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REVIEW

Immunological face of megakaryocytes

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Abstract **Megakaryocytes (MKs), which are traditionally known for their role in platelet production, are now emerging as unique immune cells with diverse capabilities. They express immune receptors, participate in pathogen recognition and response, phagocytose pathogens, contribute to antigen presentation, and interact with various immune cell types. When encountering inflammatory challenges, MKs exhibit intricate immune functions that can either promote or inhibit inflammation. These responses are mediated through mechanisms, such as the secretion of either anti-inflammatory or pro-inflammatory cytokines and release of immunomodulatory platelets according to specific conditions. This intricate array of responses necessitates a detailed exploration to determine whether the immune functions of MKs are carried out by the entire MK population or by a specific subpopulation. Breakthroughs in single-cell RNA sequencing have uncovered a unique "immune MK" subpopulation, revealing its distinct characteristics and immunoregulatory functions. This review provides latest insights into MKs' immune attributes and their roles in physiological and pathological contexts and emphasizes the discovery and functions of "immune MKs".**

Keywords megakaryocyte; platelet; immune; inflammation; heterogeneity

Introduction

Megakaryocytes (MKs) are specialized and polyploidy bone marrow cells recognized for their role in platelet production [1]. Studies on megakaryopoietic development have mainly focused on elucidating the genesis and functions of platelets, which play pivotal roles in hemostasis and coagulation [2–6]. Recent technological advances have enhanced understanding of MKs, and the remarkable versatility of MKs akin to that of platelets have been demonstrated; these MKs possess a diverse array of immune sensors and various immune functions [7–13]. Moreover, similar to various hematopoietic cell types showing heterogeneity, MKs demonstrate intricate cellular diversity, exhibiting multiple subpopulations with distinct functional attributes. These subpopulations encompass "platelet-generating MKs" primarily involved in thrombopoiesis, namely, hematopoietic stem cell (HSC) niche MKs, which modulate the quiescence and proliferation of HSCs, and immune MKs, which are engaged in immune responses and actively interact with the immune system [14–17]. The identification of immune properties and immune subpopulations within MKs have broadened our perspective on the roles of MKs in physiologic and pathological contexts. In this review, we will describe the multifaceted immune functions of MKs and elucidate their distinct immunological roles under inflammatory conditions. Additionally, we will characterize "immune MK" subsets and offer insights into their immunomodulatory roles (summarized in Fig. 1).

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Multifaceted immune roles of megakaryocytes

MKs have long been recognized for their role in platelet production, but mounting evidence suggests that they possess immunological functions under steady-state conditions and during periods of stress. Similar to other immune cells, MKs express receptors and molecules associated with immune recognition and response (Table 1), such as toll-like receptors (TLRs) 1–6, Fc gamma receptors (FcγRs), and CD40L. Cultured human megakaryocytic cell lines and endogenous mouse MKs express TLRs, which are traditionally associated with immune cells, such as monocytes, T and B lymphocytes,

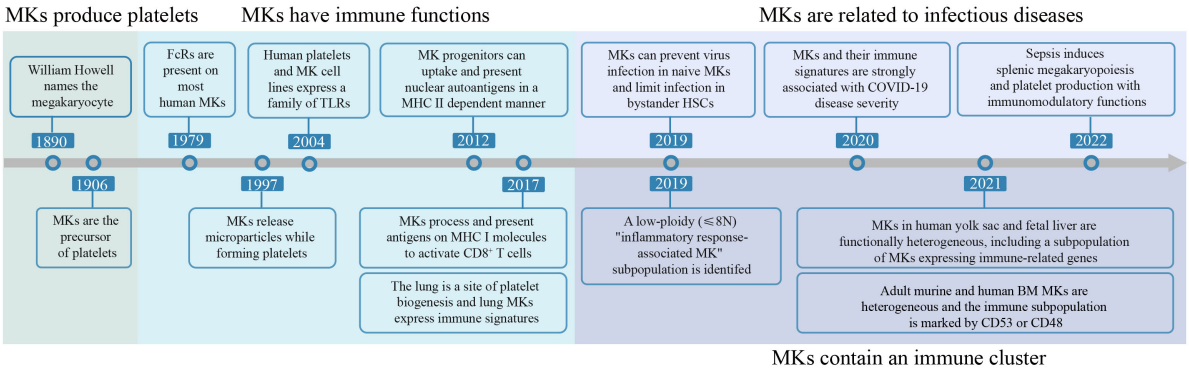

Fig. 1 Historical timeline of megakaryocyte biology: charting key contributions to understanding immunological roles and subpopulations [14–28]. MKs, megakaryocytes; FcRs, Fc-receptors; TLRs, toll-like receptors; MHC, major histocompatibility class I; MHC II, major histocompatibility class II; HSCs, hematopoietic stem cells; BM, bone marrow.

and NK cells, enabling MKs to detect pathogen-associated molecular patterns. The activation of TLRs promotes MK maturation, polyploidization, and the expression of inflammatory-related genes and proteins [29–32]. MKs also express FcγRs, which are typically found on B lymphocytes, NK cells, macrophages, and neutrophils. These receptors play a vital role in pathogen clearance by binding to antibodies attached to infected cells or pathogens [33, 34]. The presence of TLRs and FcγRs on MKs suggests their potential roles in innate and adaptive immune regulation. Furthermore, primary mouse MKs express CD40L [35, 36], which is mainly found on activated $CD4^+$ T cells, enhancing their antigen-presenting capabilities by facilitating B cell maturation and macrophage phagocytosis.

Apart from expressing immune receptors, MKs can phagocytose fungi and bacteria. Observations from bone marrow biopsy samples of patients with conditions, including pulmonary histoplasmosis and acquired immune deficiency syndrome with cryptococcal meningitis, have shown fungi enclosed within the cytoplasm of MKs, suggesting the capacity of MKs to internalize fungi [59]. However, the consequences of this phagocytosis and the predisposing factors for such occurrences remain unclear. Recent research utilizing live cell fluorescence imaging with pHrodo green *Escherichia coli* BioParticles conjugate has quantitatively measured the phagocytic activity of MKs on the basis of the acidification of ingested bacteria [16, 17, 38]. These studies have demonstrated that both bone marrow- and lung-derived MKs can phagocytose *E. coli*, internalizing the bacteria into phagolysosomes for digestion. Notably, lung-derived MKs exhibit greater efficiency in phagocytosing *E. coli* than bone marrow MKs [38, 60].

MKs can not only phagocytose antigens but also participate in antigen presentation. In a murine model of systemic lupus erythematosus, primary megakaryocyte progenitor cells (MkPs) with major histocompatibility complex class II (MHC II) molecules are the principal autoantigen-presenting cells, inciting Th17-driven autoimmunity. This phenomenon accelerates the onset of lupus and disrupts immune tolerance in healthy mice [22]. In contrast to this disease state, MkPs derived from the human $CD34^+$ hematopoietic stem and progenitor cells of healthy donors express MHC II and can stimulate the proliferation of Th17 cells by secreting various cytokines, including interleukin-1 (IL-1), IL-18, IL-6, TGFβ, and IL-23. These findings suggest that MkPs contribute to the innate immune system and enhance adaptive immunity for protection against pathogens in normal subjects [45]. Compared with MkPs, mature MKs display varying levels of major MHC I and II expression. When exposed to exogenous protein antigens, MKs efficiently engulf these proteins and generate immunogenic peptide ligands through proteolysis. These peptides are subsequently cross-presented on the surfaces of MKs in conjunction with MHC I or MHC II. This process effectively triggers the specific activation and proliferation of $CD8^+$ or $CD4^+$ T cells [16, 17, 24, 38].

MKs communicate with other immune cells and participate in immune responses (Table 2). G-CSF-mediated thrombopoietin release induces MKs and endothelial cells to secrete C-X-C chemokine receptor type 2 (CXCR2) ligands, which stimulate neutrophil motility and mobilization from the bone marrow into the bloodstream [55, 61]. Moreover, hematopoietic cells, including neutrophils, erythrocytes, lymphocytes, eosinophils, and monocytes, can transit through MKs through a process termed “emperipolesis” [62–67]. As the most commonly internalized cells, neutrophils undergo emperipolesis by MKs, facilitating the bidirectional transfer of membranes between cytoplasmic neutrophils and the demarcation membrane system of MKs. This process accelerates platelet production and leads to the formation of “hybrid platelets” bearing neutrophil membranes [68–70]. The interaction between MKs and neutrophils endows both cell types with new immune functions. Additionally, MKs release cytokines, such as

Table 1 Selected membrane immune molecules expressed by megakaryocytes

	Ligand	Murine			Human		
		Ex vivo cultured MK	Lung MK	BM MK	MK cell line	*Ex vivo* cultured MK	BM MK
CD40L	CD40	mRNA [36]		mRNA [36]	mRNA [36]		
CD74			mRNA, protein [37]	No mRNA, protein [37]			
CD80 (B7-1)	CD28, CTLA4	Protein [24]	Protein [38]	Protein [38]			
CD86 (B7-2)	CD28, CTLA4	Protein [24]	Protein [38]	Protein [38]			
FcγRI	IgG	Protein [34]		Protein [34]	No mRNA [33]		
FcγRII	IgG	No protein [34]			mRNA [33]		Protein [20]
FcγRIII	IgG	No protein [34]			No mRNA [33]		
CR1	C3b			Protein [20]			No protein [20]
IL-1R	IL-1α, IL-1β				mRNA, protein [39]	mRNA, protein [39]	
IL-6R	IL-6					mRNA [40]	No mRNA [41]
IL-21R	IL-21					mRNA, protein [42]	
IFNAR	IFNα, IFNβ				mRNA, protein [43]	mRNA, protein [43]	
IFNγR	IFNγ					Protein (MkP) [44]	
MHC I	CD8	Protein [24]					
MHC II	CD4	Protein (MkP) [22, 45]	Protein [37, 38]	Protein [45]			
CXCR4	SDF-1		mRNA [23]	mRNA, protein [17, 23]	mRNA, protein [46]	mRNA, protein [46]	
TLR1	Triacyl lipoproteins		mRNA [23]	mRNA [23]	mRNA, protein [32]		
TLR2	LPS		mRNA, protein [23, 37]	No mRNA, no protein [23]	mRNA, protein [30]		Protein [47]
TLR3	dsRNA, Poly (I:C)		mRNA [23]	mRNA [23]		mRNA, protein [29]	
TLR4	LPS	Protein [48]	mRNA, protein [23, 37]	No mRNA, no protein [23]	Protein [49]		Protein [47]
TLR5	Flagellin		mRNA [23]	mRNA [23]			
TLR6	Diacyl lipoproteins		mRNA [23]	No mRNA [23]	mRNA, protein [32]		
TLR7	ssRNA		mRNA [37]	No mRNA [37]			
TLR12	Profilin from *Toxoplasma gondii*		No mRNA [37]	mRNA [37]			
CCR7	CCL19, CCL21		Protein [38]	Protein [38]			

MkPs, megakaryocyte progenitor cells.

PF4 (CXCL4), APRIL (a proliferation-inducing ligand), and IL-6 to regulate B cell and plasma cell development and survival [52, 71]. Moreover, MKs release microparticles that transport bioactive molecules, promote megakaryocytic differentiation, and contribute to inflammatory processes [72–77]. These microparticles exert their effects on target cells either through surface ligands directly or by transferring surface receptors [78, 79]. Notably, MK-derived microparticles expressing CXCR4 can transfer this receptor to CXCR4-negative cells, thus facilitating the entry and spread of HIV strains [80]. Moreover, MKs secrete IL-1-rich microparticles into the systemic circulation, which stimulates synovial fibroblasts, contributing to arthritis susceptibility and participating in systemic inflammation [34]. Furthermore, the delivery of peroxisome proliferator-activated receptor-γ from MK and platelet microparticles to human monocytes modulates gene expression, reduces

Table 2 Selected secretory immune molecules expressed by megakaryocytes

	Receptor	Murine			Human		
		Ex vivo cultured MK	Lung MK	BM MK	MK cell line	*Ex vivo* cultured MK	BM MK
IL-1α	IL-1R1	Protein [34]			Protein [50]	Protein [34]	
IL-1β	IL-1R1, IL-1R2	Protein [34]			mRNA, protein [39, 50]	mRNA, protein [39]	mRNA, protein [51]
IL-3	IL-3R						Protein [41]
IL-6	IL-6R			Protein [52]	mRNA, protein [40, 50]	Protein [40]	mRNA, protein [41, 51]
IL-8/CXCL8	CXCR1, CXCR2				Protein		
IL-9, IL-10, IL-12, IL-13	IL-9R, IL-10R, IL-12R, IL-13R						No mRNA [41]
IL-33	IL1-R4 (ST2)			Protein [53]	Protein [53]		
IFN-α, IFN-β	IFNAR				mRNA, protein [43]	mRNA, protein [43]	
APRIL	TACI, BCMA			Protein [52]		mRNA, protein [54]	
CCL2, CCL3, CCL4, CCL6, CCL7, CCL8, CCL9, CCL12, CCL24	CCR1, CCR2, CCR3, CCR5		mRNA [23, 37]	No mRNA [23]			
CXCL1	CXCR2		mRNA [23]	mRNA, protein [23, 55]			
CXCL2	CXCR2		mRNA [23, 37]	No mRNA [23]			
CXCL3	CXCR2			mRNA [55]			
CXCL4/PF4				mRNA, protein [56]			Protein [57]
CXCL5	CXCR2		No mRNA [37]	mRNA [37]			
CXCL10, CXCL16	CXCR3, CXCR6		mRNA [23, 37]	No mRNA [23]			
CXCL13	CXCR5		mRNA [23]	mRNA [23]			
TNF-α	TNFR1, TNFR2				mRNA, protein [50]	mRNA, protein [50]	mRNA, no protein [51]
GM-CSF	GM-CSFR				Protein [50]	mRNA, protein [50]	mRNA, protein [41, 51]
TGF-β	TβR, TβRI		mRNA (fetal lung) [37]	mRNA [58]	mRNA [50]		
FGF1	FGFR			mRNA [58]			
IGF1	IGF1R		mRNA (fetal lung) [37]				

inflammatory mediator production, and enhances monocyte adherence [81]. These findings highlight MKs' considerable impact on communicating cells and their involvement in inflammatory processes.

The tissue-specific localization of MKs shapes their immunological roles, and this relationship is evident in extramedullary MKs residing in organs, such as the lungs and spleen [13–16, 82]. Adult lung MKs expressing dendritic cell markers, including CD11c [38], exhibit substantially higher TLR and chemokine expression levels than bone marrow MKs [37, 82]. Moreover, the excellent performance of these adult lung MKs in internalizing and processing antigenic proteins and bacterial pathogens underscore their involvement in immune surveillance [38, 82]. Lung MKs demonstrate heightened sensitivity to physiologic alterations associated with stress and infection, such as leukocytosis, hemorrhage, shock, and acute respiratory distress syndrome, which stimulate their production [83–92]. The elusive origin of lung MKs poses an important question, and proposed models suggest migration from bone marrow MKs *via* circulation, generation by lung HSCs, or colonization by pre-definitive hematopoietic progenitors from the yolk sac [92]. Interactions with the lung microbiome or circulating molecules are likely to influence the function of lung MKs and their platelet progeny, positioning them as key immune effector cells. Similarly, in the spleen, a crucial site for extramedullary hematopoiesis, inflammation triggers splenic MKs to produce platelets with immune functions in an

IL-3-dependent manner [28, 93]. These specialized platelets, characterized by high levels of CD40L, play a pivotal role in the activation of neutrophils and release of bactericidal neutrophil extracellular traps, enhancing microbicidal effects and contributing to overall immune function.

In summary, the immunological functions of MKs are diverse and multifaceted and intricately linked to their locations. MKs play a crucial role in innate immunity by recognizing pathogens through surface receptor molecules, initiating pathogen elimination through phagocytosis and presenting antigens to adaptive immune cells. Furthermore, MKs can amplify immune signals by interacting with other immune cell populations (Fig. 2). Given these immune properties, exploring the role of MKs in pathological or infectious scenarios holds intrigue and promise for future applications.

Immune functions of megakaryocytes in inflammation

Antiviral defenders or inflammation facilitators

The significance of MKs in the context of viral infections has attracted interest in recent years [94–98]. MKs demonstrate potent antiviral capacity because they respond to viral infections, such as those caused by influenza and dengue viruses. MKs can secrete interferons (IFNs) and upregulate IFN-induced transmembrane protein 3 (IFITM3), which is an

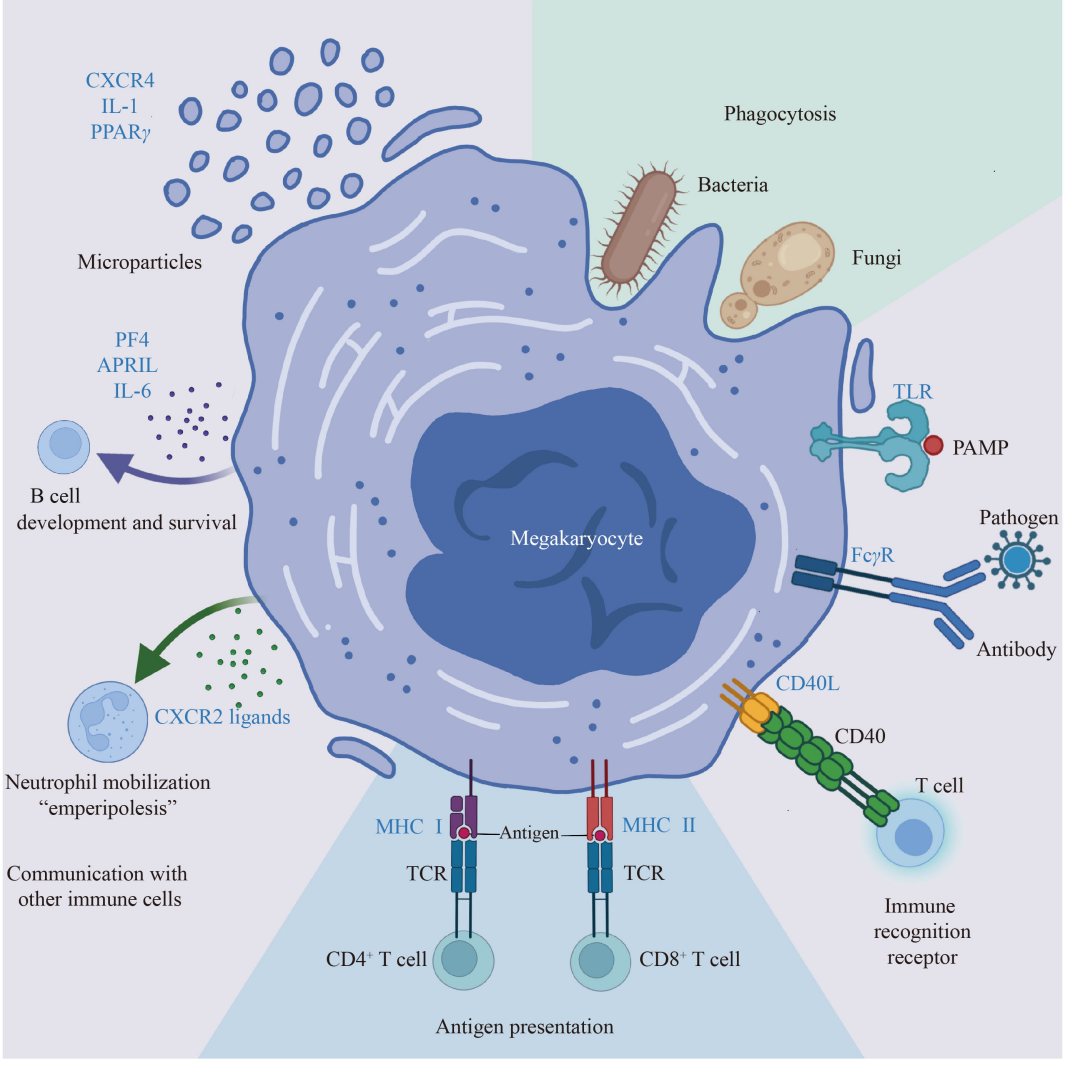

Fig. 2 Megakaryocytes respond to inflammation in a variety of ways, such as surface immune recognition receptor, phagocytosis, antigen presentation, and communication with other immune cells. TLR, toll-like receptor; PAMP, pathogen-associated molecular pattern; FcγR, Fcγ-receptor; CD40L, CD40 ligand; CXCR2 ligands, C-X-C chemokine receptor type 2 ligands; PF4, platelet factor 4; APRIL, proliferation-inducing ligand; IL-6, interleukin-6; MHC I, major histocompatibility class I; MHC II, major histocompatibility class II; CXCR4, C-X-C chemokine receptor type 4; IL-1, interleukin-1; PPARγ, peroxisome proliferator-activated receptor γ. (Created with Biorender.com.)

IFN-responsive gene that restricts viral entry and replication [99–101], thereby preventing the infection of neighboring MKs and HSCs and limiting the spread of infections [25, 102]. These findings highlight MKs' antiviral capabilities. However, three independent international multicenter studies on COVID-19 have identified a close association between MKs and disease severity. IFN-activated MKs, along with erythroid cells in peripheral blood (PB), are indicative of severe disease [26]. The severity of COVID-19 is linked to the expansion of MK-primed progenitor cells and increased expression of MK-related genes [103]. Furthermore, MKs and specific monocyte subsets are substantial sources of elevated cytokines in severe COVID-19 cases [104]. The presence of circulating MKs containing SARS-CoV-2 is a strong risk factor for mortality and multiorgan injury [95, 105]. Thus, although MKs possess antiviral capabilities through IFN secretion, an increased number of MKs is correlated with disease severity.

The immune response of MKs in viral infections plays protective or pathogenic roles (Fig. 3). This dual role may be attributed to the kinetics and quantity of pro-inflammatory and regulatory signals and the balance between these signals [106]. Initially, MKs secrete IFN signals in response to a virus's initial infection, thereby contributing to a protective innate immune response. As a disease progresses, MKs emerge as major sources of circulating calprotectin (S100A8/A9) [95], which is a potent signaling molecule and a well-established risk factor for severe diseases. Additionally, MKs release NFκB-mediated cytokines, such as IL-6 and IL-1β, which promote systemic inflammation. Under these conditions, MKs may shift toward a pathogenic innate immune response. In summary, owing to the dual immune functions of MKs in response to viral infections, further studies aimed at elucidating the mechanisms and effects of reactive immunity in MKs are crucial for manipulating MK responses under pathological conditions.

Combat bacterial infections

In response to bacterial infections, MKs with enhanced immune characteristics becomes prominent in circulation. This phenomenon is particularly notable in sepsis, which is a systemic inflammatory response syndrome primarily triggered by bacterial invasion. It results in increased vascular permeability and vasodilation, which leads to the proliferation of circulating MKs in various anatomical locations, including PB, lungs, and kidneys [107, 108]. A similar increase in circulating MKs has been observed in patients with severe tuberculosis and *Mycobacterium tuberculosis* (*Mtb*). Notably, in contrast to lymphocytes in severe diseases, inflammatory MKs increase in PB, which is characterized by an elevated cytokine profile closely linked to tuberculosis severity [109]. Thus, bacterial invasion and inflammation are the impetus behind the proliferation of immunological MKs and their secretion of cytokines.

MKs employ indirect and direct strategies to engage with bacteria (Fig. 3). The predominant indirect approach involves the release of pro-inflammatory platelets. This process is activated by bacterial infections that induce transcriptomic and proteomic changes in MKs, influencing platelet function and host responses. This process is facilitated by the upregulation of IFN-α and IFITMs in MKs, leading to increased fibrinogen

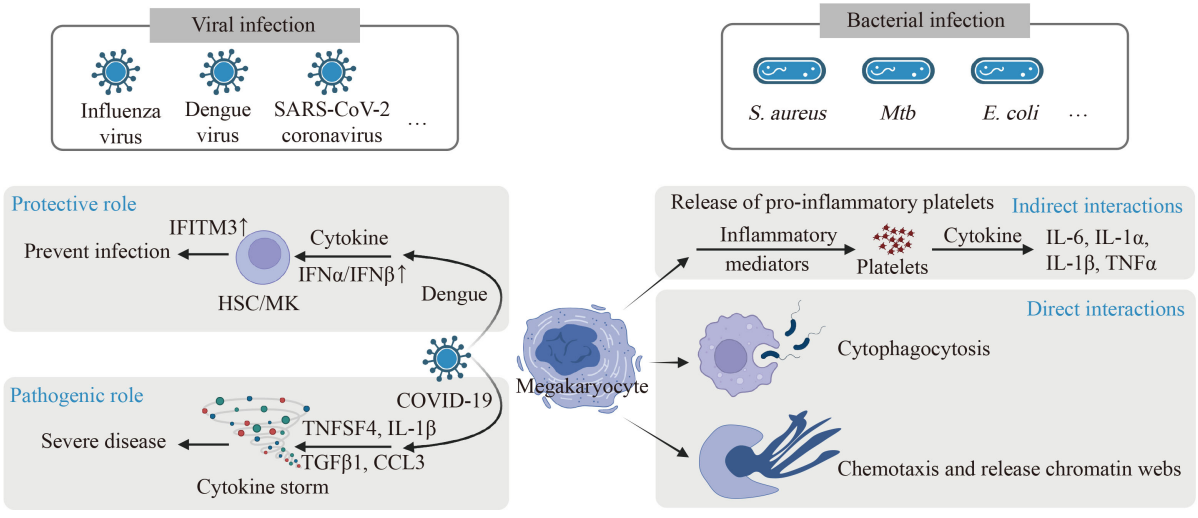

Fig. 3 Immune functions of megakaryocytes' response to viral and bacterial infections. *S. aureus*, *Staphylococcus aureus*; *Mtb*, *Mycobacterium tuberculosis*; *E.coli*, *Escherichia coli*; IFITM3, interferon induced transmembrane protein 3; HSC, hematopoietic stem cell; MK, megakaryocyte; IFNα, interferon α; IFNβ, interferon β; TNFSF4, TNF superfamily member 4; IL-1β, interleukin 1β; TGFβ1, transforming growth factor β1; CCL3, C-C motif chemokine ligand 3; IL-6, interleukin 6; IL-1α, interleukin 1α; TNFα, tumor necrosis factor α. (Created with Biorender.com.)

endocytosis and platelet hyperreactivity [110]. In a reciprocal manner, when platelets encounter pathogens as part of host defense, various inflammatory cytokines, including IL-6, IL-1α, IL-1β, and TNFα, are expressed. These cytokines exert an influence on MK proliferation and maturation, particularly in acute inflammatory conditions [111–115]. Furthermore, within the spleen, a distinct population of MKs exhibits the robust membrane expression of CD40L, producing a CD40 ligand^{hi} platelet subset with potent immunomodulatory functions [28]. In direct interactions with bacteria, MKs employ two distinct methods. The first involves cytophagocytosis, as evidenced by "immune MKs" in the bone marrow and lung MKs, which can phagocytose *E. coli* and internalize bacteria [38]. The second method involves MK undergoing chemotaxis, interacting with bacteria, and having the ability to release chromatin webs in response to pathogenic stimuli. However, further investigations are needed to elucidate the potential pro-inflammatory, pro-thrombotic, or bactericidal attributes of these MK-derived chromatin webs [108]. Interestingly, these indirect and direct strategies indicate the diversity of MKs in platelet production and immune regulation. Whether this diversity is carried out by a homogenous cellular population or distinct cell subgroups presents an intriguing question, with implications for future strategies aimed at modulating the diverse roles of MKs.

Identification and characterization of the "immune MK" subpopulation

Although advancements in single-cell technologies have allowed for the elucidation of functional diversity within numerous blood cell types [116–121], our knowledge regarding the heterogeneity of MKs has many gaps. A notable study by Wang *et al.* [27] unveiled the landscape of human embryonic MK heterogeneity and delineated the developmental trajectories of early megakaryopoiesis; they performed droplet-based single-cell RNA sequencing (scRNA-seq) using the 10X Genomics platform, analyzing cells obtained from the yolk sac at 4 weeks post-conception and fetal liver at 8 weeks post-conception; their findings revealed that fetal MKs exist as distinct transcriptional subpopulations; one subset exhibited elevated expression of genes associated with immune responses and predominantly originates from the fetal liver. This finding suggests the occurrence of MK heterogeneity during early embryonic stages.

A significant challenge remains in the *in vivo* isolation of adult MKs to study their heterogeneity. Adult MKs are notably rare, constituting a mere 0.01% of nucleated cells within the bone marrow [122]. Furthermore, the nuclei of MKs vary from diploid (2N) to higher ploidy levels, such as 4N, 8N, and even up to 128N [123, 124], reaching a diameter of over 100 μm [125]. The high ploidy MKs are fragile and releases platelets under stress generated by blood flow [126–129]. To tackle challenges in the isolation of rare, oversized, and fragile adult MKs, we developed an improved strategy that combines density gradient centrifugation, FACS sorting, manual cell selection, and fluorescence *in situ* hybridization for ploidy confirmation. Isolated MKs achieve high purity from murine and human bone marrow across a range of ploidy levels from 2N to 32N. By utilizing a modified Smart-seq2 protocol for scRNA-seq of MKs [14, 130], we discerned a low-ploidy (⩽8N) "inflammatory response-associated MK" subpopulation characterized by the heightened expression of inflammation-related genes. In the application of these techniques, our team [16] and Liu *et al.* [15] successfully isolated adult MKs and unveiled a notable aspect of cellular and functional heterogeneity within native mouse and human megakaryopoiesis. Furthermore, Liu *et al.* identified and confirmed the distinct "immune MKs" during *in vitro* human megakaryopoiesis. Importantly, both studies independently identified a novel subpopulation of "immune response-related MKs" characterized by the unique expression of surface markers CD53 (in humans and mice) or CD48 (in humans). This subpopulation exhibited a conserved gene signature and functional attributes across the two species. The identification of these specific markers not only aids in evaluating the properties of primary MKs but also facilitates the assessment of MKs derived through *in vitro* regeneration. Qin *et al.* [131] and Rodríguez *et al.* [132] used these markers to distinguish *in vitro*-derived MKs with enhanced immune functions from those biased toward thrombopoiesis; their objective was to select MKs with a high potential for platelet generation and to uncover the active factors contributing to the production of immune MKs.

The identification of immune-related MKs spans developmental stages and encompasses distinct properties [133]. During embryonic development, immune MKs exhibited enriched gene expression signatures associated with phagocytosis, antigen processing and presentation, and the expression of macrophage-specific gene C1QC. Fetal "immune MKs" were further characterized by the presence of the specific marker CD14, which is a soluble component of TLR4, playing a pivotal role in the activation of innate immune cells [134, 135]. This implies the involvement of fetal "immune MKs" in mediating the innate immune response [27]. By contrast, adult "immune MKs" displayed a mature and diverse immune profile [136], participating in innate and adaptive immunity, including pathogen recognition, phagocytosis, cell-mediated killing, antigen presentation, and neutrophil recruitment [15, 16]. In a related study, Wang *et al.* [16] explored the heterogeneity of adult MKs, focusing on the immune-modulatory role of "immune MKs"; utilizing a

mouse model afflicted by infections, they uncovered a specific subset of "CXCR4^{high} immune-MKs" that displayed an enhanced capacity for migration and played a pivotal role in the trafficking of leukocytes and phagocytosis of bacteria [17]. In contrast to surface markers, such as CD53 and CD48, which are specifically expressed on the "immune MK" subpopulation but devoid of explicit functional directions, CXCR4 is expressed in other MK subpopulations. Notably, the heightened expression of CXCR4 (CXCR4^{high}) indicates the pivotal chemotactic and recruitment capacities inherent in "immune MKs". Further investigation is warranted to elucidate the intricate relationships among these surface markers.

Remarkably, "immune MKs" predominantly manifest as low-ploidy MKs characterized by their smaller size [16]. This observation aligns with *in vivo* imaging studies, which revealed that lung-resident MKs exhibit higher expression levels of immune receptors and smaller diameters than intravascular MKs [23]. Furthermore, "immune MKs" have been identified in various tissues, displaying tissue-dependent characteristics. Comparative analysis of single-cell RNA data from bone marrow, fetal lung, and adult lung MKs, unveiled distinctive functional profiles, and bone marrow "immune MKs" are primarily involved in antigen presentation, fetal lung "immune MKs" display higher phagocytosis expression than the other MK types, and adult lung "immune MKs" exhibit strong expression in both antigen presentation and phagocytosis [16]. These findings underscore the adaptability and plasticity inherent to "immune MKs" in response to varying environmental conditions and interactions with pathogens [137].

In conclusion, the presence of the "immune MK" subpopulation exhibits a relatively conserved nature across different species (humans and mice), developmental stages (fetal and adult), and tissues (bone marrow and lung; Fig. 4).

Future directions for "immune MK"

The discovery of "immune MK" has opened up new avenues for understanding the role of MKs in various infectious diseases [138]. Interestingly, robust immune properties have been observed in platelets [12, 32, 139, 140]. However, whether a subpopulation of "immune platelets" exists or if the observed immune function is a general property of all platelets remains unclear. Understanding the immune property of platelets in the context of the recently discovered cellular heterogeneity of MK is essential. Do "immune platelets" originate from "platelet-generating MKs," which acquire immune functions after detachment from the mother MKs [134,141–143]? Alternatively, could they be generated by "immune MKs" under specific conditions (such as inflammation or pathology) and do they have inherited immune characteristics? Future exploration is warranted for these questions. Moreover, extensive studies are needed to unravel the functional roles of "immune MKs and platelets", in coordination with conventional immune cells under diverse physiologic and pathological conditions.

The developmental origin of "immune MKs" is another important question need to be addressed. Two traditional views of development paths for MKs have been proposed. One posits a stepwise path, starting with HSCs and progressing through multiple potential intermediate progenitors to ultimately yield MkPs. An alternative route

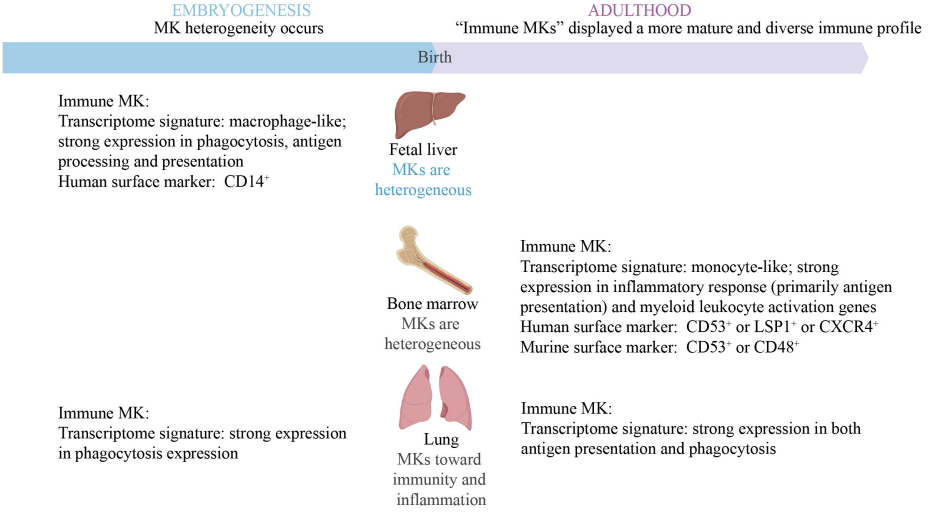

Fig. 4 Identification of the "immune MK" subpopulation in different species (mice and humans), different stages (embryogenesis and adulthood), and different tissues (fetal liver, bone marrow, and lung). LSP1, lymphocyte-specific protein 1; CXCR4, C-X-C chemokine receptor type 4. (Created with Biorender.com.)

involves a subset of MK-biased HSCs that directly differentiate into MkPs, bypassing the intermediate progenitors [25, 144–149]. Given the resemblance of the transcriptional program between "immune MKs" and monocytes, it is conceivable that "immune MKs" originate from a population of stem or progenitor cells exhibiting the characteristics of both megakaryocytic and monocytic lineages. "Immune MKs" may originate from a subset of "MK-biased HSCs" with monocytic potential, following a direct developmental route. Alternatively, they may derive from a progenitor common to both MKs and monocytes within a stepwise path [150]. A comprehensive understanding of the origins of "immune MKs" and the underlying mechanisms underlying developmental pathways during hematopoiesis necessitates further investigation.

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Compliance with ethics guidelines

Conflicts of interest Yueying Li, Kunying Chen, and Qian-Fei Wang declare that they have no conflict of interest.

This manuscript is a review article and does not involve a research protocol requiring approval by a relevant institutional review board or ethics committee.

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