Platelet Interaction with Innate Immune Cells

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Summary

Beyond their traditional role in haemostasis and thrombosis, platelets are increasingly recognised as immune modulatory cells. Activated platelets and platelet-derived microparticles can bind to leukocytes, which stimulates mutual activation and results in rapid, local release of platelet-derived cytokines. Thereby platelets modulate leukocyte effector functions and contribute to inflammatory and immune responses to injury or infection. Platelets enhance leukocyte extravasation, differentiation and cytokine release. Platelet-neutrophil interactions boost oxidative burst, neutrophil extracellular trap formation and phagocytosis and play an important role in host defence. Platelet interactions with monocytes propagate their differentiation into macrophages, modulate cytokine release and attenuate macrophage functions. Depending on the underlying pathology, platelets can enhance or diminish leukocyte cytokine production, indicating that platelet-leukocyte interactions represent a fine balanced system to restrict excessive inflammation during infection. In atherosclerosis, platelet interaction with neutrophils, monocytes and dendritic cells accelerates key steps of atherogenesis by promoting leukocyte extravasation and foam cell formation. Platelet-leukocyte interactions at sites of atherosclerotic lesions destabilise atherosclerotic plaques and promote plaque rupture. Leukocytes in turn also modulate platelet function and production, which either results in enhanced platelet destruction or increased platelet production. This review aims to summarise the key effects of platelet-leukocyte interactions in inflammation, infection and atherosclerosis.

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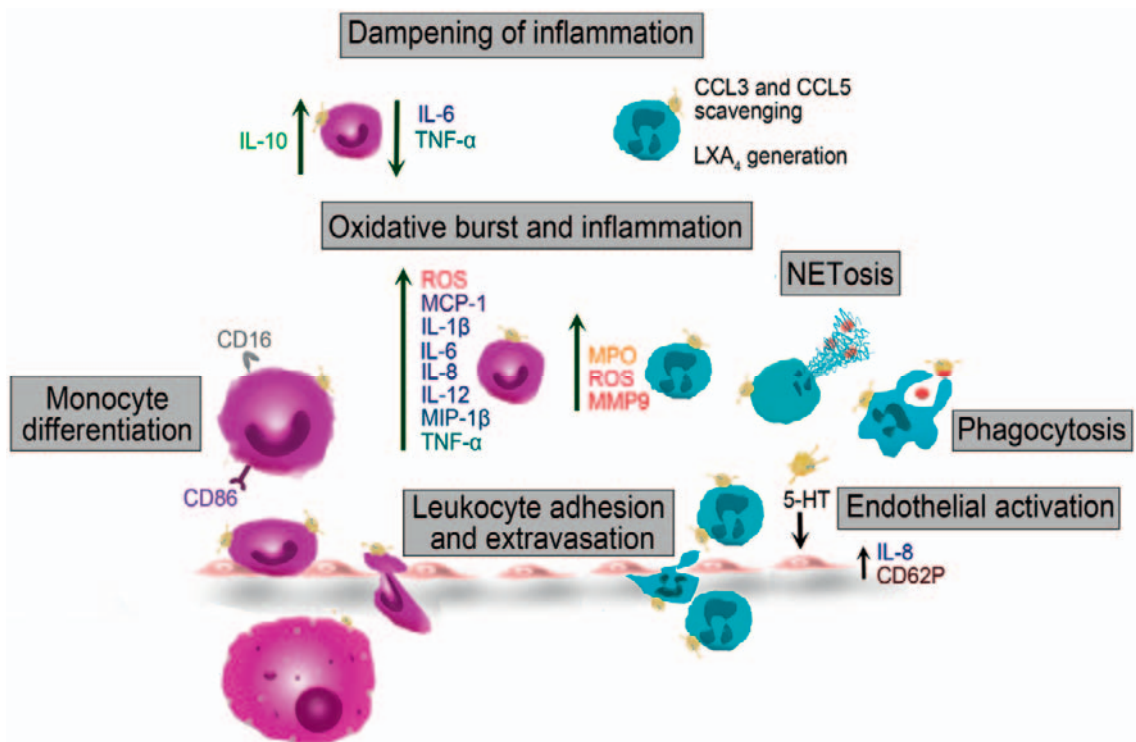## Platelets and Innate Immune Cells during Injury

Platelets are key players in haemostasis and prevent excessive bleeding upon injury. In response to vessel damage, platelets adhere and get activated at sites of injury, leading to recruitment of further platelets and thrombus formation. As injury represents a risk for infection, platelets recruit and activate leukocytes via direct cell-cell contacts and indirectly via cytokines and platelet-derived microvesicles. Activated platelets directly interact with leukocytes via P-selectin (CD62P) interaction with P-selectin glycoprotein ligand 1 (PSGL-1). This initial binding is enhanced by interaction of various other receptors, depending on the leukocyte subtype, leading to mutual activation and local cytokine release (reviewed in [1]), which modulates immune responses.

Upon activation platelets release a variety of α-granule-derived cytokines, chemokines and growth factors [2]. The mechanism of packaging inflammatory cargo into α-granules, however, is incompletely understood [3]. Cytokines can be packaged into granules during megakaryopoiesis [4] either via biosynthesis in the megakaryocyte (e.g. platelet factor 4/CXCL4) or via endocytosis from the microenvironment (e.g. albumin) in the bone marrow [3]. Despite lacking a nucleus, platelets can splice and de novo synthesise proteins from megakaryocyte-derived (pre)mRNA as shown for IL-1β and IL-18 [5, 6]. Via their open canalicular system platelets also take up factors from the circulation. Further platelets can fuse with microvesicles, which leads to intercellular exchanges of chemotactic receptors such as C-C chemokine receptor type 5 (CCR5) and chemokine (C-X-C motif) receptor 4 (CXCR4) [7, 8]. Platelet cytokine levels have been demonstrated to be elevated in cancer patients [9, 10], indicating either an active uptake of these factors by platelets or disease-related changes in megakaryopoiesis. This suggests that underlying pathologies might influence not only platelet reactivity but also their potential to modulate immune responses.

Fig. 1. Effects of platelets on innate immune cell functions during inflammation and infection.

Platelets promote monocyte and neutrophil extravasation via direct interaction and various chemokines. They further enhance leukocyte transmigration by endothelial cell activation via serotonin (5-HT) release. Platelets augment neutrophil effector functions by promoting phagocytosis and NETosis. Activated platelets further enhance oxidative burst and release of various inflammatory cytokines and enzymes of monocytes and neutrophils. Platelets direct monocytes towards a pro-inflammatory phe-

notype by inducing the expression of CD16 and CD86. However, platelets are also capable of dampening inflammatory responses by triggering IL-10 and down-regulating IL-6 and TNF-α release by monocytes. Further, neutrophils can scavenge platelet mediated CCL3 and CCL5 and platelet-neutrophil aggregates generate Lipoxin A4 (LXA4) thereby eliciting anti-inflammatory responses.

5-HT = serotonin; CCL3 = chemokine (C-C motif) ligand 3; CD16 = cluster of differentiation 16; CXCL2 = chemokine (C-X-C motif) ligand 2; LXA4 = Lipoxin A4; MCP-1 = monocyte chemoattractant protein 1; MIP-1β = macrophage inflammatory protein 1β; MMP9 = matrix metalloproteinase-9; MPO = myeloperoxidase; ROS = reactive oxygen species.

Platelet Interactions with Innate Immune Cells in Inflammation and Infection

Apart from vessel damage, a variety of pathogens as well as inflammation triggers the activation of platelets. While certain viruses and bacteria exploit platelet interactions to gain virulence advantages, platelets mediate anti-microbial effects, which are either mediated via direct interaction with pathogens, or indirectly, by orchestrating leukocyte functions. Thus, platelets are important immune modulators during inflammation and infection. An overview of the effects of platelets during inflammation and infection is depicted in figure 1 and table 1.

Anti-platelet agents not only affect platelet aggregation and thrombosis, but also target immune modulatory effects of platelets. Aspirin and novel P2Y$_{12}$ receptor antagonists such as clopidogrel, prasugrel and ticagrelor reduce interaction of platelets with leukocytes and attenuate leukocyte recruitment and effector functions. Thereby, anti-platelet medication modulates a wide range of pathologic conditions (reviewed in [1, 11]).

Leukocyte Extravasation

Platelets support leukocyte extravasation and tissue infiltration at sites of inflammation. Platelets adhere to the inflamed endothelium and mediate leukocyte rolling via glycoprotein Ib (GPIb) [12] and GPIIb/IIIa [13, 14]. Platelets enhance neutrophil rolling and firm adhesion via CD62P and chemokine CXCR2 [15-17].

Endothelial transmigration of neutrophils is elicited by binding of PSGL-1 [18] or CD11b/CD18 (MAC-1) to platelet CD62P [15], and is further enhanced by binding of CD40 to platelet-derived soluble CD40 ligand (sCD40L) [19]. Binding of activated platelets to adhering neutrophils results in polarised receptor organisation, which represents a prerequisite for intravascular migration [20].

Activated neutrophils release a number of chemokines, which attract monocytes. Activated platelets further facilitate this process. Platelets directly interact with monocytes, leading to enhanced expression of CD40, PSGL-1, CD11b and CCR2 on the monocyte surface [21, 22]. This, in turn, enhances platelet-monocyte aggregate formation and recruitment of further monocytes to the endothelium [21, 23–25]. The initial interaction between platelets and monocytes is mediated by CD62P-PSGL-1 binding [1], which is further stabilised by CD40L-MAC-1, GPVI-CD147 or via interaction of intercellular adhesion molecule 1 (ICAM-1) with platelet-bound fibrinogen [1, 25]. In addition to direct platelet-monocyte interactions, platelet-derived chemokines influence endothelial adhesion of monocytes. CXCL4, chemokine (C-C motif) ligand 5 (CCL5) and platelet-derived macrophage migration inhibiting factor (MIF) promote monocyte arrest on activated endothelial cells [26, 27]. Platelet-derived MIF and stromal cell-derived growth fac-

Table 1. Overview of the effects of platelets on innate immune cell functions

Platelet effect on leukocyte	Monocyte				Neutrophil			
	on/from platelet	on monocyte	monocyte effector function	ref.	on/from platelet	on neutrophil	neutrophil effector function	ref.
Oxidative burst and inflammation	CD40L	MAC-1	↑ MPO	23	direct interaction		↑ MPO, ROS	45, 46
	CXCL4		↑ ROS	51	sCD40L		↑ ROS	49
	CD62P, CCL5, TNFSF14	PSGL-1	↑ MCP-1, IL-1β, IL-6, IL-8, IL-12, MIP-1β, TNF-α	56, 60, 61	HMGB1		↑ MPO translocation to the plasma membrane	50
	platelets and MV		↑ C5a, TNF-α	63	direct interaction		↑ degranulation, MMP9	44, 72
	direct interaction		↑ MCP-1, IL-6, IL-8, TNF-α, IL-1β, IL-10	56, 64, 47	ATP		↓ MPO, ROS	53
	CXCL4, CCL5		↑ CXCL2	69, 70				
	CD40L	CD40	↑ IFN-α	71				
Leukocyte adhesion and extravasation	direct interaction		↑ CD40, PSGL-1, CD11b, CCR2 increased → enhanced recruitment	21, 22	CD62P, CXCR2		↑ rolling/firm adhesion	15–17
	CD62P	PSGL-1	↑ initial interaction	1	CD62P	PSGL-1, CD11b/CD18	↑ endothelial transmigration	15–17
	CD40L	MAC-1	↑ extravasation	1, 25	sCD40L	CD40	↑ transmigration	19
	GPVI	CD147	↑ extravasation	1, 25				
	platelet-bound fibrinogen	ICAM-1	↑ extravasation	1, 25				
	CXCL4, CCL5, MIF		↑ monocyte arrest on activated endothelium	26, 27				
	CXCL12		↑ recruitment	28, 29				
Monocyte differentiation	direct interaction		up-regulation of CD16 → switch from classic towards intermediate phenotype	22				
	TGF-β		up-regulation of CD16 → switch towards a non-classic or intermediate phenotype	58				
	CXCL4		CD86 expression and differentiation into macrophages	90				
	platelet-bound IgG	Fc receptor	anti-inflammatory phenotype	77				
NETosis					CD62P	PSGL-1	↑ NETosis	20, 36
					GPIbα		↑ NETosis	37
						LFA-1	↑ NETosis	38
					β-defensin, TXA$_2$, CXCL4, vWF, HMGB1		↑ NETosis + bacterial clearance	39, 40, 41

Table 1 *continued on next page*

Table 1. *Continued*

Platelet effect on leukocyte	Monocyte				Neutrophil			
	on/from platelet	on monocyte	monocyte effector function	ref.	on/from platelet	on neutrophil	neutrophil effector function	ref.
Phagocytosis					CD62P MV		phagocytosis ↑ phagocytosis ↑	31, 32, 33, 34
Dampening of inflammation	sCD40L		IL-10 ↑	75	direct interaction		LXA$_4$ ↑	79
	sCD40L CXCL4	down-regulation of CCR1, CCR2, CCR5	IL-6, TNF-α ↓	75 73	sequestering neutrophil elastase		down-regulation	44
	direct interaction, soluble mediators		NO, IL-6, TNF-α ↓	76				
	platelet-bound IgG	Fc receptors	IL-10 ↑	77				
	platelet-bound IgG		IL-1β, IL12, IL-6 ↓	77				

tor 1 (SDF-1/CXCL12) further increase monocyte recruitment via chemotaxis [28, 29].

Platelets also indirectly promote leukocyte migration via activation of endothelial cells. Platelet-derived serotonin, for example, induces secretion of endothelial Weibel-Palade bodies, leading to CD62P expression and release of IL-8, which triggers neutrophil rolling, adhesion and extravasation [30].

Phagocytosis, Neutrophil Extracellular Trap Formation and Bacterial Clearance

Platelets and platelet-derived microvesicles can directly interact with neutrophils and enhance phagocytosis of various bacteria, including *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis, Neisseria meningitides* and *Streptococcus pyogenes* [31–34], thereby contributing to bacterial clearance.

Platelets are further involved in neutrophil extracellular trap (NET) formation, an apoptotic process, leading to release of neutrophil DNA, which ensnares bacteria. Toll-like receptor 4 (TLR4)-activated platelets bind to neutrophils, sticking to the endothelium, and initiate NET formation [35]. Platelets mediate NETosis either via CD62P-PSGL-1 interactions [20, 36], platelet GPIbα [37] or neutrophil lymphocyte-function-associated-antigen-1 (LFA-1) [38]. Additionally, platelet release products, like β-defensin [39], thromboxane A_2 (TXA$_2$), CXCL4, von Willebrand factor (vWF) [40] and high-mobility group box 1 protein (HMGB1) [41], trigger NET formation and increase bacterial clearance.

However, certain bacteria have overcome platelet-mediated host defence mechanisms. *S. pyogenes*, for example, induces large, fibrinogen-associated platelet-neutrophil complexes, which reduce neutrophil chemotaxis and phagocytosis, thus supporting bacterial survival [33]. *Staphylococcus aureus* α-toxin binds to platelet A-disintegrin-and-metalloproteinase-domain-containing-protein-10 (ADAM10) leading to proteolysis of the collagen receptor GPVI. This impairs collagen-induced platelet aggregation and endothelial repair, but also induces platelet degranulation and IL-1β production by leukocytes. Thereby, *S. aureus* α-toxin exacerbates bacterial dissemination and accelerates pro-inflammatory responses, which lead to tissue damage and aggravate sepsis [42].

Oxidative Burst

Platelets modulate leukocyte oxidative burst by modulating the release of reactive oxygen species (ROS) and myeloperoxidase (MPO).

Platelets enhance MPO levels and oxidative stress in experimental models of acute colitis and immunecomplex-mediated inflammation [43, 44]. Moreover, direct interaction of platelets and neutrophils promotes MPO formation in murine models of pancreatitis [45] and lipopolysaccharide(LPS)-induced acute lung injury [46]. Viral and bacterial infections are associated with increased levels of circulating platelet-leukocyte aggregates [47], which could enhance oxidative burst also during infections [48]. Platelet-derived soluble mediators are also involved in neutrophil oxidative burst formation. Platelet sCD40L stimulates neutrophils to pro-

duce ROS [49], and platelet-derived HMGB1 triggers the translocation of MPO to the cell membrane [50].

Platelets further facilitate endogenous oxidative burst generation of monocytes to boost the destruction of phagocytosed pathogens. Platelet-induced ROS production in monocytes is modulated by direct interaction as well as secreted factors. Preventing direct platelet-monocyte interaction by blocking CD40L-MAC-1 interaction reduces the release of MPO from mouse monocytes [23]. Moreover, CXCL4 stimulation of monocytes enhances the phagocytic ability and triggers a respiratory burst [51] via activation of phosphoinositol-3-kinase, Syk and p38 [52], indicating that direct cell-cell interactions are not absolutely required for ROS induction.

However, platelets are also capable of down-regulating neutrophil ROS generation and MPO release via release of ATP from their dense granules [53], while in some pathologies platelets have no effect on MPO release at all [44].

Monocyte Differentiation

Platelets regulate monocyte functions by modulating their activation, polarisation and differentiation.

In humans, circulating monocytes can be classified in three subgroups, with distinct functions and phenotypes based on their CD14 and CD16 expression. Classical monocytes (CD14++, CD16−) are highly phagocytic cells and produce ROS, whereas the non-classical monocytes (CD14+, CD16++) patrol the endothelium and are involved in autoimmune diseases. The role of intermediate monocytes (CD14++, CD16+) is still controversial as they are associated with inflammatory diseases and release of IL-1β and TNF-α, but also with production of IL-10 [54, 55]. Platelets preferentially bind to CD16+ monocytes and may also induce a phenotypical switch of classical monocytes towards CD16+ subsets [22]. The underlying mechanism of platelet-mediated modifications of monocyte phenotypes involves activation of the nuclear factor 'kappa-light-chain-enhancer' of activated B cells (NF-κB) pathway and signal transduction via phosphorylation of Lyn kinase [56, 57].

Activated platelets release tumour-derived growth factor β (TGF-β) which also leads to up-regulation of CD16 on monocytes [58] and thus induces a switch towards intermediate and/or non-classical monocytes. In vitro, platelet-derived TGF-β promotes an inflammatory monocyte response by inducing cyclooxygenase 2 de novo synthesis via activation of the p38 MAPK pathway [59].

Pro-Inflammatory Cytokine Expression

Both direct and indirect platelet-derived signals drive leukocyte expression of pro-inflammatory cytokines. CD62P-PSGL-1 binding as well as platelet release of CCL5 and tumour necrosis factor superfamily member 14 (TNFSF14) induce monocyte expression of monocyte chemotactic protein 1 (MCP-1), TNF-α, IL-1β, IL-6, IL-8, IL-12 and macrophage inflammatory protein 1β (MIP-1β) in vitro [56, 60, 61].

Platelet-induced intracellular signalling in monocytes or macrophages is highly complex and still incompletely understood. Co-incubation with activated platelets and platelet-derived microvesicles enhances monocyte activation by inducing AKT signalling and

intracellular calcium flux [62] and leads to complement factor C5a and TNF-α release [63].

Platelet-monocyte interactions further activate the monocyte NFκB pathway to promote release of pro-inflammatory MCP-1, IL-8, TNF-α and IL-6 upon co-incubation in vitro [56, 64]. Platelet CXCL4 induces extracellular signal kinase 1 and 2 (ERK1/2) phosphorylation, which mediates survival and differentiation, and Janus kinase (JNK) signalling, which leads to production and release of cytokines and chemokines [52].

The effect of platelets on monocytic release of pro-inflammatory molecules seems to depend on the type of pathogen, as platelets differentially affect TLR4- and TLR2-mediated inflammation in vitro [65]. Platelets reduce the expression of monocyte IL-10 upon stimulation with either TLR4 or TLR2 ligands. However, platelet TLR4 stimulation enhances IL-1β, IL-6 and TNF-α expression by isolated monocytes, whereas platelet stimulation via TLR2 reduces monocyte expression of these cytokines [65]. In line with this observation, TLR2 agonist lipoteichoic acid from *S. aureus* reduces platelet degranulation and platelet-monocyte aggregate formation [66]. The immune modulatory potential of platelets is therefore dependent on the underlying pathological conditions, as platelets from patients with dengue virus infection stimulate monocytes to produce MCP-1, IL-1β, IL-8 and IL-10, while co-incubation of platelets and monocytes from healthy donors results only in increased MCP-1 release [47].

Platelets also confer pro-inflammatory effects on monocytes in vivo. Platelet inhibition reduces platelet-monocyte interaction and plasma levels of TNF-α during LPS-induced endotoxaemia in humans [67] and mice [68]. Platelet-derived CXCL4 and CCL5 are critical mediators of septic lung damage secondary to polymicrobial sepsis in a caecal ligation puncture (CLP) model as they trigger CXCL2 release of resident alveolar macrophages, thereby promoting neutrophil infiltration and tissue damage [69, 70].

Platelets further enhance IFN-α secretion of immune complex-stimulated plasmacytoid dendritic cells via CD40L-CD40 interaction [71]. Platelets also enhance neutrophil activation and degranulation, leading to an increase in matrix metalloproteinase 9 (MMP9) release [44, 72]. Thereby, platelets facilitate degradation of basement membranes by neutrophils.

During acute colitis platelet depletion is accompanied with decreased levels of CXCL2, CXCL5 and IL-6, indicating that platelets further influence inflammatory responses by acting on tissue macrophages or epithelial cells [43].

Anti-Inflammatory Effects
However, platelet function appears more multifaceted, pointing towards a finely balanced system with negative feedback mechanisms, to locally restrict inflammation. CXCL4 not only acts as an inflammatory mediator but also down-regulates the chemotactic receptors CCR1, CCR2 and CCR5 on isolated human monocytes, thereby interfering with monocyte migration [73]. Platelet-derived chrondroitin sulfate A blocks CCL5 binding to the endothelium, thereby suppressing firm monocyte adhesion in an in vitro flow model [74]. Furthermore, platelet-derived MIF has paracrine ef-

fects on platelets, by reducing ADP-induced intracellular calcium flux, thereby potentially interfering with secondary platelet activation [27]. Secretion of sCD40L also elicits anti-inflammatory effects on monocytes by increasing IL-10 expression while concomitantly down-regulating TNF-α and IL-6 following in vitro stimulation of human monocytes [75]. In line with this, co-incubation with murine platelets reduces the production and release of inflammatory nitric oxide, TNF-α and IL-6 of bone marrow-derived macrophages in vitro, indicating that platelets and their release products may attenuate inflammation during endotoxaemia [76]. Direct interaction of platelet-bound immunoglobulin G with monocytic Fc receptors drives monocytes towards an anti-inflammatory phenotype, reducing the levels of released inflammatory IL-1β, IL-12 and IL-6 while at the same time elevating IL-10 production in vitro and in an LPS-induced mouse peritonitis model [77]. In vivo data supports the dampening effect of platelets during infection, as GPIbα-IX-deficient mice show enhanced inflammatory cytokines and chemokines in a CLP sepsis model [78]. The anti-inflammatory modulation of monocyte function may prevent uncontrolled cytotoxic responses during infections.

Also platelet interactions with neutrophils can lead to anti-inflammatory effects as platelet-neutrophil aggregate formation fosters generation of lipoxin A4, which down-regulates neutrophil adhesion and extravasation [79].

Activated platelets stimulate survival of neutrophils by the release of TGF-β [80]. Apoptotic neutrophils express CCR5, which scavenges platelet-derived CCL3 and CCL5 [81], leading to down-regulation of inflammatory responses.

Moreover, platelets are capable of preventing neutrophil-induced tissue damage by sequestering neutrophil elastase [44]. Platelet-dendritic cell interactions can also diminish dendritic cell activation via scavenging heat shock protein gp96, which acts as a dendritic cell activator [82].

Considering the opposing immune modulatory effects of platelets, their role in inflammation and infection is far more complex than previously thought. Depending on the underlying pathology, site of inflammation and experimental model employed, platelets exert either pro- or anti-inflammatory effects. The underlying mechanism, which drives platelets towards an immune enhancer or results in immune dampening effects, is currently incompletely understood. Further investigations to unveil the modality of the dual role of platelets in an inflammatory setting will be eagerly anticipated.

Atherosclerosis

Platelet-leukocyte interactions modulate initiation, development and progression of atherosclerosis (summarised in fig. 2).

Atherosclerosis is associated with increased platelet activation and enhanced release of platelet-derived pro-inflammatory cytokines like IL-1β, sCD40L, CXCL4 and CCL5 [83], which promote activation of the endothelium. Activated platelets transiently adhere to endothelial cells and deposit CXCL4 and CCL5 [83], which en-

Fig. 2. Effect of platelets on innate immune cell functions during atherogenesis (gray rectangle) and plaque rupture (white rectangle). Platelets and platelet-derived chemokines promote monocyte, neutrophil and dendritic cell adhesion to inflamed endothelium and facilitate their extravasation Platelet CXCL4 further enhances survival of monocytes and neutrophils. Platelet CD40L induces ROS generation by neutrophils, which contributes to oxidation of LDL. Platelets trigger monocyte differentiation and macrophage polarization into pro-atherogenic M4 macrophages. Via CXCL4 and CXCL12 platelets enhance oxLDL uptake by monocytes and macrophages, leading to foam cell formation. Platelets promote the release of proteolytic enzymes by neutrophils and monocytes, which leads to extracellular matrix degradation and plaque destabilization. Neutrophils and dendritic cells phagocytose activated platelets, which modulates leukocyte functions. Platelet-mediated NETs contribute to additional platelet trapping and thrombus formation. CCL5 = Chemokine (C-C motif) ligand 5; CD40L = cluster of differentiation 40 ligand; CXCL4 = chemokine (C-X-C motif) ligand 4; LDL = low density lipoprotein; MIF = macrophage migration inhibitory factor; oxLDL = oxidised LDL.
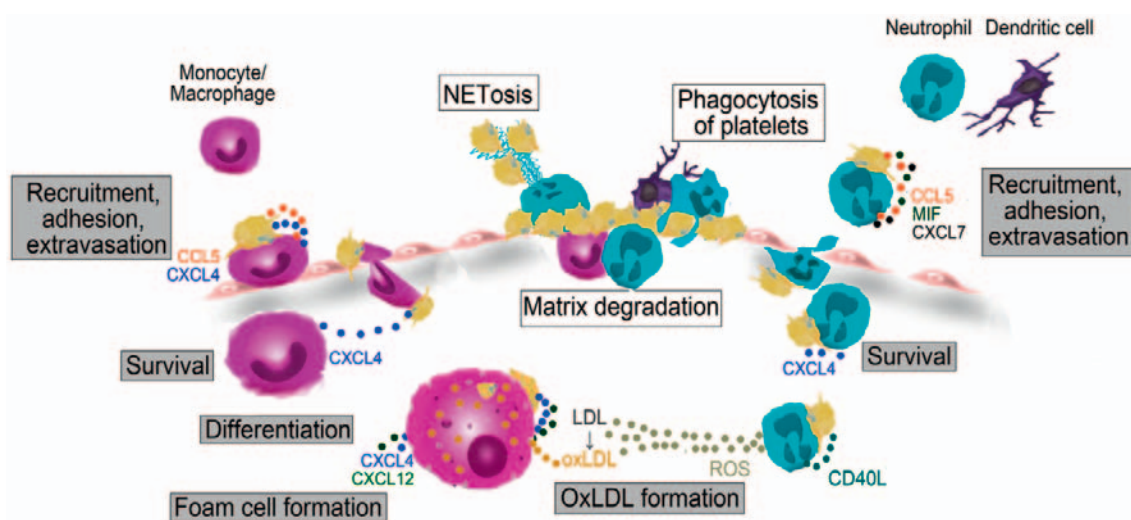
hance monocyte adhesion and tissue infiltration [22]. The pro-inflammatory microenvironment at sites of endothelial dysfunction further promotes platelet adhesion and activation [84]. This enhances the release of platelet-derived chemokines like CCL5, MIF and CXCL7, which leads to neutrophil recruitment [85, 86]. The pro-inflammatory, pro-oxidative state at atherosclerotic sites leads to modification of low-density lipoproteins (LDL). LDL oxidation is furthered by platelet release of sCD40L, which induces ROS production by neutrophils [49]. Thereby, platelets contribute to LDL oxidation and endothelial dysfunction [85]. Oxidised LDL, in turn, activates platelets, leading to platelet-neutrophil aggregate formation, which accelerates neutrophil activation and transmigration [87].

Platelet-mediated adhesion of monocytes and dendritic cells via direct cell-cell interactions at sites of atherosclerotic lesions [83, 84, 88] promotes atherogenesis [83, 89].

Platelet-derived CXCL4 prevents apoptosis of neutrophils [86] and monocytes, and induces monocyte CD86 expression and differentiation into macrophages [90]. These CXCL4-induced macrophages have distinct properties from classical M1 and alternatively activated M2 macrophages and are thus defined as M4 polarized macrophages, which exert a pro-atherogenic phenotype (reviewed in [91]).

Platelets further accelerate lipid uptake and foam cell formation by enhancing cholesterol uptake by monocytes [92–94]. This process is mediated by CXCL4, CXCL12, platelet-derived growth factor (PDGF) and phagocytosis of lipid-laden platelets, which have taken up modified LDL [22, 28, 95, 96].

Platelet depletion reduces foam cell formation [22], and CXCL4 deficiency diminishes atherosclerotic lesion development and size [97], while mice with hyper-reactive platelets show increased atherosclerotic lesion formation [84].

Plaque Rupture and Atherothrombosis

The stability of an atherosclerotic plaque is determined by inflammatory cytokines and cells present at sites of lesions. Activated platelets at atherosclerotic lesions recruit neutrophils, which secrete proteolytic enzymes like elastase, MMP8, MMP9, MPO or proteinase 3, thereby decreasing plaque stability [85, 98]. Activated platelets induce release of tissue factor-covered NETs by neutrophils at sites of plaque rupture, thus contributing to thrombus progression [85]. Lysophosphatidic acid within atherosclerotic plaques activates platelets in vitro, promoting platelet-monocyte aggregate formation, which may result in enhanced inflammatory and thrombotic stimulation in vivo [99]. Platelet-monocyte interactions further enhance matrix MMP9 production, and thereby contribute to plaque destabilisation [100, 101].

Immature myeloid dendritic cells interact with activated platelets only under low shear conditions found at sites of atherosclerotic lesions. Platelet-dendritic cell interaction damages plaque structure [102]. Neutrophils and dendritic cells can phagocytose activated platelets from preformed aggregates and thus may regulate inflammatory responses and atherothrombotic events [103, 104].

Megakaryopoiesis

Platelets are produced by megakaryocytes in the bone marrow. Megakaryocytes derive from haematopoietic stem cells, and their differentiation process is strictly regulated by a plethora of cytokines, including IL-1α, IL-1β, IL-3, IL-6, IL-8, IL-9, IL-11, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage

Table 2. Effect of innate immune cells on mega-karyopoiesis and effects of megakaryocytes and platelets on leukocyte production[a]

| | Leukocytes→ megakaryopoiesis | | MKs/platelets→ leukopoiesis |
	stimulatory	inhibitory	
IL-1α	[113]		
IL-1β	[105, 117]		[105]
IL-3			[106]
IL-6	[112,117,118]		[116]
IL-8/KC	[118]		[114]
IL-9	[119]		
IL-11	[106]		
G-CSF	[114,117]		
GM-CSF	[106,108]		[115]
TGF-β		[109]	
ROS	[111]		
Unique NK cell peptide	[109]		

[a]Macrophage and nuetrophil-derived interleukin-1α (IL-1α) induces megakaryocyte rupture, which results in rapid production of large amounts of platelets. Monocytes, macrophages, neutrophils and NK cells release IL-1β, IL-6, IL-8, IL-9, IL-11, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF) and reactive oxygen species (ROS), which stimulates haematopoietic stem cell proliferation and megakaryocyte maturation. IL-2 stimulated NK cells secrete a unique peptide that stimulates megakaryopoiesis independent of thrombopoietin (TPO). Transforming growth factor-β (TGF-β)-derived from NK cells can inhibit megakaryopoiesis. Megakaryocytes also influence leukocyte blood counts by enhancing neutrophil mobility and release into the circulation or via the release of IL-1β, IL-3, IL-6, IL-8 and GM-CSF.

colony-stimulating factor (GM-CSF) and thrombopoietin (TPO) [105-108].

A number of inflammatory states are associated with elevated platelet counts and increased blood TPO, which represents an important mediator of megakaryopoiesis and platelet production [109, 110]. The effects of innate leukocytes on megakaryopoiesis and the effect of megakaryocytes on innate leukocyte counts are summarised in table 2.

Via release of ROS, activated macrophages and neutrophils enhance haematopoietic stem cell commitment towards the megakaryocytic lineage and accelerate megakaryocyte maturation [111]. Activated leukocytes release GM-CSF, G-CSF, IL-1α, IL-1β, IL-6, IL-8, IL-11 and a unique natural killer (NK) cell peptide, thereby promoting megakaryopoiesis [105, 106, 109, 111–113]. Many leukocyte-derived cytokines induce TPO expression as well as commitment, maturation and/or rupture of megakaryocytes. This leads to increased platelet counts during acute inflammatory stimuli. Leukocytes can also diminish platelet production via release of TGF-β, which is a potent inhibitor of megakaryopoiesis and megakaryocytotic endomitosis [109].

Megakaryocytes, in turn, influence neutrophil blood counts via IL-8 and macrophage inflammatory protein 2 (MIP-2) release, which promotes neutrophil migration. Stimulation of megakaryocytes induces the release of IL-8 and MIP-2, which bind to CXCR2 to initiate vessel migration and subsequent release of neutrophils into the circulation [114]. Megakaryocyte-derived IL-1β, IL-3 and GM-CSF induce granulopoiesis [105, 115], and IL-6 treatment increases not only neutrophil and platelet counts but also monocytes and NK cell levels [116].

Disclosure Statement

The authors declared no conflict of interest.

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