Life span of monocytes and platelets: importance of interactions

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Mphi
   3.1. The mononuclear phagocyte system
   3.2. Phagocytosis
      3.2.1. Regulation of phagocytosis
      3.2.2. Phagocytosis in inflammation and wound healing
      3.2.3. Phagocytosis in human disease
   3.3. Local proliferation and (trans-) differentiation
   3.4. Apoptosis
4. Platelets
   4.1. Platelet regulation of clot formation
   4.2. Survival and death
   4.3. Phagocytosis of platelets
5. Further Interactions
6. Conclusions and perspectives
7. Acknowledgments
8. References

1. ABSTRACT

Monocytes interact and cross-talk with platelets in many settings including inflammation, hemeostasis, or vascular disorders. These interactions are important for the regulation of life span of both. During inflammatory diseases, there is a rapid targeting of monocytes and platelets to points of inflammation and endothelial injury where they lie 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 life span of monocytes and thrombocytes are described in detail. Also we depict the apoptosis of platelets and Mphi in special consideration of phagocytosis.

2. INTRODUCTION

Monocytes interact and cross-talk with platelets in many settings including inflammation, hemeostasis, 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. MPHII

3.1. The mononuclear phagocyte system

The mononuclear phagocyte system (MPS) or monocyte-macrophage system (MMS) constitutes the whole ensemble of CD34⁺ myeloid progenitor cells (bone marrow, BM) derived 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 monocytes (PBMC). Approximately 2-9% of the peripheral human blood leukocytes are PBMC, but only 40% of the available monocytes circulate while 60% migrate (3, 4). The term PBMC itself is a collective term for heterogeneous monocyctic subsets characterized by a high potential for differentiation (5, 6). Lately, particular populations even acting as pluripotent stem cells, were identified (6, 7). Until recently, the classical understanding of differentiation was that depending on the need, immature PBMC differentiate into specialized and tissue typical resident macrophages and antigen presenting cells (APC). Directly related to their achieved state of differentiation is their functional capacity enabling Mphi (monocytes/macrophages) to carry out specific roles in immunoregulation, cell mediated innate immunity against infection, malignancy or tissue repair and morphogenetic remodelling (7-14). Nevertheless, however different the offspring are, as members of the MPS they all have in common their competency for professional phagocytosis and antigen presentation (2).

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

At present, two types of phagocytic processes have been characterized: phagocytosis type I, where pseudopodia engulf particles, and type II, where invagination of the plasma membrane is involved. For both processes the actin cytoskeleton has to be reorganized through definite signaling cascades (15). After incorporation and lysis, distinct pattern of the degradation product were presented via major histocompatibility complex II (MHC-II) receptors for their immunogenic fellow combatants. But to some extent, professional APC can break the rule presenting exogenous antigens on MHC class I molecules (cross-presentation) (16).

Mphi express a broad spectrum of specific membrane receptors enabling rapid and efficient phagocytosis. Important receptors are the scavenger receptors (SR) type A and B, the LDL-receptor SR-A, receptors for vitronectin (CD36), and macroisialin (CD68), the LPS-receptor CD14, the mannose receptor (MR), and the phosphatidylosnine receptor (PSR). Complement and antibody receptors serve for the detection of opsonized pathogens, cleverly using the groundwork of the innate (complement) and adaptive (antibody) immune response (reviewed in 17 and 18). Receptor occupancy activates the monocyte and induces internalization. F-actin depolymerises from the phagosome and the vacuole becomes accessible to early endosomes. The vacuole matures, meaning it fuses with late endosomes and lysosomes (granules and vesicles containing proteolytic enzymes and oxidants), to form a phagolysosome for degradation of ingests. 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

In interaction with T-helper-1 (TH-1)-type cells, classical activated Mphi secrete a cocktail of pro-inflammatory effector molecules like IL-1, IL-6, IL-8, IL-12, and TNF-alpha in addition to prostaglandines, reactive oxygen (ROS), and nitrogen species (RNS), peroxides, leukotrienes, platelet activating factor (PAF), phospholipase, and activators of plasminogen between others. T-, B-, and NK-cells, and further monocytes are attracted and activated. The pro-inflammatory process is fully activated, and leads to local destruction. The clearance of apoptotic or necrotic cell debris is one of the “classical” tasks of Mphi. Mphi organize the affected area by phagocytosis, and by termination of the inflammatory immune response.

Previously, it has been suggested that the disposal of apoptotic cells was not accompanied by secretory or inflammatory responses until Heidenreich and others described that active and mainly immunosuppressive pathways were evoked by apoptotic cell phagocytosis, leading to enhanced production of anti-inflammatory interleukin 10 (IL-10), transforming growth factor-beta (TGF-β), prostanoids or IL-6, or even down-regulation of pro-inflammatory cytokines. Therefore, the debulking of apoptotic cells is critical. Moreover, the release of pro-inflammatory cell content into the tissue (due to a process called secondary necrosis) is avoided, and further recruitment of circulating monocytes inhibited (19-23). Not surprisingly, phagocytosis is influenced by cytokines. While interferon (INF-gamma) and IL-4 inhibit its execution, tumor necrosis factor-alpha (TNF-α) expedites it. Interestingly, the intrinsic process of phagocytosis cannot affect apoptosis of Mphi (24), whereas the uptake of certain substances can induce (25-27) or protect from cellular death (24, 28). Considering anti-inflammatory issues, phagocytosis even encourages Mphi to induce apoptosis in neutrophils (29, 30).

Usually the inflammation is limited by anti-inflammatory TH-2 activated suppressor-Mphi which show in favor of classical activated (pro-inflammatory) Mphi, a different cytokine excretion pattern including IL-1 receptor antagonist (IL-1ra) (31). These alternately activated suppressor-monocytes (aMphi) act strongly as anti-inflammatory not only by secretion of anti-inflammatory cytokines, e.g. IL-10, IL-1ra, TGF-beta, and AMAC-1. Furthermore, aMphi control the amount of pro-inflammatory activated granulocytes by induction of apoptosis via the Fas-Ligand and TNF-α production (29, 32-34). After eradication of the disquiter, T- and B- memory cells assure antigen dependent immunity. Debris are erased, healing is initiated, and in cooperation with dendritic cells self tolerance (T-cell tolerance) induced - if not, chronic inflammation and autoimmune disease prosper (35, 36).

Because monocyte/macrophage-depleted animals exhibit defective wound repair such as delays in
angiogenesis and re-epithelialization, Mphi likely contribute to both (37). In contrast, 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).

3.2.3. Phagocytosis in human disease
Atherosclerosis has been classified as a chronic inflammatory disease (42). Hence, it is not surprising that recent findings elucidated a complex role of macrophage phagocytosis in atherogenesis (25). 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 (43-46). Aside from this, the functions of macrophages/foam cells in intravascular lipid metabolism are one of the initially described characteristics in atherogenesis (47). Nowadays, the researchers learn more and more about enzymatic activities leading to angiogenesis, bleeding, coagulation, rupture, and the different actions and types of Mphi that contribute to this.

Lipid-rich plaques contain a higher microvessel density than fibrous plaques (48). Further, endothelial cells in lipid-rich plaques express increased levels of adhesion molecule for monocytes. This is a hint that microvessels are important to the recruitment of monocytes into atherosclerotic plaques. Intraplaque (neo-)vessels are susceptible to disruption. Microhemorrhage, followed by platelet and erythrocyte leaking into the lesion, occurs. Both were phagocytosed by plaque macrophages. Therefore, toxic pro-inflammatory iron accumulates, whereas the scavenging of lipoproteins leads to the formation of foam cells, and subsequently to the classical pro-inflammatory activation of these lipid-laden macrophages (18, 49). 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 (50, 51). Proteolytic matrix metalloproteinases (MMPs) regulate both integrity and composition of the extracellular matrix (ECM) (52). Activated Mphi secrete lots of MMPs and ROS which are known to activate other MMPs by inactivation of tissue inhibitors of MMPs (TIMPs). Consequently, the matrix is degraded. This excessive ECM breakdown is blamed for the progression of a stable atherosclerotic lesion to an unstable phenotype that is prone to rupture (53, 54). The subsequent vessel occlusion by thrombosis is the leading cause of sudden cardiac death and stroke in western countries.

Advanced plaques contain many apoptotic cells (AC) derived from all type of cells involved in atherosclerosis, including macrophages, T-cells, and smooth muscle cells (55). 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 (56). As mentioned before, phagocytosis of AC is known to create an anti-inflammatory environment and this induces healing. AC that are not scavenged in plaques, activate thrombin which could further induce thrombosis. This is one more reason why phagocytosis of AC inside atherosclerotic lesions might be beneficial.

Macrophages are essential for the removal of apoptotic cells from plaques, but exert strong pro-inflammatory, and therefore, atherogenic properties upon phagocytosis of lipoproteins or erythrocytes. It is no wonder that inside an atherosclerotic lesion, Mphi takes over several functions that develop with the plaque progression and/or by the location of the cell within the lesion.

Usually, tumors are effectively reduced by macrophage invasion but over the last few years, numerous studies have challenged this paradigm by showing that certain tumors seem to proliferate when surrounded and infiltrated by Mphi (57, 58). Interestingly, macrophages and monocyte chemoattractant protein-1 (MCP-1) can induce angiogenesis (57, 59-61). Macrophage recruitment is associated with the expression of MCP-1. However, besides MCP-1, there must exist further mechanisms which interact and promote tumor growth, since MCP-1 application was successfully used to treat tumors by attraction and activation of monocytes (62). One can speculate, that a subset of monocytes, namely M2, is responsible and/or maybe dysregulated (63, 64). In fact, dysregulation of MCP-1/Mphi was shown to be critical in autoimmune myocarditis (65). Otherwise alternative activation of monocytes through phagocytosis of apoptotic cells, meaning deactivation of antitumor activity, could be involved (66). But not only dysregulation of phagocytosis is involved in healing and disease. On one hand, there are viruses, i.e. the Human immunodeficiency virus, used to infect Mphi by membrane fusion, but other microorganisms (e.g., bacteria like Mycobacterium tuberculosis or protozoa like Leishmania species) even developed strategies using phagocytosis to invade Mphi. On the other hand, phagocytosis is also utilized by physicians when they apply e.g., AmBisome®, which is packed in liposomes and taken up by the infected macrophages, to treat leishmaniasis in patients.

In gout the effects of uncoated monosodium urate (MSU) crystals on articular inflammation are well described (67-69). MSU are encountered by synovial Mphi, which induces CD14 mediated the release of prostaglandins, proteases, and pro-inflammatory cytokines including TNF-alpha, IL-1β, IL-6, and IL-8 (70). Phagocytosed crystals cause lysis of the phagolysosome, release of its toxic contents and evoke cellular necrosis. Additional effects maybe caused by perforation of cell membranes.

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 remodeling, development, and immune response.

3.3. Local proliferation and (trans-)differentiation

From the 1960s to the 1980s van Furth extensively investigated the kinetics of macrophage populations in different tissues (71). He calculated a turnover time of macrophages ranging from 1 to 5 weeks while Thomas et al. measured 81 days (72-74). Due to van Furth’s work, it is generally accepted that under normal steady-state conditions, the homeostasis of tissue populations is mainly assured by monocyte recruitment and not by local proliferation. In the 1990s, Kennedy and Abbowitz used bone marrow transplantation in mice to verify this common opinion. One month after transplantation, they observed a nearly complete substitution of granulocyte-macrophage colonies in the marrow and of splenic macrophages in the host, whereas only 61% of liver and lung macrophages were obviously replaced by donor cells within the first post-transplantational year. Furthermore, donor microglial engraftment remained in only 30% of total microglia within one year (75). One can conclude 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 et al. observed self-renewal of pulmonary alveolar macrophages in radiation chimera studies (76, 77). Recently, local proliferation of microglia, pulmonary macrophages, Kupffer cells and Mphi entering grafts were described (78-81).

It was also a common agreement that the potential differentiation of monocytes is restricted to cells which serve as phagocytes and/or specialized APC. However, new findings show more and more that this is only half the story. Therefore, the field of Mphi’s differentiation is constantly becoming more complex. It was recently found that Mphi can transdifferentiate (transformation of one differentiated cell type into another (82)), a feature traditionally attributed to bone marrow-derived stem cells, into various mature cells types including non-phagocytosing myofibroblasts, endothelial cells or smooth muscle-like cells (83-85). Xie et al. showed that transdifferentiation also works the other way around. They transformed B-lymphocytes into macrophages (86). Taking these discoveries concerning transdifferentiation of Mphi into account, one should discuss the central concept of stem cell biology again. This concept states that residing stem cells within the tissue have the capacity for self-renewal and terminal differentiation into the multiple cell lineages of the respective tissue. They are replenished by precursors from the bone marrow, and in case of damage, they are driven into the process of tissue repair (87). One might add that the tissue replenishment can result also from transdifferentiating “non-precursor” cells.

The differentiation of Mphi into polarized macrophage subsets depends on the impact of the particular cytokine milieu, and leads to its remarkable versatility. In addition, the milieu is essential for monocytic survival. In other words, the supplemental lack of certain cytokines (lack of stimulation) or of nutrition (starvation) rapidly induces apoptosis in Mphi. Whereas, cellular stress, e.g., an pro-inflammatory milieu, induces expression of anti-apoptotic auto-protective survival mechanisms like hemoxygenase-1 (HO-1) and heat shock protein 70 (HSP70) (88, 89). In addition, occupancy of certain surface receptors activate Mphi protecting these activated Mphi from pro-apoptotic stimuli. Binding of endotoxin to the LPS-receptor CD14 for example was shown to inhibit apoptosis in Mphi, whereas downregulation of CD14 by anti-inflammatory IL-4 seems to promotes it (90, 91). Because the expression status of the CD14 receptor is pivotal for Mphi, its analysis (in addition to the analysis of phosphatidylserine by Annexin V staining) is a sensitive

Table 1. Selection of secreted products of activated Mphi

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Chemokines</th>
<th>Growth factors</th>
<th>Enzymes</th>
<th>Further effectors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interleukines</td>
<td>AMAC-1</td>
<td>FGF-1</td>
<td>Arginase</td>
<td>Nectins</td>
</tr>
<tr>
<td>IL-1 alpha, beta</td>
<td>IL-8</td>
<td>GM-CSF</td>
<td>MMP (1, 2, 8, 9, 12, 13)</td>
<td>ROS</td>
</tr>
<tr>
<td>IL-6, 12, 15, 18</td>
<td>MIG</td>
<td>IGF-1</td>
<td>Muramidase</td>
<td>RNS</td>
</tr>
<tr>
<td>OSM</td>
<td>IP-10</td>
<td>M-CSF</td>
<td>Myeloperoxidase</td>
<td>PAF</td>
</tr>
<tr>
<td>TNF-alpha</td>
<td>MIP, macrophage</td>
<td>PDGF</td>
<td>NADPH</td>
<td>Oxidase</td>
</tr>
<tr>
<td>Interferones</td>
<td>RANTES</td>
<td>TGF</td>
<td>Plasminogen-Activator</td>
<td></td>
</tr>
<tr>
<td>INF-25</td>
<td>VEGF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td></td>
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</tbody>
</table>

and useful parameter to discriminate apoptosis, necrosis, and vital Mphi from each other (90, 91). As mentioned above, the absence of stimuli e.g., IL1-β, TNF-α, lipopolysaccaride (LPS) or serum, Mphi render apoptotic and decay (89, 92, 93). Therefore, the life span of Mphi directly relies on their microenvironment ranging from hours to months.

### 3.4. Apoptosis

Platelets unlike monocytes do not undergo classical apoptosis. Originally, apoptosis was defined only by characteristic morphological features (94, 95). With the discovery of the molecular mechanisms, apoptosis is currently referred to a caspase-mediated cell death with common morphological changes including cell shrinkage, nuclear and cytoplasmic condensation and cellular fragmentation into membrane-bound fragments (karyopygnosis, karyorhexis, and budding). However, some variations of the stereotypical morphological changes exist depending on the cell type or stage of differentiation. These fragmented cells named “apoptotic bodies” are phagocytosed and degraded by macrophages. While the cellular and membranes integrity are initially preserved, changes in localization of receptors (e.g., CD14 expression, flip of phosphatidylserines, exposure of surface molecules designating the remains for phagocytosis) is used as early marker to recognize apoptotic bodies (96-100). Apoptotic bodies contain ribosomes, mitochondria, and chromatin fragments of a characteristic length, the latest generated by hydrolysing Ca²⁺/Mg²⁺-dependent endonucleases. The exposure of phosphatidylserines and the expression of receptors like ICAM-3 allow a quick and effective recognition and uptake of apoptotic cells by professional and non-professional phagocytes. Because of the integrity of the membranes, the apoptotic cells can be removed with little tissue disruption and without inflammation which allows a save reutilization of cellular components.

As a result of theses morphological characteristics, it was assumed that apoptosis evolves from a universal mechanism of controlled cell deletion, which complements mitosis and cytokinesis preserving a stable cell populations within tissues. This concept pursues the theory that every cell carries a genetic program for a metabolic cascade that, once activated, can lead to its demise (the history of research on apoptosis is reviewed in 98).

While apoptosis is an active, programmed procedure of autonomous cell-breakdown that avoids an arising of inflammation, necrosis has been declared as a passive, accidental cell death resulting from environmental harm followed by the uncontrolled release of pro-inflammatory cellular contents.

Apoptotic characteristics are not visible until a point of no return in the processing of programmed cell death has been passed. But by this time, preliminary biochemical cascades (mitochondrial and enzymatic sway) have already determined the fate of cells concerned. Since apoptosis is an active process, the activity of mitochondria increases after its induction, and corresponds to increased synthesis and release of apoptosis-regulating proteins. Important regulators involved into the process are members of the bcl-2 family, cyttochrom c (Cyt c, Apaf-2), Smac/DIABLO (secondary mitochondria-derived activator of caspases/direct IAP binding protein with low PI), and nitoxide. For living cells, the blockade of caspases is vital. Caspases (cysteine requiring aspartat proteases) form a system of cysteine proteases activated by subsequent cleavage. Although all caspases have homologous amino acid sequence, their structure, their specificity for aspartate and the high turnover rate in common, they significantly differ in their physiologic roles. To simplify the system, caspases can be divided into two groups: those who are centrally involved in the signal transduction leading to apoptosis (caspase-2, -3, -6, -7, -8, -9, and -10) and those related to caspase-1 (caspase-1, -4, -5, -13, and -14) who are involved in cytokine processing during inflammatory responses (101, 102). However, situations remain complex since several interactions and sideways were discovered (103-105). Caspases serve as signal mediators by cleaving more than 100 different cellular proteins. Based on functional analysis, their targets can be subdivided into six major categories: (1) proteins directly involved in the regulation of apoptosis, (2) proteins mediating/regulating apoptotic signal transduction (e.g. protein kinases), (3) structural and essential-function proteins, (4) proteins required for cellular repair, (5) proteins regulating the cell cycle and (6) proteins involved in human pathologies (104, 105).

Inhibitors of apoptosis (IAP) e.g., XIAP, c-IAP1, c-IAP2, survivin and others belong to the cell’s own defence against pro-apoptotic stimuli. They protect cells from caspase-conducted death. Necessarily, these anti-apoptotic actors have to be deactivated first, if apoptosis should be realized (104, 105).

There are two major ways of inducing apoptosis, the intrinsic (mitochondrial), and the extrinsic (membrane receptor bound) pathways (103-107). 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 and subsequently the adapter molecule FADD (Fas-associated death domain), is recruited initiating the formation of DISC (death inducing signaling complex). Consecutively, this complex activates caspase-8 (108). That 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, Ca²⁺, ceramides or pro-apoptotic members of the bcl-2 family e.g., bax, bad, and bid, can affect mitochondria, thereby activating the intrinsic cascade of apoptosis. One critical protein involved is Smac. Smac is an inhibitor of IAP and presumably released from pores within the membrane of mitochondria (109). Along with Cyt c and Apaf-1 it forms a complex called the apoptosome which activates cytosolic pro-caspase-9 (110). Caspase-9 cleaves then pro-caspase-3. 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
Life span of monocytes and platelets

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

<table>
<thead>
<tr>
<th>Mechanism or Stimulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct inhibition of transcription, translation or cell cycle (steroids, cytostatic drugs or mitotic poisons)</td>
</tr>
<tr>
<td>Starvation or absence of growth factors or hormones/cytokines (serum deprivation, GM-CSF, M-CSF, TNF-alpha, IL1beta)</td>
</tr>
<tr>
<td>Downregulation or blocking of the LPS-receptor CD14 (IL-4)</td>
</tr>
<tr>
<td>Intracellular accumulation of non-physiological proteins (cytostatic drugs, heat)</td>
</tr>
<tr>
<td>Occupation of death receptors (CD95/Fas, TNFR1, DR3/Apo3, DR4, DR5/Apo2/KILLER)</td>
</tr>
<tr>
<td>Cellular alteration (heat, hypoxia, radiation, drugs, enzymes, oxygen spezies)</td>
</tr>
<tr>
<td>Mutations or mitotic errors</td>
</tr>
<tr>
<td>Pathogenes (Mycobacterium tuberculosis, Shigella species)</td>
</tr>
<tr>
<td>Deactivation of protective factors (bcl-2 family members can be deactivated in hypoxia)</td>
</tr>
</tbody>
</table>

Abbreviations: 'GM-CSF: Granulocyte-Macrophage Colony Stimulating Factor; M-CSF: Macrophage Colony Stimulating Factor; 'TNF: Tumor necrosis factor; 'IL1beta: Interleukin 1 beta; 'LPS: Lipopolysaccharide; 'CD: Cluster of differentiation; 'TNFR1: Tumor necrosis factor receptor 1; '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

As shown by us (Figure 1), phagocytosis of platelets is a strong anti-apoptotic mechanism interacting with the caspase-dependend process of apoptosis through down-regulation of caspase-3 and -9 in monocytes (24). 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 (24, 88, 89).

Whereas the apoptosis of monocytes features all classical attributes (Figure 1), the apoptosis of anuclear platelets shows only some of the mentioned features. Taken together, there is emerging evidence that the traditional view of monocyctic replacement negating mitosis, and favoring recruitment of Mphi 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. Further, Mphi cannot only debris wounds, organise the immune response and limit the inflammation. Based on available information, they contribute to much more extent to the ‘restituto ad integrum’ or pathogenesis of disease when they are (trans-)differentiating, proliferating or inducing matrix synthesis, and angioneogenesis.

4. PLATELETS

Megakaryopoiesis is the process by which megakaryocytes (MK) proliferate, differentiate, and mature from pluripotent hematopoietic stem cells (HSC) whereas thrombopoiesis is the process by which mature MK fragment into anucleate blood platelets (111-113). The average platelet count in humans ranges from 150 x 10^9 to 400 x 10^9 per liter, though the individual level is sustained fairly constantly over time. 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 (114-117). The platelet production is controlled by thrombopoietin (TPO), an acidic glycoprotein which is synthesized in the liver, kidney, and BM (118, 119). However, factors regulating their survival remain unclear and some aspects to date known will be cover below.

4.1. Platelet regulation of clot formation

Bleeding problems associated with platelet disorders reveal their importance to haemostasis. At sites of injury, platelets adhere to the vessel wall, undergo activation, secretion, and form aggregates, building an impermeable plug which is the first line of defence against blood loss. The membrane of activated platelets provides the necessary surface initiating the extrinsic coagulation cascade which includes the generation of stabilizing thrombin and fibrin (120, 121). The complex interaction between platelets and endothelium is mediated via cellular receptors on both, the surface of platelets and endothelial cells (EC). Integrins, selectins, and adhesive proteins, such as von Willebrand factor (vWF) and fibrinogen are involved in the adhesion process (see reviews, 122-124). Moreover, the receptor-mediated cell-to-cell adhesion is accompanied by an intensive crosstalk involving paracrine signalling. Platelets release or transfer IL-1β, TGF-β, PDGF, and VEGF influencing EC function. On the other hand, EC 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 (PAF) (124). vWF binds to the GPIIb-IIIa complex on activated platelets. P-selectin/CD62P and CD40L are expressed and the platelets promote CD40L-dependently further activation of EC (125). EC respond with the production of tissue factor (TF), which is a receptor for factor VIIa and in complex the major initiator of the blood coagulation cascade (126, 127). Moreover, TF has direct pro-inflammatory effects by inducing the production of reactive oxygen (128). Therefore, and because of the induction of additional adhesion molecules, MMPs, tissue-type plasminogen activator, and cytokines (e.g., MCP-1) in/on endothelial cells, platelets participate directly in the initiation of an inflammatory response (coagulation, monocyte recruitment, and activation, and matrix remodelling) (129-132). 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 (133). 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 (134). There is increasing evidence that platelets amplify acute inflammation. The platelets and their release products seem to be important for the chemotaxis and activation of leukocytes, thereby orchestrating the healing of the wound. On the other hand, they contribute to a misleading activation of the immune system e.g., allergy, chronic inflammation (asthma, arthritis), diabetes or atherosclerosis (135-141).

In summary, blood platelets, beyond their well-recognized function in haemostasis, 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 enough 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 (142-144). The homeostasis of mature platelets is a balance between production, consumption, and disposal. While some of the mechanisms regulating thrombopoiesis have been clarified (111, 117-119, 145), the factors controlling their life span, particularly in the steady state, are still the subject of speculation.

Apoptotic processes during synthesis, activation, and in vitro storage of platelets have been demonstrated (146-152). In vitro platelets also lose function, and finally die for reasons that are poorly characterized. By definition, many events in the apoptotic pathway include nuclear processing (e.g., nuclear condensation, DNA fragmentation, and RNA synthesis). For this reason many
In the 1960s, platelet phagocytosis by macrophages is described as an alternative mechanism of foam cell formation and macrophage activation (166, 167), 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 (168). APP is stored in alpha-granules of platelets. 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 cyclooxygenase-2 (COX-2), it was found that APP-rich macrophages are activated (168). 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 (169). Notably, thrombin, leading to activation, coating or apoptosis of platelets increases the phagocytosis rate of Mphi (170). 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 (171, 172). However, we found that platelet lysates were ineffective in suppressing apoptosis in monocytes in vitro, and that platelet surface receptors or intracellular compounds released in phagolysosomes are needed (24). In contrast, Brunetti et al. observed in monocytes anti-apoptotic qualities of mediators released by platelets (173). 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 disposition of chemokines or exposure of receptors are likely enough stimulation to induce APP and Aβ release (171, 172). Moreover, it was shown in plaque that macrophages can apparently be iNOS-positive without signs of internalization. These Mphi are frequently surrounded by platelets (168).
Life span of monocytes and platelets

It is out of question that activation of monocytes is a strong survival stimulus, and effective to protect monocytes from apoptotic decay. But the key question whether phagocytosis 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 proatherogenic foam cells. Along with the progression of plaque 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 (174). 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 (175-177). 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-atherogenic, since uncontrolled phagocytosis of AC might also lead to tissue injury (178).

5. FURTHER INTERACTIONS

Several different scenarios of interaction and support between monocytes and platelets are plausible. Since their departure from the bone marrow, they share their lifespan in the bloodstream until they are attracted, activated and/or expended. But activation or death of one cell does not mean the end of interaction. Activated platelets rapidly adhere to EC and human blood leukocytes via expression of P-selectin (CD62P) interacting with their P-selectin glycoprotein (GP) ligand-1 (PSGL-1) (153, 179). 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 (125, 180). 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) (181). 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 (182).

Platelet-endothelium interactions play an important role in the development of inflammation and atherosclerosis. In EC, activated platelets induce MCP-1 secretion and surface expression of intercellular adhesion molecule-1 (ICAM-1) promoting the recruitment, adherence and extravasation of monocytes (124, 129). Furthermore, EC-adherent platelets bind and present vascular cell-derived chemokines to arrest 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 kinogen) were used as stabilizing linkers (132). Therefore, platelets participate directly in the initiation of an inflammatory response.

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 (171). 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, transferring this knowledge into a concrete anti-atherosclerotic therapy, available. 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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Life span of monocytes and platelets


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Life span of monocytes and platelets


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Life span of monocytes and platelets


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: hemeoxygenase-1; HPA-1a: human platelet antigen 1a; ITP: (Auto)immune thrombocytopenic purpura; LPS: lipopolysaccaride; MCP-1: monocyte chemotactrant 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 metalloproteinase; TNF: tumor necrosis factor; TPO: thrombopoietin; vWF: von Willebrand factor

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