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Short Review

Modulation of megakaryopoiesis and platelet production during inflammation

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ABSTRACT

Megakaryocytes (MKs) are widely known as the progenitor cells of platelets. These large, polyploid cells are a derivative of the hematopoietic stem cell (HSC), and reside in the bone marrow, lining blood vessel walls where they release their platelet progeny into circulation. Although little is known about how MKs differ under various environmental stressors, both chronic and acute inflammation alter the differentiation and molecular content of MKs. Furthermore, evidence suggests that the release of inflammatory cytokines may induce MK rupture and rapid release of platelets as a mechanism to quickly replenish diminished platelet counts in response to inflammation. Similarities between MKs and their close relatives, white blood cells, have introduced the notion that MKs may play a role in combating infection by engulfing and presenting antigens, and passing this information to circulating platelets. In addition, MKs exposed to varying bone marrow environments produce different platelets which enter circulation primed to respond to and combat inflammation, infection, or injury. This review focuses on how inflammation alters MK production, maturation, and platelet production. In addition, it introduces the idea that inflammation reprograms MKs to create different, more pathogenic platelets and leads them to take on different roles as responders to deleterious conditions. In the future, studies determining how platelets are altered in disease states may lead to novel MK- and platelet-based therapeutic targets to mitigate inflammation-related morbidity and mortality.

Megakaryocytes (MKs) are large hematopoietic cells that are primarily found in the bone marrow (BM) and give rise to platelets. MKs are derived from hematopoietic stem cells (HSCs) that terminally differentiate following signaling of the glycoprotein thrombopoietin (TPO) through its receptor c-Mpl. Once mature, MKs assemble proplatelets, extensions of the MK cytoplasm that protrude through the vessel wall into the lumen, where shear forces from blood flow facilitate conversion of the proplatelets into many smaller circulating platelets [1–3]. Platelets are classically known for their critical role in hemostasis and thrombosis and are widely accepted as the "first responders" to blood vessel damage as they rapidly bind to extracellular matrix components exposed after vascular injury and become activated. Once activated, they secrete their granule cargo and expose phosphatidylserine (PS) rich surfaces that support and propagate coagulation. However, platelets play an active role in a variety of other physiological and pathological processes, including wound healing, cancer, autoimmune disease, sepsis, and inflammation (reviewed in [4]).

Recent evidence suggests that MKs are also active players in these conditions, although their role has remained poorly understood due, in part, to difficulties in accessing them in the bone marrow. However, the connection between inflammation and MK dysfunction pre-dates the discovery of TPO; in 1987 an increase in MK progenitor formation was observed after treating mice with inflammatory agents [5]. Subsequently, Kimura and colleagues made a direct connection between interleukin (IL)-1β administration, IL-6 induction, and thrombocytosis [6]. While the relationship between inflammation and platelet production has been accepted for decades, researchers are still unraveling the complex relationship between inflammation and MK development and function. This review will examine current evidence for the emerging role of MKs as both mediators of and responders to inflammation.

Abbreviations: BM, bone marrow; CCL, C-C Motif Chemokine Ligand; HSC, hematopoietic stem cell; IL, interleukin; LPS, lipopolysaccharide; MK(s), megakaryocyte(s); pI:C, polyinosinic:polycytidylic acid; PPAR, peroxisome proliferator-activated receptors; PS, phosphatidylserine; TLR, Toll-like receptor; TPO, thrombopoietin; vWF, von Willebrand Factor; WBC, white blood cell; IFITM3, interferon induced transmembrane protein 3

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1. Effect of inflammation on MK differentiation in the BM microenvironment

Continual MK differentiation is necessary for the maintenance and renewal of circulating blood platelets, which have a lifespan of approximately 8–10 days in humans [7]. The classic model of megakaryocyte maturation states that HSCs transition through a hierarchy of increasingly differentiated cell states including multipotent progenitor, common myeloid progenitor, MK-erythroid progenitor, and MK progenitor before final differentiation into an MK [8]. The only known definitive driver of HSC differentiation into MKs is TPO, the discovery and cloning of which in 1994 allowed for efficient culturing and differentiation of MKs in vitro [9–13]. Once TPO is introduced into isolated HSCs in culture, mature MKs arise after approximately 11–12 days in human cells [14–16] and 4 days in murine cells [17–19]. However, mice lacking either TPO or the TPO receptor c-Mpl have only reduced levels of MKs and platelets, indicating that there are other mechanisms by which HSCs mature into MKs [20–23].

Clues to the factor or factors responsible for TPO-independent MK maturation may be found in the mechanisms by which inflammation affects MK development. While HSCs treated with TPO take several days to mature into MKs, platelets can be replenished within the body in a matter of hours under inflammatory conditions. This suggests that HSCs are able to quickly produce more MKs under conditions of increased platelet consumption or loss, such as in immune-mediated thrombocytopenia. The mechanisms by which this expedited platelet production occur remain under investigation, but have largely been linked to different inflammatory mediators. One process by which platelet production could be increased is through altered or accelerated differentiation of MKs. Although our current understanding of HSC differentiation dictates a hierarchical order of events, evidence for a megakaryocyte-biased population of HSCs was first shown in 2013 when Sanjuan-Pla et al. identified a functionally distinct mouse HSC subset primed for platelet-specific gene expression [24]. Using a GFP reporter driven by the MK-associated VWF gene, they showed that after transplantation, the VWF-positive HSCs exhibit a distinct lineage-biased reconstitution pattern with a strong platelet bias and very limited lymphoid potential. Additional evidence for MK-biased HSCs was provided by two reports showing that HSCs with high c-Kit expression also have an intrinsic MK lineage bias [25,26]. Further evidence suggests that $CD41^+CD42b^+$LSK cells exhibit restricted MK potential in single-cell cultures and predominant platelet production after transplantation [27].

While evidence of an MK-biased HSC population existed, the relationship of these putative populations to each other and to more classical MK progenitor populations was unknown. In a paradigm-shifting study, Haas and colleagues discovered a unique HSC compartment that becomes activated upon inflammatory stress to efficiently replenish platelets, thus identifying a functional role for MK-biased HSCs. The authors induce inflammation via polyinosinic:polycytidylic acid (pI:C) injection, mimicking a viral infection. While circulating platelet counts are initially dramatically reduced, they are restored within a few days and remained stable thereafter [8]. Haas et al. determined that the source of these platelets is a subpopulation of vWF-high HSCs that also expresses high levels of the MK marker CD41. This vWF/CD41-high population is maintained in a quiescent state in healthy subjects, but becomes activated to replenish exhausted MKs in acute inflammation. As such, this population of MK-primed HSCs essentially bypasses the normal differentiation hierarchy and differentiates directly from HSCs to MKs. Furthermore, expression of MK transcripts was found in populations of purified murine HSCs, suggesting preprogramming of undifferentiated HSCs is possible under different environmental stressors, such as inflammation [8].

In a recent paper, a tyrosyl-tRNA synthetase variant (YRS^{ACT}) was identified as another TPO-independent modulator of MK differentiation [28]. The authors show that YRS^{ACT} increases platelet counts and accelerates platelet recovery in acute models of thrombocytopenia. This effect on platelet count is attributed to two separate modes of action: skewing of HSCs to pre-determined MK progenitors ($Sca1^+$ $F4/80^+$), and the upregulation of inflammatory cytokines (IL-6 and IL-1α) previously shown to increase MK differentiation and platelet production. YRS^{ACT} is able to act directly on MK progenitors, as treatment of murine BM cultures enhances not only MK ploidy but also expansion of the unique MK-biased HSC population expressing Sca-1 and F4/80. Interestingly, this enhanced MK maturation is not exclusively a direct effect on MKs alone. YRS^{ACT} also binds Toll-like receptors (TLRs) on monocytic cells, activating the MyD88/NF-κB signaling pathway to stimulate release of IL-6 and IL-1α, which in turn upregulates megakaryopoiesis through increased TPO production. As such, it is proposed that YRS^{ACT} increases megakaryopoiesis under stress conditions in both TPO independent and dependent ways to readily replenish depleted MKs and circulating platelets [28].

While the aforementioned studies suggest that there are TPO-independent pathways to modulate MK differentiation, inflammatory conditions also produce cytokines that directly and indirectly regulate TPO production. IL-6 is an acute phase response protein that is elevated in several auto inflammatory diseases including rheumatoid arthritis, systemic lupus erythematosus, psoriasis, and Crohn's disease, in addition to type 2 diabetes [29,30] and various types of cancers [31–34]. The relationships between inflammation, increased platelet counts, and IL-6 were observed when IL-6 administration induced the formation of larger MKs with higher ploidy [35]. While it was suspected that IL-6 may act through TPO, Kaser and colleagues confirmed this in a set of elegant experiments showing that IL-6–induced thrombocytosis in mice increases hepatic TPO mRNA expression and plasma TPO levels, and the effects of IL-6 are eliminated by neutralizing TPO [36]. Furthermore, a pivotal study in *The New England Journal of Medicine* found significantly elevated levels of both IL-6 and TPO in ovarian cancer patients with thrombocytosis [32]. They ultimately determined that the thrombocytosis is driven by tumor-secreted IL-6, which signals for enhanced hepatic TPO production, thus creating a positive feedback loop [32]. As such, IL-6 is now recognized as an important mediator of TPO-driven paraneoplastic thrombocytosis.

Similar to IL-6, IL-1β production and release is upregulated under inflammatory conditions including auto-inflammatory diseases [37,38], ischemic injury [39], type 2 diabetes [40] and osteoarthritis [41], and has been tied to thrombocytosis [6,42]. Early studies revealed that after thrombocytopenia was induced in mice via whole body irradiation, IL-1β administration shows strong MK potentiation activity. It was concluded from this study that IL-1β results in upregulation of a TPO-like factor causing the existing HSC population to produce a greater proportion of MKs [43]. Then in 1990, Kimura and colleagues found that administration of IL-1β to healthy mice causes a marked thrombocytosis [6]. However, IL-1β alone does not stimulate megakaryopoiesis in vitro, suggesting that the thrombocytosis is attributed to other factors via IL-1β stimulation. Notably, they measured different factors in mouse serum after IL-1β injection and found that IL-6 was elevated, suggesting that thrombocytosis induced by IL-1β is mediated by IL-6 or a combination of IL-6 and other cytokines. Indeed, it was later found that IL-1β upregulates the expression of TPO and transcription factors c-Jun, c-Fos, GATA-1, and NF-E2 in MK cells [44]. Thus, the effects of IL-1β are likely attributed to an IL-6-mediated increase in TPO expression.

While literature has explored how circulating inflammatory cytokines act on HSC differentiation and MK maturation, less is known about the effect of MK-secreted inflammatory cytokines and their effects on surrounding cells. Cunin et al. explored this phenomenon in relation to arthritis susceptibility via reciprocal bone marrow transplantation between $Kit^{Wsh/Wsh}$ mice, that develop intensified arthritis, and $Kit^{W/Wv}$ mice, which do not have heightened susceptibility to arthritis [45]. Cunin and colleagues found that the bone marrow transplant restores arthritis susceptibility in $Kit^{W/Wv}$ mice, but note that platelet transfer does not have an effect on susceptibility. As such, the

effect was attributed to MKs, and was verified by transplantation of a CD41 negative fraction of bone marrow, which does not induce susceptibility. In vitro culture of bone marrow MKs found production of both IL-1α and IL-1β and generation of microparticles containing IL-1α and IL-1β; this is hypothesized to contribute to the exacerbated systemic IL-1 present in states of inflammation. IL-1 dependency of arthritis susceptibility was confirmed by transfer of WT and IL-1 deficient MKs to Kit^{W/W_v} mice; WT MKs restore susceptibility but IL-1 deficient MKs do not [45]. This groundbreaking study shows a platelet-independent role for MKs in inflammation.

2. Inflammation-induced alterations in platelet production from MKs

Different inflammatory states can result in thrombocytosis or thrombocytopenia, platelet counts above 450×10^9 or below 150×10^9/L, respectively. This variance in circulating platelet counts indicates either 1) modified MK production from HSCs (discussed above), 2) an altered mechanism by which platelets are produced by MKs, or 3) changes in platelet clearance. As defective platelet clearance has been studied extensively in conditions such as immune-mediated thrombocytopenia [46,47], we will focus on inflammation-induced alterations in platelet production from MKs.

While TPO largely governs the commitment of HSCs to MKs and subsequent MK maturation, it plays no role in the terminal stages of platelet production from the mature MK. To date, the mechanisms by which mature MKs are triggered to begin making proplatelets is largely unknown. However, various inflammatory mediators modify the process of proplatelet production and release. Nishimura and colleagues observed a small subset of MKs that undergo a unique rupture morphology in response to acute platelet demands after induced thrombocytopenia [48]. This MK rupture phenotype is induced by IL-1α, a proinflammatory cytokine released from endothelial cells and activated platelets. As opposed to the accepted method of platelet production in which MKs release long proplatelet extensions in the blood stream which further fragment into platelets, in this scenario, MKs rupture to make platelet-sized fragments that are directly released into the bloodstream [48]. As such, IL-1α is hypothesized to be a regulator of rapid platelet replenishment in acute injury or inflammation. However, future studies are warranted to determine the contribution of these 'ruptured' platelets to the overall platelet population and the functional role they play after their release. As they bind the same receptors, it remains largely unknown how IL-1α can mediate effects in MKs not also attributed to IL-1β. However, the differences in HSC versus MK response to these cytokines may be attributed to different receptor expression on these cell types and/or the binding affinity for IL-1α and IL-1β to each of their receptors; two receptors have been identified, deemed type I IL-1 receptor and type II IL-1 receptor, with IL-1α binding preferentially to type I and IL-1β binding preferentially to type II [49,50]. Little is known about the presence and abundance of various IL-1 receptor subtypes on the MK surface in murine and human cells. However, characterization of these receptors could lead to new discoveries in the future.

Our lab has also studied possible mechanisms of inflammatory thrombocytosis and found that C-C Motif Chemokine Ligand (CCL)5 (RANTES) can directly enhance platelet production from MKs [51]. Specifically, CCL5 signaling through its receptor CCR5 enhanced MK ploidy and proplatelet formation through pro-survival signaling downstream of BAD phosphorylation. As both CCL5 and platelet levels are increased in colitis, we then tested if CCL5 could be responsible for thrombocytosis during this condition. Indeed, we found that CCL5 signaling through CCR5 was the link between platelet count and inflammation in a murine colitis model [51]. These data present another mechanism by which inflammation, specifically CCL5, can directly increase platelet production by both enhancing MK maturation and the productivity of individual MKs in producing platelets.

It has also been proposed that inflammation and infection can modulate platelet production directly through stimulation of toll-like receptors (TLRs) on MKs. While there are limited studies addressing this hypothesis, MKs express functional TLRs, including 1,2, 3, 4, 6, and 9 [52–57], suggesting they could be active in both physiologic and pathologic conditions. This is further supported by two studies showing that TLR4-deficient mice have reduced platelet counts, suggesting TLR4 may have a role in thrombopoiesis [53,54]. In addition, Beaulieu and colleagues found that stimulation of MKs with a TLR2 agonist induces enhanced gene expression and protein levels of GP1b, CD41, MCP-1, COX2, and NFκB1, demonstrating that inflammation, through TLR2, can increase maturation of MKs. As such, the presence of functional TLRs on MKs suggests a potential role for these receptors in viral infections. However, these hypotheses need to be further explored and tested directly to discern the importance of MK TLR signaling in vivo.

It is clear that in both acute and chronic inflammatory states, cytokines have a large impact on HSC differentiation, MK fate determination, and platelet production. In acute settings where replenishing platelet counts during thrombocytopenia is necessary, rapid increases in MK count is beneficial. However, in chronic inflammatory settings, as associated with cancer and autoimmune diseases, thrombocytosis can be pathogenic and contribute to the morbidity and mortality of the disease. It is likely that the multitude of cytokines present and altered during acute and chronic inflammation do not work autonomously, but rather collectively to influence MK maturation. As such, studies comprehensively evaluating the complex combinations of cytokines and chemokines, as seen in inflammatory states in vivo, and their cooperative effects on MK maturation will revolutionize our understanding of the relationship between inflammation and MK differentiation. A better understanding of the inflammatory milieu and how it affects megakaryopoiesis may allow us to manipulate MK differentiation and platelet output to either enhance or suppress platelet counts in affected patients.

3. MKs as immune cells

Due to their common hematopoietic progenitors with white blood cells (WBCs), it is intriguing to theorize a possible intrinsic immune function for MKs and platelets. It has historically been accepted that platelets have little role in immune function and only a few studies have addressed a possible role for MKs. However, recent discoveries of shared immune receptors on platelets, MKs, and WBCs may indicate otherwise. The classic model of immune cell function states that WBCs such as neutrophils, monocytes/macrophages, and dendritic cells engulf and digest exogenous microbes via phagocytosis. The engulfed microbes are digested into proteins and ultimately small peptides that are presented by MHC class I or II molecules on the cell surface for recognition by T lymphocytes. The ability of platelets to similarly endocytose and process bacteria has long been studied but remains controversial [58–63], although a recent study suggests that platelets do not phagocytose microbes but rather gather, isolate, and sequester them [64]. This function of platelets is hypothesized to boost the activity of phagocytes, thus suggesting that platelets could play an important role in innate immune responses [64].

In a recent paper, Zufferey and colleagues looked not at platelets, but at MKs [65]. Of interest, they show that MKs are able to endocytose ovalbumin (OVA), a protein surrogate used to model foreign protein antigens that would be eliminated by immune cells in vivo. Once OVA is taken up by an MK, its immunogenic peptide ligand is generated and presented as antigen in association with MHC class I molecules on the MK cell surface. Of note, this MK presentation is then able to stimulate $CD8^+$ T-cell activation both in vitro and in vivo [65]. Although there is no evidence that MKs presenting the OVA-MHC Class I complex are sequestered and cleared from the body, Zufferey show that MKs transfer the bound OVA-MHC Class I complex to proplatelets, and therefore likely to platelets as well [65]. The implications of MKs transferring this

complex to platelets suggest that MKs can affect activation of $CD8^+$ T-cells by creating pre-primed platelets that recognize and clear pathogens rapidly during infection. Thus, MKs may play an important role in the adaptive immune response.

As the MK membrane gives rise to platelets, the majority of immune receptors on the surface of platelets are inherited from MKs. However, most studies focus on platelets and fail to examine a possible function for these receptors on MKs. One notable receptor family with a role in inflammation that is found on both platelets and MKs are the peroxisome proliferator-activated receptors (PPARβ/δ and PPARγ), commonly found on the nuclear membrane of various cell types [66]. PPARβ/δ function as transcription factors active in processes such as cellular proliferation and differentiation [67,68], which begs the question of why the receptor is found on platelets, cells that do not have nuclei or participate in the cell cycle. However, prostacyclin is a ligand for PPARβ/δ in platelets, and inhibits platelet aggregation in response to a variety of agonists likely upregulated during inflammation [66,69,70]. In addition to PPARβ/δ, MKs and platelets also have PPARγ, a receptor recognized as a target for anti-inflammatory therapies [71]. Similar to PPARβ/δ, PPARγ agonists inhibit the release of proinflammatory cytokines from platelets, thus mitigating the response to infection and injury. Of note, Akbiyik et al. discovered that PPARγ is expressed and active in human megakaryoblast cells (Meg-01 s) and human bone marrow MKs, and that PPARγ is passed from MKs to platelets. This discovery has led scientists to consider the possibility of utilizing platelets and/or MKs as targets for antidiabetic and anti-inflammatory drugs. MKs also express the critical immune co-stimulatory molecules CD40L (CD154) and CD86, which may confer upon them the ability to present antigens as described above [72–75]. Finally, MKs express and secrete several immunomodulatory cytokines and chemokines such as IL-1, TGF-β and CXCL1 [73,76–79]. These soluble immune mediators have many cellular functions related to immunomodulation such as CD8+ T cell-mediated immune suppression [80].

In addition to bacterial infection, MKs also play a role in viral infections. A recent study by Campbell et al. investigated the ability of MKs to prevent infection by dengue virus and influenza [81]. Interferon induced transmembrane protein 3 (IFITM3) is an anti-viral immune gene present on various cells that prevents viral infection by inhibiting virion particles shortly after endocytosis [82], thus regulating influenza virus replication [83,84]. Campbell et al. found that the platelet transcriptome is modified in patients infected with dengue virus or influenza, and IFITM3 is significantly upregulated on platelets isolated from dengue patients when compared to healthy controls using RNA-seq [81]. Furthermore, lower platelet IFITM3 is associated with increased virulence of the infection and mortality. When healthy subjects are administered the dengue vaccine TV003, IFITM3 markedly increases in platelets, suggesting platelets play a role in inhibiting viral infection. These studies led to question how IFITM3 is upregulated in platelets, and if it is consequentially increased in MKs as well. Previous literature states that dengue virus, which typically circulates in the bloodstream, is capable of infecting the bone marrow niche, potentially infecting MKs and their precursor cells [85–88]. Campbell and colleagues further explored this hypothesis by infecting human $CD34^+$-derived MKs with dengue virus stereotype 2 in vitro and found significant enrichment in MK *IFITM3*. In addition, MK exposure to interferons (IFNα, IFNβ, and IFNϒ) in vitro also upregulates MK IFITM3. Interestingly, MKs expressing higher amounts of IFITM3 appear resistant to dengue infection, whereas MKs deficient in IFITM3 are permissive to dengue infection. Dengue infection is associated with increased secretion of IFNα and IFNβ by MKs, which in turn increases the amount of IFITM3 expression in surrounding MKs, leading researchers to infer that MKs can inhibit dengue infection of surrounding cells. This was demonstrated by treating naïve, uninfected MKs with supernatants from dengue-infected MKs. The MKs unexposed to dengue virus show significantly increased IFITM3 and in turn reduce dengue infection of the naïve MKs as well as $CD34^+$ HSCs [81]. To interrogate whether IFITM3 alone is responsible for prevention of infection, IFITM3 was overexpressed and knocked down in Meg-01 cells (a megakaryocytic cell line). IFITM3 overexpression results in significantly reduced dengue infection in the absence of IFN stimulation, and IFITM3 knockdown results in Meg-01 cells that are more susceptible to dengue infection [81]. Taken together, it appears that MK immune function is an important and unique phenomenon. More importantly, these immune functions can be passed on to platelets, which may allow MKs to communicate with the immune system in the periphery.

4. Different MKs make different platelets

Considering the diverse and substantial effects that different inflammatory states have on MKs, it is interesting to contemplate the possibility that these MKs may give rise to platelets yielding different arrangements of alpha granule content and receptors under pathological conditions. Could accelerated differentiation of HSCs into MKs cause downstream platelets to carry less alpha granule content? Or, perhaps in the instance of infection, platelets may enter the bloodstream primed to be sequestered if presenting an MHC-I-antigen complex inherited from MKs expressing the immune complex. While an intriguing idea, few studies have addressed the hypothesis of altered platelet phenotypes in inflammation due to differences in MKs. However, there are data to support differential MK expression patterns affecting their platelet progeny. Specifically, the platelet transcriptome and corresponding protein content is changed in disease states such as dengue virus [81], myocardial infarction [89], essential thrombocythemia [90], SLE [91], and sickle cell disease [92]. Work done by multiple groups over the past decade has provided convincing evidence that MKs are indeed the source of platelet mRNA (reviewed in [93]). This supports the idea that MKs are reprogrammed during disease states to make different, more pathological, platelets.

Indeed, in sepsis, Freishtat et al. found changes in the translational profile of MKs that can be traced to circulating platelets [94]. In a murine model, MKs and platelets from mice that had undergone a cecal ligation and puncture to induce sepsis showed altered mRNA profiles; specifically, the septic MKs and platelets express the cytotoxic serine protease granzyme B. Furthermore, platelets from septic mice induce apoptosis of healthy splenocytes ex vivo, while platelets from granzyme B null (−/−) septic mice do not. Of note, granzyme B positive platelets were also found in septic children. Thus, Freishtat and colleagues propose that platelet granzyme B plays a role in sepsis-related mortality by promoting lymphoid toxicity [94]. This alteration in mRNA and subsequent protein content occurs at the MK level and is transferred into platelet progeny. This remarkable finding demonstrates that in disease and inflammation states, MKs release altered platelets, which can in turn have different physiological effects on surrounding cells upon activation.

5. Why should we care about inflammatory modulation of MKs?

Inflammation can affect the MK at almost any point between differentiation from HSCs to platelet production and phenotype (Fig. 1). Starting with impact on HSCs, the concept of committed MK progenitors is relatively new, and in the future, it will be interesting to see if MK-committed HSCs are present under normal conditions or solely under stress hematopoiesis. In addition, more studies are necessary to determine if these inflammation-induced MK-primed populations are due to HSCs 1) actively switching between progenitor subsets or 2) bypassing certain progenitor compartments altogether. Furthermore, the proposed idea of using inflammatory cytokines such as CCL5 to rescue platelet counts during thrombocytopenia needs to be further explored, but could be optimized for therapeutic use in the future. While little is known about the similarities between MKs and immune cells and the role of MKs in infection and inflammation, the shared

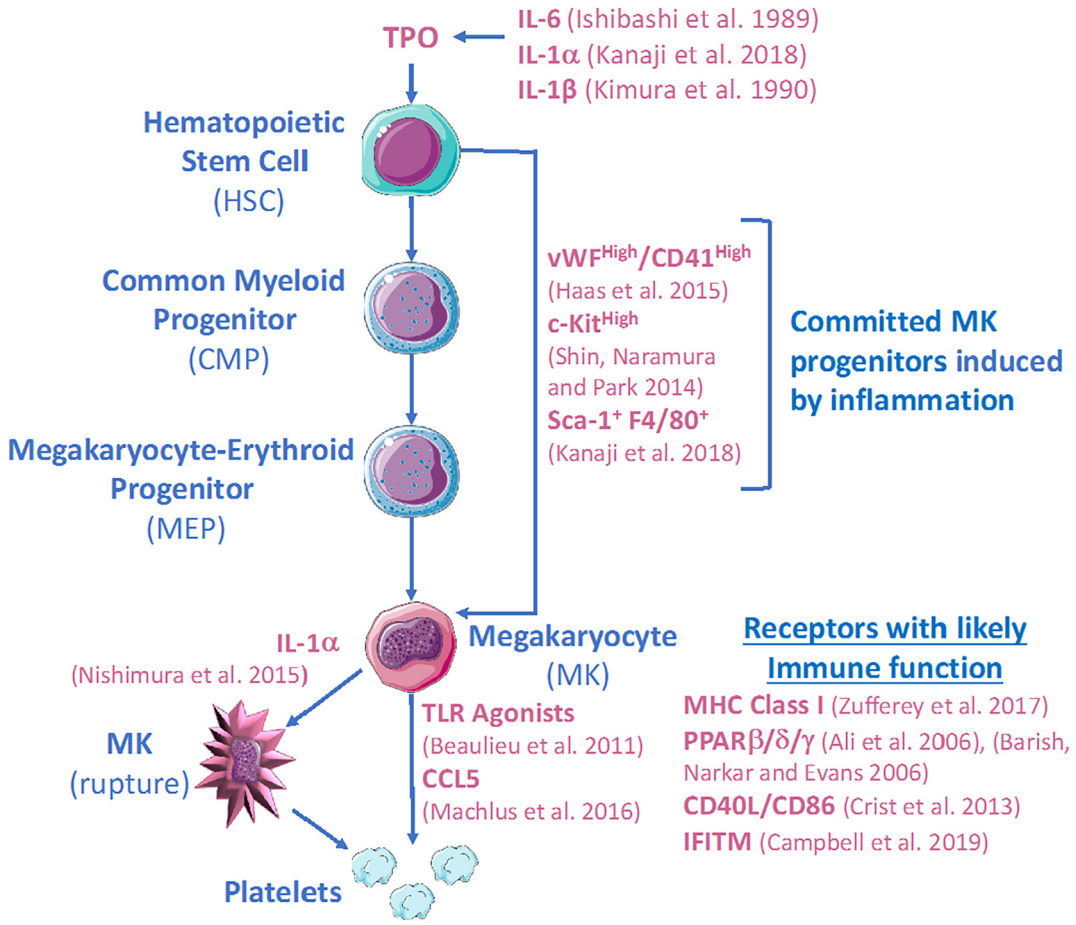

Fig. 1. Megakaryocyte differentiation and platelet production is altered in response to inflammation. Under normal conditions, TPO is the master regulator of HSC differentiation into MKs. IL-6, IL-1α and IL-1β, elevated in various inflammatory diseases, upregulate TPO production and in turn skew the HSC population to MKs. Independent of TPO, some inflammatory states induce populations of committed MK progenitors that bypass traditional stages of differentiation, including vWF^{High}/CD41^{High} cells activated in the presence of viral infection; c-Kit^{High} cells, activated in inflammatory conditions; and Sca-1^{+} F4/80^{+} cells, induced under stress conditions by the enzyme YRS^{ACT}. Downstream of MK differentiation, TLR agonists and the chemokine CCL5, which circulate in inflammation and viral infection, induce platelet production from MKs. IL-1α has also been suggested to induce MK rupture leading to the rapid release of platelets.

progenitors between WBCs and MKs may suggest that MKs take on a larger role than previously thought. The aforementioned studies have simply scratched the surface of all that remains to be discovered about MKs, and how variable these cells can be under differing environmental stressors.

The study of MKs as responders to inflammation and disease is in its nascent stages. MKs have largely been thought of as having a singular role of platelet production that is rarely, if ever modified under different conditions. However, current evidence suggests that MKs adjust during inflammation due to alterations in their differentiation from HSCs, maturation, surface receptor expression, and physiological response to environmental signals. In the future, additional studies are needed to examine developmental differences between MKs that develop in inflammatory states vs. healthy individuals, as well as at the platelet progeny stage to see if MK modifications are apparent in platelets. The novel findings discussed in this review may transform the study and development of therapeutics targeting MKs to mitigate infection and peripheral complications associated with inflammation, including thrombocytosis and thrombocytopenia. Information from these studies will allow us to make better, more effective therapeutics that target both MKs and platelets.

Authorship

GC and KRM wrote and edited the manuscript.

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Declaration of competing interest

The authors declare no relevant conflict of interest.

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