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Interactions of monocytes and platelets: implication for life

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1. ABSTRACT

Monocytes interact and cross-talk with platelets in many settings including inflammation, hemostasis, or vascular disorders. These interactions are important for the regulation of life span of both. During inflammatory diseases, there is a rapid recruitment of monocytes and platelets to the site of inflammation and endothelial injury where they act side-by-side. Adherence between monocytes/macrophages (Mphi) and platelets occur in the vessel wall and atherosclerotic plaque, but it is also shown in the blood stream where it has been called platelet satellitism. This phenomenon has been attributed to thrombotic disorders such as stroke. Furthermore, we discovered consequences for leukocyte apoptosis after the interaction with platelets. Herein, we reviewed the complex mechanism and interactions regulating the life span of both types of blood cells. We also provide a distinct focus on apoptosis of platelets and Mphi.

2. INTRODUCTION

Monocytes interact and cross-talk with platelets in many settings including inflammation, hemostasis, or vascular disorders. These interactions are important for the regulation of life span of both cell types. In this review the complex mechanism and interactions regulating life span of monocytes and thrombocytes are described in detail. Also we depict the apoptosis of platelets and Mphi in special consideration of phagocytosis.

3. MPHI

3.1. The mononuclear phagocyte system

The mononuclear phagocyte system or monocyte-macrophage system constitutes the whole ensemble of $CD34^+$ myeloid progenitor mononuclear cells sharing endocytic, morphologic, and antigenic characteristics (1,2). Environment and prevalent or emerging systemic and local

conditions (e.g., inflammation) induce activation, adherence, margination, and maturation of circulating peripheral blood mononuclear cells (PBMC). Approximately 2-9% of the peripheral human blood leukocytes are PBMC (about 5-10% of peripheral human blood leukocytes are mature monocytes) (3), but in average only about 40% of the available monocytes circulate while the rest migrate (4,5). The term PBMC itself is a collective term for heterogeneous monocytic subsets characterized by a high potential for differentiation (6,7). Lately, particular populations even acting as pluripotent stem cells, were identified (7,8). The classical understanding of differentiation comprises the maturation of immature circulating PBMC into specialized and tissue typical resident macrophages and antigen presenting cells (APC) derived from bone marrow progenitors. Three origins have been identified: macrophages can originate from the yolk sac, the fetal liver and the bone marrow during different developmental stages (9). Directly related to their differentiation state is their functional capacity to play specific roles in immunoregulation during pathogen recognition, malignancy or tissue repair and morphogenetic remodelling (10,11,12,13,14,15,16,8). Independently of their origin all MPS act as professional phagocytosis and antigen presenting cells (1).

3.2. Phagocytosis

Phagocytosis, the uptake of particles larger than 0.5 μm, is one of the strategies of cells to internalize particles and solutes. Professional phagocytes are characterized by their professional phagocytic receptors which are able to ingest particles even when expressed in non-phagocytic cells.

3.2.1. Regulation of phagocytosis

Phagocytosis can be divided in type I, involving the engulfment of particles via pseudopodia, and type II, referring to complement-dependant invagination of the plasma membrane (17). After incorporation and lysis, distinct products of the degradation process are presented via major histocompatibility complex II (MHC-II) receptors. But to some extent, professional APC can break the rule presenting exogenous antigens on MHC class I molecules (cross-presentation) (18).

Mphi express a broad spectrum of specific membrane receptors enabling rapid and efficient phagocytosis. Important receptors are the scavenger receptors type A and B, comprising a group of receptors (CD204, MARCO, CD36, CD68 etc.) which recognize modified oxidized LDL and have been implicated in host defence (19). Receptor occupancy activates the monocyte and can lead to internalization of (opsonized) pathogens. Phagosomes are formed using actin remodelling and acidification of the phagosome lumen kills unwanted intruders. During this event the maturing vacuole drastically changes its composition, keeping its size, via fusion and secession with endosomes of distinct developmental stages (early and late endosomes) and lysosomes containing acidic material (proteolytic enzymes and oxidants). The maturation process ends in the generation of a microbicidal phagolysosome capable of degrading ingested material. Processed antigens are mainly presented via major MHC-II to recruit T- and B-cells for activation of the primary immune response.

3.2.2. Phagocytosis in inflammation and wound healing

The microenvironment helps determine distinct populations of macrophages classified as M1 and M2. The M2 macrophages can further be subdivided into M2a, M2b and M2c depending on the cytokine milieu (20). In the presence of IFNg and/or LPS, classical activated macrophages (M1) evolve and promote host-defense via secretion of a pro-inflammatory effector molecule cocktail including IL-1, IL-6, IL-8, IL-12, IL-23, CXCL10, CCL5 and TNF-alpha (20,21). M1 control pathogen infiltration by producing reactive oxygen species (ROS), nitric oxide (NO), leukotrienes (22), platelet activating factor (PAF), prostaglandins (23), and plasminogen activator enzymes among others. Secretion of these cytokines and chemokines results in recruitment and activation of T-, B-, NK-cells, and inflammatory monocytes. The removal of apoptotic or necrotic cell debris is one of the "classical" tasks of M1.

Clearance of cells (apoptotic or (secondary) necrotic) induces immunoregulatory pathways. The subsequent immune response, either pro- or antiflammatory, is dependent on many different factors such as the type of cell, the stage of the dying cell, the type of cell death, mechanism of uptake and the microenvironment. Phagocytosis of early apoptotic cells induces an anti-inflammatory response leading to inhibition of monocyte recruitment and tolerance, uptake of late apoptotic and necrotic cells typically induces inflammation promoting autoimmunity (24,25,26,27,28,29). Phagocytosis is influenced by cytokines, for exampleIFN-g and IL-4 inhibit phagocytosis, tumor necrosis factor-alpha (TNF-α) expedites it. Interestingly, the intrinsic process of phagocytosis cannot affect apoptosis of Mphi (30), whereas the uptake of certain substances can induce (31,32,33) or protect from cellular death (34,30). Considering anti-inflammatory issues, phagocytosis even encourages Mphi to induce apoptosis in neutrophils (35,36).

Monocyte/macrophage-depleted animals exhibit defective wound repair such as delays in angiogenesis and re-epithelialization, suggesting an important role for Mphi in these processes (37). Administration of Mphi into wounds resulted in considerably improved healing (38,39). It is of note, that the presence of Mphi not only creates an aseptic wound-milieu but initiates proliferation and synthesis of new matrix components (40), whereas the incidence of keloid decreases (41). These Mphi are characterized as low IL-12 producers and can be subdivided into three groups M2a, M2b and M2c. M2a macrophages known as alternatively activated macrophages, differentiate in the presence of IL-4, IL-13 or IL-21 (20) and participate in tissue repair. In contrast to classical activated pro-inflammatory M1 macrophages, M2a phagocytes exhibit an anti-inflammatory cytokine profile enabeling them to limit and terminate inflammation. M2a's act via secretion of cytokines involved in wound healing, angiogenic and immune-regulatory cytokines and

growth factors such as IL-10, TGF-beta and VGEF. Regulation also happens through upregulation of arginase-1 expression which causes a shift from NO synthases towards ornithine production and collagen synthesis (42) and through limitation of pro-inflammatory activated granulocytes via induction of apoptosis through Fas-ligand and TNF-α production (35,43,44,45). Debris is eliminated, healing is initiated, and in cooperation with dendritic cells self- tolerance (T-cell tolerance) is induced. If termination of inflammation is not regulated, chronic inflammation and autoimmune diseases can arise (46,47). Immune complexes and ligands of IL-1R and TLRs lead to the differentiation of M2b, while M2c macrophages are induced via IL-10, TGFbeta or glucocorticoids. These subsets have also regulatory functions.

3.2.3. Phagocytosis in human disease

Atherosclerosis has been classified as a chronic inflammatory disease due to the accumulation of white blood cells and formation of atheromatous plaques (48). Hence, it is not surprising that findings to date elucidated a complex role of macrophage phagocytosis in atherogenesis (33). Much of the previous investigations on atherogenesis focused on the mechanisms by which monocytes are attracted and tethered to the endothelial layer emphasising the role of different receptors (49,50,51,52) and the functions of macrophages/foam cells in intravascular lipid metabolism (53). Nowadays, we learn more and more about enzymatic activities leading to angiogenesis, bleeding, coagulation, rupture, and the different actions and types of Mphi involved in this process.

The enrichment of modified lipids such as oxidized LDL on the arterial wall, but also a change in blood rheology or inflammation, results in the activation of endothelial cells (54). This stimulation leads to an increased expression of adhesion molecules for monocytes and T cells such as P- and E-selectin, VCAM and ICAM (55) facilitating the recruitment of these cells. Mature macrophages phagocytose oxidized LDL mainly via the scavenger receptors A, lectin-like oxLDL receptor, and CD36 leading to accumulation of lipid droplets in the cytoplasm and causing the transformation into foam cells (56), and subsequently to the classical pro-inflammatory activation of these lipid-laden macrophages (57,58). However, despite the detrimental effects of foam cell formation on atherogenesis, pharmacological approaches to suppress foam cell generation through inhibition of acyl-coenzyme A:cholesterol acyltransferase-1 (ACAT1) failed, and resulted paradoxically in increased atherosclerosis (59,60).

An alternative recruitment of monocytes into these lipid-rich plaques occurs via the vasa vasorum, a network of microvessels (61). Activated macrophages produce and secrete proangiogenic factors inducing neovascularization most likely under hypoxic and inflammatory conditions leading to vulnerable intraplaque (neo-)vessels. Microhemorrhage and subsequent cell leakage results in iron accumulation and phagocytosis of these cells by plaque macrophages turning into foam cells (61). Subsequent aggregation of these foam cells results in the onset of a necrotic core while concurrently deposition of extracellular matrix components and smooth muscle cell recruitment expedites the fibrous cap formation and the progression and vulnerability of the plaque.

Advanced plaques contain many apoptotic cells (AC) derived from all types of cells involved in atherosclerosis, including macrophages, foam cells, T-cells, and smooth muscle cells (62). Within the lesion inductors of apoptosis, i.e. hypoxia, growth factor withdrawal, high concentrations of free cholesterol and oxidised LDL, production of pro-apoptotic cytokines such as TNF-α or the release of excessive amounts of ROS/RNS by macrophages in addition to direct cell to cell interactions (e.g., binding of Fas Ligand to Fas) are present in abundance (63). As mentioned before, phagocytosis of AC also known as efferocytosis creates an anti-inflammatory environment via IL-10 and TGF-b and PGE2 production inducing repair (64). AC that are not scavenged in plaques become secondarily necrotic after loss of membrane integrity, accumulate in the growing plaque and contribute to the development of inflammation and via activation of thrombin to thrombosis. This elucidates the importance and beneficial effects of efficient phagocytosis of AC inside atherosclerotic lesions.

Tumors were originally thought to be effectively reduced by macrophage invasion. However, over the last few years, conflicting results have challenged this paradigm by showing that different macrophage subsets can be linked with either protective or pathogenic roles in tumor growth (9,65,21,66). M1 macrophages are part of the anti-tumor response through inflammatory cytokine production and counteracting the immunosuppressive and protumoral activities of M2 and regulatory subsets. However, tumors are capable of inducing differentiation of tumor-associated macrophages (TAM) changing their immunologically active state into a M2-like immunosuppressive and tumor promoting phenotype. Nevertheless, it is generally accepted that macrophages play an important regulatory role in tumor progression, metastasis, invasion and angiogenesis (67,68).

Interestingly, monocyte chemoattractant protein-1 (MCP-1) also known as CCL2 is a chemokine involved in monocyte/macrophage recruitment and has been implied in angiogenesis (69,70,65,71). Although it has angiogenic and protumoral effects, MCP-1 application was successfully used to treat tumors by attraction and activation of monocytes (72). This apparently contradictory statement could be explained by the different macrophage subsets involved. M2 macrophages could be responsible and/or maybe dysregulated (73,74). In fact, dysregulation of MCP-1/Mphi was shown to be critical in autoimmune myocarditis (75,76). Otherwise alternative activation of monocytes through phagocytosis of apoptotic cells, meaning deactivation of antitumor activity, could be involved (77).

Dysregulation of phagocytosis promotes the development of many more different diseases such as atopic dermatitis, alzheimer´s disease (78,79) and fungal infection (80). In gout the effects of uncoated monosodium

Table 1. Selection of secreted products of activated Mphi

Cytokines	Chemokines	Growth factors	Enzymes	Further effectors
Interleukines	AMAC-1 [1]	FGF [2]	Arginase	Nectins
IL [3]-1 alpha, beta, ra,	IL-8	GM-CSF [4]	MMP [5] (1, 2, 8, 9, 12, 13)	ROS [6]
IL-6, 12, 15, 18, 23, 32	MIG [7]	IGF-1 [8]	Muraminidase	RNS [9]
OSM [10]	IP-10 [11]	M-CSF [12]	Myeloperoxidase	PAF [13], LTB_4 [14]
TNF-alpha [15]	MIP [16]-1alpha, beta	PDGF [17]	NADPH [18]-Oxidase	PGE_2 [19]
PAF	RANTES [20]	TGF [21]-beta	Plasminogen-Activator	
Interferones		VEGF [22]	Superoxiddismutase	
INF [23]-alpha, beta				
IL-10				

The strict division in cytokines, chemokines and growth factor is rather classical and serves for better demonstration only
Abbreviations: [1]AMAC: alternative activated macrophage associated chemokine; [2]FGF: fibroblast growth factor; [3]IL: Interleukin; [4]GM-CSF: Granulocyte-Macrophage Colony Stimulating Factor; [5]MMP: matrix metalloprotease; [6]ROS: reactive oxygen species; [7]MIG: monokine induced by INF-gamma; [8]IGF: insulin like growth factor; [9]RNS: reactive nitrogen species; [10]OSM: onkostatin M; [11]IP: interferon-inducible protein; [12]M-CSF: Macrophage Colony Stimulating Factor; [13]PAF: platelet activating factor; [14]LTB: leukotriene B; [15]TNF: tumor necrosis factor; [16]MIP: macrophage inflammatory protein; [17]PDGF: platelet-derived growth factor; [18]NADPH: nicotinamide adenine dinucleotide phosphate; [19]PGE: prostaglandin; [20]RANTES: regulated on activation, normal T cell expressed and secreted; [21]TGF: transforming growth factor; [22]VEGF: Vascular Endothelial Growth Factor; [23]INF: Interferon

urate crystals on articular inflammation are well described (81,82,83,84). Urate crystals are encountered by synovial Mphi, which induces CD14 mediated release of prostaglandins, proteases, and pro-inflammatory cytokines including TNF-alpha, IL-1β, IL-6, and IL-8 (85). Phagocytosed crystals cause lysis of the phagolysosome, release of its toxic contents and evoke cellular necrosis. Additional effects may be caused by perforation of cell membranes.

Phagocytosis plays obviously an important role in healing and disease but it is also an essential process targeted by viruses, bacteria, parasites and drugs. The Human immunodeficiency virus infects $CD4^+$ Mphi via membrane fusion, while bacteria like *Mycobacterium tuberculosis* or protozoa such as Leishmania species even developed strategies using phagocytosis to invade Mphi. However, the mechanism of phagocytosis is also utilized by physicians for drug delivery when they apply e.g., AmBisome ®, which is Amphotericin B packed in liposomes and taken up by the infected macrophages, to treat leishmaniasis in patients.

Taken together, receptor binding/activation of Mphi initiates various biological functions such as phagocytosis, subsequent intracellular dismantling of pathogens, production of ROS/RNS, production and release of inflammatory messengers (Table 1), cell mediated cytotoxicity, and enhancement of antigen presentation. Phagocytosis itself drives Mphi in dependency of the absorbed materials into certain modes of activation. Phagocytosis is pivotal for uptake and degradation of pathogenes, debris and senescent cells also taking part in tissue remodelling, development, and immune response.

3.3. Local proliferation and (trans-)differentiation

From the 1960s to the 1980s Dr. van Furth and collaborators extensively investigated the kinetics of macrophage populations in different tissues (86) detectingthat under normal steady-state conditions, the homeostasis of tissue populations is mainly assured by monocyte recruitment and not by local proliferation. However in the 1990s, Kennedy and Abkowitz performed bone marrow transplantation in mice (87) drawing the conclusion that tissue macrophages turn over more slowly than previously thought or one can assume that under certain conditions, local proliferation takes place. In contrast to van Furth, Tarling observed self-renewal of pulmonary alveolar macrophages in radiation chimera studies (88,89). Recently, local proliferation of microglia, pulmonary macrophages, Kupffer cells and Mphi entering grafts were described (90,91,92,93). Furthermore, different groups have reported a proliferation-inducing effect of IL-4 on M2 (94) and also M1 macrophages which might be associated with the expression of macrophage-activating factor (c-MAF) (95).

The differentiation of Mphi into polarized macrophage subsets depends also on the milieu. Lack of certain stimulatory cytokines or of nutrients rapidly induces apoptosis of Mphi. A pro-inflammatory milieu, induces the expression of anti-apoptotic and survival molecules like hemoxygenase-1 (HO-1) and heat shock protein 70 (HSP70) (96,97). In addition, binding of endotoxin to CD14 inhibits apoptosis in Mphi, whereas downregulation of CD14 by anti-inflammatory IL-4 promotes apoptosis (98,99).

3.4. Apoptosis

Platelets unlike monocytes do not undergo classical apoptosis. There are two major ways of inducing apoptosis, the intrinsic (mitochondrial), and the extrinsic (membrane receptor bound) pathways (100,101,102,103,104). For examples of apoptosis-inducing conditions in Mphi see table 2. Important receptors involved in the extrinsic induction of apoptosis are the Fas *(CD95/APO-1*) and the death receptor TNFR1. FasL binds to its receptor which multimerizes and induces recruitment of the adapter molecule *FADD* (Fas-associated death domain). This initiates the formation of *DISC (*death inducing signalling complex). Consecutively, this complex activates caspase-8 (105,106,107). Caspase-8 in turn

Table 2. Mechanisms and stimuli inducing apoptosis in Mphi (examples)

Direct inhibition of transcription, translation or cell cycle (steroids, cytostatic drugs or mitotic poisons)
Starvation or absence of growth factors or hormones/cytokines (serum depletion, GM-CSF [1], M-CSF [2], TNF-alpha [3], IL1beta [4])
Downregulation or blocking of the LPS-receptor [5] CD14 [6] (IL-4)
Intracellular accumulation of non-physiological proteins (cytoststic drugs, heat)
Occupation of death receptors (CD95/Fas, TNFR1 [7], DR3/Apo3 [8], DR4, DR5/Apo2/KILLER)
Cellular alteration (heat, hypoxia, radiation, drugs, enzymes, oxygen species)
Mutations or mitotic errors
Pathogenes (Mycobacterium tuberculosis, Shigella species)
Deactivation of protective factors (bcl-2 family members can be deactivated in hypoxia)

Abbreviations: [1]GM-CSF: Granulocyte-Macrophage Colony Stimulating Factor; M-CSF: [2]Macrophage Colony Stimulating Factor; [3] TNF: Tumor necrosis factor; [4]IL1beta: Interleukin 1 beta; [5]LPS: Lipopolysaccharide; [6]CD: Cluster of differentiation; [7]TNFR1: Tumor necrosis factor receptor 1; [8]DR: Death receptor

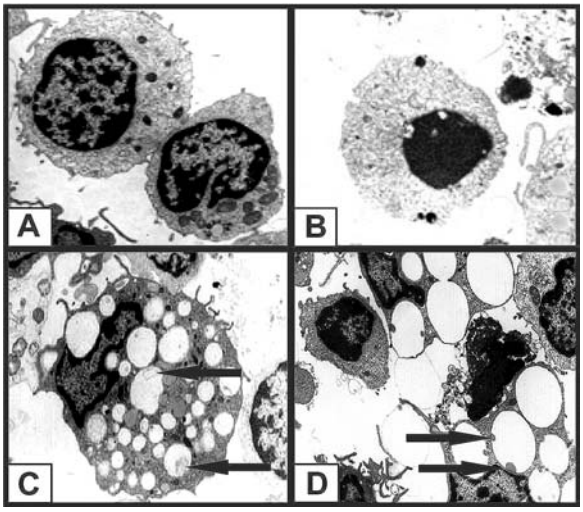

Figure 1. The transmission electron microscope (TEM) reveals complete ingestion of platelets. (A) Two monocytes that maintained normal morphology after a 60-h culture in 5 % FCS-containing medium. (B) Typical changes of apoptotic monocytes after serum deprivation for 60 h. Dense nuclear condensation, cytoplasmic vacuolization, shrinkage, and rounding is visible. Investigating monocytes after co-culture with platelets showed that monocytes ingested platelets (arrows) in huge amounts and, despite culturing them in 0.2 % FCS-containing medium no signs of apoptosis occurred (C). In contrast, monocytes exerted typical signs of apoptosis after ingestion of latex beads (arrow, D). Magnification: x7200.

stimulates amongst others the main effector caspase-3 and a pro-apoptotic member of the bcl-2 family named BID. In parallel several factors including ROS, caspases, Ca^{2+}, ceramides or BID, can inhibit anti-apoptotic Bcl2 and affect mitochondria, thereby activating the intrinsic or mitochondrial cascade of apoptosis. The activation of the intrinsic pathway leads to the efflux of Smac and the assembly of Bax-Bak inducing cytochrome c release from the mitochondria. Smac, one critical protein, is an inhibitor of IAP (108). Cyt c, Apaf-1 and dATP/ATP form a complex called the apoptosome which recruits and activates cytosolic pro-caspase-9 (106,109). Caspase-9 cleaves then downstream caspases such as pro-caspase-3 and -7. Active caspase-3 cleaves a diverse repertoire of substrates including the DNAase-inhibitor (ICAD/DFF45) contributing to DNA-fragmentation, the anti-apoptotic members of the bcl-2 family, proteins participating in DNA-repairing and in the regulation of the cytoskeleton (100,101). In addition, the issue of caspase-mediated death is related to the expression of pro-apoptotic proteins, like p53, p21, apoptosis inducing factor (AIF) or endonucleases. All of them could affect the integrity of the mitochondria (101,102). It is well known that impairment of mitochondria disrupts the electron transport chain, which leads to a loss of ATP and of function resulting in a massive release of ROS. Although, activation of caspases mostly ends with cellular demise, their blocking can delay but not stop the finalizing process, as demonstrated when antagonists of apoptosis, like *bcl-2* or *bcl-xL,* were added to dying cells (100,103). As we have shown, phagocytosis of platelets is a strong anti-apoptotic mechanism interacting with the caspase-dependent process of apoptosis through down-regulation of caspase-3 and -9 in monocytes (30). Furthermore, we could not only observe the down-regulation of pro-apoptotic effectors, but additionally identified the up-regulation of anti-apoptotic proteins like hemeoxygenase-1 (HO-1) and heat shock protein 70 (HSP70). They act as important survival mechanisms antagonising apoptosis, and protecting Mphi from stress related cell damage (96,30,97).

Whereas the apoptosis of monocytes features all classical attributes like cell shrinkage, nuclear and cytoplasmic condensation, cellular fragmentation into membrane-bound fragments (karyopyknosis, karyrhexis) and membrane blebbing (Figure 1), apoptosis of anuclear platelets shows only some of the mentioned features (see below). Taken together, there is emerging evidence that the traditional view of monocytic replacement without local proliferation but Mphi recruitment has to be revised. Stem cell-like Mphi exist, and, unmatched by any other cell type, Mphi are pluripotent showing an enormous plasticity of differentiation and function diversity. Mphi cannot only clear debris and apoptotic cells and induce wound healing, organise the immune response and limit the inflammation. Based on latest research, they contribute to a greater extent to the '*restitutio ad integrum'* or pathogenesis of disease when (trans-)differentiating, proliferating or inducing matrix synthesis, and angioneogenesis than it was initially assumed.

4. PLATELETS

Platelets, also known as thrombocytes, originate from their precursor megakaryocytes and are small anucleated cell fragments involved in hemostasis and immune response. They evolve from two processes called megakaryocytopoiesis followed by thrombopoiesis. During megakaryocytopoiesis, pluripotent hematopoietic stem cells (HSC) proliferate, differentiate, and mature into megakaryocytes (MK). In the process of thrombopoiesis mature MK fragment into anucleated blood platelets (110,111,112,113). The average platelet count in humans ranges from 150 x 10^9 to 400 x 10^9 per liter. Assuming a life span of roughly 9-10 days, an average blood volume of five liters, and 1/3 of platelets pooled within the spleen (extensively assessed by radiolabel and splenectomy studies in the 1960s and 1970s), the adult offers a daily production of approximately 1 x 10^{11} platelets to maintain its homeostasis. If needed, the production level can increase by more than twentyfold (114,115,116,117).

Until recently platelets only have been assigned to play a role in hemostasis but more and more evidence show that they might also play an important role in regulating the immune response to pathogens. Interestingly, platelets express a wide range of receptors known to be involved in innate immunity such as TLR1-9, complement receptors and Fc receptors (118,119,120). The platelet production is controlled by thrombopoietin (TPO), an acidic glycoprotein which is synthesized in the liver, kidney, and BM (121,122). However, factors regulating their survival remain unclear and some aspects to date known will be reviewed below.

4.1. Platelet regulation of clot formation

Bleeding problems associated with platelet disorders reveal their importance in hemostasis. At sites of injury, platelets adhere to the vessel wall, undergo activation, secrete granule content, and aggregate, building an impermeable plug. This is the first line of defence against blood loss. When the vascular wall is injured, components of the subendothelial extracellular matrix are exposed inducing retardation of migrating platelets. The interaction of the adhesive molecule von Willebrand factor (vWF) and the adhesive platelet integrin receptor complex glycoprotein (GP) Ib-V-IX induces the initial tethering of the platelet to the ECM under high fluid shear stress. This leads to further receptor interactions (see reviews, (123,124,125)) of a broad range of integrins and selectins and subsequent activation of the platelets (126). The membrane of activated platelets provides the necessary surface initiating the extrinsic coagulation cascade including the generation of stabilizing thrombin and fibrin (127,128). Platelet activation is further associated with cytoskeleton remodelling, change in morphology and the release of granule content including adhesive molecules like vWF, fibrinogen, adenosine diphosphate, serotonin and thromboxane A2. All this purposes stable adhesion to the endothelium and platelet activation and thrombi formation (123).

Integrins play an important role in stabilizing the adhesion of activated platelets. Three β1 and two β3 of the heterodimeric transmembrane proteins are found on these anuclear cells with αIIbβ3 (also known as GPIIb/IIIa) being the integrin most abundantly expressed on the surface. After platelet activation, αIIbβ3 undergoes a conformational change shifting into a high-affinity state allowing efficient ligand binding and bidirectional signalling (126,129). The ligands of αIIbβ3 such as fibrinogen, fibrin, vWF, CD40L, fibronectin, vitronectin and thrombospondin-1 are all involved in platelet activation, inter-platelet bridging and thrombi stability. The receptor-mediated cell-to-cell adhesion is accompanied by an intensive crosstalk involving paracrine signalling. Platelets release a broad range of mediators such as cytokines (IL-1β, TGF-β etc.), chemokines (IL-8, CCL3, CCL7, CXCL7, PF4 etc) and growth factors (PDGF, VEGF) influencing and recruiting different cell types ranging from neutrophils, monocytes, lymphocytes and endothelial cells (CD40L-dependently activation of EC (130)). The cell-cell interaction via CD40 and CD40L between platelets and ECs induces upregulation of different adhesion molecules, cytokines and chemokines. On the other hand ECs are also able to influence and inhibit platelet function through e.g., nucleoside triphosphate diphosphohydrolases, prostacyclin, and NO (to avoid untimely activation in the steady state) or activate platelets through e.g., vWF, and platelet-activating factor (125). Tissue factor (TF) present in the subendothelial tissue and normally segregated from blood can be expressed by endothelial cells and mononcytes through platelet activation (131,132). It is a glycosylated transmembrane protein functioning as receptor for factor VIIa. The complex of receptor and ligand binding constitutes the major initiator of the blood coagulation cascade (133,134,135). Moreover, TF has direct pro-inflammatory effects by inducing the production of reactive oxygen (136). The mode of action of platelets is very diverse participating indirectly or directly in the initiation of an inflammatory response (coagulation, monocyte recruitment, and activation, and matrix remodelling) (137,138,139,140).

Intravascular thrombus formation by platelets is important for the physiological stop of blood loss, but it is critically responsible for the morbidity and mortality of arterial vascular diseases (141). Hence, anti-platelet drugs are an integral part of the prophylaxis, and the therapy of myocardial infarction, stroke, and diseases regarding to the peripheral arterial systems (142). There is increasing evidence that platelets amplify acute inflammation. Platelets and their release products seem to be important for the chemotaxis and activation of leukocytes, capture and killing of pathogens, thereby orchestrating the healing of the wound and defense against pathogens (118). On the other hand, they contribute to a misleading activation of the immune system e.g., allergy, chronic inflammation (asthma, arthritis, multiple sclerosis), diabetes or atherosclerosis (143,144,145,146,147,148,149).

In summary, blood platelets, beyond their well-recognized function in hemostasis, play a crucial and active role in inflammatory responses. As a result of direct interactions with leukocytes and endothelial cells, and, through the release of pro-inflammatory mediators, they

promote the recruitment of circulating leukocytes to immune-reactive points of interest.

4.2. Survival and death

Until today, the factors controlling the life span of platelets are not well understood. It is known that overall platelet function diminishes with aging and that *in vivo* senescent platelets show a significant reduced responsiveness to physiological agonists, whereas younger platelets are more reactive (150,151,152,153). The homeostasis of mature platelets is a balance between production, consumption, and disposal. While some of the mechanisms regulating thrombopoiesis have been clarified (115,121,122,154,111), the factors controlling their life span, particularly in the steady state, are still the subject of speculation. It has been controversially discussed whether extrinsic or intrinsic factors contribute to platelet death. Some pursue the opinion that aging of platelets is associated with the accumulation of damage throughout platelet life time such as stress, thrombi, shear, or temperature fluctuations (155,156). When a damage threshold is reached, platelets are cleared via phagocytosis. On the other hand, this is opposed by the view of an intrinsic apoptosis pathway leading to programmed platelet death (157,158).

Apoptotic processes during synthesis, activation, and *in vitro* storage of platelets have been demonstrated (159,160,161,162,163,164,165). *In vitro* platelets cell death has promoted controversial views. Cell death of platelets involves loss of mitochondrial integrity (associated with prolonged storage of platelets) similarly found in nucleated cells (159,162). Platelet apoptosis involves Apaf-1, caspase-9, caspase-3, and proteins of the Bcl-2 family, which are key regulators of the intrinsic pathway of apoptosis (159,166,167) and also form platelet-derived micorparticles via an apoptosis-like process (168). In addition, anti-apoptotic (Bcl-2, Bcl-x_L) and pro-apoptotic (Bax, Bak) members were detected (159). Interestingly, human platelets exhibit apoptotic events after agonist stimulation or under shear stress (167). Because these agonists include collagen and thrombin, it was suggested that their death is associated with blood coagulation (169). While low concentrations of thrombin can activate platelets, higher concentration generated during coagulation induce programmed cell death via ROS-dependent activation of caspases-3 and -9, cytochrome c release and phosphatidylserine exposure (170,171). In addition, Brown *et al.* showed a caspase-independent form of PCD in platelets (172). Recently, Bcl-x_L was identified as a life-limiting timer in platelets. Its amount is gradually declining with platelet aging (173,157,158).

4.3. Phagocytosis of platelets

Platelets invade the atherosclerotic lesion via leakage and/or rupture of ingrown microvessels where they lie side by side to plaque macrophages. Detection and clearance of platelets by macrophages involves class A scavenger receptors, PS receptors, recognition of CD36 (174) and mild fat globule-epidermal growth factor 8 (MFGE8) (175,176).

Immune thrombocytopenic purpura (ITP) is a bleeding disorder caused by auto-antibodies directed against own platelets (against cell-specific glycoproteins (GPIIb-IIIa, GPIb-IX and others)) leading to enhanced platelet clearance through Fc-receptor mediated phagocytosis by resident tissue Mphi mainly in liver and the spleen. In other cases, intramedullar destruction of antibody-coated platelets by Mphi or the inhibition of megakaryocytopoiesis or possibly complement-mediated lysis occurs (177). In the specific case of fetal/neonatal alloimmune thrombocytopenia, where platelets were opsonised by maternal antibodies and phagocytosed via FC-receptors, platelets target molecule was identified, namely human platelet antigen 1a (HPA-1a) (178). Most of the understanding of the pathophysiology of antibody-mediated platelet destruction today is deduced from *in vivo* studies in patients suffering from ITP. After labeling with an allo-antibody, platelets bind to Fcγ receptors (FcγR) on Mphi, mainly from spleen and liver, and are then destroyed via phagocytosis (178). Based on this knowledge, different therapeutic strategies (specific and unspecific) were developed. Strategies established or still under evaluation, range from removal of allo-antibodies (plasmapheresis or immunoadsorption), to immunosuppression (to inhibit the production of antibodies and to limit phagocytosis). They also include the saturation of the phagocytosis or impeding of antibody production by intravenous immunoglobulin, and splenectomy (reduction of Mphi) in combination with platelet transfusion but only under life threatening conditions. The specific blockade of the FcγRI-receptor of Mphi by antibodies is another therapeutical approach (177). Mphi mainly express FcγRI and FcγRII (a subset displays FcγRIII), but only inhibition of the FcγRI was effective to suppress phagocytosis of platelets in fetal/neonatal alloimmune thrombocytopenia (179,178). In contrast, patients with ITP responded to a monoclonal antibody against the FcγRIIIa receptor (180). Other strategies focus on the deactivation of T- or B-cells (anti-CD154-respectively anti-CD20-antibodies) (181,177).

Since the 1960s, platelet phagocytosis by macrophages is described as an alternative mechanism of foam cell formation and macrophage activation (182,183), whereas the direct linkage of platelet phagocytosis and macrophage activation via processing of platelet-derived amyloid precursor protein (APP) and generation of β-amyloid (Aβ)-like peptides was recently shown (184). APP is stored in alpha-granules of platelets and plays an important role in the pathogenesis of atherosclerosis and also Alzheimer's disease (185,184). Its accumulation in atherosclerotic plaques macrophages is related to platelet phagocytosis in the field of susceptible neo-vessels. Confirmed by analysis of inducible nitric oxide synthase (iNOS), TNF-alpha, and cyclooxigenase-2 (COX-2), it was found that APP-rich macrophages are activated (184). Nevertheless, the uptake of platelets from APP-knockout mice failed to activate macrophages. Therefore, APP or derived fragments were considered to be effector-molecules that induce macrophage activation after platelet phagocytosis. Interestingly, non-steroidal anti-inflammatory drugs (NSAIDs), and HMG-CoA reductase inhibitors (statins), two classes of pharmaceuticals affecting APP processing and Aβ formation in Alzheimer's disease, reduce macrophage activation after platelet phagocytosis

and inhibit formation of Aβ-containing peptides (186). Notably, thrombin, leading to activation, coating or apoptosis of platelets increases the phagocytosis rate of Mphi (187). In addition, platelets display multiple adhesion molecules and receptors supporting leukocyte arrest and facilitating recruitment of leukocytes into sites of vascular inflammation. Otherwise, platelets not only bind to leukocytes, enhancing their contact with the endothelium, but also secrete mediators, triggering monocyte arrest and inducing extravasation and migration (138,188). However, we found that platelet lysates were ineffective in suppressing apoptosis in monocytes *in vitro,* and platelet surface receptors or intracellular compounds released in phagolysosomes are needed (30). In contrast, Brunetti observed in monocytes anti-apoptotic qualities of mediators released by platelets (189). The micromilieu or differences in activation of platelets *in* and *ex vivo* could be responsible for anti-apoptotic failure of lysates or supernatants from cultured thrombocytes. In part for this reason, the phagocytosis of platelets might be effective but not essential to activate Mphi. Degranulation in terms of platelet activation (e.g., coagulation after intraplaque microhemorrhage) and the consecutive paracrine desposition of chemokines or exposure of receptors are likely enough stimulation to induce APP and Aβ release (188,138). Moreover, it was shown in plaque that macrophages can apparently be iNOS-positive without signs of internalization. These Mphi are frequently surrounded by platelets (184). However, the question whether, besides being a strong monocytic survival stimulus, phagocytosis of platelets in atherosclerosis is protective or destructive, still remains unanswered.

In early lesions the oxygen supply is high and the amounts of erythrocytes and thrombocytes are still low, because neo-vascularisation did not yet take place. Therefore, the total value of apoptotic material is marginal and phagocytosis is focused on lipoproteins generating pro-atherogenic foam cells. Along with the progression of plaques the micromilieu impoverishes and the burden of particles, necrotic and apoptotic debris increases. As described, only phagocytosis of AC can be beneficial for plaque stability. Unfortunately the combination of oxidative stress and cytoplasmic saturation with indigestible material impairs the phagocytosis of AC by Mphi in atherosclerotic plaques (190). Thus, the benefits resulting from uptake of AC would be overpowered by pro-inflammatory stimuli associated with phagocytosis of lipoproteins, platelets or erythrocytes, unless platelets are driven to be *in vitro* like and afterwards phagocytosed. We observed very effective anti-inflammatory activation and better survival of cultured human monocytes co-incubated with platelets. As a consequence of this, stimulation of phagocytosis in general may actually advance rather than limit plaque progression. Nevertheless, clearance of AC is a desirable process, and if possible, a selective stimulation of the uptake of AC might slow down the plaque progression. Approaches promoting phagocytosis of AC have been undertaken by administration of lipoxins, statins or azithromycin (191,192,193). Until now, the indirect way of stimulating phagocytosis via manipulation of platelets is untested and unexplored. Future studies are necessary to decide whether this strategy can be successfully used as anti-atherogen, since uncontrolled phagocytosis of AC might also lead to tissue injury (194).

5. FURTHER INTERACTIONS

Several different scenarios of interaction and support between monocytes and platelets are plausible. Since their emergence in the bone marrow, they share their lifespan in the bloodstream until they are attracted, activated and/or expanded. Activated platelets rapidly adhere to the endothelium and human blood leukocytes via expression of P-selectin (CD62P) interacting with their P-selectin glycoprotein (GP) ligand-1 (PSGL-1) (166,195). Adherence of activated platelets to leukocytes is a key event in the sequence of thrombus formation. Cell to cell interaction of platelet and monocyte induces the production of tissue factor, the major initiator of blood coagulation (130,196). Induction of monocyte TF depends predominantly on P-selectin/PSGL-1 binding and to a minor degree on the interaction of leukocyte CD40 with the platelet CD40 ligand (CD40L) (197). Adherence between Mphi and platelets occurs in the vessel wall and plaque, but it is also shown in the blood stream where it has been called platelet satellitism (198).

Platelet-endothelium interactions play an important role in the development of inflammation and atherosclerosis. In the endothelium, activated platelets induce MCP-1 secretion and surface expression of intercellular adhesion molecule-1 (ICAM-1) promoting the recruitment, adherence and extravasation of monocytes (137,125). Furthermore, platelets adherent to the endothelium bind and present vascular cell-derived chemokines to recruit circulating mononuclear cells. Moreover, platelets provide a sticky surface for leukocyte tethering and subsequent firm adhesion. Monocytes adhere to platelets using a Mac-1-dependent (CD11b/CD18, alphaMbeta2) mechanism. In addition, junctional adhesion molecule-C (JAM-C, JAM-3) and ICAM-2 as well as bridging proteins (fibrinogen or kininogen) were used as stabilizing linkers (140). Therefore, platelets participate directly in the initiation of an inflammatory response.

Platelets are involved in immunological processes after organ transplantation, e.g. it has been shown that there may be a correlation of platelet number within transplanted kidney function. A negative correlation between number of platelets and number of lymphocytes and a positive correlation between platelet count and number of other immunocompetent cells could be shown in kidney allograft recipients (199).

Pharmacological interventions change besides their influence on the pure number of platelets and leukocytes (e.g. corticosteroids, mycophenolate, calcineurin inhibitors etc) the interaction of platelets and leukocytes. This provides information about possible molecular mechanisms inducing rejection of allografts or cardiovascular complications. E.g., immunosuppressive therapy regimens impact on platelet CD62 expression and PAC1 (expression markers of platelet degranulation), and

aggregation in renal transplant patients (200,201). Interestingly, anti-inflammatory effects of the P2Y(12) receptor antagonist clopidogrel due to inhibition of the expression of platelet activation markers inhibiting the interaction of platelets and leukocytes had been observed in renal transplant patients (202).

Platelets interact with pathogens in a variety of clinical situations. After contact with bacteria and spirochetes platelets aggregate through crossreactive immunodeterminants and plasma proteins, and have the ability to recognize pathogens via Toll-like receptors (188). Therefore, they are co-workers for monocytes in the detection of and defence against microorganisms.

6. CONCLUSIONS AND PERSPECTIVES

Complex interactions between platelets and monocytes referring to various important clinical situations have been discovered. Platelets and Mphi stimulate each other via direct and indirect (e.g., via endothelial cells) cross talk resulting in reciprocal functional modulation, cooperation, and survival. Adherence of activated platelets to monocytes initiates thrombus formation and platelet recruitment by activated leukocytes plays an important role in modulating an inflammatory reaction. Platelets and platelet-derived mediators have been found to activate and modulate leukocyte apoptosis, whereas the phagocytosis of platelets is involved in crucial pro- and anti-inflammatory processes. Until now, there is unfortunately no therapeutical breakthrough available transferring this knowledge into a concrete anti-atherosclerotic therapy. However, preliminary approaches promoting anti-inflammatory activity of Mphi through the use of statins or antibiotics have been started but still need more evaluation. Therefore, further understanding of the specific interactions between platelets and Mphi would be appreciated because it may lead to the development of novel therapeutic strategies in immunology and vascular medicine.

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Abbreviations: AIF: apoptosis inducing factor; AC: apoptotic cell; ACAT1: acyl-coenzyme A:cholesterol acyltransferase-1; APC: antigen presenting cells; APP: amyloid precursor protein; BM: bone marrow; CD: cluster of differentiation; Cyt c: cytochrom c; DISC: death inducing signaling complex; EC: endothelial cell; ECM: extracellular matrix; FADD: Fas-associated death domain; HO-1: hemoxygenase-1; HPA-1a: human platelet antigen 1a; HSP70: heat shock protein 70; IAP: inhibitor of apoptosis; IL: interleukin; INF: interferon; ITP: (Auto)immune thrombocytopenic purpura; LPS: lipopolysaccaride; MCP-1: monocyte chemoattractant protein-1; MHC: major histocompatibility complex; MMP: matrix metalloproteinase; Mphi: monocytes/macrophages; MPS: mononuclear phagocyte system; MSU: monosodium urate; PF4: platelet activating factor 4; PBMC: circulating peripheral blood monocytes; PSR: phosphatidylserine receptor; RANTES: regulated on activation, normal T cell expressed and secreted; RNS: reactive nitrogen species; ROS: reactive oxygen species; Smac: secondary mitochondria-derived activator of caspases; SR: scavenger receptor; TEM: transmission electron microscope; TF: tissue factor; TGF: transforming growth factor; TH-1: T-helper-1-type cell; TIMP: tissue inhibitors of matrix metalloproteinase; TNF: tumor necrosis factor; TPO: thrombopoietin; vWF: von Willebrand factor

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