CRITICAL CARE

REVIEW

Bench-to-bedside review: Platelets and active immune functions - new clues for immunopathology?

Olivier Garraud[1,2,*], Hind Hamzeh-Cognasse[1], Bruno Pozzetto[1], Jean-Marc Cavaillon[3] and Fabrice Cognasse[1,2]

Abstract

Platelets display a number of properties besides the crucial function of repairing damaged vascular endothelium and stopping bleeding; these are exploited to benefit patients receiving platelet component transfusions, which might categorize them as innate immune cells. For example, platelets specialize in pro-inflammatory activities, and can secrete a large number of molecules, many of which display biological response modifier functions. Platelets also express receptors for non-self-infectious and possibly non-infectious danger signals, and can engage infectious pathogens by mechanisms barely explained beyond observation. This relationship with infectious pathogens may involve other innate immune cells, especially neutrophils. The sophisticated interplay of platelets with bacteria may culminate in sepsis, a severe pathology characterized by significant reductions in platelet count and platelet dysfunction. How this occurs is still not fully understood. Recent findings from in-depth platelet signaling studies reveal the complexity of platelets and some of the ways they evolve along the immune continuum, from beneficial functions exemplified in endothelium repair to deleterious immunopathology as in systemic inflammatory response syndrome and acute vascular diseases. This review discusses the extended role of platelets as immune cells to emphasize their interactions with infectious pathogens sensed as potentially dangerous.

*Correspondence: Olivier.garraud@efs.sante.fr
[2]EFS Auvergne-Loire and GIMAP-EA 3064, Université de Saint-Etienne, Faculté de Médecine, 15 rue Ambroise Paré, 42023 Saint-Etienne cedex 2, France
Full list of author information is available at the end of the article

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Introduction

Besides being essential to hemostasis, blood platelets have been recently shown to be able to bind infectious agents or engulf them. Clinical observations suggest that thrombocytopenia usually worsens during infection, implying both central and peripheral causes. Platelets constitutively express molecules that are classically acknowledged to function in primary hemostasis. Platelet-associated molecules comprise more than 1,000 proteins: 69 are membrane proteins [1]. Platelets are highly active in shedding their surface molecules [1]. Besides their seminal role in hemostasis, and because they display molecules otherwise known to intervene in immune responses, platelets have been recently shown to actively - and not only passively - participate in immunity.

The immune system is classically viewed as a duality of innate and adaptive immunity, and despite their unique functions they share common intricate properties. The definition of what is unique in innate immunity is imprecise, and may be approached by opposition to what is unique to adaptive immunity. Generally, innate immune cells express invariant receptors, as opposed to adaptive immune cells, which express rearranged receptors [2]. Myeloid originating cells - a family to which megakaryocytes belong - express several receptor families with various functions and platelets have inherited many properties of their ancestry regarding expression of such receptors. Indeed, they can: i) express non-mutated ligands that bind pathogens or pathogen-derived structures; ii) express invariant receptors that ease the ingestion of a variety of pathogens by different mechanisms to destroy them or inhibit pathogenicity; iii) secrete a wide array of molecules to communicate with other cells in the microenvironment or at distance to mediate local actions such as inflammation; iv) respond to signaling molecules to activate machinery in a polarized manner, either locally or at a distance to fight danger or to repair tissue damage. Expression of all four properties characterizes so-called innate immune cells from non-immune cells, such as endothelial and epithelial cells, that can exert partial immune responses.

Until recently, platelets would have been considered as non-immune cells with some immune functions.

Platelets express non-mutated receptors that bind pathogens and pathogen-derived structures, which in turn activate them

Pathogen sensors and ligands on and in platelets

In the mid-1970s, it was shown that platelets could bind lipopolysaccharides (LPSs) using a lipid A moiety [3,4]. Further, pathogen recognition receptors (PRRs) other than classical receptors (CRs and FcRs) were discovered intracellularly and on the surface of human and mouse platelets in 2004/2005. Significantly, most Toll-like receptor (TLR) family members are expressed on and/or in platelets [5-8]. Thus, it is likely that other PRR protein families will also be identified.

Functional PRRs (CRs, FcRs for Igs, TLRs and possibly other receptors) [6] allow platelets to bind foreign microbial invaders and products derived from microbes. This allows platelets to participate in danger sensing, which is typical of innate cells. LPSs from Gram-negative bacteria that bind cell surface MD2/TLR4 complexes and bacterial lipopeptides (or synthetic tri- or diacylpeptides, Pam3CSK4 and Pam2CSK4), which are classical ligands for TLR2 in dimeric association with TLR1 or TLR6, are widely used to investigate physio-pathological interactions of pathogen-associated molecular patterns (PAMPs; infectious danger signatures).

The ligation of pathogen-derived moieties to platelet sensors leads to platelet activation

LPS-TLR4/MD2 binding is not passive because it is accompanied by rearrangement of other platelet surface molecules, or their membrane expression from the cytosol, such as CD40-ligand (CD40L), a prominent platelet molecule secreted in large quantities upon appropriate stimulation [9]. Whether LPS directly induces platelet activation is still controversial. However, ligation of LPS to human platelet-expressed TLR4 might activate platelets and mobilize other membrane and cytoplasmic molecules. Previously, we demonstrated that LPS from various strains of Gram-negative bacteria could bind TLR4 on human platelets and elicit soluble CD40L secretion dose-dependently, suggesting an adaptive function of platelets within the environment [9]. The consistent failure of several leading groups in the platelet biology field to find evidence of direct and substantial platelet activation after LPS-TLR4/MD2 binding, in particular when testing mouse cells [6], implies that intermediate steps are required. Such intermediate steps for human platelets may include the need for absorption, from plasma, of soluble CD14 on the platelet surface, which is essential in human systems [9]. Studies on direct contacts between platelets and Gram-negative bacteria that display LPS [6,10,11] indicate that platelets can be activated differentially depending on the precise stimulus applied, and that platelets - following their activation - secrete elective cytokine profiles [12]; despite the fact that this evidence is experimental, and may barely be extrapolated to real life conditions, it seems rather strong [13].

Further elucidation of these processes is required to make firm conclusions regarding this controversial issue, and consistent proof of this assumption may be extremely relevant in clinics because of the disputed role of platelets in sepsis and questions regarding therapeutic intervention in life-threatening sepsis patients.

Platelet sensors of infectious moieties and thrombosis

TLR2 is a functional receptor in platelets, as revealed by experimental ligation of the synthetic ligand Pam3CSK4. Subsequent stimulation of TLR2 by Pam3CSK4 and protease-activated receptor 1 (PAR1), an inducer of platelet secretion and aggregation, induced NF-κB phosphorylation within the platelet inflammasome and differential platelet cytokine and serotonin release [14] (Damien P, Cognasse F, Payrastre B, Spinelli S, Blumberg N, Phipps R, McNichol A, Pozzettto B, Garraud O, Hamzeh-Cognasse H, submitted). Recently, Risitano and colleagues [15] demonstrated *in vitro* and *in vivo* in a murine model the visual and functional transfer of RNA associated with platelet-like particles from wild-type to TLR2-deficient mice. However, laboratory findings obtained from *ex vivo* experiments or *in vivo* mouse models must be extrapolated to human pathophysiological situations.

In addition to TLR4 and TLR2, TLR9, an intracellular pathogen sensor, is consistently described in association with mouse and human platelets. Panigrahi and colleagues [16] recently demonstrated that platelet TLR9 was functional and linked oxidative stress, innate immunity, and thrombosis. Simultaneously, Thon and colleagues [17] demonstrated TLR9 was stored in a newly defined intracellular compartment in platelets, remarkably extending the basic knowledge of platelet physiology, and helping define a novel mechanism of platelet TLR9 organization and signaling.

How do platelets fight danger? The microbicidal potency of platelets

Platelets activate cellular machinery to sense and fight danger by using sensors in the form of TLRs, among other PRRs (Table 1). Further, platelets have differential functional programs depending on the bacterial and viral danger they face and can adapt their response to the nature and extent of the stress/danger by favoring specific cytokine secretion [18]. This can be considered characteristic of innate immune cells [6,19,20].

Table 1. Proposed exogenous and endogenous ligands for principal Toll-like receptors

Receptor	Synthetic ligands	Natural ligands	Ligand origin
TLR1-2	Pam3CSK4	Triacyl lipopeptides	Bacteria and mycobacteria
TLR2		Lipoprotein/lipopeptide	Various pathogens
		Peptidoglycan	Gram-positive bacteria
		Lipoteichoic acid	Gram-positive bacteria
		Lipoarabinomannan	Mycobacteria
		Phenol-soluble modulin	*Staphylococcus epidermidis*
		Glycoinositol phospholipids	*Trypanosoma cruzi*
		Glycolipids	*Treponema maltophilia*
		Porins	*Neisseria*
		Atypical lipopolysaccharide	*Leptospira interrogans*
		Atypical lipopolysaccharide	*Porphyromonas gingivalis*
		Zymosan	Fungi
		HSP 70	Host
		Necrotic or dying cells	Host
		Versican	Host
		Antiphospholipid antibodies	Host
		Amyloids	Host
TLR2-6	FSL1, MALP2, Pam2CSK4	Diacyl lipopeptides	Mycoplasma
TLR2-4		HMGB1	Host
		Biglycan	Host
TLR4		Lipopolysaccharide	Gram-negative bacteria
		Taxol	Plants
		Fusion protein	Respiratory syncytial virus
		Envelope protein	Mouse mammary-tumor virus
		?	*Chlamydia pneumoniae*
		HSP 60	Host
		HSP 70	Host
		Type III repeat extra domain A of fibronectin	Host
		Oligosaccharides of hyaluronic acid	Host
		Polysaccharide fragments of heparan sulfate	Host
		Fibrinogen	Host
		Necrotic or dying cells	Host
		MRP-8/14	Host
		F-EDA	Host
		Hyaluronan	Host
		Tenascin-C	Host
		mmLDL	Host
		OxPAPC	Host
		OxLDL	Host
		AGE-LDL	Host
		Surfactant protein A	Host
		b-Defensin	Host
TLR6		Diacyl lipopeptides	*Mycoplasma*
		Lipoteichoic acid	Gram-positive bacteria
		Zymosan	Fungi
TLR9	CpG-A, CpG-B, CpG-C ODNs	CpG DNA	Bacteria

AGE-LDL, advanced glycation end-product of low-density-lipoprotein; F-EDA, extra domain A of fibronectin; HSP, heat-shock protein; mmLDL, minimally modified low density lipoprotein; MRP, myeloid-related protein; OxLDL, oxidized low-density lipoprotein; OxPAPC, oxidation products of 1-palmitoyl-2-arachidonoyl-sn-glycerol-2-phosphatidylcholine; ?, unknown.

Of particular note, platelets exert direct microbicidal activity and they can also secrete microbicidal peptides/proteins, such as thrombocidin-1, thrombocidin-2, platelet microbicidal protein-1 (PMP-1) and β-defensin 1, among others [10]. Platelets may contribute indirectly to microbe destruction by transporting surface-bound immune complexes or complement fractions or Igs with antibody activity. Moreover, platelets facilitate the

antimicrobial activity of neutrophils [20] and macrophages [6]. Antimicrobial peptides and proteins can also aid or complement mechanisms used by platelets to eradicate infectious pathogens. For instance, *Staphylococcus aureus* uses two clumping factors (Clf A and Clf B), two fibronectin-binding proteins (Fnbp A and Fnbp B), protein A, and SSL5, and it binds antibody and complement. Consequently, this bacterium is well equipped to interact with platelets under both static and shear conditions and to trigger platelet activation as a result [11].

Expression of PRRs - in particular TLRs - on and in platelets is still puzzling: a path for a clue?

The presence of TLRs - and possibly of other danger sensors - on and in platelets is indeed intriguing, as those PRRs, despite their capacity of linking a variety of PAMPs, are paramount in sensing blood borne infectious pathogens, such as bacteria and viruses. The relationship between bacteria and platelets is extremely complex; platelets secrete antibacterial products but bacterial invasion and sepsis in turn severely impair platelet production, availability and functioning. However, platelets - which are by definition essentially circulating cells - are not aimed at being sentinels of immunity as may be immature dendritic cells, for example, positioned at entry points of pathogens into the organism; why are platelets thus equipped with such a well formed apparatus for sensing pathogens [22]? Perhaps an interesting path resides in the relationship of platelets with bacteria in the oral cavity. Of interesting note, autologous platelets are now used in surgery wounds by many dentists in order to prevent excess bleeding during dental avulsion, and dentists report decreased levels of infection (A Nurden, personal communication). On the one hand, platelets in breached oral mucosae and on exposed vascular endothelia are exquisitely reactive to bacteria sensing as we (and others [11,23]) have reported. On the other hand, as bacteria are extremely frequent in the oral cavity and can inflict severe damage to organs in cases of dental-originating bacteriemia [24,25], one can wonder as to whether this interaction between platelets and bacteria in the oral cavity is truly beneficial to the host; this question remains to be investigated.

Platelets can ingest a variety of pathogens and interact with phagocytic cells

Platelets and infectious pathogens inside

Early observations revealed that platelets can harbor infectious pathogens (viruses [26], bacteria [10], and parasites [27]) on their surface, using CRs, FcRs for Igs and TLRs [19,25,28]. Other receptors bind HIV (CLEC-2, CXCR4 and DC-Sign), HCV (GPVI), and Coxsackie adenovirus [11,26]. Furthermore, platelets can engulf infectious pathogens: HIV [26] and bacteria [11] have been found inside platelets [26,29]. Even passive pathogen entry is now acknowledged as necessitating molecular and mutual interactions. Phagocytosis of antibody-opsonized particles is primarily mediated by the actin-based cytoskeleton and is a dynamic process involving a complex mix of proteins, including actin, Arp2/3 complex, Rho-family GTPases, filament-capping proteins, tropomyosin, Rho kinase, and myosin II [30].

Hypotheses on infectious pathogen entry inside platelets

The precise mechanism(s) of pathogen engulfment within platelets is not yet known, but may be non-classical phagocytosis as platelets do not contain a complete phagosome ultrastructure, although they possess important and crucial factors such as *syk* [30] and extracellular FcγRIIa, a molecule with an intracellular immunoreceptor tyrosine-based activation motif (ITAM) that is important for immune complex clearance [31]. Platelets can increase phagocytosis of periodontal pathogens by neutrophils, and may directly engulf bacteria such as *Porphyromonas gingivalis* and *S. aureus* [29]. While challenging for microbiologists, this discovery is extremely stimulating for cell biologists, as it highlights that a mechanism of entry is involved that may be selective, since not all types of virus or bacterium have been observed within platelets.

Therefore, it is important to study further the infectivity of pathogens within platelets. On the one hand, platelet engulfment of pathogens may not result in bacterial killing, because platelet vacuoles lack structures for killing; on the other hand, however, engulfment may succeed in bacterial killing since - upon activation - platelets can release β-defensins that have an antimicrobial effect. Last, engulfment of bacteria by platelets may enable pathogens to escape immune surveillance.

Platelet and engulfment of infectious pathogens: what next?

The destiny of engulfed microbes within platelets remains unclear: it may be different depending on the specific pathogen or the microbe's intrinsic properties, such as infectivity. When a microbe is ingested by a phagocytic cell, there are several possible outcomes for the microbe, the four main possibilities being: i) it is destroyed or lysed; ii) it is embedded within the structure like in a sanctuary, where it is hidden or protected from external defense elements; iii) it is not destroyed but to the contrary develops, with two sub-options - its development is limited to the host cell containment capacity or it exceeds the host cell capacity and bursts out, freeing the newly formed infectious pathogens that are ready to carry on invading new host cells and so on (until a negative signal for multiplication is given); iv) it behaves as a Trojan horse, modifying the host cell in such a

manner that it acts as a carrier to transport the invader to immune sites or other compartments [10,11,24,25,28, 32-34]. None of these options has been demonstrated thus far in platelets infected by parasites, bacteria or viruses. The presence of infectious pathogens within platelets thus remains a mystery; no role for propagating viruses such as HIV has been identified so far, while platelets and HIV clearly have mutual interactions and platelets participate in HIV-induced pathology [35]. Thus, *stricto sensu*, platelets do not appear to be fully competent for phagocytosis and their trapping of infectious pathogens remains unsolved.

Platelet assistance to neutrophils: neutrophil extracellular traps

Platelets can aid phagocytosis by professional leukocytes. Interestingly, platelets can help the formation of neutrophil extracellular traps (NETs) that link inflammation and thrombosis (NETs contribute to thrombus formation by interacting with platelets) [36]. Clark and colleagues [37] described a mechanism of platelet-neutrophil interactions leading to improved bacterial trapping, in which activated platelets adhered to immobilized neutrophils in a model of endotoxemia and sepsis. The cell-cell mechanism involved may depend upon the properties of platelets because platelet cell fixation modified platelet-neutrophil interactions. While some groups hypothesized that this type of platelet-neutrophil interaction depended in part on LPS-induced platelet activation via platelet TLR4 expression [36,37], others claimed no evidence of direct TLR4 platelet activation [7,8], in contrast to TLR2 [38] or TLR9 [16,17] platelet activation. NETs catch platelets and maintain their aggregation, indicating they are substrates for platelet adhesion and that they provide stimuli for platelet activation. Platelets may bind to NETs directly or indirectly. Purified histones associate with the platelet surface *in vitro*, particularly via TLRs [39,40]. Platelets also bind double- and single-stranded DNA *in vitro* [39]. Interactions of NETs with platelets may lead to a vicious loop of NET formation and platelet activation, because platelets, if pre-stimulated with LPS or collagen, induce NET release by neutrophils [37]. Despite controversy regarding the role of platelet TLR4/MD2 activation by LPS, bacterial residues might induce the binding of platelets to adherent neutrophils in pulmonary sinusoids, and cause sustained neutrophil activation and NET formation. Indeed, LPS, even at high concentrations, is unable to induce NET formation directly from neutrophils, suggesting that platelets are necessary for rapid LPS-induced neutrophil NET formation. In addition, several investigators have demonstrated that neutrophil-induced NET formation can occur in response to LPS and other agonists in the absence of platelets [41]. NETs contain proteolytic activity that can trap and kill microbes in tissues [37]. Furthermore, platelets and polymorphonuclear leukocytes interact in dynamic conditions. Formation of close interactions between neutrophils and platelets, a condition necessary for active phagocytosis, is expanded under inflammatory conditions, in which platelets participate substantially [21]. Formation of a synapse between neutrophils and platelets, a requirement for effective phagocytosis, is magnified under inflammatory conditions, where platelets undergo substantial and persistent activation [42].

Platelets secrete molecules that regulate inflammation

Platelet association with a panoply of biological response modifiers

Platelets are characterized in part by their association with a large number of molecules (Table 2), of which more than 300 are secreted. However, these data should be interpreted with caution, as platelet molecules may originate from three sources: i) inherited from lineage cells (megakaryocyte); ii) absorbed from the environment; iii) secreted *de novo* (a recently identified puzzling issue that is unexpected in non-nucleated cells devoid of nucleic acid and DNA, except mitochondrial DNA and RNA absorbed from lysed cells such as tumor cells) [6,19]. Consequently, the number of molecules of platelet origin and the repertoire of proteins present on platelet membranes is debatable. Activation processes allow the recruitment of molecules onto the cell surface (P-selectin or CD62P is initially located intracellularly within the α granule) [43]. Conversely, many molecules are internalized during activation or shed in membrane-derived microparticles (MPs). Thus, during activation there is a change in platelet phenotype with the appearance of novel molecules and disappearance of others, some changes being transient and others permanent. During activation, proteins/glycoproteins can be proteolytically cleaved from the cell surface. However, this shedding process is distinct from secretion, resulting from mobilization of molecules from storage granules with or without transient expression at the surface (as for sCD40L and sCD62P).

The secretion activity of platelets

In the circulation, each platelet contains about 35 α granules and 5 dense bodies. α Granules enclose an array of immunomodulatory soluble factors, comprising important chemokines, such as platelet factor-4 (PF4; CXCL4), β-thromboglobulin (β-TG; an isoform of CXCL7), regulated upon activation normal T cell expressed and secreted (RANTES; CCL5), and macrophage inflammatory protein-1-α (MIP-1α; CCL3) (Table 2). All these molecules have major roles in many fields of innate immunity.

Table 2. Diversity of proposed platelet-associated molecules with known immune functions

Molecule	Classification	Functions	Cellular targets	Reference
ADAM10 (CDw156c)	Enzyme - protease	Shedding of surface receptors in platelets	Platelet, B-cells, malignant cell types, endothelial cells	PMID:20644114
ADAM17 (CD156b)	Enzyme - protease	Shedding of surface receptors in platelets	Platelets	PMID:20644114
ADAMTS13	Enzyme - protease	(Sub)endothelial VWF cleavage	Platelets, endothelial cells	PMID:19389207
Angiogenin	Enzyme - RNAse	Angiogenesis	Endothelial cells	PMID:18279456
Angiopoietin-1	Growth factor	Angiopoiesis	Endothelial cells	PMID:22071944
Angiopoietin-related growth factor	Growth factor	Angiopoiesis	Endothelial cells	PMID:22071944
Angiostatin	Enzyme - protease	Angiogenesis	Endothelial cells	PMID:22512504
Basigin	Enzyme - protease	Spermatogenesis (*P. falciparum* binding)	Epithelial cells, endothelial cells, leukocytes, erythrocytes	PMID:21320284
BDNF	Growth factor	Neurogenesis	Neurons	PMID:15585351
Beta-thromboglobulin	Chemokine	Chemotaxis	Fibroblasts, neutrophils	PMID:11138777
bFGF	Growth factors	Angiogenesis	Fibroblasts, endothelial cells	PMID:21142700
BMP2	Cytokine	Osteogenesis	Osteoblast	PMID:22808271
BMP6	Cytokine	Osteogenesis	Mesenchymal stem cells	PMID:19413738
CCL17	Chemokine	Chemotaxis	T cells	PMID:18723831
CCL3 (also known as PF4)	Chemokine	Chemotaxis and angiogenesis	Monocytes, neutrophils	PMID:12851650
CCL4	Chemokine	Chemotaxis	Natural killer cells, monocytes, leukocytes	PMID:12851650
CCL5	Chemokine	Chemotaxis	T cells, eosinophils, basophils, leukocytes	PMID:12851650
CCL7	Chemokine	Chemotaxis	Monocytes, macrophages	PMID:12851650
CRP	Inflammatory marker	Modulator of innate and adaptive immunity	Endothelial cells, platelets, polymorpho-nuclear leukocytes, monocytes	PMID:15492312
CTGF	Growth factor	Angiogenesis	Endothelial cells, fibroblasts, monocytes, chondrocyte	PMID:15598883
CXCL4	Chemokine	Angiogenesis	Neutrophils, monocytes, endothelial cells, fibroblasts	PMID:1718005
CXCL5	Chemokine	Chemotaxis	Neutrophils, epithelial cells	PMID:12851650
CXCL7	Chemokine	Chemotaxis	Fibroblasts, neutrophils, monocytes	PMID:18723831
EGF	Growth factor	Chemotaxis	Epidermal cells, epithelial cells, fibroblasts	PMID:1572403
Endostatin	Cytokine	Mitogen	Endothelial cells	PMID:21680800
Endothelial cell-selective adhesion molecule	Immunoglobulin superfamily	Angiogenesis	Neutrophils, monocytes, endothelial cells	PMID:17723134
Gas-6	Growth factor	Angiogenesis	Vascular smooth muscle cells, endothelial cells	PMID:10648841
HMGB1	Cytokine	Angiogenesis	Neutrophil, monocytes, dendritic cells, macrophages	PMID:11154118
IGFBP3	Growth factor	Modulator of innate and adaptive immunity	Fibroblasts	PMID:7679986
IL-1-α	Cytokine	Modulator of innate and adaptive immunity and angiogenesis	Endothelial cells, dendritic cells, monocytes, macrophages, B and T cells	PMID:7631154
IL-1-β	Cytokine	Modulator of innate and adaptive immunity and angiogenesis	Endothelial cells, dendritic cells, monocytes, macrophages, B and T cells	PMID:7631154
IL-7	Cytokine	Modulator of innate and adaptive immunity	T and B cells, natural killer cells, macrophages, monocytes	PMID:12742982
IL-8	Chemokine	Modulator of innate and adaptive immunity	Neutrophils, B cells, endothelial cells, macrophage	PMID:14713510

Continued overleaf

Table 2. Continued

Molecule	Classification	Functions	Cellular targets	Reference
Osteoprotegerin	Cytokine	Modulator of innate and adaptive immunity	Osteoclasts	PMID:14585373
PAI-1	Enzyme - protease	Chemotaxis	Lymphocytes, neutrophils	PMID:17884148
PDGF	Growth factor	Osteogenesis	Epithelial cells, endothelial cells, smooth muscle cells, fibroblasts, chondrocytes	PMID:2835772
Soluble CD40 ligand	Cytokine	Angiogenesis and chemotaxis	Endothelial cells	PMID:12186789
Soluble CD62P	Cytokine	Angiogenesis and chemotaxis	B cells, dendritic cells, platelets	PMID:9496730
TGF-β	Growth factor	Modulator of innate and adaptive immunity	Neutrophils, monocytes	PMID:11012227
Thrombocidin-1	Chemokine, antimicrobial factor	Cellular interaction	Epithelial cells, endothelial cells, B cells	PMID:10877842
Thrombocidin-2	Chemokine, antimicrobial factor	Modulator of innate and adaptive immunity	*Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Lactococcus lactis,* fungicidal for *Cryptococcus neoformans*	PMID:10877842
Thrombospondin-1	Cytokine	Modulator of innate and adaptive immunity	*Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Lactococcus lactis,* fungicidal for *Cryptococcus neoformans*	PMID:16263699
Tissue inhibitor of metalloproteinases-1	Enzyme - protease	Modulator of innate immunity	Endothelial cells, neutrophils, vascular smooth muscle cells, fibroblasts	PMID:19410025
Tissue inhibitor of metalloproteinases-2	Enzyme - protease	Inflammation	Keratinocytes, endothelial cells	PMID:19410025
Tissue inhibitor of metalloproteinases-3	Enzyme - protease	Angiogenesis	Keratinocytes, endothelial cells	PMID:19410025
Tissue inhibitor of metalloproteinases-4	Enzyme - protease	Angiogenesis	Keratinocytes, endothelial cells	PMID:19410025
VEGF	Growth factor	Angiogenesis	Endothelial cells	PMID:21680800

ADAM, a disintegrin and metalloproteinase; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; BDNF, brain-derived neurotrophic factor; bFGF, basic fibroblast growth factor; BMP, bone morphogenetic protein; CCL, C-C motif ligand; CRP, C-reactive protein; CTGF, connective tissue growth factor; CXCL, C-X-C motif ligand; EGF, epidermal growth factor; Gas-6, growth arrest-specific 6; HMGB1, high-mobility group protein B1; IGFBP3, insulin-like growth factor-binding protein 3; IL, interleukin; PAI, plasminogen activator inhibitor; PDGF, platelet derived growth factor; TGF, transforming growth factor; VEGF, vascular endothelial growth factor; VWF, von Willebrand factor.

A selection of platelet secreted molecules important in immune process regulation

Platelet factor-4

PF4 prevents apoptosis of monocytes and initiates their differentiation into macrophages, induces neutrophil adhesion to non-stimulated endothelium and granule-content release [44], favors neutrophil, monocyte, and fibroblast chemotaxis, and eosinophil adherence, induces basophil release of histamine, and controls multiple T-cell activities, including inhibition of monocyte-dependent and CD3/CD28 antibody ligation-induced T-cell proliferation and activation. It has a complex role in the regulation of T-cell responses (regulatory T cells ($CD4^+CD25^+$) and non-regulatory T cells ($CD4^+CD25^-$)) and inhibits $CD4^+CD25^-$ T-cell proliferation, but stimulates proliferation of $CD4^+CD25^+$ regulatory T cells [45].

β-Thromboglobulin

β-TG comprises sets of proteins, actually proteolytic products of inactive precursors, that stimulate or inhibit neutrophils, depending on their processing [46], and attract neutrophils, but not monocytes [47], in the vasculature.

RANTES

RANTES is consistently released by thrombin-stimulated platelets. It is less pleiotropic than other chemokines, but it is highly efficient in recruiting monocytes, T cells and eosinophils [48]. It contributes to inflammation and atheromatogenesis by recruiting circulating monocytes that bind altered microvascular and arterial endothelium by triggering shear-resistant monocyte arrest [49].

Macrophage inflammatory protein-1α

MIP-1α has histamine-releasing activity for basophils and is chemotactic for T lymphocytes. It is an important mediator of virus-induced inflammation *in vivo* and plays a role in cellular, but not humoral, responses to coxsackie virus B3. During infection, MIP-1α may be required for the efficient recruitment of T cells to sites of viral infection [50].

Thus, if platelets can secrete chemokines, the latter can exert reciprocal actions on platelets. RANTES and MIP-1α stimulate platelets to increase calcium signals, to aggregate, and to release granular contents.

Soluble CD40 ligand

Other cytokine- and chemokine-like molecules secreted by platelets are leading immunomodulatory agents, either in physiology or in pathology, such as CD40L [51,52]. The identification of CD40L as a major product, both quantitatively and qualitatively, of platelets was important because it was the first evidence that platelets abundantly secreted factors not involved in hemostasis. The role of soluble CD40L (sCD40L) in acute transfusion reactions, first in non-hemolytic febrile transfusion reactions and then in transfusion related acute lung injury, is intriguing. We and other groups established *ex vivo* models of blood cells obtained from healthy donors to explore the extent to which intact or lysed platelets could be transfused, and investigated biological response modifiers from supernatants and determined how they affected different cell types, including B lymphocytes, T lymphocytes, dendritic cells, monocytes, and epithelial cells (Figure 1). The accumulation of inflammatory cytokines and chemokines during platelet component storage increased the inflammatory effects of leukocytes and endothelial cells to levels comparable to those observed during pathological conditions. We provided evidence for a role of sCD40L in acute transfusion reaction cases in which there was complete excretion of sCD40L from platelets into the supernatant such that no sCD40L was left within the platelets, in contrast to that observed in control patient samples [53,54]. We extended this finding to a series of other cytokines previously unassociated with platelets, IL-27 and OX40L [55].

Microparticles at the interface of procoagulant activity and inflammation

The 'microparticle' phenomenon

In 1967, Wolf described small membrane fragments referred to as 'platelet dust' that were released after platelet activation [56]. This 'platelet dust' (now acknowledged as MPs) retain procoagulant activity similar to activated platelets [56]. MPs of various cell types have been detected in circulating blood [57,58], including from leukocytes, erythrocytes, endothelial cells and platelets. The most abundant circulating MPs are platelet-derived MPs, less than 1.0 μm in diameter, which represent approximately 70 to 90% of all circulating MPs [59]. The stimulation and activation of each cell type can induce increased levels of cytoplasmic calcium associated with the translocation of phosphatidylserine and phosphatidylethanolamine from the inner to the outer leaflet of the cell membrane and activation of calpain, which facilitates MP shedding by cleaving cytoskeletal filaments [60]. MPs are involved in ischemic stroke, metastasis, tumor development, and inflammatory and neurodegenerative diseases [60].

Platelet microparticles

Platelet MPs are membrane vesicles shed by platelets after activation that transports platelet molecules (glycoprotein IIb/IIIa, Ib and P-selectin). The interface between blood cells and arterial vessel walls determines the progression of atherosclerotic plaques and thrombotic complications [61]. Evidence suggests a role of activated platelets and platelet-derived MPs in disease development by interaction with leukocytes, endothelial cells and smooth muscle cells [62]. During inflammation, platelet-derived MPs can activate antigen-presenting cells, modulate dendritic cell activation, increase T-cell responses, induce B-cell production of IgG antibodies, and enhance germinal center formation in cooperation with T cells [60].

The role of platelets in sepsis

Sepsis is frequently associated with unexplained thrombocytopenia

Sepsis provides an interesting model for deciphering the role of platelets beyond hemostatic functions. Thrombocytopenia is a frequent occurrence in sepsis, implying platelets are involved in the pathophysiology [63,64]. Generally, platelet counts in sepsis patients markedly decrease during the first 4 days [65]. Conversely, sepsis is a significant risk factor for thrombocytopenia in critically ill patients, and the severity of sepsis correlates with decreased platelet counts [66]. During bacterial infection, direct interactions between platelets and bacteria, leading to enhanced macrophagy [67], may contribute to thrombocytopenia. Platelet TLR4 was found to lead to neutrophil-dependent pulmonary sequestration in response to LPS [8]. Interestingly, they demonstrated the *in vivo* formation of platelet aggregates in response to LPS [68]. However, mechanisms that may link impaired central platelet production and peripheral overconsumption and/or destruction are poorly understood.

Hypotheses for sepsis-associated thrombocytopenia: the coagulation path

Platelet consumption may also be important in patients with sepsis because of their activation and subsequent sequestration on endothelium or increased binding to circulating neutrophils [69]. Platelet adhesion to capillaries requires NADPH oxidase, inducible nitric oxide synthase, P-selectin and activated coagulation (platelet adhesion, fibrin deposition, and blood flow stoppage in capillaries) [70]. In addition to endotoxin and inflammatory cytokines, the ongoing generation of thrombin (a

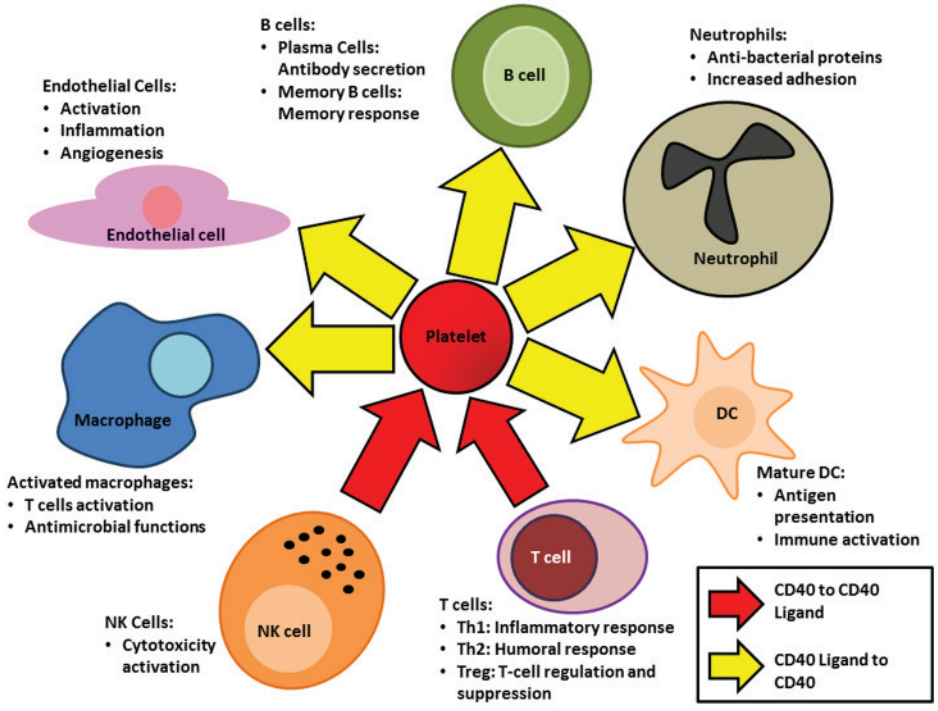

Figure 1. Hypothetical mechanisms by which platelet membrane CD40/CD40L and soluble CD40L might regulate interactions between immune cells. Platelets can interact with numerous immune cells such as B cells, T cells, neutrophils, macrophages, endothelial cells, natural killer (NK) cells and dendritic cells (DC). Interactions with these cells can induce numerous immune-related events as shown. Th, T-helper; Treg, regulatory T cell.

potent activator of platelets *in vivo*) and the release of platelet activating factor activates platelets. The contribution of platelet activating factor in sepsis was demonstrated in animal models [71], although targeted therapeutic interventions to antagonize it led to disappointing results in human sepsis [72]. Coagulation is closely associated with severe systemic inflammatory responses occurring in sepsis patients [73], but therapeutic approaches targeting coagulation that were beneficial in animal models were generally unsuccessful in humans.

Further hypotheses for sepsis-associated thrombocytopenia: the inflammatory path

Intriguingly, decreased plasma levels of RANTES seem to parallel thrombocytopenia [74]. Besides this, tissue factor, a prominent product of platelets [75,76] and activated monocytes [77], functions in coagulation and inflammation by exposing procoagulant phospholipid binding sites for factors Va and Xa. Thrombospondin-1 contributed to mortality in experimental models [78]. Of note, there is a balance between levels of pro- and anti-inflammatory cytokines (the latter lead to a so-called cytokine storm). Transforming growth factor-β1 is an important counter-regulatory, anti-inflammatory cytokine in sepsis and represents a prime candidate for factors that mediate sepsis resolution; it also prevents leukocyte apoptosis [79].

Conclusion

Platelets are thought to sense danger by recognizing subendothelial molecules following vascular endothelium damage, and following activation they engage repair systems by secreting appropriate adhesion and healing molecules. Platelets can sense other forms of danger, in particular those that are infectious in nature as they express numerous specialized (TLRs) and less specialized but highly functional receptors (FCR for Igs/antibodies and complement factor receptors). Platelets can be activated by infectious pathogen moieties and by immune complexes formed around infectious pathogens. The nature of the infectious danger activates platelets, directing them to secrete specific biological response modifiers comprising cytokine and chemokine-like molecules. Platelets also engage membrane and secreted factors that interact with neighboring and distant immune cells to eliminate immune complexes, characteristic of innate immunity. These complex responses might even alter adaptive immune responses, at least in experimental models. During sepsis syndrome, platelets

are particularly mobilized along a spectrum ranging from hyperactivation to exhaustion, and this may be an attractive target for new drug treatment approaches, once the finely tuned mechanisms are identified.

Abbreviations
CD40L, CD40-ligand; IL, interleukin; LPS, lipopolysaccharide; MIP, macrophage inflammatory protein; MP, microparticle; NET, neutrophil extracellular trap; PAMP, pathogen-associated molecular pattern; PF, platelet factor; PRR, pathogen recognition receptor; RANTES, regulated upon activation normal T cell expressed and secreted; sCD40L, soluble CD40 ligand; TG, thromboglobulin; TLR, Toll-like receptor.

Competing interests
The authors declare that they have no competing interests.

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Author details
[1]Université de Lyon, GIMAP, EA3064, F-42023-A, Saint Etienne, France. [2]EFS Auvergne-Loire and GIMAP-EA 3064, Université de Saint-Etienne, Faculté de Médecine, 15 rue Ambroise Paré, 42023 Saint-Etienne cedex 2, France. [3]Unit Cytokines & Inflammation, Institut Pasteur, Paris 75015, France.

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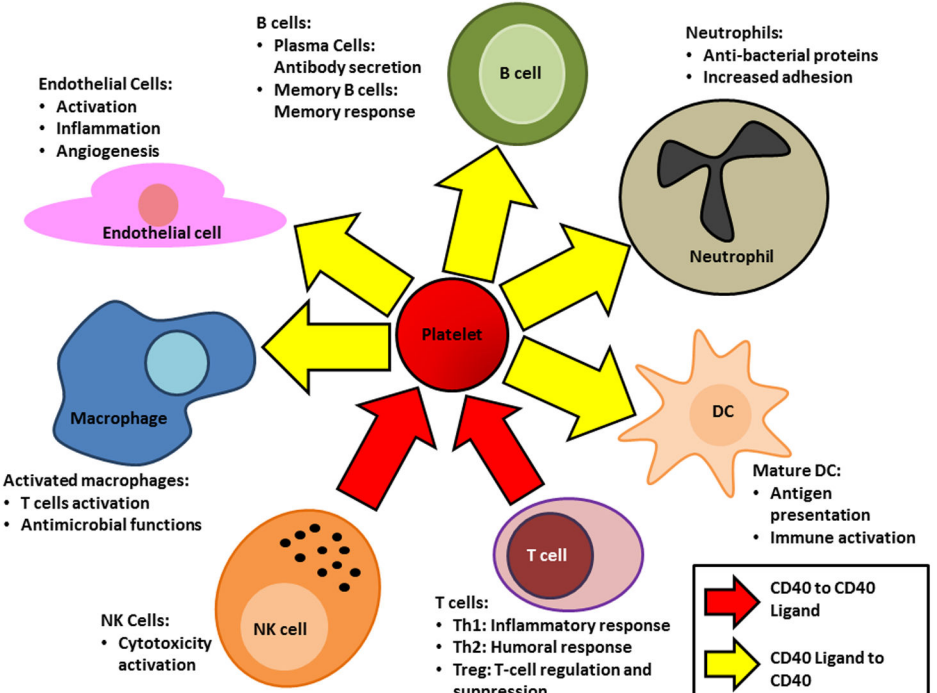
B cells:
• Plasma Cells: Antibody secretion
• Memory B cells: Memory response
B cell
Neutrophils:
• Anti-bacterial proteins
• Increased adhesion
Endothelial Cells:
• Activation
• Inflammation
• Angiogenesis
Endothelial cell
Neutrophil
Platelet
Macrophage
DC
Activated macrophages:
• T cells activation
• Antimicrobial functions
Mature DC:
• Antigen presentation
• Immune activation
T cell
NK Cells:
• Cytotoxicity activation
NK cell
T cells:
• Th1: Inflammatory response
• Th2: Humoral response
• Treg: T-cell regulation and suppression
CD40 to CD40 Ligand
CD40 Ligand to CD40

Figure 1