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Emerging Concepts of Megakaryocytes Distribution and Role in Inflammatory Joint Disease

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Author's contribution

As a sole author of this mini review manuscript I declare that I am the only person who prepared it. I also declare that I read and approved the final manuscript including the galley proof.

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ABSTRACT

Inflammatory joint diseases are characterised by significant bone and cartilage destruction, leading to disability and reduced life quality. Some of these illnesses have a systemic character which leads to life-threatening conditions. The exact mechanisms that are involved in joint pathology are still not yet known, as a variety of cell types are implicated in them. Increasing evidence shows that megakaryocytes and platelets are key players in joint inflammation and bone remodeling, and in the accompanying systemic disorders such as thrombocytosis. Here we summarise the available data on the megakaryopoiesis, megakaryocyte distribution and discuss some aspects of their involvement in inflammatory joint diseases.

Keywords: Megakaryocytes; megakaryopoiesis; inflammatory joint diseases; arthritis.

1. INTRODUCTION

There are over 100 different types of inflammatory joint diseases, generally named "arthritis". Despite inflammation, as their common feature, most arthritic diseases are accompanied by a variety of pathologic changes in other tissues. They can be caused by trauma, wear

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and tear, joint infection, or have systemic and autoimmune nature. Different cell types are involved in joint inflammation but the complexity of their interactions and contribution to pathology is still not well understood. Increasing data points on megakaryocytes (MKs) to be key players in inflammatory joint disease manifestations. Megakaryocytes are large polyploid cells that reside in the bone marrow and are precursors for platelets [1]. They are the largest cells in bone marrow, as their size varies from 20-100 μm, and are the rarest ones accounting nearly 0.01% of nucleated bone marrow cells [2,3]. Actually, the size of megakaryocytes grows during their development, as their chromosomes duplicate several times because of a process called endomitosis. Megakaryopoiesis starts from the commitment of multipotent hematopoietic stem cells toward megakaryocyte-committed progenitors, the proliferation and differentiation of these megakaryocyte progenitors, the polyploidisation of megakaryocyte precursors and the maturation of megakaryocytes (MKs) [4]. Mature MK produce platelets by a dynamic and regulated process of cytoplasmic fragmentation, called proplatelet formation, and consisting of long pseudopodial elongations that protrude the endothelium and break in the blood flow. Platelets (thrombocytes) are small (1 to 3 μm in diameter), membrane-bounded, anucleate cytoplasmic fragments with a discoid shape that function in continuous surveillance of blood vessels, blood clotting, and repair of injured tissue. It has been shown in numerous studies and reviews that megakaryocytes and platelets also play an unexpected role in several other processes such as acute phase response and inflammation, innate immunity, neoangiogenesis and tumour metastasis [5-9]. In recent reviews, some of the nonhemostatic roles of platelets, particularly with respect to inflammation and immunity have been outlined briefly and comprehensibly. Especially, their ability to secrete many immunomodulatory cytokines and chemokines, to express TLRs, MHC I molecules, and to produce membrane microparticles under different environmental stresses or CD40L that bind to receptors on endothelial cells triggering inflammatory reactions [10-13]. In the current review, we aimed to track the hitherto known findings of the distribution of megakaryocytes and proplatelets in arthritic diseases, as increasing data shows that their number in affected tissues corresponds to the severity of inflammation.

2. ORIGIN OF MEGAKARYOCYTES AND PLATELETS IN THE ORGANISM

According to the monophyletic theory of hematopoiesis, all the blood cells are derived from a common progenitor hematopoietic stem cell (HSC). In bone marrow descendants of HSC differentiate into two major colonies of multipotent progenitor cells: the common lymphoid progenitor (CLP) and common myeloid progenitor (CMP) cells [14,15]. CMP cells continue their development as they branch to granulocyte/monocytes progenitor cells and megakaryocyte /erythrocyte progenitor cells [16]. The megakaryocyte /erythrocyte progenitor cell lineage is committed to megakaryopoiesis after stimulation with the hematopoietic growth factor thrombopoietin that reacts to its receptor Mpl. Actually, this growth factor stimulates the self renewal of HSCs and induces transcription factors for the expression of proteins that commit them to the platelet lineage [17]. The fact that thrombopoietin promotes the growth and development of MKs is used in *in vitro* studies of cell culture systems that remodel MK differentiation, maturation, proplatelet extension, and platelet production. Thus it becomes possible to study the mechanisms that regulate these processes [18,19]. During their maturation, MKs grow dramatically in size, become polyploid through endomitosis, develop highly invaginated membrane system, and are filled with platelet-specific granules [20]. At the end of their maturation, they express MK/platelet-specific tubulin isoform β1 and their development depends on the transcription factor NF-E2 [21]. In mature megakaryocytes, cytoplasm is packed into multiple long processes called proplatelets that extrude the nucleus aside. An MK can extend up to 20 proplatelets, each branching repeatedly. Platelets form at the edges of the proplatelets [22]. Summarised platelet formation can be conventionally divided into two phases. The first phase is the maturation and development of MK and takes days requiring MK-specific growth factors. During this time, massive nuclear proliferation and enlargement of the MK cytoplasm occur. The MK is filled with cytoskeletal proteins, platelets specific granules, and a sufficient amount of membrane needed to perform the platelet assembly process. The second phase can be completed within hours, while MKs generate platelets by remodelling their cytoplasm first into preplatelets and then into proplatelets, which undergo subsequent events to generate discoid platelets. In humans, the time required for MKs to complete polyploidisation,

mature, and release platelets is about 5 days. Once released into the bloodstream platelets survive 7–10 days.

3. NOVEL INSIGHTS FOR MEGAKARY-OPOIESIS LOCATION

Until recently it was believed that there is a cellular migration during megakaryopoiesis. The essence of this suggestion is that blood cell precursors migrate from an endosteal niche towards the vessel sinusoids during maturation [23-25]. Some data suggest that up-regulation of the cytokine SDF-1 and its receptor CXCR4 may be important for the migration of MKs to the vascular niche [23,26]. The vascular niche is comprised of extracellular matrix proteins, such as fibronectin, fibrinogen, collagen IV, and von Willebrand factor, which in conjunction with the chemokine-mediated interaction of progenitors allow MKs to relocate to a microenvironment that is both permissive and instructive for the late stages of MK maturation and proplatelet formation [23].

In 2017 Stegner et al. reported their results which clearly contradict the current idea of cellular migration during megakaryopoiesis and support a modified model that shows that MKs at sinusoids are replenished by sinusoidal precursors rather than cells from a distant periostic niche. Using imaging of MKs in the intact BM, they showed that MKs can be found within the entire BM, without a bias towards bone-distant regions. They applied contemporary technology such as two-photon microscopy and in-situ light-sheet fluorescence microscopy combined with computational simulations and thus revealed slow MK migration, limited intervascular space and a vessel-biased MK pool [27]. In the same studies, these authors showed that the classical integrin-dependent cell migration is not required during megakaryopoiesis as they blocked the chemokine receptor CXCR4 by using Plerixafor (AMD3100). This finding was supported by the fact that WHIM syndrome (warts, hypogammaglobulinemia, infections, and myelokathexis syndrome), which is caused in some patients by hyperreactive CXCR4 signalling, is not associated with altered platelet counts [28] . According to previous research of another group MK distribution probably is the consequence of the dense microvasculature and presumably perisinusoidal HSCs [29]. It appears likely that MK precursor cells reside in close proximity to the vasculature, allowing the rapid generation of new MKs upon platelet demand [27]. These authors proposed that the vessel biased distribution of MKs could be supported by MK polarisation (e.g., via 'directed growth') which could be triggered by contact with adhesive molecules and could affect the production of platelets. They also assumed the possibility that two pools of MKs exist: one of the pools residing at the vessel to rapidly produce platelets on demand, and another pool positioned more distant from the vasculature and consisting of quiescent or potentially megakaryocytic progenitor cells. According to these authors, the model of two MK pools could explain the fact that the therapies to increase platelet count are not fully effective. They suggest further studies to elucidate how to activate the quiescent MK pool to accelerate platelet production [27].

4. BIDIRECTIONAL INTERACTION OF MEGAKARYOCYTES AND THEIR SURROUNDING MICROENVIRONMENT

Increasing data show that MK and proplatelet formation is dependent on the microenvironment. MK cultured *in vitro* can form proplatelets in suspension, which shows that direct interaction with bone marrow cells is not necessary for this process. In their natural microenvironment in bone marrow *in vivo* MK produce platelets more efficiently than *in vitro*. This suggests that the bone marrow microenvironment plays an important role in stimulating and enhancing proplatelet formation and platelet release [4].

The vasculature niche of bone marrow is comprised of extracellular matrix (ECM) proteins, such as collagen IV, fibronectin, fibrinogen, and von Willebrand factor, which combined with chemokine-mediated interaction of progenitors allow MKs to relocate to a microenvironment that is important for the late stages of MK maturation and proplatelet formation [23]. Later it was found in particular that among bone marrow ECM components, fibronectin, type IV collagen and laminin are the most abundant around bone marrow sinusoids and constitute a peri-cellular matrix surrounding megakaryocytes [30]. Megakaryocytes express components of the basement membrane and these molecules contribute to the regulation of megakaryocyte development and bone marrow ECM homeostasis both *in vitro* and *in vivo* [30].

Evidence for the important role of the microenvironment in vasculature niche for MKs maturation and proplatelet formation is obtained

from *in vitro* studies and studies of different disease in human that are the result of abnormal MK and platelet production. A disease called Bernard-Soulier syndrome is characterised by thrombocytopenia with giant platelets and functional defects like defective platelet adhesion to subendothelium and reduced platelet aggregation leading to macrothrombocytopenia [31]. In Bernard-Soulier syndrome there are genetic lesions of the platelet membrane glycoprotein complex GPIb–IX–V, which contains the binding site for von Willebrand factor, a plasma glycoprotein important for platelet adhesion to the endothelium [32]. The MKs of these patients do not extend proplatelets *in vitro* [33]. These data suggest that the proper interaction of the platelet membrane glycoprotein complex GPIb–IX–V with von Willebrand factor is one of the mechanisms necessary for normal proplatelet formation.

Bone marrow sinusoids contain the extracellular matrix protein fibrinogen which enhances proplatelet formation *in vitro* [34]. MKs bind to fibrinogen through integrin $\alpha_{IIb}\beta_3$, which stimulates proplatelet formation and is important for their release. This statement is disproved after studying a disease called Glanzmann's thrombasthenia characterised by either absent or nonfunctioning integrin $\alpha_{IIb}\beta_3$. Despite the mutations of the gene for integrin $\alpha_{IIb}\beta_3$, these patients still have a normal level of circulating platelets. It was concluded that integrin $\alpha_{IIb}\beta_3$ enhances proplatelet formation but is not necessary for it, so the effect of fibrinogen in the bone microenvironment is not critical for MKs development.

Another abundant protein in the hematopoietic microenvironment is fibronectin. It is known to be a proliferative stimulus for hematopoietic stem cells [35]. Fibronectin is important for megakaryocytopoiesis, proliferation, and differentiation through adhesion to fibronectin receptors VLA-4 (very late antigen 4) and VLA-5 [36-38]. On the other hand, fibronectin is expressed by the MKs themselves and regulates their development and bone marrow homeostasis both *in vitro* and in vivo [30].

Bone marrow cells are in contact not only with the vasculature and its microenvironment but also with the components of the bone. Osteoblasts, osteoclasts and osteocytes are the main cellular subsets in bone that maintain its structure and homeostasis. Osteoclasts degrade mineralised bone and osteocytes regulate and maintain the bone homeostasis. Actually the components of the bone, and its microenvironment respectively, are produced by osteoblasts. Thus osteoblasts from a specific osteoblastic niche in which collagen I is the most abundant component, as bone composition was studied in details long ago [39]. It was found that MK bind to collagen through integrin molecules which inhibits proplatelet formation [40,41]. It is suggested that osteoblastic environment inhibits the last stages of MKs maturation and proplatelet formation [42]. These authors developed a model for studying MK function in the bone marrow microenvironment by differentiating human mesenchymal stem cells into osteoblasts and then co-culturing this osteoblast with hematopoietic stem cells and MKs [42]. In this study the authors found that hematopoietic stem cells forma specific niche that is characterised by collagen I deposition, creating a quiescent microenvironment conducive for hematopoietic stem cells to differentiate to the megakaryocytic lineage without completing their maturation and without extending proplatelets. The model of Palotta et all is particularly useful for developing suitable *in vitro* systems to study and manipulate MKs differentiation and maturation in both normal and diseased states. This model also supports the last hypothesis of Stegner et al, 2017 [27] about the two MK pools, as it shows the presence of quiescent microenvironment for the MK hematopoietic stem cells. In support of this hypothesis is the review of Machlus and Italiano who summarised the data for megakaryocytes development and platelet formation and concluded that the studies of the MK surrounding suggest a model in which the osteoblastic niche provides an environment that allows MKs to mature and develop, whereas the vascular niche enhances proplatelet formation [4].

5. MEGAKARYOCYTES AND PLATELETS AS KEY PLAYERS IN BONE HOMEOSTASIS

Increasing data show that MKs participate in the mechanisms of bone homeostasis in health and disease, but it is still controversial and needs more detailed study. MKs can express different molecules in different conditions and thus they are capable of playing dual and opposing roles in bone remodelling, especially in osteoclastogenesis. When MKs express osteoprotegerin (OPG) it is supposed that they could inhibit osteoclast development [43]. On the other hand, when they express receptor activator of nuclear factor κB ligand (RANKL), they could

enhance osteoclast development [44]. Activated platelets also enhance osteoclastogenesis as they are a source of RANKL [45]. On the contrary, there are some studies showing that platelets suppress osteoclastogenesis as they are a source of OPG [46]. An *in vitro* study using anti-OPG antibodies and MKs from OPG-deficient mice showed that actually, OPG was not responsible for the MK-mediated inhibition of OC development, and it was suggested that an unidentified factor(s) is present in MK cellular media that inhibits OC development [47]. Another *in vitro* study shows that MKs inhibit osteoclast formation and activity influencing primarily the osteoclasts precursors [48]. All this data reported in the literature is controversial and needs an extensive additional study to elucidate the precise mechanisms by which MKs and platelets affect bone remodelling and homeostasis. Moreover, most of the studies are performed *in vitro*, outside the organism, which may be a reason for the inappropriate selection of experimental components. Despite the increasing evidence for interactions between MKs and osteoblasts in experimental models, it has not been reported any data about the effects of these interactions in human. There is a lot of information about the MKs functions in inflammatory disorders, it has to be better understood how they contribute to disease development. The results could be applied to manipulate MKs functions for elaboration proper therapeutic approaches.

Clinical studies demonstrate that platelets produced by MKs are involved in bone remodelling under acute and physiologic conditions [49]. It is reported that around the fractures and microfractures of bone are localised platelet aggregates that release Platelet-Derived Growth Factor (PDGF), Vascular Endothelial Growth Factor (VEGF), Insulin-Like Growth Factor 1 (IGF-1) or Transforming Growth Factor 2 (TGFβ), all known to recruit osteogenic cells [50]. Other bone remodelling modulators produced by the platelets are reported to be Thromboxane A2 (TxA2) and Prostaglandins [49].

Monocytes and neutrophils are the most abundant cells in the blood. Monocytes interact with platelets through P-selectin. It is expressed on the platelet plasma membrane and was initially called platelet activation-dependent granule external membrane protein (PADGEM) or Granule Membrane Protein 140 (GMP-140) [51]. It was demonstrated *in vivo* in a primate arteriovenous shunt model that platelet P-selectin immobilises leukocytes at the site of the lesion [52,53]. Genetic proof of the role of P-selectin in the platelet interactions with leukocytes was provided by the generation of P-selectin knock –out mice [54]. It was found that the interaction of P-selectin and its receptor PSGL-1 not only attract monocytes but also induce tissue factor-bearing microparticle formation and monocyte pro-inflammatory changes [55]. The major source of circulating microparticles are the activated platelets [56,57]. Activated microparticles are lipid membrane vesicles of 0.1-1 μm in size. Platelet microparticles are present at increased numbers in the joint space of patients with rheumatoid arthritis and have a pro-inflammatory function, as they induce synovial fibroblast cytokine responses that depend on IL-1α and IL-1β [58].

6. MEGAKARYOCYTES AND PLATELETS IMPLICATION IN INFLAMMATORY JOINT DISEASE

Inflammation is a complex process that is a result of the cooperation of a variety of soluble factors and cells. Increasing data points on the importance of platelets as key players in inflammation and bone marrow immunity [10-13]. MKs have been described as cells that link the innate and adaptive immunity [59]. Early MK precursors are MHC class II positive, quickly losing its expression while still maintaining high expression levels of MHC class I. Using cerebral malaria model it was demonstrated that platelets express T cell co-stimulatory molecules, process and present antigen in MHC class I, and directly activate naïve T cells in a platelet-dependent manner [60]. Moreover, it has been demonstrated that mature $CD34^-$ MHC class II^- CD41+ MKs can act as potent antigen presenting cells for CD8+ T cells exposing a wide array of peptide antigens on their surface in association with MHC class I and that during *in vitro* thrombopoiesis these MHC class I –protein complexes were transferred from MKs to pro-platelets [61]. It was generally considered that inflammatory joint diseases, especially those with autoimmune character, such as rheumatoid arthritis, are CD4+ T cell dependent. Increasing data points on participation also of CD8+ T-cells, especially of the recently described tissue-resident population of CD8+ T-cells which by interaction with antigen-presenting cells might have a key role in diseasing pathology [62].

Considering these findings it could be proposed that during inflammation, MKs could contribute to CD8+ cells engagement in arthritis.

The role of platelets in inflammation can be demonstrated by understanding how platelets interact with pathogens [11]. It has been described that platelets are able to sense damage and presence of pathogens through TLRs, Ig- and complement receptors [12]. The importance of TLRs for megakaryopoiesis and thrombopoiesis was demonstrated using mice with a deletion in the locus for the TLR4 gene. In this experiment, the lack of TLR4 resulted in decreased platelet production, turnover, and thrombin-stimulated expression of P-selectin [63]. Other authors reported that MKs maturation also depends on TLR2 and its ligand Pam3CSK4 [64]. It was shown that human and murine platelets express TLR2, TLR4, and TLR9 and that platelet-associated TLR4 expression may significantly modulate LPS-induced thrombocytopenia and TNF-α production *in vivo* [65]. The importance of TLRs for MKs maturation and platelets production suggests that they could participate in joint inflammation including that caused by some microorganisms, such as *Staphilococcus auteus*, *Candida albicans*, *Borrelia burgdorferi*, etc.

Among receptors expressed by MKs and platelets are Fc ones. FcγR (Fc gamma receptors) are primary targets for autoantibody-mediated effects and an important issue is how the FcγR pathway is affected in autoimmune disorders such as rheumatoid arthritis. It has been described that FcγRI (CD64), II (CD32), IIb (CD32b) and III (CD16) are involved in disease development as dysregulation of these receptors is detected in the patients with rheumatoid arthritis [66]. FcγR expressed on megakaryocytes are important for the pathophysiology of immune complex-mediated thrombocytopenias [67]. FcγR expressed by megakaryocytes are of membrane-bound and soluble forms and excessive binding of immune complexes leads to platelet activation and thrombosis or increased platelet clearance and thrombocytopenia [68]. A role of MKs and platelets in perpetuating allergic inflammation was suggested after it was found that FcεR is expressed in the cytoplasm of human megakaryocytes and on the surface of platelets [69]. Binding antigen-antibody complexes could activate MKs and platelets, thus implicating them in joint inflammation.

CD40L is another proinflammatory factor expressed and released by megakaryocytes and platelets. It is a type II protein-ligand member of the tumour necrosis factor (TNF) superfamily that modulates the adaptive immune response and is implicated in the initiation, progression, and secondary pathology associated with many chronic inflammatory and autoimmune diseases [70]. Expression of CD40L in megakaryocytes can be regulated by upstream mechanisms involving transcription factor NFATc2 and EGR-1 which was suggested to impact the inflammatory activity of platelets and this could be a potential therapeutic target for modulation of platelet-mediated inflammation in disease [71].

Megakaryocytes and platelets produce various chemokines and cytokines involved in the inflammatory processes. They express TGF-β1 which was proposed to participate in a feedback mechanism for regulation of megakaryopoiesis and thrombopoiesis [72]. These authors described that when released by damaged MKs or platelets, TGF-β1 stimulates thrombopoietin synthesis in bone marrow stromal cells and commits them to the megakaryocyte lineage and to the expression of TGF-β1 receptors which makes them susceptible for further suppression by TGF-β1 [72]. On the other hand, TGF-β is described to be a critical cytokine required for inducing regulatory T cells phenotype. Actually, depending on the concentration, condition and the context, TGF-β determines whether the response to be directed toward Treg or Th17 cell types [73]. Small concentrations of TGF-β combined with inflammatory cytokines such as IL-6 and IL-21 could promote Th17 responses, while its high concentrations are favourable for ~~a~~ Treg response [73]. It was reported that imbalance of Treg/Th17 axes in peripheral blood samples is typical in rheumatoid arthritis patients, as higher numbers of Th17 correlate with disease severity [74].

As MKs counts are increased during rheumatoid arthritis, it could be suggested that they can be involved in maintaining the balance of Treg/Th17. In arthritis, it was reported that in chronic inflammation in obesity and infections binding of IL1β to its receptor IL1R1 on MKs enhances their maturation and stimulates the RNA profiles in the developing platelets to be proinflammatory and prothrombotic [75].

Another important feature of MKs and platelets suggesting them to be involved in inflammation is their ability to produce microparticles [76,77].

Microparticles are small membrane fragments that are shed in circulation by a variety of cell types including platelets, endothelial cells, leukocytes, and erythrocytes [78]. Megakaryocyte-derived microparticles and microparticles generated from activated platelets may differ in their mRNA content and in their expression of platelet activation markers such as P-selectin [79]. Microparticles derived from activated platelets are capable to activate the complement system [80,81]. Levels of platelet-derived microparticles are increased in blood and synovial fluid of patients affected with rheumatoid arthritis [58,82]. It was reported that platelet depletion in murine models of rheumatoid arthritis attenuated the disease [58]. Summed up, platelet microparticles can carry cytokines and chemokines, functional enzymes, lipid mediators like thromboxane A2, surface receptors, microRNA, autoantigens, transcription factors like STAT molecules [10]. It was suggested that distinguishing platelet-derived microparticles from megakaryocyte-derived ones could be useful in evaluating the effects of disease states and for monitoring them for diagnostic and prognostic purposes [79]. Future comparisons of the 2 microparticle populations (MK and platelet ones) will assess these possibilities.

Some of the key immune features of MKs that make them important for the inflammatory joint disease are listed in the following table:

Rheumatoid arthritis (RA) is a classic example of chronic inflammatory joint disease, leading to the destruction of articular cartilage and bone erosion. It is considered to be of autoimmune and systemic character as it involves not only the joint tissues but also the entire organism. Thrombocytosis is one of the RA specific accompanying and complicating features. Although it is correlated with disease activity, the exact pathogenetic mechanisms that cause increased MK and platelet counts in RA are still unknown. It is suggested that proinflammatory pleiotropic cytokines of RA also have megakaryocytopoietic/thrombopoietic properties [83]. There is little evidence that MK and platelets are directly involved in joint inflammation in RA. The number of platelets and platelet-derived proteins in the synovium and synovial fluid in arthritic joints is increased [84-86]. In RA, activated platelets, alone or together with other inflammatory cells and mediators, may play a significant role in thrombus formation, synovial microcirculation, and destruction of cartilage [87-89]. There is a report showing that platelets in synovial fluid have higher levels of membrane component platelet factor 4, which suggests migration of circulating platelets and targeted action against rheumatoid joints [84]. Other studies suggested that the contents of synovial fluid during inflammation may recruit platelets from the circulation and facilitate their proinflammatory and prothrombotic effects in the synovium [90]. Actually during rheumatoid inflammation synovitis may be aggravated by the inflammatory and immune mediators that are released by MK and platelets.

Table 1. Immune features of MKs pointing on their involvement in inflammatory joint diseases

Features of MKs	Effect on inflammatory joint disease
Present peptides in complex with MHC class I [60-62]	Involve CD8+ T cells in inflammation
Express TLRs[63-65]	Activation upon binding of pathogen-associated molecules
Express FcγR[66-69]	Activation upon binding of Ag-Ab complexes
Express and release CD40L[70, 71]	Initiation, progression and secondary pathology of inflammation
Expression of TGF-β1[72-74]	Feedback mechanism for megakaryopoiesis and thrombopoiesis; The balance of Treg/Th17 cells
Production of microparticles[10, 58, 77-82]	Dissemination of variety of proinflammatory molecules

Acute infections, some of which affecting inflammatory joint disease as *C. albicans, B. burgdorferi, etc. ,* are associated with systemic inflammation which was reported to trigger the release of immunomodulatory agents and the interaction of platelets with neutrophils to facilitate the formation of neutrophil extracellular traps (NETs) [13,91]. This is followed by a rapid consumption of platelets leading to a transient thrombocytopenia [92,93].

Very recently, it was reported an increased count of MKs with already formed platelets in synovium and cartilage in ZIA, which is a mouse model of arthritis similar to RA in human [94]. According to this study, the count of MKs and proplatelets in ZIA depends on the influence of the complement

system. The authors suggest further studies to elucidate the participation of complement in MK-dependent mechanisms [94]. In this study, an abundance of MKs in the synovium and cartilage was described and it was suggested that ZIA could be an appropriate model to reveal the role of MKs in arthritis and its pathology. Based on this finding, the authors assumed that in ZIA platelets by releasing cytokines and chemokines may recruit more inflammatory cells like neutrophils to the sites of inflammation. Also, they supposed that MKs, osteoblasts, and osteoclasts may interact with each other in their natural environment during joint inflammation, as dysregulation of the MK lineage corresponds with bone pathology both in mice and in humans [94].

Unlike RA, thrombocytosis rarely appears in osteoarthritis. According to some reports high platelet counts associated with markers of synovial leukocyte activation and rheumatoid factor are found in RA, but not in OA [86,95]. Data about the involvement of MKs and platelets in other inflammatory joint disease is rather scarce and further studies in this direction could yield important information for their pathogenesis and treatment.

Synovial inflammation is a typical feature for RA and is characterised by diffuse inflammatory infiltrates of T cells, B cells, macrophages, and dendritic cells. Also, there are clusters of lymphoid follicular aggregates comprising T cells, B cells, and dendritic cells, and structures with germinal centre-like reactions, and, rarely, granulomatous lesions. Thus, the synovial microenvironment appears optimal for supporting antigen requisition, storage, processing, and presentation by APCs to T cells [96]. Rheumatoid arthritis is considered to be a chronic inflammatory disease with features of autoimmunity. CD4+ cells and cytokines that they produce have crucial roles in this illness, which is comprehensively described in a review [96]. Moreover, the activation status and proinflammatory potential of CD8+ T cell subsets observed in the RA patients strongly suggests that a population of local and systemic cytotoxic effector T cells plays a role in this disease [97]. The finding that MKs can be potent antigen presenting cells for CD8+ T cells [60,61] could be a reason to further investigate the possible importance of this interaction in arthritis.

BM is considered to be the general niche of MKs in the organism, but they can also be found in the spleen [98] and in the capillary beds in the lungs [99]. Light-sheet fluorescence microscopy showed that the translocation of BM-derived mature MKs into different organs is unlikely to be a dominant phenomenon [27]. Actually, these authors studied MKs in healthy conditions. The purpose of the presence of MKs in organs is still not extensively studied neither in health nor in disease. Some data about MK distribution and count in organisms in different conditions is scattered in the literature. Preliminary and not yet published data obtained in our laboratory confirms the presence of MKs in the spleen. Moreover, it shows that in inflammatory joint disease, such as the mouse models zymosan-induced arthritis (ZIA) and collagenase-induced osteoarthritis (CIOA), the count of MKs in bone marrow and spleen was dramatically increased compared to healthy animals.

These recent findings support the hypothesis that MKs or their progenitors reside in the organs and are able to produce more MKs and proplatelets upon demand or even to participate in inflammatory processes by expressing certain regulatory molecules. MKs may be key players in the development of the systemic character of some inflammatory joint disease. The increase of MKs, proplatelets and platelets number in the affected tissues and in organs outside them, such as the spleen, is a finding that puts the question whether MKs migrate there or appear in these sites as a result of the commitment of resident MK precursor cells located in the organs. Some reports show that hematopoietic stem cells are present in the spleen of mice [100] and human [101]. Moreover, these hematopoietic stem cells are similar to the bone marrow ones but differ in their behaviour [102].

7. CONCLUSION

The precise mechanisms of megakaryopoiesis, MKs development and platelet activation in arthritis are not fully understood. There are lots of future perspectives of MKs and platelets investigations directed towards their function in the organism during health and disease. The finding that MKs count increases not only in the affected joints but also in immune organs, such as the spleen, could be a milestone for elucidating the systemic manifestations of the inflammatory joint disease that are considered to be of autoimmune character. It is plausible that inhibition of MKs and platelets with subsequent decrease of MK and platelet-derived inflammatory markers may have a beneficial

effect on the course of treatment of inflammatory joint disease.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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