive immunity: unique delivery of CD154 signal by platelet-derived membrane vesicles. *Blood* (2008) **111**(10):5028–36. doi:10.1182/blood-2007-06-097410
37. Zhang X, Liu Y, Gao Y, Dong J, Mu C, Lu Q, et al. Inhibiting platelets aggregation could aggravate the acute infection caused by *Staphylococcus aureus*. *Platelets* (2011) **22**(3):228–36. doi:10.3109/09537104.2010.543962
38. Verschoor A, Neuenhahn M, Navarini AA, Graef P, Plaumann A, Seidlmeier A, et al. A platelet-mediated system for shuttling blood-borne bacteria to CD8 [alpha]+ dendritic cells depends on glycoprotein GPIb and complement C3. *Nat Immunol* (2011) **12**(12):1194–201. doi:10.1038/ni.2140
39. Cognasse F, Hamzeh-Cognasse H, Lafarge S, Delezay O, Pozzetto B, McNicol A, et al. Toll-like receptor 4 ligand can differentially modulate the release of cytokines by human platelets. *Br J Haematol* (2008) **141**(1):84–91. doi:10.1111/j.1365-2141.2008.06999.x
40. Thon JN, Peters CG, Machlus KR, Aslam R, Rowley J, Macleod H, et al. T granules in human platelets function in TLR9 organization and signaling. *J Cell Biol* (2012) **198**(4):561–74. doi:10.1083/jcb.201111136
41. Elzey BD, Tian J, Jensen RJ, Swanson AK, Lees JR, Lentz SR, et al. Platelet-mediated modulation of adaptive immunity. A communication link between innate and adaptive immune compartments. *Immunity* (2003) **19**(1):9–19. doi:10.1016/S1074-7613(03)00177-8
42. Mariani E, Filardo G, Canella V, Berlingeri A, Bielli A, Cattini L, et al. Platelet-rich plasma affects bacterial growth in vitro. *Cytotherapy* (2014) **16**(9):1294–304. doi:10.1016/j.jcyt.2014.06.003
43. Fitzgerald JR, Foster TJ, Cox D. The interaction of bacterial pathogens with platelets. *Nat Rev Microbiol* (2006) **4**(6):445–57. doi:10.1038/nrmicro1425
44. Yeaman MR. Platelets in defense against bacterial pathogens. *Cell Mol Life Sci* (2010) **67**(4):525–44. doi:10.1007/s00018-009-0210-4
45. Speth C, Rambach G, Lass-Flörl C. Platelet immunology in fungal infections. *Thromb Haemost* (2014) **112**(4):632–9. doi:10.1160/TH14-01-0074
46. Weyrich AS, Denis MM, Schwertz H, Tolley ND, Foulks J, Spencer E, et al. mTOR-dependent synthesis of Bcl-3 controls the retraction of fibrin clots by activated human platelets. *Blood* (2007) **109**(5):1975–83. doi:10.1182/blood-2006-08-042192
47. Rondina M, Schwertz H, Harris E, Kraemer B, Campbell R, Mackman N, et al. The septic milieu triggers expression of spliced tissue factor mRNA in human platelets. *J Thromb Haemost* (2011) **9**(4):748–58. doi:10.1111/j.1538-7836.2011.04208.x
48. Schwertz H, Tolley ND, Foulks JM, Denis MM, Risenmay BW, Buerke M, et al. Signal-dependent splicing of tissue factor pre-mRNA modulates the thrombogenicity of human platelets. *J Exp Med* (2006) **203**(11):2433–40. doi:10.1084/jem.20061302
49. Brown GT, McIntyre TM. Lipopolysaccharide signaling without a nucleus: kinase cascades stimulate platelet shedding of proinflammatory IL-1β-rich microparticles. *J Immunol* (2011) **186**(9):5489–96. doi:10.4049/jimmunol.1001623
50. Gambaryan S, Kobsar A, Rukoyatkina N, Herterich S, Geiger J, Smolenski A, et al. Thrombin and collagen induce a feedback inhibitory signaling pathway in platelets involving dissociation of the catalytic subunit of protein kinase A from an NFκB-IκB complex. *J Biol Chem* (2010) **285**(24):18352–63. doi:10.1074/jbc.M109.077602
51. Gidlöf O, van der Brug M, Öhman J, Gilje P, Olde B, Wahlestedt C, et al. Platelets activated during myocardial infarction release functional miRNA, which can be taken up by endothelial cells and regulate ICAM1 expression. *Blood* (2013) **121**(19):3908–17. doi:10.1182/blood-2012-10-461798
52. Assinger A. Platelets and infection – an emerging role of platelets in viral infection. *Front Immunol* (2014) **5**:649. doi:10.3389/fimmu.2014.00649
53. Chaipan C, Soilleux EJ, Simpson P, Hofmann H, Gramberg T, Marzi A, et al. DC-SIGN and CLEC-2 mediate human immunodeficiency virus type 1 capture by platelets. *J Virol* (2006) **80**(18):8951–60. doi:10.1128/JVI.00136-06
54. Pate KAM, Lyons CE, Dorsey JL, Shirk EN, Queen SE, Adams RJ, et al. Platelet activation and platelet-monocyte aggregate formation contribute to decreased platelet count during acute simian immunodeficiency virus infection in pig-tailed macaques. *J Infect Dis* (2013) **208**(6):874–83. doi:10.1093/infdis/jit278
55. Singh MV, Davidson DC, Jackson JW, Singh VB, Silva J, Ramirez SH, et al. Characterization of platelet-monocyte complexes in hiv-1-infected individuals: possible role in HIV-associated neuroinflammation. *J Immunol* (2014) **192**(10):4674–84. doi:10.4049/jimmunol.1302318
56. Iannacone M, Sitia G, Narvaiza I, Ruggeri ZM, Guidotti LG. Antiplatelet drug therapy moderates immune-mediated liver disease and inhibits viral clearance in mice infected with a replication-deficient adenovirus. *Clin Vaccine Immunol* (2007) **14**(11):1532–5. doi:10.1128/CVI.00298-07
57. Iannacone M, Sitia G, Isogawa M, Whitmire JK, Marchese P, Chisari FV, et al. Platelets prevent IFN-α/β-induced lethal hemorrhage promoting CTL-dependent clearance of lymphocytic choriomeningitis virus. *Proc Natl Acad Sci U S A* (2008) **105**(2):629–34. doi:10.1073/pnas.0711200105
58. Iannacone M, Sitia G, Isogawa M, Marchese P, Castro MG, Lowenstein PR, et al. Platelets mediate cytotoxic T lymphocyte-induced liver damage. *Nat Med* (2005) **11**(11):1167–9. doi:10.1038/nm1317
59. Lang PA, Contaldo C, Georgiev P, El-Badry AM, Recher M, Kurrer M, et al. Aggravation of viral hepatitis by platelet-derived serotonin. *Nat Med* (2008) **14**(7):756–61. doi:10.1038/nm1780
60. Goncalves R, Zhang X, Cohen H, Debrabant A, Mosser DM. Platelet activation attracts a subpopulation of effector monocytes to sites of *Leishmania* major infection. *J Exp Med* (2011) **208**(6):1253–65. doi:10.1084/jem.20101751

61. Vaheri A, Strandin T, Hepojoki J, Sironen T, Henttonen H, Makela S, et al. Uncovering the mysteries of hantavirus infections. *Nat Rev Microbiol* (2013) **11**(8):539–50. doi:10.1038/nrmicro3066
62. Zarbock A, Polanowska-Grabowska RK, Ley K. Platelet-neutrophil-interactions: linking hemostasis and inflammation. *Blood Rev* (2007) **21**(2):99–111. doi:10.1016/j.blre.2006.06.001
63. Frenette PS, Denis CV, Weiss L, Jurk K, Subbarao S, Kehrel B, et al. P-selectin glycoprotein ligand 1 (PSGL-1) is expressed on platelets and can mediate platelet-endothelial interactions in vivo. *J Exp Med* (2000) **191**(8):1413–22. doi:10.1084/jem.191.8.1413
64. Lam FW, Burns AR, Smith CW, Rumbaut RE. Platelets enhance neutrophil transendothelial migration via P-selectin glycoprotein ligand-1. *Am J Physiol Heart Circ Physiol* (2011) **300**(2):H468–75. doi:10.1152/ajpheart.00491.2010
65. Wong CH, Jenne CN, Petri B, Chrobok NL, Kubes P. Nucleation of platelets with blood-borne pathogens on Kupffer cells precedes other innate immunity and contributes to bacterial clearance. *Nat Immunol* (2013) **14**(8):785–92. doi:10.1038/ni.2631
66. Cao TM, Takatani T, King MR. Effect of extracellular pH on selectin adhesion: theory and experiment. *Biophys J* (2013) **104**(2):292–9. doi:10.1016/j.bpj.2012.12.005
67. Kuijper P, Torres HG, Van Der Linden J, Lammers J, Sixma J, Koenderman L, et al. Platelet-dependent primary hemostasis promotes selectin and integrin-mediated neutrophil adhesion to damaged endothelium under flow conditions. *Blood* (1996) **87**(8):3271–81.
68. Page C, Pitchford S. Neutrophil and platelet complexes and their relevance to neutrophil recruitment and activation. *Int Immunopharmacol* (2013) **17**(4):1176–84. doi:10.1016/j.intimp.2013.06.004
69. Assinger A, Laky M, Schabbauer G, Hirschl AM, Buchberger E, Binder BR, et al. Efficient phagocytosis of periodontopathogens by neutrophils requires plasma factors, platelets and TLR2. *J Thromb Haemost* (2011) **9**(4):799–809. doi:10.1111/j.1538-7836.2011.04193.x
70. Grommes J, Alard J-E, Drechsler M, Wantha S, Mörgelin M, Kuebler WM, et al. Disruption of platelet-derived chemokine heteromers prevents neutrophil extravasation in acute lung injury. *Am J Respir Crit Care Med* (2012) **185**(6):628–36. doi:10.1164/rccm.201108-1533OC
71. Hartwig H, Drechsler M, Lievens D, Kramp B, von Hundelshausen P, Lutgens E, et al. Platelet-derived PF4 reduces neutrophil apoptosis following arterial occlusion. *Thromb Haemost* (2014) **111**(3):562–4. doi:10.1160/TH13-08-0699
72. Gawaz M, Fateh-Moghadam S, Pilz G, Gurland HJ, Werdan K. Platelet activation and interaction with leucocytes in patients with sepsis or multiple organ failure. *Eur J Clin Invest* (1995) **25**(11):843–51. doi:10.1111/j.1365-2362.1995.tb01694.x
73. Andrews RK, Arthur JF, Gardiner E. Neutrophil extracellular traps (NETs) and the role of platelets in infection. *Thromb Haemost* (2014) **112**(4):659–65. doi:10.1160/TH14-05-0455
74. Clark SR, Ma AC, Tavener SA, McDonald B, Goodarzi Z, Kelly MM, et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat Med* (2007) **13**(4):463–9. doi:10.1038/nm1565
75. McDonald B, Urrutia R, Yipp BG, Jenne CN, Kubes P. Intravascular neutrophil extracellular traps capture bacteria from the bloodstream during sepsis. *Cell Host Microbe* (2012) **12**(3):324–33. doi:10.1016/j.chom.2012.06.011
76. Kraemer BF, Campbell RA, Schwertz H, Cody MJ, Franks Z, Tolley ND, et al. Novel anti-bacterial activities of β-defensin 1 in human platelets: suppression of pathogen growth and signaling of neutrophil extracellular trap formation. *PLoS Pathog* (2011) **7**(11):e1002355. doi:10.1371/journal.ppat.1002355
77. Jenne CN, Wong CH, Zemp FJ, McDonald B, Rahman MM, Forsyth PA, et al. Neutrophils recruited to sites of infection protect from virus challenge by releasing neutrophil extracellular traps. *Cell Host Microbe* (2013) **13**(2):169–80. doi:10.1016/j.chom.2013.01.005
78. Caudrillier A, Kessenbrock K, Gilliss BM, Nguyen JX, Marques MB, Monestier M, et al. Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury. *J Clin Invest* (2012) **122**(7):2661. doi:10.1172/JCI61303
79. Rossaint J, Herter JM, Van Aken H, Napirei M, Döring Y, Weber C, et al. Synchronized integrin engagement and chemokine activation is crucial in neutrophil extracellular trap-mediated sterile inflammation. *Blood* (2014) **123**(16):2573–84. doi:10.1182/blood-2013-07-516484
80. van Gils JM, Zwaginga JJ, Hordijk PL. Molecular and functional interactions among monocytes, platelets, and endothelial cells and their relevance for cardiovascular diseases. *J Leukoc Biol* (2009) **85**(2):195–204. doi:10.1189/jlb.0708400
81. Stephen J, Emerson B, Fox KA, Dransfield I. The uncoupling of monocyte-platelet interactions from the induction of proinflammatory signaling in monocytes. *J Immunol* (2013) **191**(11):5677–83. doi:10.4049/jimmunol.1301250
82. Passacquale G, Vamadevan P, Pereira L, Hamid C, Corrigall V, Ferro A. Monocyte-platelet interaction induces a pro-inflammatory phenotype in circulating monocytes. *PLoS One* (2011) **6**(10):e25595. doi:10.1371/journal.pone.0025595
83. Scull CM, Hays WD, Fischer TH. Macrophage pro-inflammatory cytokine secretion is enhanced following interaction with autologous platelets. *J Inflamm (Lond)* (2010) **7**:53. doi:10.1186/1476-9255-7-53
84. Halvorsen B, Smedbakken LM, Michelsen AE, Skjelland M, Bjerkeli V, Sagen EL, et al. Activated platelets promote increased monocyte expression of CXCR5 through prostaglandin E2-related mechanisms and enhance the anti-inflammatory effects of CXCL13. *Atherosclerosis* (2014) **234**(2):352–9. doi:10.1016/j.atherosclerosis.2014.03.021
85. Xiang B, Zhang G, Guo L, Li X-A, Morris AJ, Daugherty A, et al. Platelets protect from septic shock by inhibiting macrophage-dependent inflammation via the cyclooxygenase 1 signalling pathway. *Nat Commun* (2013) **4**:2657. doi:10.1038/ncomms3657
86. Gudbrandsdottir S, Hasselbalch HC, Nielsen CH. Activated platelets enhance IL-10 secretion and reduce TNF-α secretion by monocytes. *J Immunol* (2013) **191**(8):4059–67. doi:10.4049/jimmunol.1201103
87. Li N. Platelet-lymphocyte cross-talk. *J Leukoc Biol* (2008) **83**(5):1069–78. doi:10.1189/jlb.0907615
88. Diacovo TG, Catalina MD, Siegelman MH, Von Andrian UH. Circulating activated platelets reconstitute lymphocyte homing and immunity in L-selectin-deficient mice. *J Exp Med* (1998) **187**(2):197–204. doi:10.1084/jem.187.2.197
89. Zamora C, Cantó E, Nieto JC, Ortiz MA, Diaz-Torné C, Diaz-Lopez C, et al. Functional consequences of platelet binding to T lymphocytes in inflammation. *J Leukoc Biol* (2013) **94**(3):521–9. doi:10.1189/jlb.0213074
90. León-Ponte M, Ahern GP, O'Connell PJ. Serotonin provides an accessory signal to enhance T-cell activation by signaling through the 5-HT7 receptor. *Blood* (2007) **109**(8):3139–46. doi:10.1182/blood-2006-10-052787
91. Katsounas A, Schlaak JF, Lempicki RA. CCL5: a double-edged sword in host defense against the hepatitis C virus. *Int Rev Immunol* (2011) **30**(5–6):366–78. doi:10.3109/08830185.2011.593105
92. Elzey BD, Ratliff TL, Sowa JM, Crist SA. Platelet CD40L at the interface of adaptive immunity. *Thromb Res* (2011) **127**(3):180–3. doi:10.1016/j.thromres.2010.10.011
93. Liu CY, Battaglia M, Lee SH, Sun Q-H, Aster RH, Visentin GP. Platelet factor 4 differentially modulates CD4+ CD25+ (regulatory) versus CD4+ CD25-(nonregulatory) T cells. *J Immunol* (2005) **174**(5):2680–6. doi:10.4049/jimmunol.174.5.2680
94. Shi G, Field DJ, Ko KA, Ture S, Srivastava K, Levy S, et al. Platelet factor 4 limits Th17 differentiation and cardiac allograft rejection. *J Clin Invest* (2014) **124**(2):543. doi:10.1172/JCI71858
95. Danese S, de la Motte C, Reyes BMR, Sans M, Levine AD, Fiocchi C. Cutting edge: T cells trigger CD40-dependent platelet activation and granular RANTES release: a novel pathway for immune response amplification. *J Immunol* (2004) **172**(4):2011–5. doi:10.4049/jimmunol.172.4.2011
96. Langer HF, Daub K, Braun G, Schönberger T, May AE, Schaller M, et al. Platelets recruit human dendritic cells via Mac-1/JAM-C interaction and modulate dendritic cell function in vitro. *Arterioscler Thromb Vasc Biol* (2007) **27**(6):1463–70. doi:10.1161/ATVBAHA.107.141515
97. Hagihara M, Higuchi A, Tamura N, Ueda Y, Hirabayashi K, Ikeda Y, et al. Platelets, after exposure to a high shear stress, induce IL-10-producing, mature dendritic cells in vitro. *J Immunol* (2004) **172**(9):5297–303. doi:10.4049/jimmunol.172.9.5297
98. Hamzeh-Cognasse H, Cognasse F, Palle S, Chavarin P, Olivier T, Delézay O, et al. Direct contact of platelets and their released products exert different effects on human dendritic cell maturation. *BMC Immunol* (2008) **9**(1):54. doi:10.1186/1471-2172-9-54
99. Nakanishi T, Inaba M, Inagaki-Katashiba N, Tanaka A, Vien PTX, Kibata K, et al. Platelet-derived RANK ligand enhances CCL17 secretion from dendritic

cells mediated by thymic stromal lymphopoietin. *Platelets* (2014):1–7. doi:10.3109/09537104.2014.920081
100. Kissel K, Berber S, Nockher A, Santoso S, Bein G, Hackstein H. Human platelets target dendritic cell differentiation and production of proinflammatory cytokines. *Transfusion* (2006) **46**(5):818–27. doi:10.1111/j.1537-2995.2006.00802.x
101. Panigrahi S, Ma Y, Hong L, Gao D, West XZ, Salomon RG, et al. Engagement of platelet toll-like receptor 9 by novel endogenous ligands promotes platelet hyperreactivity and thrombosis. *Circ Res* (2013) **112**(1):103–12. doi:10.1161/CIRCRESAHA.112.274241
102. Massberg S, Brand K, Grüner S, Page S, Müller E, Müller I, et al. A critical role of platelet adhesion in the initiation of atherosclerotic lesion formation. *J Exp Med* (2002) **196**(7):887–96. doi:10.1084/jem.20012044
103. Michelson AD, Barnard MR, Krueger LA, Valeri CR, Furman MI. 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* (2001) **104**(13):1533–7. doi:10.1161/hc3801.095588
104. Koenen RR, von Hundelshausen P, Nesmelova IV, Zernecke A, Liehn EA, Sarabi A, et al. Disrupting functional interactions between platelet chemokines inhibits atherosclerosis in hyperlipidemic mice. *Nat Med* (2009) **15**(1):97–103. doi:10.1038/nm.1898
105. Strüßmann T, Tillmann S, Wirtz T, Bucala R, von Hundelshausen P, Bernhagen J, et al. Platelets are a previously unrecognised source of MIF. *Thromb Haemost* (2013) **110**(5):1004–13. doi:10.1160/TH13-01-0049
106. Daub K, Seizer P, Stellos K, Krämer BF, Bigalke B, Schaller M, et al. Oxidized LDL-activated platelets induce vascular inflammation. *Semin Thromb Hemost* (2010) **36**(02):146–56. doi:10.1055/s-0030-1251498
107. Burger PC, Wagner DD. Platelet P-selectin facilitates atherosclerotic lesion development. *Blood* (2003) **101**(7):2661–6. doi:10.1182/blood-2002-07-2209
108. Lievens D, Zernecke A, Seijkens T, Soehnlein O, Beckers L, Munnix IC, et al. Platelet CD40L mediates thrombotic and inflammatory processes in atherosclerosis. *Blood* (2010) **116**(20):4317–27. doi:10.1182/blood-2010-01-261206
109. Mause SF, Weber C. Microparticles protagonists of a novel communication network for intercellular information exchange. *Circ Res* (2010) **107**(9):1047–57. doi:10.1161/CIRCRESAHA.110.226456
110. Gawaz M, Brand K, Dickfeld T, Pogatsa-Murray G, Page S, Bogner C, et al. Platelets induce alterations of chemotactic and adhesive properties of endothelial cells mediated through an interleukin-1-dependent mechanism. Implications for atherogenesis. *Atherosclerosis* (2000) **148**(1):75–85. doi:10.1016/S0021-9150(99)00241-5
111. Lindemann S, Tolley ND, Dixon DA, McIntyre TM, Prescott SM, Zimmerman GA, et al. Activated platelets mediate inflammatory signaling by regulated interleukin 1β synthesis. *J Cell Biol* (2001) **154**(3):485–90. doi:10.1083/jcb.200105058
112. Gawaz M, Langer H, May AE. Platelets in inflammation and atherogenesis. *J Clin Invest* (2005) **115**(12):3378–84. doi:10.1172/JCI27196
113. Lievens D, von Hundelshausen P. Platelets in atherosclerosis. *Thromb Haemost* (2011) **106**(5):827. doi:10.1160/TH11-08-0592
114. Simon DI. Inflammation and vascular injury: basic discovery to drug development. *Circ J* (2011) **76**(8):1811–8. doi:10.1253/circj.CJ-12-0801
115. Simon DI, Chen Z, Xu H, Li CQ, Dong JF, McIntire LV, et al. Platelet glycoprotein Ibα is a counterreceptor for the leukocyte integrin Mac-1 (CD11b/CD18). *J Exp Med* (2000) **192**(2):193–204. doi:10.1084/jem.192.2.193
116. Manthey HD, Zernecke A. Dendritic cells in atherosclerosis: functions in immune regulation and beyond. *Thromb Haemost* (2011) **106**(5):772. doi:10.1160/TH11-05-0296
117. Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. *Nat Immunol* (2010) **11**(9):785–97. doi:10.1038/ni.1923
118. Acosta J, Qin X, Halperin J. Complement and complement regulatory proteins as potential molecular targets for vascular diseases. *Curr Pharm Des* (2004) **10**(2):203–11. doi:10.2174/138161204343441
119. Giannakopoulos B, Passam F, Rahgozar S, Krilis SA. Current concepts on the pathogenesis of the antiphospholipid syndrome. *Blood* (2007) **109**(2):422–30. doi:10.1182/blood-2006-04-001206
120. Peerschke E, Yin W, Grigg S, Ghebrehiwet B. Blood platelets activate the classical pathway of human complement. *J Thromb Haemost* (2006) **4**(9):2035–42. doi:10.1111/j.1538-7836.2006.02065.x
121. Peerschke EI, Yin W, Ghebrehiwet B. Platelet mediated complement activation. *Adv Exp Med Biol* (2008) **632**:81–91.
122. Peerschke EI, Yin W, Ghebrehiwet B. Complement activation on platelets: implications for vascular inflammation and thrombosis. *Mol Immunol* (2010) **47**(13):2170–5. doi:10.1016/j.molimm.2010.05.009
123. Bäck J, Huber Lang M, Elgue G, Kalbitz M, Sanchez J, Nilsson Ekdahl K, et al. Distinctive regulation of contact activation by antithrombin and C1-inhibitor on activated platelets and material surfaces. *Biomaterials* (2009) **30**(34):6573–80. doi:10.1016/j.biomaterials.2009.07.052
124. Hamad OA, Nilsson PH, Wouters D, Lambris JD, Ekdahl KN, Nilsson B. Complement component C3 binds to activated normal platelets without preceding proteolytic activation and promotes binding to complement receptor 1. *J Immunol* (2010) **184**(5):2686–92. doi:10.4049/jimmunol.0902810
125. Patzelt J, Mueller K, Breuning S, Karathanos A, Schleicher R, Seizer P, et al. Expression of anaphylatoxin receptors on platelets in patients with coronary heart disease. *Atherosclerosis* (2015) **238**(2):289–95. doi:10.1016/j.atherosclerosis.2014.12.002
126. Verschoor A, Langer HF, Pan R, Wang J, Nardi M, Li Z, et al. Crosstalk between platelets and the complement system in immune protection and disease. *Thromb Haemost* (2013) **110**(5):910–9. doi:10.1160/TH13-02-0102
127. McCarthy MJ, Loftus IM, Thompson MM, Jones L, London NJ, Bell PR, et al. Angiogenesis and the atherosclerotic carotid plaque: an association between symptomatology and plaque morphology. *J Vasc Surg* (1999) **30**(2):261–8. doi:10.1016/S0741-5214(99)70136-9
128. von Birgelen C, Klinkhart W, Mintz GS, Papatheodorou A, Herrmann J, Baumgart D, et al. Plaque distribution and vascular remodeling of ruptured and nonruptured coronary plaques in the same vessel: an intravascular ultrasound study in vivo. *J Am Coll Cardiol* (2001) **37**(7):1864–70. doi:10.1016/S0735-1097(01)01234-7
129. Tenaglia AN, Peters KG, Sketch MH Jr, Annex BH. Neovascularization in atherectomy specimens from patients with unstable angina: implications for pathogenesis of unstable angina. *Am Heart J* (1998) **135**(1):10–4. doi:10.1016/S0002-8703(98)70336-9
130. Slevin M, Turu MM, Rovira N, Luque A, Baldellou M, Krupinski J, et al. Identification of a 'snapshot' of co-expressed angiogenic markers in laser-dissected vessels from unstable carotid plaques with targeted arrays. *J Vasc Res* (2009) **47**(4):323–35. doi:10.1159/000265566
131. Kolodgie FD, Gold HK, Burke AP, Fowler DR, Kruth HS, Weber DK, et al. Intraplaque hemorrhage and progression of coronary atheroma. *N Engl J Med* (2003) **349**(24):2316–25. doi:10.1056/NEJMoa035655
132. Virmani R, Kolodgie FD, Burke AP, Finn AV, Gold HK, Tulenko TN, et al. Atherosclerotic plaque progression and vulnerability to rupture angiogenesis as a source of intraplaque hemorrhage. *Arterioscler Thromb Vasc Biol* (2005) **25**(10):2054–61. doi:10.1161/01.ATV.0000178991.71605.18
133. Hutter R, Valdiviezo C, Sauter BV, Savontaus M, Chereshnev I, Carrick FE, et al. Caspase-3 and tissue factor expression in lipid-rich plaque macrophages evidence for apoptosis as link between inflammation and atherothrombosis. *Circulation* (2004) **109**(16):2001–8. doi:10.1161/01.CIR.0000125526.91945.AE
134. Fernández-Ortiz A, Badimon JJ, Falk E, Fuster V, Meyer B, Mailhac A, et al. Characterization of the relative thrombogenicity of atherosclerotic plaque components: implications for consequences of plaque rupture. *J Am Coll Cardiol* (1994) **23**(7):1562–9. doi:10.1016/0735-1097(94)90657-2
135. Toschi V, Gallo R, Lettino M, Fallon JT, Gertz SD, Ferna A, et al. Tissue factor modulates the thrombogenicity of human atherosclerotic plaques. *Circulation* (1997) **95**(3):594–9. doi:10.1161/01.CIR.95.3.594
136. Badimon JJ, Lettino M, Toschi V, Fuster V, Berrozpe M, Chesebro JH, et al. Local inhibition of tissue factor reduces the thrombogenicity of disrupted human atherosclerotic plaques effects of tissue factor pathway inhibitor on plaque thrombogenicity under flow conditions. *Circulation* (1999) **99**(14):1780–7. doi:10.1161/01.CIR.99.14.1780
137. Day SM, Reeve JL, Pedersen B, Farris DM, Myers DD, Im M, et al. Macrovascular thrombosis is driven by tissue factor derived primarily from the blood vessel wall. *Blood* (2005) **105**(1):192–8. doi:10.1182/blood-2004-06-2225
138. Bhattacharjee G, Ahamed J, Pedersen B, El-Sheikh A, Mackman N, Ruf W, et al. Regulation of tissue factor-mediated initiation of the coagulation cascade by cell surface grp78. *Arterioscler Thromb Vasc Biol* (2005) **25**(8):1737–43. doi:10.1161/01.ATV.0000173419.31242.56

139. Sakakura K, Nakano M, Otsuka F, Ladich E, Kolodgie FD, Virmani R. Pathophysiology of atherosclerosis plaque progression. *Heart Lung Circ* (2013) **22**(6):399–411. doi:10.1016/j.hlc.2013.03.001
140. Shah PK, Falk E, Badimon JJ, Fernandez-Ortiz A, Mailhac A, Villareal-Levy G, et al. Human monocyte-derived macrophages induce collagen breakdown in fibrous caps of atherosclerotic plaques. Potential role of matrix-degrading metalloproteinases and implications for plaque rupture. *Circulation* (1995) **92**(6):1565–9.
141. Geng Y-J, Libby P. Evidence for apoptosis in advanced human atheroma. Colocalization with interleukin-1 beta-converting enzyme. *Am J Pathol* (1995) **147**(2):251.
142. Ehara S, Kobayashi Y, Yoshiyama M, Shimada K, Shimada Y, Fukuda D, et al. Spotty calcification typifies the culprit plaque in patients with acute myocardial infarction an intravascular ultrasound study. *Circulation* (2004) **110**(22):3424–9. doi:10.1161/01.CIR.0000148131.41425.E9
143. Maldonado N, Kelly-Arnold A, Vengrenyuk Y, Laudier D, Fallon JT, Virmani R, et al. A mechanistic analysis of the role of microcalcifications in atherosclerotic plaque stability: potential implications for plaque rupture. *Am J Physiol Heart Circ Physiol* (2012) **303**(5):H619–28. doi:10.1152/ajpheart.00036.2012
144. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation* (2002) **105**(9):1135–43. doi:10.1161/hc0902.104353
145. Ridker PM, Thuren T, Zalewski A, Libby P. Interleukin-1β inhibition and the prevention of recurrent cardiovascular events: rationale and design of the canakinumab anti-inflammatory thrombosis outcomes study (CANTOS). *Am Heart J* (2011) **162**(4):597–605. doi:10.1016/j.ahj.2011.06.012
146. Ridker PM. Testing the inflammatory hypothesis of atherothrombosis: scientific rationale for the cardiovascular inflammation reduction trial (CIRT). *J Thromb Haemost* (2009) **7**(1):332–9. doi:10.1111/j.1538-7836.2009.03404.x
147. Gaziano JM, Greenland P. When should aspirin be used for prevention of cardiovascular events? *JAMA* (2014) **312**(23):2503–4. doi:10.1001/jama.2014.16047
148. Verheugt FW, Gersh BJ. Aspirin beyond platelet inhibition. *Am J Cardiol* (2002) **90**(1):39–41. doi:10.1016/S0002-9149(02)02383-4
149. Cyrus T, Sung S, Zhao L, Funk CD, Tang S, Praticò D. Effect of low-dose aspirin on vascular inflammation, plaque stability, and atherogenesis in low-density lipoprotein receptor-deficient mice. *Circulation* (2002) **106**(10):1282–7. doi:10.1161/01.CIR.0000027816.54430.96
150. Tous M, Ferré N, Vilella E, Riu F, Camps J, Joven J. Aspirin attenuates the initiation but not the progression of atherosclerosis in apolipoprotein E-deficient mice fed a high-fat, high-cholesterol diet. *Basic Clin Pharmacol Toxicol* (2004) **95**(1):15–9. doi:10.1111/j.1742-7843.2004.pto950104.x
151. Paul-Clark MJ, van Cao T, Moradi-Bidhendi N, Cooper D, Gilroy DW. 15-epi-lipoxin A4-mediated induction of nitric oxide explains how aspirin inhibits acute inflammation. *J Exp Med* (2004) **200**(1):69–78. doi:10.1084/jem.20040566
152. Kopp E, Ghosh S. Inhibition of NF-kappa B by sodium salicylate and aspirin. *Science* (1994) **265**(5174):956–9. doi:10.1126/science.8052854
153. Steinhubl SR, Badimon JJ, Bhatt DL, Herbert J-M, Lüscher TF. Clinical evidence for anti-inflammatory effects of antiplatelet therapy in patients with atherothrombotic disease. *Vasc Med* (2007) **12**(2):113–22. doi:10.1177/1358863X07077462
154. Ikeda Y, Shimada K, Teramoto T, Uchiyama S, Yamazaki T, Oikawa S, et al. Low-dose aspirin for primary prevention of cardiovascular events in Japanese patients 60 years or older with atherosclerotic risk factors: a randomized clinical trial. *JAMA* (2014) **312**(23):2510–20. doi:10.1001/jama.2014.15690
155. Huynh K. Atherosclerosis: low-dose aspirin failed to improve cardiovascular outcomes. *Nat Rev Cardiol* (2015) **12**(1):3. doi:10.1038/nrcardio.2014.194
156. Li M, Zhang Y, Ren H, Zhang Y, Zhu X. Effect of clopidogrel on the inflammatory progression of early atherosclerosis in rabbits model. *Atherosclerosis* (2007) **194**(2):348–56. doi:10.1016/j.atherosclerosis.2006.11.006
157. Afek A, Kogan E, Maysel-Auslender S, Mor A, Regev E, Rubinstein A, et al. Clopidogrel attenuates atheroma formation and induces a stable plaque phenotype in apolipoprotein E knockout mice. *Microvasc Res* (2009) **77**(3):364–9. doi:10.1016/j.mvr.2009.01.009
158. Quinn MJ, Bhatt DL, Zidar F, Vivekananthan D, Chew DP, Ellis SG, et al. Effect of clopidogrel pretreatment on inflammatory marker expression in patients undergoing percutaneous coronary intervention. *Am J Cardiol* (2004) **93**(6):679–84. doi:10.1016/j.amjcard.2003.11.048
159. Ramadan R, Dhawan SS, Syed H, Pohlel FK, Binongo JN, Ghazzal ZB, et al. Effects of clopidogrel therapy on oxidative stress, inflammation, vascular function, and progenitor cells in stable coronary artery disease. *J Cardiovasc Pharmacol* (2014) **63**(4):369–74. doi:10.1097/FJC.0000000000000057
160. Azar RR, Kassab R, Zoghbi A, Aboujaoudé S, El-Osta H, Ghorra P, et al. Effects of clopidogrel on soluble CD40 ligand and on high-sensitivity C-reactive protein in patients with stable coronary artery disease. *Am Heart J* (2006) **151**(2):521.e1–4. doi:10.1016/j.ahj.2005.10.021
161. Klinkhardt U, Bauersachs R, Adams J, Graff J, Lindhoff-Last E, Harder S. Clopidogrel but not aspirin reduces P-selectin expression and formation of platelet-leukocyte aggregates in patients with atherosclerotic vascular disease. *Clin Pharmacol Ther* (2003) **73**(3):232–41. doi:10.1067/mcp.2003.13
162. Xiao Z, Théroux P. Clopidogrel inhibits platelet-leukocyte interactions and thrombin receptor agonist peptide-induced platelet activation in patients with an acute coronary syndrome. *J Am Coll Cardiol* (2004) **43**(11):1982–8. doi:10.1016/j.jacc.2003.10.071
163. Waehre T, Damås J, Pedersen T, Gullestad L, Yndestad A, Andreassen A, et al. Clopidogrel increases expression of chemokines in peripheral blood mononuclear cells in patients with coronary artery disease: results of a double-blind placebo-controlled study. *J Thromb Haemost* (2006) **4**(10):2140–7. doi:10.1111/j.1538-7836.2006.02131.x
164. Nagy B Jr, Miszti-Blasius K, Kerenyi A, Clemetson KJ, Kappelmayer J. Potential therapeutic targeting of platelet-mediated cellular interactions in atherosclerosis and inflammation. *Curr Med Chem* (2012) **19**(4):518–31. doi:10.2174/092986712798918770
165. Subbanagounder G, Leitinger N, Shih PT, Faull KF, Berliner JA. Evidence that phospholipid oxidation products and/or platelet-activating factor play an important role in early atherogenesis in vitro and in vivo inhibition by WEB 2086. *Circ Res* (1999) **85**(4):311–8. doi:10.1161/01.RES.85.4.311
166. Ewing MM, de Vries MR, Nordzell M, Pettersson K, de Boer HC, van Zonneveld AJ, et al. Annexin A5 therapy attenuates vascular inflammation and remodeling and improves endothelial function in mice. *Arterioscler Thromb Vasc Biol* (2011) **31**(1):95–101. doi:10.1161/ATVBAHA.110.216747
167. Hamad OA, Bäck J, Nilsson PH, Nilsson B, Ekdahl KN. Platelets, complement, and contact activation: partners in inflammation and thrombosis. *Adv Exp Med Biol* (2012) **946**:185–205. doi:10.1007/978-1-4614-0106-3_11
168. Ricklin D, Lambris JD. Complement-targeted therapeutics. *Nat Biotechnol* (2007) **25**(11):1265–75. doi:10.1038/nbt1342
169. Ricklin D, Lambris JD. Complement in immune and inflammatory disorders: therapeutic interventions. *J Immunol* (2013) **190**(8):3839–47. doi:10.4049/jimmunol.1203200
170. Hill A, Hillmen P, Richards SJ, Elebute D, Marsh JC, Chan J, et al. Sustained response and long-term safety of eculizumab in paroxysmal nocturnal hemoglobinuria. *Blood* (2005) **106**(7):2559–65. doi:10.1182/blood-2005-02-0564
171. Röth A, Hock C, Konik A, Christoph S, Dührsen U. Chronic treatment of paroxysmal nocturnal hemoglobinuria patients with eculizumab: safety, efficacy, and unexpected laboratory phenomena. *Int J Hematol* (2011) **93**(6):704–14. doi:10.1007/s12185-011-0867-y
172. Lindberg S. Prognostic utility of the soluble CD40 ligand in acute coronary syndrome. *Coron Artery Dis* (2014) **25**(7):548–9. doi:10.1097/MCA.0000000000000155
173. Setianto BY, Hartopo AB, Achadiono DN, Gharini PP. Association between levels of circulating soluble CD40 ligand on admission and in-hospital events among acute coronary syndrome patients. *Acta Med Indones* (2011) **43**(2):82–7.
174. Pusuroglu H, Akgul O, Erturk M, Uyarel H, Bulut U, Akkaya E, et al. Predictive value of elevated soluble CD40 ligand in patients undergoing primary angioplasty for ST-segment elevation myocardial infarction. *Coron Artery Dis* (2014) **25**(7):558–64. doi:10.1097/MCA.0000000000000142
175. Zhao W, Zhang F, Li Z, Yu H, Li Z, Gao W. Soluble CD40 ligand is associated with angiographic severity of coronary artery disease in patients with acute coronary syndrome. *Chin Med J* (2014) **127**(12):2218–21. doi:10.3760/cma.j.issn.0366-6999.20133159
176. Pamukcu B, Lip GY, Snezhitskiy V, Shantsila E. The CD40-CD40L system in cardiovascular disease. *Ann Med* (2011) **43**(5):331–40. doi:10.3109/07853890.2010.546362
177. Ridker PM, Buring JE, Rifai N. Soluble P-selectin and the risk of future cardiovascular events. *Circulation* (2001) **103**(4):491–5. doi:10.1161/01.CIR.103.4.491

178. Kisucka J, Chauhan AK, Zhao B-Q, Patten IS, Yesilaltay A, Krieger M, et al. Elevated levels of soluble P-selectin in mice alter blood-brain barrier function, exacerbate stroke, and promote atherosclerosis. *Blood* (2009) **113**(23):6015–22. doi:10.1182/blood-2008-10-186650

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 08 December 2014; accepted: 19 February 2015; published online: 06 March 2015.

*Citation: Nording HM, Seizer P and Langer HF (2015) Platelets in inflammation and atherogenesis. Front. Immunol. **6**:98. doi: 10.3389/fimmu.2015.00098*
This article was submitted to Inflammation, a section of the journal Frontiers in Immunology.

frontiers in
IMMUNOLOGY

REVIEW ARTICLE
published: 02 February 2015
doi: 10.3389/fimmu.2015.00028

Platelet transfusion – the new immunology of an old therapy

Moritz Stolla[1], Majed A. Refaai[1], Joanna M. Heal[1], Sherry L. Spinelli[1], Olivier Garraud[2], Richard P. Phipps[1,3,4,5] and Neil Blumberg[1]*

[1] *Department of Pathology and Laboratory Medicine, School of Medicine and Dentistry, University of Rochester Medical Center, Rochester, NY, USA*
[2] *Etablissement Francais du Sang Auvergne-Loire, Universite de Lyon, Saint-Etienne, France*
[3] *Department of Microbiology and Immunology, School of Medicine and Dentistry, University of Rochester Medical Center, Rochester, NY, USA*
[4] *Department of Environmental Medicine, School of Medicine and Dentistry, University of Rochester Medical Center, Rochester, NY, USA*
[5] *Department of Medicine, School of Medicine and Dentistry, University of Rochester Medical Center, Rochester, NY, USA*

Edited by:
Heiko Mühl, University Hospital Goethe University Frankfurt, Germany

Reviewed by:
Bo Shen, University of Illinois at Chicago, USA
Angelo A. Manfredi, Vita Salute San Raffaele University, Italy

****Correspondence:***
Neil Blumberg, Department of Pathology and Laboratory Medicine, School of Medicine and Dentistry, University of Rochester Medical Center, Box 608, 601 Elmwood Avenue, Rochester, NY 14642, USA
e-mail: neil_blumberg@urmc.rochester.edu

Platelet transfusion has been a vital therapeutic approach in patients with hematologic malignancies for close to half a century. Randomized trials show that prophylactic platelet transfusions mitigate bleeding in patients with acute myeloid leukemia. However, even with prophylactic transfusions, as many as 75% of patients, experience hemorrhage. While platelet transfusion efficacy is modest, questions and concerns have arisen about the risks of platelet transfusion therapy. The acknowledged serious risks of platelet transfusion include viral transmission, bacterial sepsis, and acute lung injury. Less serious adverse effects include allergic and non-hemolytic febrile reactions. Rare hemolytic reactions have occurred due to a common policy of transfusing without regard to ABO type. In the last decade or so, new concerns have arisen; platelet-derived lipids are implicated in transfusion-related acute lung injury after transfusion. With the recognition that platelets are immune cells came the discoveries that supernatant IL-6, IL-27 sCD40L, and OX40L are closely linked to febrile reactions and sCD40L with acute lung injury. Platelet transfusions are pro-inflammatory, and may be pro-thrombotic. Anti-A and anti-B can bind to incompatible recipient or donor platelets and soluble antigens, impair hemostasis and thus increase bleeding. Finally, stored platelet supernatants contain biological mediators such as VEGF and TGF-β1 that may compromise the host versus tumor response. This is particularly of concern in patients receiving many platelet transfusions, as for acute leukemia. New evidence suggests that removing stored supernatant will improve clinical outcomes. This new view of platelets as pro-inflammatory and immunomodulatory agents suggests that innovative approaches to improving platelet storage and pre-transfusion manipulations to reduce toxicity could substantially improve the efficacy and safety of this long-employed therapy.

Keywords: platelets, transfusion, immune response, transfusion reaction, storage, thrombosis, bleeding

INTRODUCTION

Since their discovery, platelets have displayed a remarkable development from a pro-thrombotic and pro-hemostatic cell fragment to a surprisingly versatile immune-thrombotic cell. The plethora of findings continues to surprise and excite at the same time. In concordance, an old therapy, i.e., platelet transfusion, either for prophylaxis or acute bleeding has been in transition as well. Initially thought as an "easy fix" for thrombocytopenia, platelet transfusions are now considered a double-edged sword at best. Despite all the controversies, platelet transfusions are a clinical necessity and do save lives every day worldwide. However, the majority of platelet transfusions are administered for prophylactic purposes, and for chronic conditions, rather than acute hemorrhage. It is presently unclear if platelet transfusions are effective in settings of acute hemorrhage. Moreover, the following questions are open and continue to evolve: Do platelet transfusions work the way we think? What are the downsides to this therapy? Some reports already highlight a pro-thrombotic side effect of platelet transfusion, leaving the thrombocytopenic patient, who is already at risk for bleeding with an increased risk of thrombosis and bleeding at the same time.

Abbreviations: NET, neutrophil extracellular trap; PMP, platelet microparticles; sPs, soluble P-selectin.

PLATELETS – NOVEL FINDINGS IN IMMUNITY AND INFLAMMATION

We briefly summarize a selection of the most relevant findings in regards to an inflammatory and defensive role of platelets from the recent literature.

Platelets are anucleate cell fragments, derived from megakaryocytes and circulate with an average number of 150,000–300,000/μl in humans. Their role in thrombosis and hemostasis has been well described. Their role in inflammation, however, was

discovered only relatively recently and has constantly been evolving ever since. From an evolutionary perspective, platelets likely originated from a versatile cell type with both strong hemostatic and defensive cell properties (1, 2). Their role in hemostasis is therefore closely intertwined with their sentinel and inflammatory properties. Activation of platelet surface receptors leads to inside-out signaling, which is followed by integrin activation, which in turn, is crucial for thrombus formation (3). In addition to G-protein coupled receptors (mainly protease-activated receptors, or PARs), platelets also possess immunoreceptor tyrosine-based activation motif (ITAM), e.g., Fc-receptors, glycoprotein VI, and C-type lectin-like receptor 2 (CLEC2). Fc-receptors allow for immunoglobulin and immune complex binding, while GPVI (the major collagen receptor) and CLEC2 are known to be important for vascular integrity in inflammation (4). Toll-like receptors (TLR) are present on platelets as well, although there are conflicting reports about their functionality (5–8). A recent report indicated that platelets were able to discriminate between different LPS isoforms by differential cytokine secretion by mononuclear cells, including IL-6, TNF-α, and IL-8 (9). In addition, platelets express the coxsackievirus and adenovirus receptor (CAR). Of note, coxsackievirus 1 and 3 induced P-selectin exposure and phosphatidyl serine exposure in platelets, albeit independently of CAR. The presence of platelets led to lower coxsackievirus 1 and 3 titers, to less myocardial virus load and to better survival in mice infected with the virus. To a similar conclusion came authors of another recent paper, showing how platelets protect the host during encephalomyocarditis virus (EMCV) infection via TLR7. Platelet depletion led to reduced survival, and transfusion of WT platelets into TLR7-deficient mice improved survival and was accompanied by a drop in platelet count (10). Another virus, which has recently been described to interact with platelets, is the H1N1 influenza virus. During infection with H1N1, platelets show activation of their surface receptors, lipid mediator generation, and release of microparticles. Interestingly, immunized subjects showed circulating immune complexes of virus and IgG, which were able to activate platelets via FcγRIIa. Alternatively, H1N1 virus was able to activate platelets via thrombin generation (11).

Platelets express high-mobility group protein 1 (HMGB1) (12). Upon activation, HMGB1 is translocated from the cytoplasm to the outer plasma membrane. In its extracellular location, HMGB1 has been implicated in inflammatory, proliferative, and migratory processes (13). TLR2, 4, 9, the receptor for advanced glycolation end-products (RAGE) and Mac-1 have been described in the inflammatory actions of HMGB1 (14–16), while ultimately these signals lead to NF-κb activation. While HMGB1 has not been investigated in the context of platelet transfusion, it is entirely conceivable that HMGB1 accumulates during platelet storage and triggers inflammatory sequela upon transfusion.

We and others have demonstrated that although platelets are anucleate cell fragments they express an armamentarium of transcription factors including the NF-κb transcriptional regulatory system, Bcl-3, and PPARγ (17–22). The current concept is that these factors act in non-transcriptional ways in platelets, e.g., by modulating the response to activation, even though they act as traditional transcription factors in megakaryocytes (17, 21).

Although platelets do not possess a nucleus, there is evidence that platelets can synthesize IL-1β in substantial amounts upon activation. In a resting state, platelets contain the pre-mRNA of IL-1β. However, when activated, they synthesize pro-IL-1β protein, which is further processed and ends up as the mature form of the cytokine IL-1β (23, 24). One of the earlier discoveries in platelet biology was the tendency of platelets to "stick" to leukocytes when activated and in generalized inflammatory conditions. All subclasses of leukocytes have been described to adhere to platelets (25–27). However, the majority of studies describe myeloid leukocytes as the platelet-binding partner. The interaction between platelet and leukocyte mainly depends on the P-selectin – PSGL-1, and fibrinogen – αIIbβ3, or directly to GPIb and αMβ2 (28).

The initial contact between leukocyte and platelet is established via platelet P-selectin and leukocyte PSGL-1. On the molecular level, P-selectin binds in a stereospecific manner to the N-terminal region of PSGL-1, by recognizing a motif with tyrosine sulfate residues, fucose, galactose, and sialic residues on a core-2 *O*-glycan (29). Overall, the interaction between P-selectin and PSGL-1 has a very rapid association/dissociation rate, facilitating the rapid capture, tethering, and rolling under high flow and shear conditions (30). Of note, this initial step of tethering and rolling does not require any leukocyte activation signals, but subsequent steps of firm adhesion and transmigration require signaling events that ultimately lead to integrin activation. Specifically, Nef-associated factor 1 (Naf-1, downstream of PSGL-1) is phosphorylated by Src-family kinase (SFK), and leads to Mac-1 (αMβ2) activation. In addition, neutrophils show LFA-1 (αLβ2) activation, while monocytes and lymphocytes show β1 and β2 activation (31, 32). Integrin outside-in signaling via SFK then leads to phosphorylation of proline-rich tyrosine kinase 2 (Pyk2), which is followed by a sustained leukocyte activation with stabilization of the integrin bond and a delayed inflammatory response including NF-κb activation. Furthermore, if circulating as heterotypic aggregates, they can facilitate leukocyte deposition at sites of inflammation and vascular injury and have been described in a plethora of diseases (33–36).

Notably, platelets contain substantial amounts of CD40L (formally known as CD154), a protein with a significant role in T-cell-dependent isotype switching and generation of antibody subclasses by B-cells. Platelets can stimulate neutrophils, T-cells, and endothelial cells via CD40L (37–39). In addition to interacting with leukocytes, platelets closely interact with endothelial cells. Inflammatory conditions are usually accompanied by a certain degree of vascular leakiness. Interestingly, thrombocytopenic patients usually do not bleed with platelet counts above 10,000/μl unless they develop inflammation. This contributory role of platelets to vascular integrity seems to mainly depend on platelet ITAM receptors (4, 40–42). In a similar way, platelets seem to protect the vasculature in tumors and prevent bleeding inside of tumors (43, 44). Surprisingly, platelets have also been found to enhance vascular permeability during inflammation, perhaps due to their secretion of VEGF, serotonin, and other similar factors. These seemingly contradictory results might reflect model, species, and site-specific differences, but it might also depend on a differential release of mediators, specifically serotonin has been shown to be crucial for the induction of vessel permeability by platelets (45).

How the differential contribution to vessel integrity and leakiness is regulated *in vivo* remains to be investigated. Examining vascular integrity during dengue virus infection, Hottz et al. found that platelets contribute to vascular leakage by releasing IL-1β-rich microparticles after assembly of nucleotide-binding domain leucin-rich repeat containing protein (NLRP3), and are thus likely to contribute to hemorrhage frequently observed during infection with this virus (46). Other virus-induced hemorrhagic fevers also critically involve platelets (47). Recent studies also highlight the ability of platelets and platelet-like particles to transfer microRNA to leukocytes and endothelial cells. Even though this has not been shown to be the case in an inflammatory setting, it is an interesting mechanism to transfer "long distance" information and contribute to vascular homeostasis (48). An intriguing recent study by Massberg et al. suggested that platelets serve as "first sensors" for endothelial damage and potential invaders. Platelets adhered to and activated neutrophils, which in turn released nucleosomes and serine proteases in order to trigger the intrinsic and extrinsic coagulation pathway. This localized promotion of thrombosis by platelets and neutrophils in order to trap invaders in the microcirculation is a novel example of how closely intertwined innate defense mechanisms and thrombotic processes are (49, 50).

One of the most striking recent findings is how neutrophils produce extracellular traps, or "neutrophil extracellular traps (NETs)" in order to cause thrombosis and thereby trap invading microbes. While neutrophils are the major inducer of NETs by ejecting their nucleus, there is emerging evidence that platelets assist neutrophils in this process: platelet β-defensin-1 released after stimulation with *Staphylococcus* α-toxin was found to be critically involved in the formation of NETs under flow conditions *in vitro* and *in vivo* (51). Earlier, an elegant study by Clark et al. showed that TLR4 on platelets binds to circulating LPS and mediates neutrophil–platelet binding with subsequent neutrophil activation and NET formation in mice and humans. The authors were able to show that NET formation helped trapping bacteria. Similar to the study mentioned above by Massberg et al., platelets function as a "sensor" for circulating LPS and facilitate neutrophil activation (52). Of note, platelets were also critically involved in NET formation in a mouse model of transfusion-related acute lung injury (53, 54). A somewhat under-recognized cell organelle in platelets has recently been shown to contribute to the inflammatory response by platelets: by releasing mitochondria, either free or coated by microvesicles, they provided the substrate for secreted phospholipase A_2-IIa (PLA_2-IIa), which in turn produces lysophospholipids, fatty acids, and mtDNA to activate leukocytes (55).

PLATELET STORAGE AND INFLAMMATION

Prolonged storage leads to changes in platelets mostly associated with platelet activation. Contents of the alpha granules are secreted which leads to a gradual increase in P-selectin and CD40L membrane expression. Consistently, the number of platelets attached to leukocytes increases over time during storage (56). A substantial amount of P-selectin and CD40L are shed into the plasma as soluble P-selectin (sPS) and sCD40L, respectively (57, 58) (**Figure 1**). CD40L and its interaction with neutrophils and endothelial cells have been implicated in the development of non-antibody-mediated transfusion-associated acute lung injury (TRALI) (39). Of note, platelets act in concert with T-cells to mediate B-cell stimulation and function as a "first wave" of B-cell activation (37). Specifically, after a 3-day incubation with platelets, B cells showed an increased production of IgG1, IgG2, and IgG3, but not IgG4, IgA, or IgM (37). Interestingly, activation of dendritic cells by platelets has proven to be independent of sCD40L. While platelets do stick to dendritic cells, dendritic cell activation is mediated by nucleotides like ADP and ATP (59). Platelet CD40L has also been involved in host defense against *Listeria monocytogenes* in a mouse model (60). In addition to its role in inflammation, platelet CD40L has also been implicated in more traditional roles of platelets such as thrombus formation and thrombus stabilization (61). In addition to P-selectin and CD40L, multiple other platelet alpha and dense granule substances are found in the supernatant: e.g., β-thromboglobulin, platelet factor 4, and serotonin (62).

Stored platelets also exhibit an increase in phosphatidylserine (PS) exposure (**Figure 1**), which can be assessed via annexin V binding. Of note, the degree of platelet activation does not correlate with P-selectin expression, platelet count increment, or function *in vivo*. P-selectin-expressing platelets that were transfused into primates and rabbits rapidly lost their surface P-selectin but continued to function and to circulate *in vivo* (63–65).

Platelet microparticles (PMP) are released from platelets upon activation or apoptosis. Platelet concentrates contain PMPs in the supernatant, reflecting activation during collection, and/or storage (**Figure 1**). Apheresis collection is associated with less PMP formation when compared to platelets separated from whole blood donations (66, 67). PMPs have been shown to be critically involved in inflammatory conditions like rheumatoid arthritis (68). Infusion of activated platelets and microparticles causes early atherosclerotic lesions (fatty streaks) and exacerbate atherosclerosis in mice and diabetic patients (69–71). Furthermore, PMPs enable platelets to transport RNA, cytokines, or chemokines to other cells and tissues, as they are small enough to exit the circulation and to enter the surrounding tissues. This has been shown for RANTES (72), IL-1α (68), IL-1β (24, 73), PPARγ (74), retinoid X transcription factor (74), and CD40L (75, 76), albeit not under transfusion conditions. Human cytomegalovirus (HCMV) activates platelets via TLR2 and triggers ADP release and subsequent platelet leukocyte aggregate formation *in vitro* and *in vivo*, which might explain how this virus might contribute to atherosclerotic lesion formation (77).

We were recently able to show that the composition of microparticles can be altered by genetic engineering. Using lentiviral technology, we introduced green fluorescent protein (GFP) and increased PPARγ expression in megakaryocytic cell lines and primary megakaryocytes. Platelets and microparticles generated by modified megakaryocytes were internalized by a monocytic cell line and altered the expression of a target protein of PPARγ. This is a potentially useful tool in altering and investigating transcellular communications (78). To what extend PMP contribute to the hemostatic properties of platelet transfusions has not been yet investigated. Moreover, PMPs function as messengers to convey pro-inflammatory information to endothelial cells and leukocytes. No study has investigated the pro-inflammatory role of PMPs after transfusion yet, but it seems likely that there are some systemic effects to the recipient. Stored platelets also contain

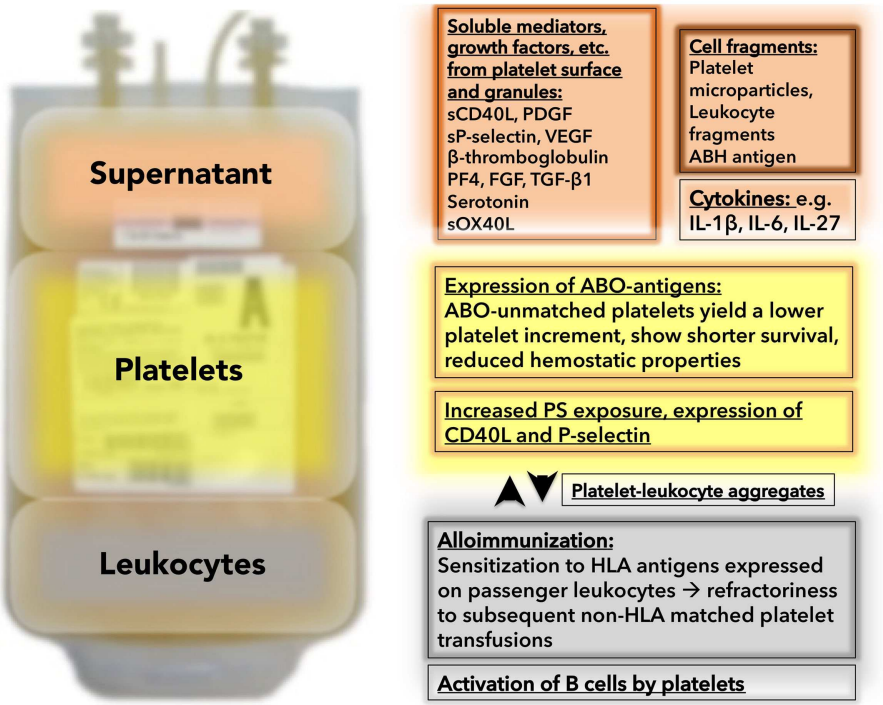


FIGURE 1 | Unwanted passengers and events during platelet storage: Besides platelets, platelet concentrates contain "unwanted passengers" in the form of soluble mediators in the supernatant (in the case of unwashed platelet concentrates – which are still the routine product in virtually all US hospitals) and leukocytes (in the case of non-leukoreduced platelet concentrates – which are still an available product in some US hospitals). The supernatant (red background) contains substances which are secreted or shed into the plasma during storage containing soluble mediators, like sCD40L, PDGF, or sP-selectin. In addition, cytokines and cell fragments can be found. The platelet themselves (yellow background) change during storage. They become hyporesponsive (not depicted), and degranulate with increased expression of alpha granule contents (e.g., P-selectin). Platelets express ABO antigens, which lead to suboptimal results and complications if transfused into ABO-unmatched recipients. A marker for platelet activation is platelet leukocyte aggregate formation (white background). With prolonged storage, the number of platelet leukocyte aggregates increase. Leukocytes in the stored bag (gray background) release cytokines, which can induce febrile transfusion reactions. Passenger leukocytes lead to sensitization to HLA antigens with subsequent refractoriness.

minute fragments of leukocytes that have been involved in HLA-sensitization and platelet refractoriness in pre-clinical studies (79) (**Figure 1**).

Adding some clinical relevance to the aforementioned pre-clinical *in vitro* and lab findings, we were able to show that washing red cells and platelets (i.e., removing the supernatant) is associated with less inflammatory response in high-risk pediatric populations compared to non-washed platelet transfusions (80). Furthermore, the patients in the study-arm needed fewer transfusions and there was a trend to lower mortality in the study-arm (washed units). Larger clinical trials are needed to determine if this finding is clinically significant (80).

TRANSFUSION REACTIONS AND IMMUNOMODULATION

Although platelet transfusions are generally well-tolerated, they cause more transfusion reactions than any other blood product (81). The adverse events range from simple allergic reactions to severe anaphylactic reactions, febrile-non-hemolytic reactions, transfusion-associated sepsis, and TRALI. Several strategies have been shown to reduce the rate of transfusion reactions, e.g., leukocyte reduction, washing, and ABO matching. As described above, stored platelets release sCD40L and accumulate CD40L on their surface. In fact, platelet concentrates from apheresis and whole blood collection demonstrated the highest sCD40L concentrations compared to all other blood products (39). Platelet concentrates involved in TRALI had significantly higher sCD40L levels compared to uninvolved ones. *In vitro* sCD40L was able to prime the PMN oxidase rapidly suggesting that sCD40L could be critically involved in non-antibody-mediated TRALI (39). But, its role might not be limited to that; earlier data from our group suggested that sCD40L could induce febrile transfusion reactions by activating cyclooxygenase-2 and thereby producing prostaglandin E_2 (82). Of note, the concentrations that were required to produce PGE_2 *in vitro* are easily met and even exceeded by one order of magnitude after transfusion of unwashed platelets. In addition, cytokine accumulation (e.g., IL-1β, IL-6, IL-27) and accumulation of soluble OX40 ligand in the stored platelet bag has been shown to contribute to febrile non-hemolytic reactions (83, 84) (**Figure 1**). Washing and leukoreduction was protective and helped to reduce the incidence of febrile transfusion reactions (85–87). In addition to sCD40L, cytokines, and sPS, platelet-derived growth factor (PDGF) accumulates during platelet storage (88). Moreover,

other growth factors like VEGF, FGF-2, and TGF-β1 have been shown to accumulate during platelet storage (89–91) (**Figure 1**). Upon transfusion into patients with (hematologic-) malignancies, these growth factors may promote cancer growth and antagonize growth factor-targeted therapies (90). Overall, there is good reason to believe that the number of described soluble mediators is not complete; as a recent study showed the presence of 1048 proteins in the supernatant, including 69 membrane proteins (10 had been shown to be shed from platelets before). However, this data warrants further validation under storage conditions since platelets were activated with agonists instead of activating them by prolonged storage (92). We previously investigated the platelet proteome under storage conditions, using mass spectrometry and found that 117 proteins changed during storage conditions. Notably, 22 out of 117 proteins were previously described in platelets and two-thirds of these 22 were associated with alpha granules, supporting degranulation as one of the major events during storage (93).

In an intriguing study, Yazer et al. investigated if a febrile non-hemolytic transfusion reaction renders the patient more susceptible for the development of alloantibodies to subsequent red blood cell products, and found a significantly higher rate of sensitization in the study-arm (94). This result corroborated recent findings in animal models (95, 96) and is presumably due to cytokines in the platelet unit that are high enough to induce a febrile transfusion reaction and generate a humoral immune response which predisposes the recipient to more effective antibody production (97). The idea that transfusions could alter the immune system of the recipient other than through alloimmunization stems from the discovery that allogeneic transfusions enhanced kidney graft acceptance in transplant recipients (98). Shortly afterwards, an association between colorectal cancer recurrence and transfusion was noted and confirmed in retrospective studies (99–101). In concordance was another subsequent finding that transfusions are associated with infections perioperatively in cancer patients (102). Although all of the above mentioned initial work on transfusion-mediated immunomodulation was done on red blood cell transfusions, it is likely that soluble mediators that accumulate in platelet concentrates have an immunomodulatory effect upon transfusion into the recipient as well. Specifically, sCD40L and similar mediators could alter host defense in a way that tumor defense is impaired by shifting the immunity toward a type-2 immune function (103, 104). Our group demonstrated that sCD40L stimulates PGE_2 production and induces COX-2 (58, 82, 105, 106). Another possible mechanism may be that sCD40L functions as a proliferation and survival factor for the circulating leukemic cells. It is likely that washing platelet concentrates before transfusions improves survival in leukemic patients (107, 108). We were recently able to replicate our previous findings in younger patients with acute myeloid leukemia with a striking survival benefit of almost 100% in patients with favorable risk AML on a washed transfusion protocol and a short-term mortality of nearly 0% in patients with any type of AML in the treatment group (washed protocol, unpublished observations). Washing the platelet concentrate is an inexpensive and relatively easy way to get rid of the supernatant. It goes along with a mild activation and a minor loss of platelet numbers prior to transfusion. However, these modest downsides are disproportionate as compared to the potential benefits to patients in terms of survival.

Interestingly, the rate of TRALI is highest in platelet transfusions. Although it has never been convincingly demonstrated that transfused platelets are causally related to the development of TRALI, a recent discovery linked recipient platelets to the pathogenesis in antibody-mediated TRALI. Aspirin treatment and platelet depletion prevented TRALI in a mouse model (54). Furthermore, two papers linked NETs to the pathogenesis of TRALI and one of them linked the occurrence of NETs directly to platelets (53, 109).

ABO MATCHING FOR PLATELET TRANSFUSIONS

The ABO blood group system is still the most important system in transfusion medicine since it was first discovered by Karl Landsteiner roughly a century ago. ABO antigens are both integral to and passively adsorbed onto the red blood cell surface. The discovery that platelets also express ABO antigens was made in the 1950s (110). More recently, they have been localized on integrins αIIbβ3 and α2β1 (111, 112). One of the earliest clinical discoveries was that non-ABO-matched platelets transfusions yield a lower platelet count increment (113). This was further substantiated in multiple randomized and observational studies; in addition ABO-matched platelet transfusion protected better from bleeding and led to less frequent refractoriness (114–116). *In vitro*, co-incubation of A, B, or AB platelets with O plasma with differing titers of anti-A and anti-B inhibited platelet aggregation and was also associated with other anticoagulant properties, delivering a potential explanation for the reduced hemostatic activity of non-ABO-matched platelet transfusions (117). The lower platelet increment is speculatively due to clearance of antibody-coated platelets and platelets coated with immune complexes by phagocytic cells. We showed previously, that circulating ABO immune complexes that were isolated from patients after non-ABO-matched transfusion were able to adhere to platelets via the Fc-receptor and complement receptors, cC1q and gC1q. In addition, *in vitro* formed ABO immune complexes showed the same ability to bind platelets via the aforementioned receptors. Inhibition of the receptors with blocking antibodies reduced the amount of bound immune complexes by 67–99%. Together these findings provide an additional potential mechanism for platelet clearance after non-ABO-matched transfusion and overall for the importance of ABO-matched transfusions clinically (118).

In a cohort trial investigating the effect of ABO matching platelets in patients undergoing cardiac surgery, we showed that patients who received at least one ABO-mismatched pool of platelet had significantly longer hospital stays, more days of fever, and more RBC transfusions. Notably, recipients of ABO-identical platelets had one-quarter the mortality, fewer mean days of antibiotics and hours in the ICU than patients receiving ABO-mismatched transfusions. Maybe due to the smaller number of patients, these latter differences were not significant (119). Our institution switched to universal ABO-matched platelet and cryoprecipitate transfusion in 2005, and this approach led to improved clinical outcomes and reduced transfusion requirements in surgical patients (120). Furthermore, this approach has also proven feasible from a blood management and administrative standpoint

(121). **Table 1** summarizes all findings from trials that were performed by our group, at our institution with regards to ABO matching and platelets transfusions.

ALLOIMMUNIZATION TO HLA

Patients who receive frequent platelet transfusions sometimes become less responsive to platelet transfusion, a phenomenon termed "refractoriness." There are immune-mediated mechanisms of platelet refractoriness and non-immune-mediated mechanisms. Overall, the non-immune platelet removal is more common than immune-mediated removal (122). However, in light of the focus of this review article, we will focus on the immune-mediated mechanisms. The most common reason to develop immune-mediated platelet refractoriness is the development of antibodies to foreign HLA A, B (class I MHC), which are expressed on platelets and most other cell types, or the development of platelet-specific antibodies. Interestingly, in case of the sensitization to HLA antigens, transfusion of leukocyte containing blood products is associated with a higher rate of immunization, and consistently, universal leukoreduction leads to a significant reduction in platelet refractoriness (123) (**Figure 1**). This along with others lead to the conclusion that transfusing a small amount of leukocytes with red blood cells or platelets is more immunogenic than the transfused platelets themselves. Our institution's policy is to transfuse only leukoreduced and ABO-identical platelets, and our internal data suggest that this leads to fewer incidences of platelet refractoriness (<1% of patients). One potential solution when alloimmunization has occurred and refractoriness is evident is to expend the money necessary and engage in the time-consuming process of finding an HLA-matched donor. This can be facilitated by HLA-matching or by a test that determines the antibody specificity and selects donors based on their lack of the corresponding antigens (124).

PLATELET TRANSFUSION AND THROMBOSIS

Inflammation and thrombosis are closely intertwined; it is therefore conceivable that platelet transfusions not only lead to inflammation, but also thrombosis. Indeed, there is evidence, mainly coming from an observational study, that transfusions are associated with thrombosis. A retrospective study investigated the associations between transfusions and venous thromboembolism, arterial thromboembolism, and mortality in hospitalized patients with cancer. All three outcomes were significantly more common in patients that received platelet transfusion (125). However, more studies, preferably randomized, controlled trials are needed to show this association is indeed causal. More data is available for red blood cell transfusions (126, 127), while overall clinical data on this subject is still rather scarce.

It is one of the issues of platelet transfusions that the hemostatic potential of the stored platelets decrease over time in the storage bag. On the other hand, the amount of soluble pro-inflammatory and pro-thrombotic mediators in the supernatant increases. These mediators presumably partially compensate for the loss of platelet function, but it is plausible that they contribute to thrombosis as well. Platelet-derived microparticles and sCD40L have been shown to be involved in thrombosis under pre-clinical, experimental conditions and are most likely amongst the culprits that mediate thrombosis (128–131).

Overall it appears, as if fresher platelets (<3 days of storage) are preferable due to reduced cytokine load and are likely less

Table 1 | Studies investigating the role of ABO-group matching in platelet transfusions.

Author	Study type/question	Result	Year	Journal	Reference
Heal et al.	*In vitro* study investigating the role of circulating immune complexes after ABO-non-identical transfusion	Circulating immune complexes adhere to platelets via Fc-receptor and complement receptors and provide a potential mechanism for platelet clearance	1996	Vox sanguinis	(118)
Blumberg et al.	Retrospective cohort-study investigating patients undergoing cardiac surgery	Patients who received ABO-mismatched platelets had a longer hospital stay, more fever, and more RBC transfusion. Furthermore, there was a trend toward a 75% reduction in mortality with ABO-identical platelets	2001	Transfusion	(119)
Refaai et al.	Retrospective analysis of non-ABO-identical platelet transfusion and the effect on transfusion requirements and other clinical parameters	ABO-identical transfusions might lead to lower transfusion requirements and better clinical outcome	2011	Vox sanguinis	(120)
Henrichs et al.	Feasibility trial, designed to answer if uniform ABO-identical platelet transfusion is doable in a tertiary care hospital setting	97% of patients received ABO-identical platelets. There was an unexpected reduction in febrile and allergic reactions. In addition, there was a reduction in RBC alloimmunization and HLA platelet requirements	2012	Transfusion	(121)
Refaai et al.	*In vitro* study investigating the effect of anti-A and anti-B on platelet function and clot formation	Anti-A and anti-B inhibit platelet aggregation and reduce clot formation in various *in vitro* assays	2013	Transfusion	(117)

The table summarizes the in vitro and clinical studies by our group at the University of Rochester investigating the role of the ABO blood group system in platelet transfusions.

pathogenic. More clinical studies are needed to evaluate the hemostatic improvement and/or impairment, as well as the thrombotic risk as a function of storage time.

AUTHOR CONTRIBUTIONS

MS and NB wrote a draft of the manuscript and revised it critically for important intellectual content. MR, SS, JH, and RP provided important feedback and revised the manuscript critically for important intellectual content. All authors approved the final version of the manuscript.

ACKNOWLEDGMENTS

Moritz Stolla received a training grant from the American Society of Hematology together with Neil Blumberg.

REFERENCES

1. Semple JW, Italiano JE Jr, Freedman J. Platelets and the immune continuum. *Nat Rev Immunol* (2011) **11**(4):264–74. doi:10.1038/nri2956
2. Vieira-de-Abreu A, Campbell RA, Weyrich AS, Zimmerman GA. Platelets: versatile effector cells in hemostasis, inflammation, and the immune continuum. *Semin Immunopathol* (2012) **34**(1):5–30. doi:10.1007/s00281-011-0286-4
3. van de Vijver E, De Cuyper IM, Gerrits AJ, Verhoeven AJ, Seeger K, Gutiérrez L, et al. Defects in Glanzmann thrombasthenia and LAD-III (LAD-1/v) syndrome: the role of integrin beta1 and beta3 in platelet adhesion to collagen. *Blood* (2012) **119**(2):583–6. doi:10.1182/blood-2011-02-337188
4. Boulaftali Y, Hess PR, Getz TM, Cholka A, Stolla M, Mackman N, et al. Platelet ITAM signaling is critical for vascular integrity in inflammation. *J Clin Invest* (2013) **123**(2):908–16. doi:10.1172/JCI65154
5. Aslam R, Speck ER, Kim M, Crow AR, Bang KW, Nestel FP, et al. Platelet Toll-like receptor expression modulates lipopolysaccharide-induced thrombocytopenia and tumor necrosis factor-alpha production in vivo. *Blood* (2006) **107**(2):637–41. doi:10.1182/blood-2005-06-2202
6. Cognasse F, Hamzeh H, Chavarin P, Acquart S, Genin C, Garraud O. Evidence of Toll-like receptor molecules on human platelets. *Immunol Cell Biol* (2005) **83**(2):196–8. doi:10.1111/j.1440-1711.2005.01314.x
7. Ståhl AL, Svensson M, Mörgelin M, Svanborg C, Tarr PI, Mooney JC, et al. Lipopolysaccharide from enterohemorrhagic *Escherichia coli* binds to platelets through TLR4 and CD62 and is detected on circulating platelets in patients with hemolytic uremic syndrome. *Blood* (2006) **108**(1):167–76. doi:10.1182/blood-2005-08-3219
8. Garraud O, Berthet J, Hamzeh-Cognasse H, Cognasse F. Pathogen sensing, subsequent signalling, and signalosome in human platelets. *Thromb Res* (2011) **127**(4):283–6. doi:10.1016/j.thromres.2010.10.015
9. Berthet J, Damien P, Hamzeh-Cognasse H, Arthaud CA, Eyraud MA, Zéni F, et al. Human platelets can discriminate between various bacterial LPS isoforms via TLR4 signaling and differential cytokine secretion. *Clin Immunol* (2012) **145**(3):189–200. doi:10.1016/j.clim.2012.09.004
10. Koupenova M, Vitseva O, MacKay CR, Beaulieu LM, Benjamin EJ, Mick E, et al. Platelet-TLR7 mediates host survival and platelet count during viral infection in the absence of platelet-dependent thrombosis. *Blood* (2014) **124**(5):791–802. doi:10.1182/blood-2013-11-536003
11. Boilard E, Paré G, Rousseau M, Cloutier N, Dubuc I, Lévesque T, et al. Influenza virus H1N1 activates platelets through FcgammaRIIA signaling and thrombin generation. *Blood* (2014) **123**(18):2854–63. doi:10.1182/blood-2013-07-515536
12. Rouhiainen A, Imai S, Rauvala H, Parkkinen J. Occurrence of amphoterin (HMG1) as an endogenous protein of human platelets that is exported to the cell surface upon platelet activation. *Thromb Haemost* (2000) **84**(6):1087–94. Available from: http://th.schattauer.de/en/contents/archive/issue/892/manuscript/2627/show.html
13. Bianchi ME. HMGB1 loves company. *J Leukoc Biol* (2009) **86**(3):573–6. doi:10.1189/jlb.1008585
14. Tian J, Avalos AM, Mao SY, Chen B, Senthil K, Wu H, et al. Toll-like receptor 9-dependent activation by DNA-containing immune complexes is mediated by HMGB1 and RAGE. *Nat Immunol* (2007) **8**(5):487–96. doi:10.1038/ni1457
15. Lotze MT, Tracey KJ. High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. *Nat Rev Immunol* (2005) **5**(4):331–42. doi:10.1038/nri1594
16. Orlova VV, Choi EY, Xie C, Chavakis E, Bierhaus A, Ihanus E, et al. A novel pathway of HMGB1-mediated inflammatory cell recruitment that requires Mac-1-integrin. *EMBO J* (2007) **26**(4):1129–39. doi:10.1038/sj.emboj.7601552
17. Akbiyik F, Ray DM, Gettings KF, Blumberg N, Francis CW, Phipps RP. Human bone marrow megakaryocytes and platelets express PPARgamma, and PPARgamma agonists blunt platelet release of CD40 ligand and thromboxanes. *Blood* (2004) **104**(5):1361–8. doi:10.1182/blood-2004-03-0926
18. Gambaryan S, Kobsar A, Rukoyatkina N, Herterich S, Geiger J, Smolenski A, et al. Thrombin and collagen induce a feedback inhibitory signaling pathway in platelets involving dissociation of the catalytic subunit of protein kinase A from an NFkappaB-IkappaB complex. *J Biol Chem* (2010) **285**(24):18352–63. doi:10.1074/jbc.M109.077602
19. Liu F, Morris S, Epps J, Carroll R. Demonstration of an activation regulated NF-kappaB/I-kappaBalpha complex in human platelets. *Thromb Res* (2002) **106**(4–5):199–203. doi:10.1016/S0049-3848(02)00130-5
20. Spinelli SL, Casey AE, Pollock SJ, Gertz JM, McMillan DH, Narasipura SD, et al. Platelets and megakaryocytes contain functional nuclear factor-kappaB. *Arterioscler Thromb Vasc Biol* (2010) **30**(3):591–8. doi:10.1161/ATVBAHA.109.197343
21. Spinelli SL, Maggirwar SB, Blumberg N, Phipps RP. Nuclear emancipation: a platelet tour de force. *Sci Signal* (2010) **3**(144):e37. doi:10.1126/scisignal.3144pe37
22. Weyrich AS, Dixon DA, Pabla R, Elstad MR, McIntyre TM, Prescott SM, et al. Signal-dependent translation of a regulatory protein, Bcl-3, in activated human platelets. *Proc Natl Acad Sci U S A* (1998) **95**(10):5556–61. doi:10.1073/pnas.95.10.5556
23. Denis MM, Tolley ND, Bunting M, Schwertz H, Jiang H, Lindemann S, et al. Escaping the nuclear confines: signal-dependent pre-mRNA splicing in anucleate platelets. *Cell* (2005) **122**(3):379–91. doi:10.1016/j.cell.2005.06.015
24. Lindemann S, Tolley ND, Dixon DA, McIntyre TM, Prescott SM, Zimmerman GA, et al. Activated platelets mediate inflammatory signaling by regulated interleukin 1beta synthesis. *J Cell Biol* (2001) **154**(3):485–90. doi:10.1083/jcb.200105058
25. Li N. Platelet-lymphocyte cross-talk. *J Leukoc Biol* (2008) **83**(5):1069–78. doi:10.1189/jlb.0907615
26. van Gils JM, Zwaginga JJ, Hordijk PL. Molecular and functional interactions among monocytes, platelets, and endothelial cells and their relevance for cardiovascular diseases. *J Leukoc Biol* (2009) **85**(2):195–204. doi:10.1189/jlb.0708400
27. Zarbock A, Polanowska-Grabowska RK, Ley K. Platelet-neutrophil-interactions: linking hemostasis and inflammation. *Blood Rev* (2007) **21**(2):99–111. doi:10.1016/j.blre.2006.06.001
28. Smyth SS, McEver RP, Weyrich AS, Morrell CN, Hoffman MR, Arepally GM, et al. Platelet functions beyond hemostasis. *J Thromb Haemost* (2009) **7**(11):1759–66. doi:10.1111/j.1538-7836.2009.03586.x
29. Leppanen A, White SP, Helin J, McEver RP, Cummings RD. Binding of glycosulfopeptides to P-selectin requires stereospecific contributions of individual tyrosine sulfate and sugar residues. *J Biol Chem* (2000) **275**(50):39569–78. doi:10.1074/jbc.M005005200
30. McEver RP, Cummings RD. Perspectives series: cell adhesion in vascular biology. Role of PSGL-1 binding to selectins in leukocyte recruitment. *J Clin Invest* (1997) **100**(3):485–91. doi:10.1172/JCI119556
31. Wang HB, Wang JT, Zhang L, Geng ZH, Xu WL, Xu T, et al. P-selectin primes leukocyte integrin activation during inflammation. *Nat Immunol* (2007) **8**(8):882–92. doi:10.1038/nature05671
32. Evangelista V, Pamuklar Z, Piccoli A, Manarini S, Dell'elba G, Pecce R, et al. Src family kinases mediate neutrophil adhesion to adherent platelets. *Blood* (2007) **109**(6):2461–9. doi:10.1182/blood-2006-06-029082
33. Diacovo TG, Roth SJ, Buccola JM, Bainton DF, Springer TA. Neutrophil rolling, arrest, and transmigration across activated, surface-adherent platelets via sequential action of P-selectin and the beta 2-integrin CD11b/CD18. *Blood* (1996) **88**(1):146–57.
34. Ghasemzadeh M, Hosseini E. Platelet-leukocyte crosstalk: linking proinflammatory responses to procoagulant state. *Thromb Res* (2013) **131**(3):191–7. doi:10.1016/j.thromres.2012.11.028

35. Rinder HM, Bonan JL, Rinder CS, Ault KA, Smith BR. Activated and unactivated platelet adhesion to monocytes and neutrophils. *Blood* (1991) **78**(7):1760–9.
36. Schulz C, Schäfer A, Stolla M, Kerstan S, Lorenz M, von Brühl ML, et al. Chemokine fractalkine mediates leukocyte recruitment to inflammatory endothelial cells in flowing whole blood: a critical role for P-selectin expressed on activated platelets. *Circulation* (2007) **116**(7):764–73. doi:10.1161/CIRCULATIONAHA.107.695189
37. Cognasse F, Hamzeh-Cognasse H, Lafarge S, Chavarin P, Cogné M, Richard Y, et al. Human platelets can activate peripheral blood B cells and increase production of immunoglobulins. *Exp Hematol* (2007) **35**(9):1376–87. doi:10.1016/j.exphem.2007.05.021
38. Henn V, Slupsky JR, Gräfe M, Anagnostopoulos I, Förster R, Müller-Berghaus G, et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. *Nature* (1998) **391**(6667):591–4. doi:10.1038/35393
39. Khan SY, Kelher MR, Heal JM, Blumberg N, Boshkov LK, Phipps R, et al. Soluble CD40 ligand accumulates in stored blood components, primes neutrophils through CD40, and is a potential cofactor in the development of transfusion-related acute lung injury. *Blood* (2006) **108**(7):2455–62. doi:10.1182/blood-2006-04-017251
40. Boulaftali Y, Hess PR, Kahn ML, Bergmeier W. Platelet immunoreceptor tyrosine-based activation motif (ITAM) signaling and vascular integrity. *Circ Res* (2014) **114**(7):1174–84. doi:10.1161/CIRCRESAHA.114.301611
41. Goerge T, Ho-Tin-Noe B, Carbo C, Benarafa C, Remold-O'Donnell E, Zhao BQ, et al. Inflammation induces hemorrhage in thrombocytopenia. *Blood* (2008) **111**(10):4958–64. doi:10.1182/blood-2007-11-123620
42. Ho-Tin-Noe B, Demers M, Wagner DD. How platelets safeguard vascular integrity. *J Thromb Haemost* (2011) **9**(Suppl 1):56–65. doi:10.1111/j.1538-7836.2011.04317.x
43. Ho-Tin-Noe B, Carbo C, Demers M, Cifuni SM, Goerge T, Wagner DD. Innate immune cells induce hemorrhage in tumors during thrombocytopenia. *Am J Pathol* (2009) **175**(4):1699–708. doi:10.2353/ajpath.2009.090460
44. Ho-Tin-Noe B, Goerge T, Wagner DD. Platelets: guardians of tumor vasculature. *Cancer Res* (2009) **69**(14):5623–6. doi:10.1158/0008-5472.CAN-09-1370
45. Cloutier N, Pare A, Farndale RW, Schumacher HR, Nigrovic PA, Lacroix S, et al. Platelets can enhance vascular permeability. *Blood* (2012) **120**(6):1334–43. doi:10.1182/blood-2012-02-413047
46. Hottz ED, Lopes JF, Freitas C, Valls-de-Souza R, Oliveira MF, Bozza MT, et al. Platelets mediate increased endothelium permeability in dengue through NLRP3-inflammasome activation. *Blood* (2013) **122**(20):3405–14. doi:10.1182/blood-2013-05-504449
47. Zapata JC, Cox D, Salvato MS. The role of platelets in the pathogenesis of viral hemorrhagic fevers. *PLoS Negl Trop Dis* (2014) **8**(6):e2858. doi:10.1371/journal.pntd.0002858
48. Risitano A, Beaulieu LM, Vitseva O, Freedman JE. Platelets and platelet-like particles mediate intercellular RNA transfer. *Blood* (2012) **119**(26):6288–95. doi:10.1182/blood-2011-12-396440
49. Massberg S, Grahl L, von Bruehl ML, Manukyan D, Pfeiler S, Goosmann C, et al. Reciprocal coupling of coagulation and innate immunity via neutrophil serine proteases. *Nat Med* (2010) **16**(8):887–96. doi:10.1038/nm.2184
50. Ruf W, Ruggeri ZM. Neutrophils release brakes of coagulation. *Nat Med* (2010) **16**(8):851–2. doi:10.1038/nm0810-851
51. Kraemer BF, Campbell RA, Schwertz H, Cody MJ, Franks Z, Tolley ND, et al. Novel anti-bacterial activities of beta-defensin 1 in human platelets: suppression of pathogen growth and signaling of neutrophil extracellular trap formation. *PLoS Pathog* (2011) **7**(11):e1002355. doi:10.1371/journal.ppat.1002355
52. Clark SR, Ma AC, Tavener SA, McDonald B, Goodarzi Z, Kelly MM, et al. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat Med* (2007) **13**(4):463–9. doi:10.1038/nm1565
53. Caudrillier A, Kessenbrock K, Gilliss BM, Nguyen JX, Marques MB, Monestier M, et al. Platelets induce neutrophil extracellular traps in transfusion-related acute lung injury. *J Clin Invest* (2012) **122**(7):2661–71. doi:10.1172/JCI61303
54. Looney MR, Nguyen JX, Hu Y, Van Ziffle JA, Lowell CA, Matthay MA. Platelet depletion and aspirin treatment protect mice in a two-event model of transfusion-related acute lung injury. *J Clin Invest* (2009) **119**(11):3450–61. doi:10.1172/JCI38432
55. Boudreau LH, Duchez AC, Cloutier N, Soulet D, Martin N, Bollinger J, et al. Platelets release mitochondria serving as substrate for bactericidal group IIA secreted phospholipase A2 to promote inflammation. *Blood* (2014) **124**(14):2173–83. doi:10.1182/blood-2014-05-573543
56. Cognasse F, Hamzeh-Cognasse H, Lafarge S, Acquart S, Chavarin P, Courbil R, et al. Donor platelets stored for at least 3 days can elicit activation marker expression by the recipient's blood mononuclear cells: an in vitro study. *Transfusion* (2009) **49**(1):91–8. doi:10.1111/j.1537-2995.2008.01931.x
57. Kostelijk EH, Fijnheer R, Nieuwenhuis HK, Gouwerok CW, de Korte D. Soluble P-selectin as parameter for platelet activation during storage. *Thromb Haemost* (1996) **76**(6):1086–9.
58. Kaufman J, Spinelli SL, Schultz E, Blumberg N, Phipps RP. Release of biologically active CD154 during collection and storage of platelet concentrates prepared for transfusion. *J Thromb Haemost* (2007) **5**(4):788–96. doi:10.1111/j.1538-7836.2007.02412.x
59. Hamzeh-Cognasse H, Cognasse F, Palle S, Chavarin P, Olivier T, Delézay O, et al. Direct contact of platelets and their released products exert different effects on human dendritic cell maturation. *BMC Immunol* (2008) **9**:54. doi:10.1186/1471-2172-9-54
60. Elzey BD, Schmidt NW, Crist SA, Kresowik TP, Harty JT, Nieswandt B, et al. Platelet-derived CD154 enables T-cell priming and protection against *Listeria monocytogenes* challenge. *Blood* (2008) **111**(7):3684–91. doi:10.1182/blood-2007-05-091728
61. André P, Prasad KS, Denis CV, He M, Papalia JM, Hynes RO, et al. CD40L stabilizes arterial thrombi by a beta3 integrin – dependent mechanism. *Nat Med* (2002) **8**(3):247–52. doi:10.1038/nm0302-247
62. Snyder EL, Hezzey A, Katz AJ, Bock J. Occurrence of the release reaction during preparation and storage of platelet concentrates. *Vox Sang* (1981) **41**(3):172–7. doi:10.1111/j.1423-0410.1981.tb01032.x
63. Berger G, Hartwell DW, Wagner DD. P-Selectin and platelet clearance. *Blood* (1998) **92**(11):4446–52.
64. Michelson AD, Barnard MR, Hechtman HB, MacGregor H, Connolly RJ, Loscalzo J, et al. In vivo tracking of platelets: circulating degranulated platelets rapidly lose surface P-selectin but continue to circulate and function. *Proc Natl Acad Sci U S A* (1996) **93**(21):11877–82. doi:10.1073/pnas.93.21.11877
65. Krishnamurti C, Maglasang P, Rothwell SW. Reduction of blood loss by infusion of human platelets in a rabbit kidney injury model. *Transfusion* (1999) **39**(9):967–74. doi:10.1046/j.1537-2995.1999.39090967.x
66. Rank A, Nieuwland R, Liebhardt S, Iberer M, Grützner S, Toth B, et al. Apheresis platelet concentrates contain platelet-derived and endothelial cell-derived microparticles. *Vox Sang* (2011) **100**(2):179–86. doi:10.1111/j.1423-0410.2010.01385.x
67. Sloand EM, Yu M, Klein HG. Comparison of random-donor platelet concentrates prepared from whole blood units and platelets prepared from single-donor apheresis collections. *Transfusion* (1996) **36**(11–12):955–9. doi:10.1046/j.1537-2995.1996.36111297091737.x
68. Boilard E, Nigrovic PA, Larabee K, Watts GF, Coblyn JS, Weinblatt ME, et al. Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science* (2010) **327**(5965):580–3. doi:10.1126/science.1181928
69. Huo Y, Schober A, Forlow SB, Smith DF, Hyman MC, Jung S, et al. Circulating activated platelets exacerbate atherosclerosis in mice deficient in apolipoprotein E. *Nat Med* (2003) **9**(1):61–7. doi:10.1038/nm810
70. Massberg S, Brand K, Grüner S, Page S, Müller E, Müller I, et al. A critical role of platelet adhesion in the initiation of atherosclerotic lesion formation. *J Exp Med* (2002) **196**(7):887–96. doi:10.1084/jem.20012044
71. Nomura S, Suzuki M, Katsura K, Xie GL, Miyazaki Y, Miyake T, et al. Platelet-derived microparticles may influence the development of atherosclerosis in diabetes mellitus. *Atherosclerosis* (1995) **116**(2):235–40. doi:10.1016/0021-9150(95)05551-7
72. Mause SF, von Hundelshausen P, Zernecke A, Koenen RR, Weber C. Platelet microparticles: a transcellular delivery system for RANTES promoting monocyte recruitment on endothelium. *Arterioscler Thromb Vasc Biol* (2005) **25**(7):1512–8. doi:10.1161/01.ATV.0000170133.43608.37
73. Brown GT, McIntyre TM. Lipopolysaccharide signaling without a nucleus: kinase cascades stimulate platelet shedding of proinflammatory IL-1beta-rich microparticles. *J Immunol* (2011) **186**(9):5489–96. doi:10.4049/jimmunol.1001623
74. Ray DM, Spinelli SL, Pollock SJ, Murant TI, O'Brien JJ, Blumberg N, et al. Peroxisome proliferator-activated receptor gamma and retinoid X receptor transcription factors are released from activated human platelets and shed in

microparticles. *Thromb Haemost* (2008) **99**(1):86–95. doi:10.1160/TH07-05-0328
75. Elzey BD, Ratliff TL, Sowa JM, Crist SA. Platelet CD40L at the interface of adaptive immunity. *Thromb Res* (2011) **127**(3):180–3. doi:10.1016/j.thromres.2010.10.011
76. Sprague DL, Elzey BD, Crist SA, Waldschmidt TJ, Jensen RJ, Ratliff TL. Platelet-mediated modulation of adaptive immunity: unique delivery of CD154 signal by platelet-derived membrane vesicles. *Blood* (2008) **111**(10):5028–36. doi:10.1182/blood-2007-06-097410
77. Assinger A, Kral JB, Yaiw KC, Schrottmaier WC, Kurzejamska E, Wang Y, et al. Human cytomegalovirus-platelet interaction triggers toll-like receptor 2-dependent proinflammatory and proangiogenic responses. *Arterioscler Thromb Vasc Biol* (2014) **34**(4):801–9. doi:10.1161/ATVBAHA.114.303287
78. Sahler J, Woeller C, Spinelli S, Blumberg N, Phipps R. A novel method for overexpression of peroxisome proliferator-activated receptor-gamma in megakaryocyte and platelet microparticles achieves transcellular signaling. *J Thromb Haemost* (2012) **10**(12):2563–72. doi:10.1111/jth.12017
79. Blajchman MA, Bardossy L, Carmen RA, Goldman M, Heddle NM, Singal DP. An animal model of allogeneic donor platelet refractoriness: the effect of the time of leukodepletion. *Blood* (1992) **79**(5):1371–5.
80. Cholette JM, Henrichs KF, Alfieris GM, Powers KS, Phipps R, Spinelli SL, et al. Washing red blood cells and platelets transfused in cardiac surgery reduces postoperative inflammation and number of transfusions: results of a prospective, randomized, controlled clinical trial. *Pediatr Crit Care Med* (2012) **13**(3):290–9. doi:10.1097/PCC.0b013e31822f173c
81. Heddle NM, Klama LN, Griffith L, Roberts R, Shukla G, Kelton JG. A prospective study to identify the risk factors associated with acute reactions to platelet and red cell transfusions. *Transfusion* (1993) **33**(10):794–7. doi:10.1046/j.1537-2995.1993.331094054613.x
82. Phipps RP, Kaufman J, Blumberg N. Platelet derived CD154 (CD40 ligand) and febrile responses to transfusion. *Lancet* (2001) **357**(9273):2023–4. doi:10.1016/S0140-6736(00)05108-4
83. Heddle NM, Klama L, Singer J, Richards C, Fedak P, Walker I, et al. The role of the plasma from platelet concentrates in transfusion reactions. *N Engl J Med* (1994) **331**(10):625–8. doi:10.1056/NEJM199409083311001
84. Hamzeh-Cognasse H, Damien P, Nguyen KA, Arthaud CA, Eyraud MA, Chavarin P, et al. Immune-reactive soluble OX40 ligand, soluble CD40 ligand, and interleukin-27 are simultaneously oversecreted in platelet components associated with acute transfusion reactions. *Transfusion* (2014) **54**(3):613–25. doi:10.1111/trf.12378
85. Hetland G, Mollnes TE, Bergh K, Hogasen K, Bergerud UE, Solheim BG. Effect of filtration and storage of platelet concentrates on the production of the chemotaxins C5a, interleukin 8, tumor necrosis factor alpha, and leukotriene B4. *Transfusion* (1998) **38**(1):16–23. doi:10.1046/j.1537-2995.1998.38198141493.x
86. Wang RR, Triulzi DJ, Qu L. Effects of prestorage vs poststorage leukoreduction on the rate of febrile nonhemolytic transfusion reactions to platelets. *Am J Clin Pathol* (2012) **138**(2):255–9. doi:10.1309/AJCP5H7EKZTGGBKZ
87. Vo TD, Cowles J, Heal JM, Blumberg N. Platelet washing to prevent recurrent febrile reactions to leucocyte-reduced transfusions. *Transfus Med* (2001) **11**(1):45–7. doi:10.1046/j.1365-3148.2001.00280.x
88. Cognasse F, Boussoulade F, Chavarin P, Acquart S, Fabrigli P, Lamy B, et al. Release of potential immunomodulatory factors during platelet storage. *Transfusion* (2006) **46**(7):1184–9. doi:10.1111/j.1537-2995.2006.00869.x
89. Kunz D, Luley C, Heim MU, Bock M. Transforming growth factor beta is increased in plasma of patients with hematologic malignancies after transfusion of platelet concentrates. *Transfusion* (1998) **38**(2):156–9. doi:10.1046/j.1537-2995.1998.38298193097.x
90. Kanter J, Khan SY, Kelher M, Gore L, Silliman CC. Oncogenic and angiogenic growth factors accumulate during routine storage of apheresis platelet concentrates. *Clin Cancer Res* (2008) **14**(12):3942–7. doi:10.1158/1078-0432.CCR-07-4824
91. Mohle R, Green D, Moore MA, Nachman RL, Rafii S. Constitutive production and thrombin-induced release of vascular endothelial growth factor by human megakaryocytes and platelets. *Proc Natl Acad Sci U S A* (1997) **94**(2):663–8. doi:10.1073/pnas.94.2.663
92. Fong KP, Barry C, Tran AN, Traxler EA, Wannemacher KM, Tang HY, et al. Deciphering the human platelet sheddome. *Blood* (2011) **117**(1):e15–26. doi:10.1182/blood-2010-05-283838
93. Springer DL, Miller JH, Spinelli SL, Pasa-Tolic L, Purvine SO, Daly DS, et al. Platelet proteome changes associated with diabetes and during platelet storage for transfusion. *J Proteome Res* (2009) **8**(5):2261–72. doi:10.1021/pr800885j
94. Yazer MH, Triulzi DJ, Shaz B, Kraus T, Zimring JC. Does a febrile reaction to platelets predispose recipients to red blood cell alloimmunization? *Transfusion* (2009) **49**(6):1070–5. doi:10.1111/j.1537-2995.2009.02116.x
95. Hendrickson JE, Desmarets M, Deshpande SS, Chadwick TE, Hillyer CD, Roback JD, et al. Recipient inflammation affects the frequency and magnitude of immunization to transfused red blood cells. *Transfusion* (2006) **46**(9):1526–36. doi:10.1111/j.1537-2995.2006.00946.x
96. Hendrickson JE, Chadwick TE, Roback JD, Hillyer CD, Zimring JC. Inflammation enhances consumption and presentation of transfused RBC antigens by dendritic cells. *Blood* (2007) **110**(7):2736–43. doi:10.1182/blood-2007-03-083105
97. Heal JM, Phipps RP, Blumberg N. One big unhappy family: transfusion alloimmunization, thrombosis, and immune modulation/inflammation. *Transfusion* (2009) **49**(6):1032–6. doi:10.1111/j.1537-2995.2009.02182.x
98. Opelz G, Terasaki PI. Poor kidney-transplant survival in recipients with frozen-blood transfusions or no transfusions. *Lancet* (1974) **2**(7882):696–8. doi:10.1016/S0140-6736(74)93268-1
99. Burrows L, Tartter P, Aufses A. Increased recurrence rates in perioperatively transfused colorectal malignancy patients. *Cancer Detect Prev* (1987) **10**(5–6):361–9.
100. Blumberg N, Agarwal MM, Chuang C. Relation between recurrence of cancer of the colon and blood transfusion. *Br Med J* (1985) **290**(6474):1037–9. doi:10.1136/bmj.290.6474.1037
101. Burrows L, Tartter P. Effect of blood transfusions on colonic malignancy recurrent rate. *Lancet* (1982) **2**(8299):662. doi:10.1016/S0140-6736(82)92764-7
102. Tartter PI, Quintero S, Barron DM. Perioperative blood transfusion associated with infectious complications after colorectal cancer operations. *Am J Surg* (1986) **152**(5):479–82. doi:10.1016/0002-9610(86)90207-2
103. Babcock GF, Alexander JW. The effects of blood transfusion on cytokine production by TH1 and TH2 lymphocytes in the mouse. *Transplantation* (1996) **61**(3):465–8. doi:10.1097/00007890-199602150-00026
104. Kirkley SA, Cowles J, Pellegrini VD Jr, Harris CM, Boyd AD, Blumberg N. Cytokine secretion after allogeneic or autologous blood transfusion. *Lancet* (1995) **345**(8948):527. doi:10.1016/S0140-6736(95)90627-4
105. Blumberg N, Phipps RP, Kaufman J, Heal JM. The causes and treatment of reactions to platelet transfusions. *Transfusion* (2003) **43**(2):291–2. doi:10.1046/j.1537-2995.2003.t01-2-00362.x
106. Blumberg N, Gettings KF, Turner C, Heal JM, Phipps RP. An association of soluble CD40 ligand (CD154) with adverse reactions to platelet transfusions. *Transfusion* (2006) **46**(10):1813–21. doi:10.1111/j.1537-2995.2006.00979.x
107. Blumberg N, Heal JM, Liesveld JL, Phillips GL, Rowe JM. Platelet transfusion and survival in adults with acute leukemia. *Leukemia* (2008) **22**(3):631–5. doi:10.1038/sj.leu.2404920
108. Blumberg N, Heal JM, Rowe JM. A randomized trial of washed red blood cell and platelet transfusions in adult acute leukemia [ISRCTN76536440]. *BMC Blood Disord* (2004) **4**(1):6. doi:10.1186/1471-2326-4-6
109. Thomas GM, Carbo C, Curtis BR, Martinod K, Mazo IB, Schatzberg D, et al. Extracellular DNA traps are associated with the pathogenesis of TRALI in humans and mice. *Blood* (2012) **119**(26):6335–43. doi:10.1182/blood-2012-01-405183
110. Gurevitch J, Nelken D. ABO groups in blood platelets. *Nature* (1954) **173**(4399):356. doi:10.1038/173356a0
111. Santoso S, Kiefel V, Mueller-Eckhardt C. Blood group A and B determinants are expressed on platelet glycoproteins IIa, IIIa, and Ib. *Thromb Haemost* (1991) **65**(2):196–201.
112. Ogasawara K, Ueki J, Takenaka M, Furihata K. Study on the expression of ABH antigens on platelets. *Blood* (1993) **82**(3):993–9.
113. Aster RH. Effect of anticoagulant and ABO incompatibility on recovery of transfused human platelets. *Blood* (1965) **26**(6):732–43.
114. Pavenski K, Warkentin TE, Shen H, Liu Y, Heddle NM. Posttransfusion platelet count increments after ABO-compatible versus ABO-incompatible platelet transfusions in noncancer patients: an observational study. *Transfusion* (2010) **50**(7):1552–60. doi:10.1111/j.1537-2995.2010.02602.x
115. Slichter SJ, Davis K, Enright H, Braine H, Gernsheimer T, Kao KJ, et al. Factors affecting posttransfusion platelet increments, platelet refractoriness,

and platelet transfusion intervals in thrombocytopenic patients. *Blood* (2005) **105**(10):4106–14. doi:10.1182/blood-2003-08-2724
116. Heal JM, Rowe JM, McMican A, Masel D, Finke C, Blumberg N. The role of ABO matching in platelet transfusion. *Eur J Haematol* (1993) **50**(2):110–7. doi:10.1111/j.1600-0609.1993.tb00150.x
117. Refaai MA, Carter J, Henrichs KF, Davidson DC, Pollock SJ, Casey AE, et al. Alterations of platelet function and clot formation kinetics after in vitro exposure to anti-A and -B. *Transfusion* (2013) **53**(2):382–93. doi:10.1111/j.1537-2995.2012.03718.x
118. Heal JM, Masel D, Blumberg N. Interaction of platelet fc and complement receptors with circulating immune complexes involving the AB0 system. *Vox Sang* (1996) **71**(4):205–11. doi:10.1046/j.1423-0410.1996.7140205.x
119. Blumberg N, Heal JM, Hicks GL Jr, Risher WH. Association of ABO-mismatched platelet transfusions with morbidity and mortality in cardiac surgery. *Transfusion* (2001) **41**(6):790–3. doi:10.1046/j.1537-2995.2001.41060790.x
120. Refaai MA, Fialkow LB, Heal JM, Henrichs KF, Spinelli SL, Phipps RP, et al. An association of ABO non-identical platelet and cryoprecipitate transfusions with altered red cell transfusion needs in surgical patients. *Vox Sang* (2011) **101**(1):55–60. doi:10.1111/j.1423-0410.2010.01464.x
121. Henrichs KF, Howk N, Masel DS, Thayer M, Refaai MA, Kirkley SA, et al. Providing ABO-identical platelets and cryoprecipitate to (almost) all patients: approach, logistics, and associated decreases in transfusion reaction and red blood cell alloimmunization incidence. *Transfusion* (2012) **52**(3):635–40. doi:10.1111/j.1537-2995.2011.03329.x
122. Levin MD, de Veld JC, van der Holt B, van 't Veer MB. Immune and nonimmune causes of low recovery from leukodepleted platelet transfusions: a prospective study. *Ann Hematol* (2003) **82**(6):357–62. doi:10.1007/s00277-003-0648-7
123. Seftel MD, Growe GH, Petraszko T, Benny WB, Le A, Lee CY, et al. Universal prestorage leukoreduction in Canada decreases platelet alloimmunization and refractoriness. *Blood* (2004) **103**(1):333–9. doi:10.1182/blood-2003-03-0940
124. Petz LD, Garratty G, Calhoun L, Clark BD, Terasaki PI, Gresens C, et al. Selecting donors of platelets for refractory patients on the basis of HLA antibody specificity. *Transfusion* (2000) **40**(12):1446–56. doi:10.1046/j.1537-2995.2000.40121446.x
125. Khorana AA, Francis CW, Blumberg N, Culakova E, Refaai MA, Lyman GH. Blood transfusions, thrombosis, and mortality in hospitalized patients with cancer. *Arch Intern Med* (2008) **168**(21):2377–81. doi:10.1001/archinte.168.21.2377
126. Tan TW, Farber A, Hamburg NM, Eberhardt RT, Rybin D, Doros G, et al. Blood transfusion for lower extremity bypass is associated with increased wound infection and graft thrombosis. *J Am Coll Surg* (2013) **216**(5):1005–14.e2. doi:10.1016/j.jamcollsurg.2013.01.006
127. Tollefson MK, Karnes RJ, Rangel L, Carlson R, Boorjian SA. Blood type, lymphadenectomy and blood transfusion predict venous thromboembolic events following radical prostatectomy with pelvic lymphadenectomy. *J Urol* (2014) **191**(3):646–51. doi:10.1016/j.juro.2013.10.062
128. Blumberg N, Spinelli SL, Francis CW, Taubman MB, Phipps RP. The platelet as an immune cell-CD40 ligand and transfusion immunomodulation. *Immunol Res* (2009) **45**(2–3):251–60. doi:10.1007/s12026-009-8106-9
129. Prasad KS, Andre P, Yan Y, Phillips DR. The platelet CD40L/GP IIb-IIIa axis in atherothrombotic disease. *Curr Opin Hematol* (2003) **10**(5):356–61. doi:10.1097/00062752-200309000-00006
130. Nomura S, Okamae F, Abe M, Hosokawa M, Yamaoka M, Ohtani T, et al. Platelets expressing P-selectin and platelet-derived microparticles in stored platelet concentrates bind to PSGL-1 on filtrated leukocytes. *Clin Appl Thromb Hemost* (2000) **6**(4):213–21. doi:10.1177/107602960000600406
131. Siljander P, Carpen O, Lassila R. Platelet-derived microparticles associate with fibrin during thrombosis. *Blood* (1996) **87**(11):4651–63.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 03 November 2014; accepted: 14 January 2015; published online: 02 February 2015.
*Citation: Stolla M, Refaai MA, Heal JM, Spinelli SL, Garraud O, Phipps RP and Blumberg N (2015) Platelet transfusion – the new immunology of an old therapy. Front. Immunol. **6**:28. doi: 10.3389/fimmu.2015.00028*
This article was submitted to Inflammation, a section of the journal Frontiers in Immunology.