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# Monocyte-endothelial cell interactions in vascular and tissue remodeling

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Monocytes are circulating leukocytes of innate immunity derived from the bone marrow that interact with endothelial cells under physiological or pathophysiological conditions to orchestrate inflammation, angiogenesis, or tissue remodeling. Monocytes are attracted by chemokines and specific receptors to precise areas in vessels or tissues and transdifferentiate into macrophages with tissue damage or infection. Adherent monocytes and infiltrated monocyte-derived macrophages locally release a myriad of cytokines, vasoactive agents, matrix metalloproteinases, and growth factors to induce vascular and tissue remodeling or for propagation of inflammatory responses. Infiltrated macrophages cooperate with tissue-resident macrophages during all the phases of tissue injury, repair, and regeneration. Substances released by infiltrated and resident macrophages serve not only to coordinate vessel and tissue growth but cellular interactions as well by attracting more circulating monocytes (e.g. MCP-1) and stimulating nearby endothelial cells (e.g. TNF- $\alpha$ ) to expose monocyte adhesion molecules. Prolonged tissue accumulation and activation of infiltrated monocytes may result in alterations in extracellular matrix turnover, tissue functions, and vascular leakage. In this review, we highlight the link between interactions of infiltrating monocytes and endothelial cells to regulate vascular and tissue remodeling with a special focus on how these interactions contribute to pathophysiological conditions such as cardiovascular and chronic liver diseases.

## KEYWORDS

monocyte, macrophage, endothelial cell, angiogenesis, tumor, cardiovascular diseases, liver diseases.

## 1 Introduction

Monocytes and monocyte-derived macrophages (MDM) are plastic cells from the innate immune system that exhibit essential and distinct roles in homeostasis, immune response, inflammation and tissue repair (1). They contribute to a wide spectrum of diseases and are therefore attractive therapeutic targets (2–4). Potential therapeutic interventions involving monocyte or MDM modulation require an in-depth understanding of the mechanisms that govern their ontogeny, tissue infiltration, activation, and phenotype adaptation to the microenvironment. Monocytes can transdifferentiate to macrophages under extreme circumstances but generally do not significantly contribute to the majority of tissue macrophage populations in physiological conditions or in some particular inflammatory disorders (1). Most tissue macrophages are derived from embryonic precursors, established before birth, and maintained by self-renewal in adults (5, 6). Monocytes comprise ~4% of mice and 10% of humans blood nucleated cells with substantial reservoirs in the spleen and lungs that can be rapidly recruited to damaged tissues (7, 8). Circulating monocytes display a characteristic short half-life of around 20 hours (6), which is prolonged when they do transdifferentiate into macrophages to assist in establishing tissue-resident mononuclear phagocyte population (9). Indeed, monocytes appear as short-lived plastic cells meant to protect against pathogens or to harmonize vascular and tissue remodeling upon recruitment driven by chemokines and monocyte adhesion molecules (10). Therefore, monocytes are dynamic cellular components that can complete the functions of tissue-resident mononuclear phagocytes on demand. The greatest number of tissue-resident macrophages are configured as hepatic Kupffer cells (KCs). KCs are the most abundant tissue macrophages in mammals, representing the 80–90% of total tissue macrophages (11). In physiological conditions, there is only a small number of MDM in the hepatic portal space (12). Mouse MDM can be distinguished from KCs by their differential expression on cell surface markers such as CD11b, F4/80, Ly6C, and macrophage colony-stimulating factor 1 receptor (CSF1R) (13). Human MDM are typically identified as CD14<sup>+</sup>, CC-chemokine receptor 2 (CCR2)<sup>+</sup> cells (13). The liver is the first line defense against foreign molecules in particular those ingested. It is a dynamic filter and the core of body metabolism with a unique capacity among organs to regenerate to a physiological size even after two-thirds of its mass has been removed. This effect appreciated even in Greek mythology is the hallmark of health and as with Tityus and Prometheus regeneration occurs with partial hepatectomy. Excessive injury or significant disease can hamper such repair. Here, we discuss how monocyte-endothelial cell interactions and MDM participate in the regulation of angiogenesis and regeneration in liver diseases.

Injured cells release specific signals identified as damage-associated molecular patterns (DAMPs) that activate the immune system similarly to pathogen-associated molecular patterns (PAMPs), small molecular motifs released from bacteria or viruses (14). These endogenous molecules (calcium-binding proteins, structural and extracellular matrix (ECM) proteins, etc.)

exhibit a wide array of cellular functions in homeostasis and as injury signals in a complex integrated network checking and balancing tissue repair and further damage. Tissue-specific macrophage subpopulations sense these signals and trigger endothelial cells (ECs), monocytes, and other immune cells to contain injury and initiate an immune response. Infiltrated MDM generally display a pro-inflammatory phenotype (M1-like) involving secretion of cytokines such as interleukin 1 (IL-1) and tumor necrosis factor (TNF- $\alpha$ ) for inflammation propagation, and IL-12 for T helper 1 (TH1) lymphocyte activation and induction of the adaptive immune response (15). M1-like macrophages also release reactive oxygen and nitrogen species aimed at elimination of possible biological aggressors. However, all these cocktails of cytokines and free radicals also produce substantial collateral tissue damage to the host during the reaction against the insult. To prevent harmful effects to the tissue, regulatory mechanisms activate and promote macrophage apoptosis or polarization to a M2-like anti-inflammatory and pro-regenerative phenotype that facilitates wound healing (16). Indeed, damaged epithelial cells release alarmins, which induce IL-4 and IL-13 secretion by T-helper lymphocytes and other immune cells. Both IL-4 and IL-13 are major regulators of macrophage polarization to an anti-inflammatory M2-like phenotype (15). M2-like macrophages release vascular or fibroproliferative growth factors such as vascular endothelial growth factors (VEGFs) or transforming growth factor (TGF $\beta$ 1), respectively (17). VEGF-A, for example, stimulates angiogenesis and vascular leakage (18). TGF $\beta$ 1 induces fibroblast differentiation to myofibroblasts or phenomena of epithelial or endothelial to mesenchymal transitions (19, 20) that promote the synthesis of ECM components or tissue inhibitors of metalloproteinases (TIMP). The balance of M1/M2 profiles on MDM is crucial to understand prognostic in chronic diseases and especially in the context of liver cirrhosis (21) or in atherosclerosis and cardiovascular disease (22). In cardiovascular diseases, M1-like macrophages characterize progression lesions while regressing plaques are enriched in M2 macrophages (22). However, macrophage heterogeneity in atherosclerotic plaques may have only a partial semblance to M1-like and M2-like macrophage phenotypes. Indeed, it is yet necessary to identify gene-expression profiles and transcriptional pathways that underlie the distinctiveness and diversity of MDM in cardiovascular diseases.

## 2 Monocyte-endothelial cell interactions: molecular pathways

Monocytes interact with ECs under physiological or pathophysiological conditions to orchestrate inflammation, angiogenesis, or tissue remodeling (23). Indeed, the migration of monocytes from the circulation to peripheral organs during an inflammatory response depends on their interaction with ECs. These interactions are orchestrated and controlled by chemoattractant and adhesion molecules that allow monocyte trafficking (24). The initial rolling of monocytes along the activated endothelium results in a firm adhesion that eventually

culminates with their transmigration at inflammation sites where they transdifferentiate into macrophages or dendritic cells (23).

## 2.1 Monocyte-attracting chemokines

Circulating monocytes have been classified into different subsets based on the chemokine receptors they express and the presence of specific surface molecules (25). In humans, monocytes are classified according to the presence of CD14 and CD16 on their surface (26, 27). CD14<sup>+</sup>CD16<sup>-</sup> are known as classical monocytes, which are the most abundant in the bloodstream. Alternatively, CD14<sup>+</sup>CD16<sup>+</sup> are referred as intermediate monocytes and, CD14<sup>+</sup>CD16<sup>++</sup>, as non-classical patrolling monocytes. However, in mice, only two subsets have been identified (28). One of these populations corresponds to CD14<sup>+</sup> CD62 ligand (CD62L)<sup>+</sup> CC-chemokine receptor 2 (CCR2)<sup>+</sup>, which is known as LY6C<sup>hi</sup> or inflammatory monocytes (23, 29). The second population is similar to CD16<sup>+</sup>CCR2<sup>-</sup> monocytes in humans, and it is known as LY6C<sup>low</sup> or patrolling monocytes, that express high levels of CX3C-chemokine receptor 1 (CX3CR1) and low levels of CCR2 and LY6C (24).

Recruitment of LY6C<sup>hi</sup> monocytes from the bone marrow is mediated by the binding of CC-chemokine ligand 2 (CCL2) (30), also known as monocyte chemoattractant protein-1 (MCP-1), and CCL7 (or MCP-3) to CCR2 (31, 32). Most cells express CCL2 in response to pro-inflammatory cytokines in infections (33). Thus, after many infections, circulating levels of CCL2 increase in both the serum and inflamed tissues, where CCL2 binds to CCR2 that is expressed on certain cell types (23). CCL7 expression is also

stimulated by infections and contributes to LY6C<sup>hi</sup> monocyte recruitment (23). Both CCL2 and CCL7 have shown important roles on monocyte recruitment, although the mechanism of action is still unclear (31, 32). Conversely, recruitment and survival of LY6C<sup>low</sup> monocytes is mediated by the binding of CX3C-chemokine ligand 1 (CX3CL1), also known as fractalkine (FKN), to CX3CR1 (34).

Monocytes also express other CC-chemokine receptors, such as CCR1 and CCR5 (35) that bind to various cytokines including CCL3 (also known as MIP1 $\alpha$ ) and CCL5 (also denominated RANTES) (29). Both receptors display specialized roles in monocyte recruitment. CCR1 mediates monocyte arrest in fluid shear stress generated by blood flow. CCR5 is involved in monocyte spreading. They both contribute to transendothelial chemotaxis towards CCL5 gradients (36). However, none of these receptors have shown redundancy in cell recruitment during inflammation, which has implications in the development and progression of many diseases, including atherosclerosis and rheumatoid arthritis (29, 37, 38). This may be a consequence of the wide spectrum of cells expressing these chemokine receptors (39), so determining the specific role they play in monocyte recruitment is complex (23).

Other chemokines have also been suggested to play a role on monocyte recruitment (Table 1). Some examples are CCR6 (42), CCR7 (43), CCR8 (44), and CXC-chemokine receptor 2 (CXCR2) (45). Circulating monocytes express low levels of CCR6 and do not respond to CCL20, which explains that CCR6 does not display a significant role on the extravasation of monocytes from the circulation to the tissues, but it does on the migration or function of monocytes in inflammation (46, 47).

TABLE 1 Chemokines and chemokine receptors involving monocytes and endothelial cells.

Receptor	Chemokine ligands	Receptor expressed on	Ligands mostly expressed/secreted by	Reference
CCR1	CCL3 (MIP-1 $\alpha$ ), CCL5 (RANTES), CCL7 (MCP-3), CCL14 (HCC1)	Monocytes, T cells, dendritic cells	Monocytes macrophages, endothelial cells, T cells, dendritic cells, neutrophils, epithelial cells, fibroblasts	(40, 41)
CCR2	CCL2 (MCP-1), CCL8 (MCP-2), CCL7 (MCP-3), CCL13 (MCP-4), CCL16 (HCC4)	Monocytes, endothelial cells, dendritic cells	Monocytes, macrophages, endothelial cells, T cells, epithelial cells, fibroblasts	(40, 41)
CCR5	CCL3 (MIP-1 $\alpha$ ), CCL4 (MIP-1 $\beta$ ), CCL5 (RANTES), CCL11 (eotaxin), CCL14 (HCC1), CCL16 (HCC4)	Monocytes, macrophages, endothelial cells, T cells, dendritic cells	Monocytes, macrophages, endothelial cells, T cells, epithelial cells, leukocytes	(40, 41)
CCR8	CCL1 (I309)	Monocytes, T cells, dendritic cells	Monocytes, T cells, mast cells	(40, 41)
CXCR1	CXCL8 (IL-8), CXCL6 (GCP2)	Monocytes, neutrophils	Macrophages, endothelial cells, T cells, epithelial cells, fibroblasts	(40, 41)
CXCR2	CXCL8 (IL-8), CXCL1 (GRO $\alpha$ ), CXCL2 (GRO $\beta$ ), CXCL3 (GRO $\gamma$ ), CXCL5 (ENA-78), CXCL6 (GcP2)	Monocytes, microvascular endothelial cells, neutrophils	Monocytes, macrophages, endothelial cells, T cells, epithelial cells, fibroblasts, mast cells	(40, 41)
CXCR3-B	CXCL4 (PF4), CXCL9 (MIG), CXCL10 (IP-10), CXCL11 (I-TAC)	Microvascular endothelial cells, T cells, natural killer cells	Monocytes, endothelial cells, T cells, neutrophils, epithelial cells, fibroblasts, cancer cells	(40, 41)
CX3CR1	CX3CL1 (fractalkine)	Monocytes, macrophages, T cells, dendritic cells, smooth-muscle cells, natural killer cells	Endothelial cells, T cells	(40, 41)

## 2.2 Monocyte-attracting adhesion molecules

Monocyte migration to inflammatory sites is a multistep process involving many molecules (Table 2). The initial tethering and rolling of monocytes along the inflamed endothelium are mediated by selectins. Selectins are cell-surface proteins that interact with glycoprotein ligands to allow monocytes to bind weakly and reversibly to cytokine-activated ECs (24, 62). The selectins involved in this process are L-selectin (CD62L), P-selectin (CD62P) and E-selectin (CD62E). L-selectin, expressed on circulating monocytes, interacts with specific fucosylated sialoglycoproteins expressed on lymph node venules and inflamed or injured vascular endothelium (48, 49, 63). P-selectin glycoprotein ligand-1 (PSGL-1), P-selectin glycoprotein ligand-1 (PSGL-1), expressed on monocytes, interacts with P-selectin and E-selectin expressed on inflamed endothelium (50). Then, PSGL-1 can also interact with circulating monocytes expressing L-selectin and amplify the monocyte recruitment (52). This initial adhesion mediated by selectins reduces the rolling velocity of monocytes and allows the cells to interact with chemokines that are bound to inflamed ECs (64). ECs are activated by inflammatory cytokines such as TNF- $\alpha$  or IL-1 $\beta$ . This activation induces the expression of adhesion molecules such as E- and P-selectin, intercellular adhesion molecule 1 (ICAM1/CD54) and vascular cell-adhesion molecule 1 (VCAM1/CD106) that participate in monocyte migration (65).

Chemokines can bind to transmembrane heparan sulphate proteoglycans on the luminal surface of vascular ECs to be

presented to monocytes (66). The main proteoglycans identified as chemokine-binders include CD44, syndecan 1 and syndecan 4 (which bind CCL5) and syndecan 2 (which interacts with CXCL8) (66). Chemokines bind to G-protein-coupled receptors (GPCRs) of monocytes and induce inside-out signals that result in integrin activation. GPCRs activate specific Gi and Gq heterotrimeric proteins and their downstream effectors. Two key guanosine triphosphatases (GTPases), RhoA and Rap1, have been implicated in chemokine activation of integrins (67, 68). Rap1 is a small GTPase of the RAS family that cycles between an inactive GDP-bound form and an active GTP-bound form (54). Activated Rap1 binds RAPL, and the complex activates the integrin by binding to the alpha-chain of integrin (69). Rap1 also binds to Rap-interacting adapter molecule (RIAM), which recruits talin (70). Talin is a cytoskeletal protein that binds to the beta-chain of the integrins, thereby triggering integrin activation (71, 72). Integrin activation induces conformational changes initiated at the alpha and beta subunit cytoplasmic tails and transmitted to their extracellular domain (73). This inside-out signaling activates integrins such as lymphocyte function-associated antigen 1 (LFA1; also known as  $\alpha$ L $\beta$ 2-integrin and CD11a-CD18) and very late antigen 4 (VLA4; also known as  $\alpha$ 4 $\beta$ 1-integrin, and CD49d-CD29). Activated  $\beta$ 2 and  $\alpha$ 4-integrins ensure the arrest of monocytes and the formation of firm adhesions by binding ICAM1 and VCAM1 respectively, which are expressed by inflamed endothelial cells (54) [Table 2]. Other integrins such as the macrophage receptor 1 (Mac1; also known as  $\alpha$ M $\beta$ 2-integrin and CD11b-CD18) and  $\alpha$ X $\beta$ 2-integrin are also activated *via* this signaling pathway (56).

TABLE 2 Monocyte adhesion molecules involved in transendothelial migration.

Adhesion molecule	Ligands	Ligand expressed on	Function	Reference
L-selectin (CD62L)	Fucosylated sialoglycoproteins	Endothelial cell	Rolling	(48, 49)
PSGL-1	P-selectin (CD62P) E-selectin (CD62E)	Endothelial cell	Rolling	(50, 51)
PSGL-1	L-selectin	Monocyte	Monocyte recruitment	(52, 53)
LFA1 ( $\alpha$ L $\beta$ 2-integrin or CD11a-CD18)	ICAM1/CD54	Endothelial cell	Arrest	(24, 54, 55)
VLA4 ( $\alpha$ 4 $\beta$ 1-integrin or CD49d-CD29)	VCAM1/CD106	Endothelial cell	Arrest	(24, 54, 55)
Mac1 ( $\alpha$ M $\beta$ 2-integrin or CD11b-CD18)	ICAM1/CD54 among others	Endothelial cell	Arrest	(56)
LFA1 ( $\alpha$ L $\beta$ 2-integrin or CD11a-CD18)	JAM-A	Endothelial cell	Transmigration	(57)
VLA4 ( $\alpha$ 4 $\beta$ 1-integrin or CD49d-CD29)	JAM-B	Endothelial cell	Transmigration	(58)
Mac1 ( $\alpha$ M $\beta$ 2-integrin or CD11b-CD18)	JAM-C	Endothelial cell	Transmigration	(59)
$\alpha$ X $\beta$ 2-integrin (CD11c-CD18)	JAM-C	Endothelial cell	Transmigration	(59)
PECAM1 (CD31)	PECAM1	Endothelial cell	Transmigration	(60)
CD99	CD99	Endothelial cell	Transmigration	(61)



## 2.3 Monocyte transmigration

The firm adhesion of a monocyte to the vascular endothelium results in a morphological and phenotypical change known as polarization (Figure 1) (74). Polarization involves the formation of two different regions: the lamellipodia at the leading edge and the uropod at the tail of the monocyte (74). Polarization involves reorganization of the cytoskeletal proteins, intracellular regulatory molecules, chemoattractant receptors and integrins (75). F-actin changes from radially symmetric around the cell to accumulated in the leading edge (76). Chemokine receptors redistribute to the leading edge, while other adhesion molecules, such as CD44, accumulate at the uropod (75). In addition, high affinity integrins mobilize to the leading edge and low affinity integrins to the uropod (54). Attachment of integrins at the leading edge and detachment at the uropod occurs during migration (77). Several signaling pathways contribute to polarization including Rho family GTPases, GTPase Rap1, protein kinases, and lipid kinases (75). GTPase Rap1 has also been described as a key molecule in integrin activation and redistribution during leukocyte polarization (54). RAP1 and RAPL control the polarized recruitment of integrin clusters to the lamellipodium (69). RHOA, another member of the RAS superfamily, also participates in integrin clustering by activating RHO-associated coiled-coil containing protein kinase 1

(ROCK1), which phosphorylates the actin cytoskeleton (24). GPCR downstream signals also activate PI3K, which participates in monocyte polarization *via* activation of the atypical protein kinase C- $\zeta$  (PKC- $\zeta$ ) and formation of the polarity complex (consisting of partitioning defective 6 (PAR6)/PKC- $\zeta$ /lethal giant larvae, LGL). PKC- $\zeta$  signaling facilitates integrin lateral mobility (after integrin is in its high affinity form) due to the mobilization of new lipid membrane to the leading edge (78). The polarized recruitment of integrins clusters to the lamellipodium results in polarized adhesion and then migration (24). After polarization, monocytes migrate to the interendothelial junctions.

Adjacent ECs are connected by a wide array of endothelial junctions: tight junctions, adherens junctions and gap junctions (79). Tight and adherens junctional transmembrane proteins mediate cell adhesion by homophilic interactions and form a zipper-like structure along the cell border. This adhesion is reorganized during monocyte transendothelial migration (79). Indeed, tight and adherens junctional proteins play a critical role in this process (Table 2) (24). Tight junctions are localized at the apical site of the interendothelial junctions and form a close contact between adjacent ECs. Tight junctions are composed of occludins, claudins and junctional adhesion molecules (JAMs), but only JAMs have been described to participate directly in the transendothelial migration of monocytes (80). Three members of the JAM family

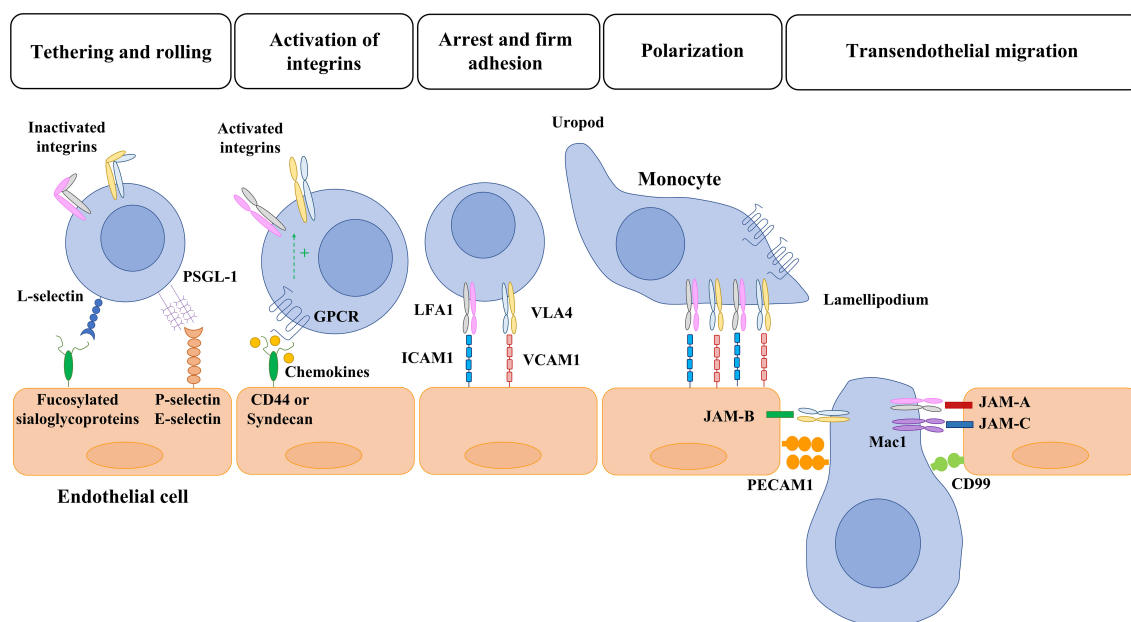


FIGURE 1

Overview of monocyte-endothelial cell interaction and transmigration. Monocyte migration to inflammatory sites is a multistep process with many molecules involved. First, selectins mediate the initial tethering and rolling of monocytes along the cytokine-activated endothelial cells. Then, the monocyte interacts with chemokines that are bound to transmembrane heparan sulphate proteoglycans (CD44 and syndecan) expressed in the endothelium. The activation of GPCR leads to the activation of integrins and the consequent monocyte arrest mediated by the interaction of LFA1 and VLA4 with ICAM1 and VCAM, respectively. Once a monocyte establishes firm adhesion to the vascular endothelium, it undergoes a morphological change known as polarization, in which chemokine receptors and activated integrins redistribute to the leading edge. After polarization, monocytes migrate toward interendothelial junctions and then transmigrate into the underlying tissues. The members of the JAM family expressed by endothelial cells (JAM-A, JAM-B, JAM-C) interact with the activated integrins of monocytes (LFA1, VLA4, Mac1) and allow the transmigration through tight junctions. Lastly, PECAM-1 (CD31) and CD99 hemophilic engagement and endothelial retraction lead to monocyte extravasation. PSGL: P-selectin glycoprotein ligand-1; GPCR: G-protein-coupled receptors; LFA1: lymphocyte function-associated antigen 1; VLA4: very late antigen 4; ICAM1: intercellular adhesion molecule 1 (ICAM1/CD54); VCAM: vascular cell-adhesion molecule 1; Mac1: macrophage receptor 1; PECAM: platelet/endothelial cell-adhesion molecule 1.

have been described: JAM-A, JAM-B, and JAM-C. JAMs from ECs bind to monocyte integrins. LFA1 has been identified as a ligand for JAM-A (57). JAM-B has been described to bind to  $\alpha 4\beta 1$ -integrin and JAM-C to  $\alpha M\beta 2$ -integrin (also known as MAC1 and CD11b-CD18) and  $\alpha X\beta 2$ -integrin (also known as CD11c-CD18) (58, 59). Moreover, JAM-C is specifically required to prevent reverse transmigration of monocytes back to the vascular lumen (81). The interaction between the members of the JAM family and the integrins expressed by monocytes allows the transmigration through tight junctions (Figure 1). Then, monocytes transmigrate through the adherens junctions. The main component of adherens junctions is the vascular endothelial cadherin (VE-Cadherin), an endothelial-specific protein anchored to the cytoskeleton and responsible for endothelial tightness against leakage (24). VE-cadherin plays an important role on the control of vascular permeability and integrity but does not interact with monocyte proteins to facilitate transmigration. In contrast, molecules present in adherens junctions such as platelet/endothelial cell-adhesion molecule 1 (PECAM1) and CD99 participate in this process by homophilic interactions (24, 60, 61) (Figure 1). VE-cadherin is crucial to regulate endothelial permeability because it participates in adherens junctions dismantling of and EC retraction, which is required to complete the transmigration of monocytes (60). The clustering of selectins and/or VCAM/ICAM induces an activation of RhoA and an increase in intracellular free calcium within the endothelial cells (82). This results in the activation of the calmodulin-dependent enzyme myosin light chain kinase (MLCK), thereby causing a conformational change in myosin II (83) and the phosphorylation of the VE-cadherin cytoplasmic tail (82). ICAM-1 engagement also results in the activation of Src and PYK2 kinases, which also phosphorylate VE-Cadherin (84). The phosphorylation on Tyr658 and Tyr731 induce the dissociation of VE-cadherin from the cytoskeleton and allow the splitting of EC junctions (82). These changes lead to the increase in vascular permeability and allow monocyte extravasation (60). Monocytes mostly transmigrate through junctions between adjacent ECs (paracellular transmigration) although monocytes can also migrate through ECs (transcellular transmigration) by the formation of vesiculo-vacuolar organelles (85).

The reorganization of interendothelial junctions during inflammation is temporally and spatially regulated by inflammatory mediators and leukocyte transendothelial migration (86). These changes are reversible, and endothelium quiescence and vascular permeability are restored once the triggering cause is removed. However, in some pathological conditions such as chronic inflammation and atherosclerosis, the ECs remain activated and the interendothelial junctions become instable (87). This instability causes an impairment in the endothelial barrier function leading to uncontrolled leukocyte migration and vascular leakage. Defects in the organization of endothelial cell junctions can lead to vascular malformations, vascular fragility and rupture, and appearance of hemorrhages and edema (88). Loss of barrier integrity is a common feature in several vascular disorders including anaphylaxis, diabetic microangiopathy, angioedema, or cancer and metastasis (85). Endothelial junctions not only mediate adhesion but also trigger intracellular signals that communicate cell

position, limit growth and apoptosis. They are essential to maintain vascular integrity and homeostasis (88). Furthermore, the reorganization of interendothelial junctions and loosening of cell-cell adhesion is also required for other physiological processes such as angiogenesis (85).

### 3 Monocyte-endothelial cell interactions in angiogenesis and vasculogenesis

Angiogenesis is the process of formation of new endothelium-lined channels from pre-existing blood vessels (89). Vasculogenesis refers to the creation of new blood vessels, mainly in the embryo, involving differentiation of angioblasts or endothelial progenitor cells (90). In adults, blood vessel formation may be defined by either angiogenesis or arteriogenesis depending on the initial trigger and the final vessel structure. Whereas angiogenesis is usually induced by hypoxia, arteriogenesis is induced by physical forces and, among them, primarily by increased fluid shear stress. Arteriogenesis entails the remodeling of pre-existing arterio-arteriolar anastomoses *via* recruitment of smooth muscle cells to fully established and functional arteries (89). In contrast, angiogenesis (capillary sprouting) results in higher capillary density but without the presence of vascular smooth muscle cells. Increasing evidence implicates blood monocytes in the selection of vascular sprouting points and assistance in vascular bridging (90, 91). Indeed, monocytes and MDM release a combination of chemokines, growth factors, and proteases that may simultaneously participate in immune cell attraction, basement membrane degradation, and endothelial proliferation (92, 93).

#### 3.1 Monocyte-endothelial cell interactions in angiogenesis

Vascular sprouting or angiogenesis is one, but not the only, process of blood vessel formation in the adult. Ordered angiogenesis has long been considered a critical mechanism for optimal wound healing. Little is known about the molecular basis by which leading ECs at vascular sprouts (endothelial “tip” cells) are designated to grow and elongate to form new blood vessels. Some investigations show that direct interactions of monocytes with ECs may be a driving force to stimulate endothelial proliferation (93, 94) and to mediate the fusion of endothelial tip cells (95). Indeed, some investigators have demonstrated that circulating monocytes are selectively recruited to certain regions of regenerating livers after hepatectomy (Figure 2A), particularly surrounding sprouting points (91) (Figure 2B).

Monocyte recruitment initiates in portal areas and expands to the rest of hepatic tissue in correlation with vasodilation and the expression of the inducible form of nitric oxide synthase (iNOS). iNOS is upregulated in injury and induces the synthesis of the vasodilator and proangiogenic substance NO (96). Indeed, angiogenesis initiates with vasodilation, a process involving NO

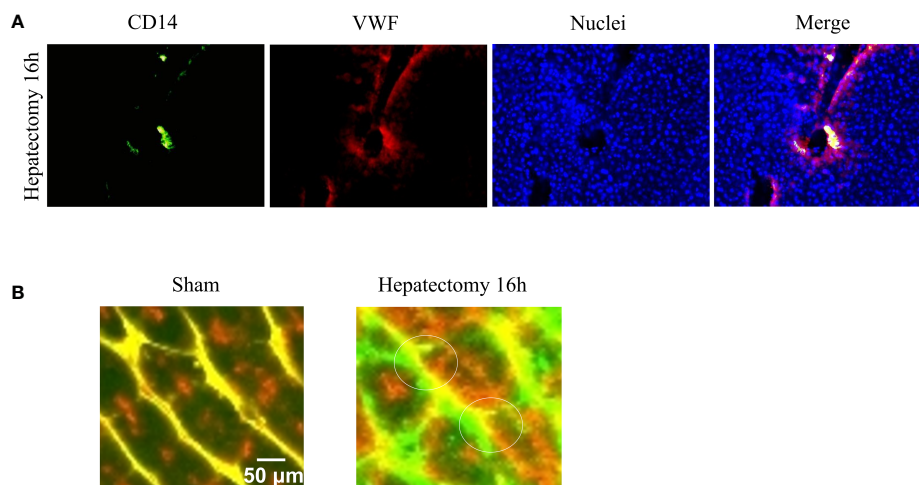


FIGURE 2

Monocyte-endothelial cell interactions and vascular sprouting occurring after hepatectomy. **(A)** Images of vessels from liver sections identified by staining for von Willebrand factor (VWF, in red) and recruited monocytes by staining for CD14 (in green). Nuclei were stained by DAPI (in blue). Initial contacts of recruited monocytes (in yellow) take place in portal areas. **(B)** Amplification of multiphoton images (vessels in green and yellow, macrophages in red), visualized vascular buds (white circles) surrounded by spread recruited macrophages 16 hours after hepatectomy. Images reprinted with permission from “Melgar-Lesmes, P.; et al. Monocyte-endothelial cell interactions in the regulation of vascular sprouting and liver regeneration in mouse. *J Hepatol.* 2015; 63 4):917-25. doi: 10.1016/j.jhep.2015.05.011”.

(89). Then, vascular permeability increases in response to proangiogenic factors such as VEGF, plasma proteins extravasate and pave the ground with a provisional path for the migration of ECs. In this scenario, vascular permeability is affected by the formation of fenestrations, vesiculo-vacuolar organelles and the relocation of PECAM-1 and VE-cadherin, which implicates Src kinases (97, 98). Although angiogenesis requires increased permeability, vascular leakage, and release of permeability factors such as VEGF, it needs to be finely regulated to avoid water and sodium imbalance, circulatory collapse, intracranial hypertension, or ascites (18) among other pathological conditions. Either VEGF or other proangiogenic factors such as angiopoietins may be released by monocytes and MDM to regulate vascular permeability (92, 99). Angiopoietin 1 (Ang-1), a ligand of the endothelial Tie2 receptor, is an endogenous inhibitor of vascular permeability *via* strengthening of endothelial junctions (100). In contrast, angiopoietin 2 (Ang-2) is a partial agonist/antagonist of Tie2, thus promoting vascular leakage (101, 102). Indeed, Ang-2 is involved in the processes of detachment of smooth muscle cells and loosening of the ECM (103). In addition, MDM release different matrix metalloproteinases (MMP) that may activate or liberate a myriad of growth factors (bFGF, VEGF, IGF-1, etc) retained within the extracellular matrix (104, 105).

Substances released by parenchymal cells (damage-associated molecular patterns) and resident macrophages (e.g. TNF- $\alpha$ ) during tissue injury serve not only to coordinate vessel and tissue growth, but also cellular interactions, attracting circulating monocytes (e.g. MCP-1) and inducing neighboring ECs to expose monocyte adhesion molecules such as those described in the section 2.2 of this review (106) (Figure 3).

Circulating monocytes detect attracting signals and interact with ECs to initiate endothelial disruption and monocyte translocation to

local sprouting points. It has been demonstrated that the number of interactions of recruited monocytes with liver vascular network during regeneration is directly associated with phosphorylation and disruption of VE-cadherin connections (91). This uncoupling of inter-EC connectivity mediated by VE-cadherin is critical to the plasticity of the selected endothelial tip cell and for EC migration and elongation (107). MDM also serve as chaperones of endothelial sprouting by locally secreting proliferative factors, such as Wnt5a and Ang-1 and the stalk cell stabilizer Notch1 (Figure 4).

The release of Wnts from infiltrating monocytes and, namely, the contribution of Wnt5a and non-canonical ligands is especially important to harmonize angiogenesis in inflamed vessels (108).. Recruited monocytes are important beyond the priming phase of angiogenesis and throughout the process of tissue regeneration. Indeed, leukocyte adhesion molecules such as P-selectin, CCR2, and VCAM-1 upregulate during tissue regeneration and maintain the number of rolling interactions and the likelihood of recruiting monocytes. Specifically, the adhesion molecule ICAM-1 is upregulated after hepatectomy, but its gene expression is significantly downregulated when monocyte interactions and VE-cadherin phosphorylation are maxima. This points to a precise regulation of this adhesion molecule by monocyte interactions in accordance with the requirements of endothelial sprouting and the subsequent hepatic mass expansion. In fact, ICAM-1 activation is an important signaling pathway to trigger vascular sprouting (109, 110) and arteriogenesis (111). ICAM-1 is then an excellent candidate to understand the role of direct interactions of monocytes with endothelium to regulate vascular and tissue regeneration. Indeed, gene suppression of any of the subunits of the monocyte receptor Mac-1 (CD11b/CD18) disrupts wound healing in mice (112, 113). Moreover, suppression of the CD11b gene drops survival in mice undergoing partial hepatectomy and hinders vascular and liver mass

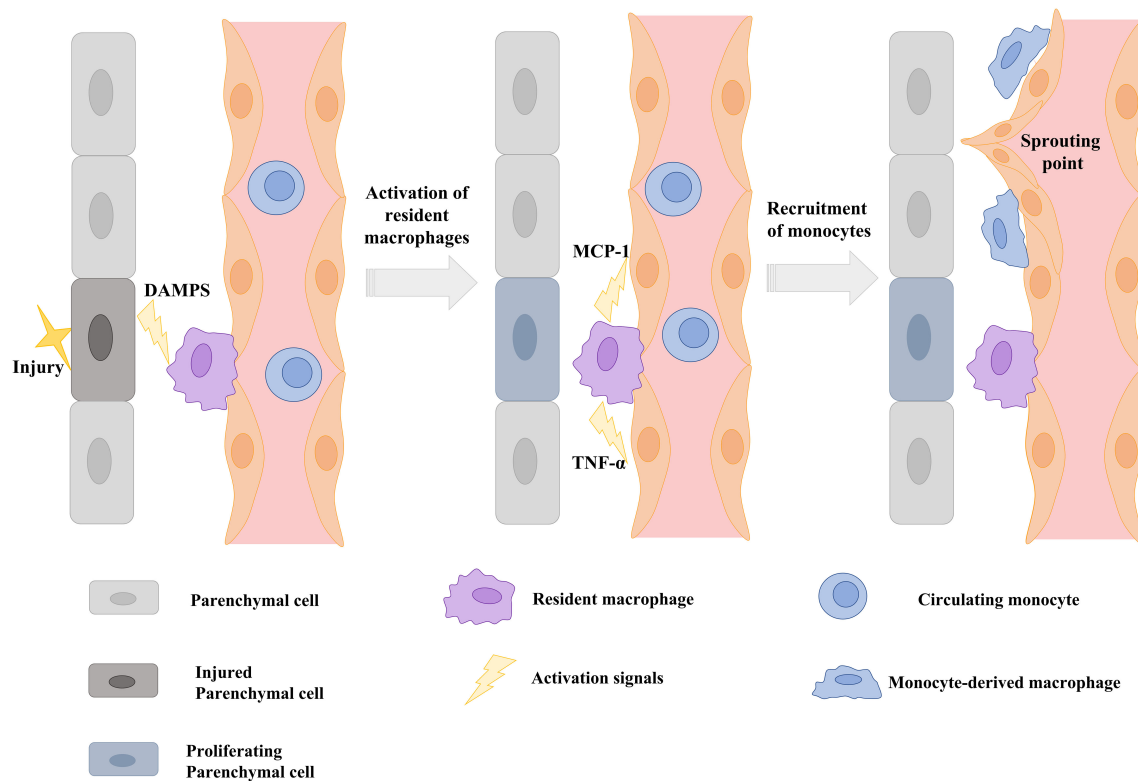


FIGURE 3

Schematic illustration showing that signals from injured parenchymal cells stimulate the release of cytokines and chemokines from resident macrophages and the induction of monocyte adhesion molecules on the endothelium to stimulate vascular sprouting. DAMPS: Danger-associated molecular patterns; TNF- $\alpha$ : tumor necrosis factor alpha; MCP-1: Monocyte chemoattractant protein-1. Scheme modified and adapted from "Melgar-Lesmes, P.; et al. Monocyte-endothelial cell interactions in the regulation of vascular sprouting and liver regeneration in mouse. *J Hepatol.* 2015 Oct;63 (4):917-25. doi: 10.1016/j.jhep.2015.05.011".

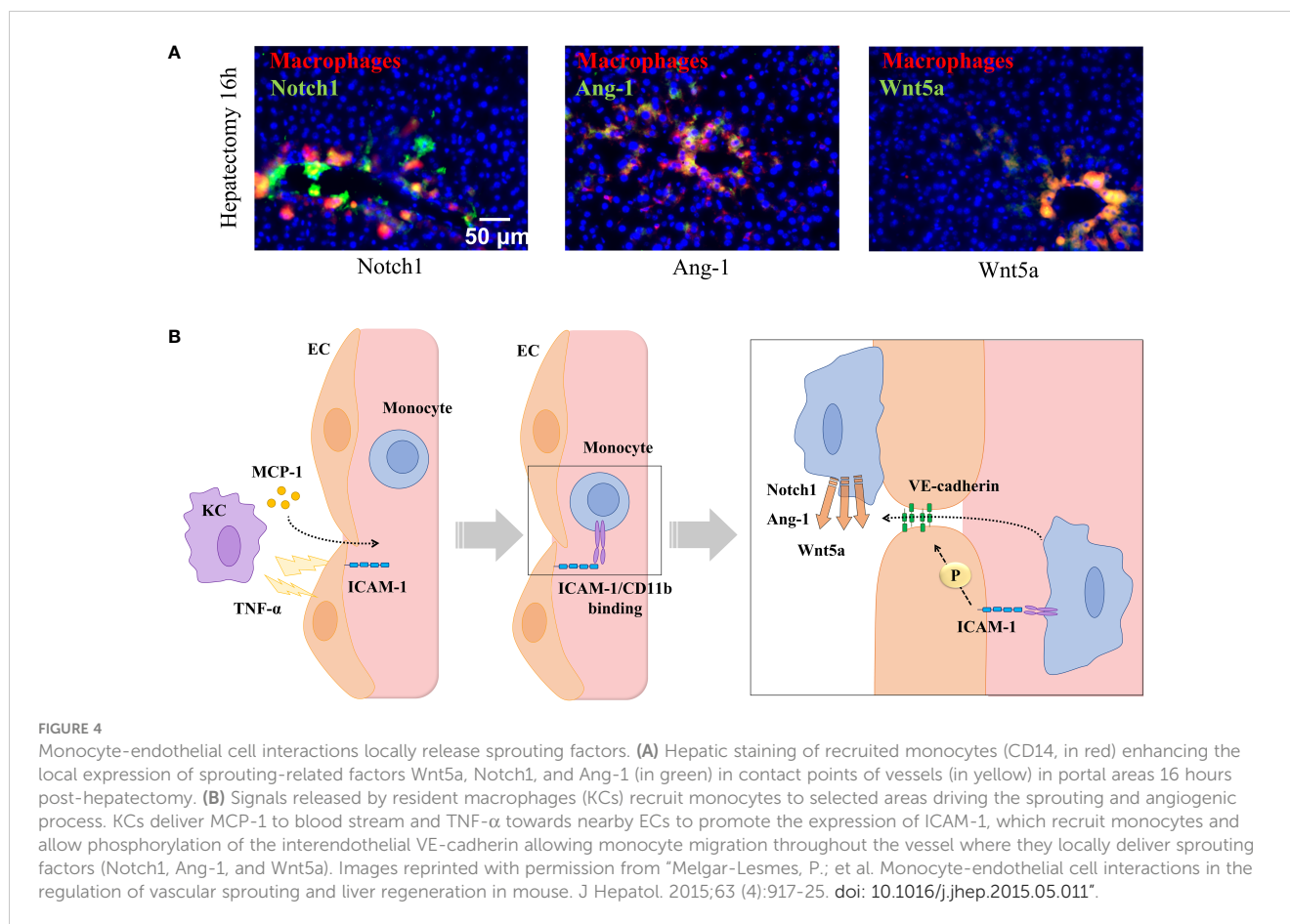
regeneration in line with a reduced infiltration of circulating monocytes into the hepatic vascular network (91). Interestingly, the lack of CD11b also induces TNF- $\alpha$  expression, which can also directly promote VE-cadherin phosphorylation to replace the missing effects of monocyte-endothelial cell interactions (114). However, this increase in TNF- $\alpha$  is followed by an intense vascular leakage and a disorganized and aberrant vascular network after hepatectomy (91). Indeed, exacerbated increase of TNF- $\alpha$  by monocytes or MDM is associated to suppressive effects on wound healing in mice (115). To regulate these vascular disorders, tissue macrophages, such as KC, may progressively move into vessel walls from their static state and location in an attempt to replace the role of monocyte-EC interactions in the control of vascular and tissue growth in ICAM-1 KO mice (91). Thus, macrophage interaction with ICAM-1 in endothelium seems a critical step in the regulation of the endothelium integrity and in the control of TNF- $\alpha$  expression during angiogenesis after hepatectomy.

### 3.2 Monocyte-endothelial cell interactions in vasculogenesis

The molecular basis of vasculogenesis (in the embryo and from endothelial progenitors) diverges from that of pathological

angiogenesis in the adult. The formation of the vascular network in the embryonic phase requires several sequential steps combining both mechanisms, vasculogenesis and angiogenesis. Vasculogenesis gives rise to the heart and the initial embryonic vascular plexus and surrounding membranes comprising the yolk sac circulation (116). Angiogenesis accounts for the expansion and remodeling of this vascular tree *via* endothelial sprouting and intussusceptive microvascular growth. However, little is known of any possible direct implication of monocyte or macrophage-EC interactions to drive vasculogenesis. The fact is that the macrophage is one of the first blood cell lineages to originate during embryonic development. Macrophages generate and grow surrounded by ECs and vasculogenesis occurs in parallel with hematopoiesis (116). Studies in mice have revealed that embryonic macrophages arise during three different waves of hematopoiesis: primitive hematopoiesis, erythro-myeloid progenitor (EMP) generation, and definitive hematopoietic stem cell-mediated hematopoiesis (116). Macrophages are then distributed through most developing organs (117). These initial macrophages derived during primitive hematopoiesis behave as a mediator between neovascularization and organ architecture during fetal organogenesis (118). Further recent findings using inducible *Csf1r* promoter-driven-Cre ROSAYFP reporter mice support a direct relationship between blood and endothelial lineages (119). These authors found that





circulating EMP contribute to ECs in vascular network in multiple tissues (liver, brain, heart, lung, and yolk sac), and their interaction continues throughout adulthood (119). Other authors have found evidence on the active role of macrophages to promote vasculogenesis during retinal neovascularization (120). They have shown that macrophages boost the recruitment and differentiation of bone marrow-derived cells (BMCs). Mechanistically, they found that specifically M2-like macrophages affected the migration and activation of BMCs *via* secretion of VEGF and stromal cell-derived factor-1 (SDF-1). This is consistent with another investigation that demonstrated a higher recruitment of BMCs by SDF-1 and hepatocyte growth factor (HGF), released by the interaction between macrophages and matrix-embedded endothelial cells (MEECs) during liver regeneration (121). Namely, HGF stimulated the expression of the receptors CXCR4 and CXCR7 (122), that promoted the mobilization of endothelial progenitors to the blood stream and their recruitment into injured liver. In turn, incorporated endothelial progenitors secreted more HGF promoting positive feedback and the formation of new blood vessels that irrigated the implants and the ischemic tissue (121).

Vascular development is one of the earliest organogenesis events in embryonic development. A primitive vascular tree provides a basic path for circulating cells and guarantees the supply of nutrients. EC differentiation arises during gastrulation when cells invaginate to form the mesoderm. This process occurs around the embryonic day E7.25 in different clusters of cells in the

extra-embryonic yolk sac, called blood islands (123). Blood islands are disposed of primitive hematopoietic cells in the center and aligned endothelial cells in the periphery (124). Primitive hematopoiesis produces unipotent myeloid progenitors that may uniquely generate the macrophage lineage (124), bipotent progenitors of erythrocytes, and megakaryocytes (125). Then, these progenitors are mobilized to the blood circulation around E8.0 (124). The first embryonic-derived macrophages are detected in the yolk sac at E9.0 (126). Yolk sac-derived macrophages colonize first the developing brain by E9.5 and then the rest of the embryonic tissues by E12.5 (123, 124). Although primitive macrophages are generated directly from progenitors without going through a monocyte intermediate (126), it remains elusive the possible interplay with endothelium during this phase to understand positioning and growth of the fetal vascular tree during organogenesis. Both vasculogenesis and macrophage generation occur in parallel in space and time, but how direct interactions of nascent macrophages or monocytes with ECs contribute to vasculogenesis need further investigation.

### 3.3 Monocyte-endothelial cell interactions in tumor angiogenesis

Infiltration of circulating monocytes to the tumor microenvironment (TME) is critical for tumor angiogenesis (127).



It is well-known that inflammation is a starting event to attract and recruit monocytes and a driving force for monocyte-endothelial cell interactions and extravasation. For this reason, cancer cells use these endogenous mechanisms to stimulate an inflammatory milieu and release angiogenic factors that stimulate the endothelium to expose adhesion molecules, which enable adhesion and extravasation of proangiogenic monocytes (128). There are different markers described for these proangiogenic monocytes that cancer cells recruit to the TME. Different authors have found that human CD16<sup>+</sup> patrolling monocytes promote angiogenesis and boost the expansion of human colorectal carcinoma xenografts (128, 129). These CD16<sup>+</sup> monocytes seem to be recruited to the TME by cancer-released inflammatory cytokines and angiogenic factors (TNF- $\alpha$ , IL-1 $\beta$ , IL13, VEGF, etc.). In contrast, other investigations have described that inflammatory GR1<sup>+</sup> monocytes are the proangiogenic monocyte subset that supports the growth of primary tumors (130, 131). In any case, once proangiogenic monocytes are attached to inflamed endothelium within the TME, these monocytes are locally retained by endothelial CX3CL1/Fractalkine released by ECs in response to interferon gamma (IFN- $\gamma$ ), which is present in the inflammatory locus (132). CX3CL1 is a distinctive CX3C chemokine that is anchored to the cell membrane to allow leucocyte adhesion (133, 134), although it is also present in a soluble form that exhibits monocyte and lymphocyte chemotaxis properties (135). Furthermore, CX3CL1 also facilitates vascular extravasation of monocytes in lung tumor metastasis (136).

Hypoxia is a driving force of angiogenesis in tumors (137, 138). Some investigations have described that hypoxia, *via* hypoxia-inducible factor-1 (HIF-1), enhances the expression of proangiogenic factors such as VEGF, Ang-1 and Ang-2 in endothelial and cancer cells (139, 140), and promotes the synthesis of CXCR4 (a receptor for CXCL12) and Tie2 (angiopoietin receptor) on macrophages, which allows the interstitial migration of proangiogenic macrophages inside the tumor (128, 141, 142). Numerous tumor-derived chemokines are critical for recruiting monocytes into the tumor milieu and to promote the transition of monocytes to tumor-associated macrophages (TAM). These include chemokines such as CCL3 (macrophage inflammatory protein, MIP1 $\alpha$ ), CCL2 (MCP-1) and CCL4 (MIP1 $\beta$ ), interleukins (IL-6 and IL-1 $\beta$ ) and cytokines (colony stimulating factor 1 (CSF-1) (143–145). Monocytes recruited into the tumor are then further reprogrammed by cancer cells to display a proangiogenic and immunotolerant M2-like macrophage phenotype (146). Indeed, tumor-associated macrophages (TAMs) are the most abundant immunosuppressive cells in the TME. They play a fundamental role in the tumor initiation, growth, and progression (147). M2-like TAM, under hypoxic milieu, also release proangiogenic factors such as VEGF and placental growth factor (PIGF), and chemokines such as CCL2 and CXCL9, that regulate the expansion of peritumoral vascular network (143, 145). Additionally, TAMs are also involved in immunosuppression of CD8<sup>+</sup> T cells and natural killer (NK) cells, a major mechanism of anti-tumor immunity (148, 149).

The proangiogenic activity of TAM is also mediated by MMP activity, which contribute to the release of matrix-bound growth

factors such as VEGF. For example, active MMP-9 is produced by mouse and human proangiogenic Tie2<sup>+</sup> monocytes (150). The release of different matrix-bound growth factors from TME by active MMP-9 represents one of the most challenging mechanisms for current therapies to interfere with tumor angiogenesis (141). Indeed, digestion and delivery of angiogenic factors by MMPs is a vicious circle for tumor angiogenesis. Tumor tissues synthesize and accumulate MMPs and growth factors in the extracellular matrix, and simultaneously induce the arrival of more proangiogenic monocytes. Then, these monocytes infiltrate and transdifferentiate into TAM and secrete additional MMP-9 and growth factors to the TME, as suggested by previous studies in mice (151, 152).

Hypoxic TAMs show deep variations in the expression of numerous metabolic genes because they are compelled to adjust their metabolism to low oxygen pressure to maintain the energy needs (153). Cytokines are also important effectors on macrophage metabolism, inducing a wide array of metabolic changes. For example, pro-inflammatory M1-like macrophages change their metabolism toward increased anaerobic glycolysis, pentose phosphate pathway activation, and protein and fatty acid synthesis (154). In contrast, cytokines such as IL-4, IL-10, and IL-13 released by M2-like macrophages lead to a phenotype that more closely resembles the characteristics of TAMs (displaying enriched oxidative phosphorylation and stable glycolysis) (155). It is known that metabolic changes influence angiogenic and immunosuppressive properties of hypoxic TAMs. One signaling pathway governing these events is REDD1, a negative regulator of mTOR (156). Indeed, REDD1-mediated inhibition of mTOR hampers glycolysis in TAMs and reduces their excessive angiogenic response promoting the formation of anomalous blood vessels. Hence, TAMs lacking REDD1 promote the formation of smoothly aligned, pericyte-covered, functional vessels preventing vascular leakage, hypoxia, and metastases. TAMs deficient in REDD1 are highly glycolytic and drain glucose from TME affecting nearby ECs. This hinders vascular hyperactivation and stimulates the formation of quiescent vascular junctions (156). This functional link between TAM metabolism and tumor angiogenesis could be exploited in the future for the design of novel anti-angiogenic therapies for cancer.

## 4 Monocyte-endothelial cell interactions in tissue remodeling

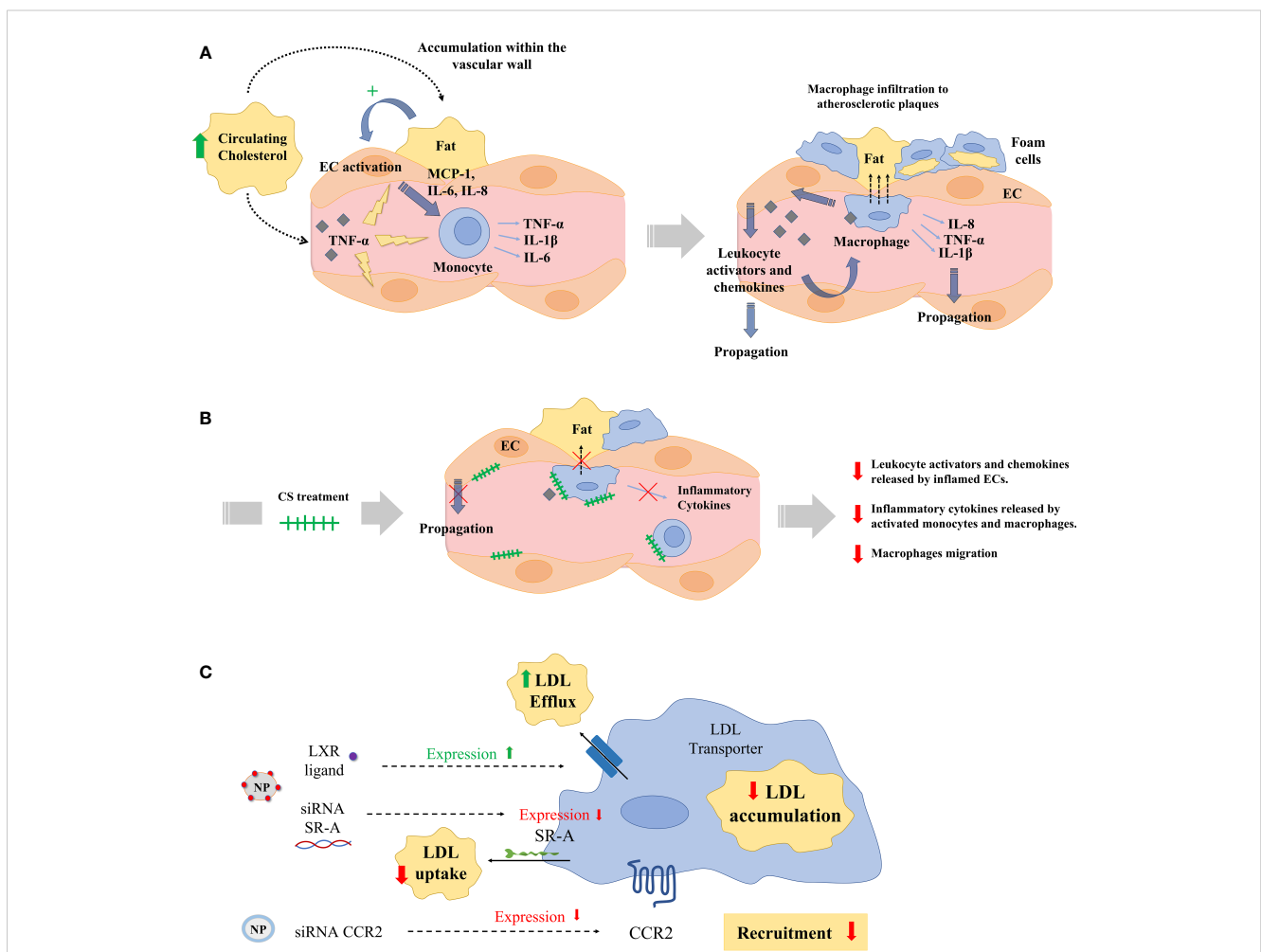
### 4.1 Modulation of monocytes and MDM in atherosclerosis and cardiovascular diseases

#### 4.1.1 Monocytes and MDM in atherogenesis

CVDs are the leading cause of death and disability worldwide (157). The main etiological factor for CVD is atherosclerosis (158). Atherosclerosis predominantly affects the coronary circulation and increases the risk of myocardial infarction and stroke, but shear stress caused by atheroma plaques may also affect the peripheral and cerebrovascular circulation (159, 160). Atherosclerosis is currently understood as a chronic inflammatory disorder

involving many immune cell types (161). Namely, MDM were the first inflammatory cells identified in the atherosclerotic plaques (162). Monocytes also contribute substantially to the different stages of atherosclerosis and myocardial infarction including initiation, progression, thrombus formation, and scarring (163, 164). Innate immune response is initially activated during early arterial injury, which induces the recruitment of bone marrow-derived monocytes into the intima (165). Hypercholesterolemia and clonal hematopoiesis of indeterminate potential are known risk factors in atherosclerosis *via* boosting the recruitment of mononuclear phagocytes into atheroma plaques (166). Monocytes that infiltrate into the intima of injured vessels transdifferentiate to inflammatory macrophages and can proliferate and promote plaque progression (Figure 5A). Monocyte and macrophage subsets secrete chemokines and cytokines that may stabilize or destabilize the plaque (167).

Generally, M1-like inflammatory macrophages increase the risk for unstable plaques and a class of M2-like resolving macrophages seem to promote plaque stabilization *via* efferocytosis, collagen production, and TGF- $\beta$  production (168, 169). However, it is important to emphasize that there are multiple subpopulations of macrophages coexisting within the atheroma plaque with distinct genetic markers and functions, such as resident-like anti-inflammatory, inflammatory, and TREM2<sup>high</sup> macrophages (170). ECs in injured vascular walls also release an array of chemokines that are commonly classified in four subgroups: CXC, CC, C, and CX3C chemokines. CX3CL1, also known as fractalkine, is the only member of the CX3C chemokine family. CX3CL1 and other chemokines such as CXCL12 may bind either to heparan sulfate (HS) on ECs or to their chemokine receptors in monocytes (171). Therefore, these chemokines may attract, retain, or induce



**FIGURE 5**  
**(A)** Hypercholesterolemia, high blood pressure, or disrupted flow patterns lead to LDL accumulation within the vascular wall. This accumulation activates endothelial cells (ECs), which recruit and activate monocytes by the secretion of chemokines and monocyte activators (MCP-1, IL-6, IL-8). Then, monocytes infiltrate atherosclerotic lesions, differentiating into macrophages and ultimately into foam cells. Macrophages and foam cells deliver pro-inflammatory mediators (TNF- $\alpha$ , IL-1 $\beta$ ), which decisively participate in the propagation of the inflammatory response and plaque progression. **(B)** Exogenous administration of CS disrupts the release of leukocyte activators and chemokines from aortic ECs inflamed with TNF- $\alpha$  and interferes with the release of inflammatory cytokines in activated monocytes and macrophages, and with their migration. **(C)** Numerous nanoparticles (NPs) have been used to target and treat macrophages in experimental atherosclerosis. Nanoparticles have aimed at reducing low density lipoprotein (LDL) accumulation in macrophages either reducing the macrophage expression of LDL scavenger receptors (SRs) using siRNA or delivering the liver-x-receptor (LXR) ligand to increase the expression of cholesterol transporters. Other NPs have been designed to deliver siRNA against the expression of the chemokine receptor 2 (CCR2) and reduce monocyte recruitment to atherosclerotic plaques.

monocyte transdifferentiation into macrophages and foam cells thereby displaying a major role on the progression of atheroma plaques and atherosclerosis (172, 173).

#### 4.1.2 Monocytes and MDM in angiogenesis and inflammation

Angiogenesis and inflammation are close collaborators in tissue remodeling following vascular injury and atherosclerosis. One of the initial signatures of early atherosclerosis is the appearance of a dysfunctional endothelium (174). Some of the factors affecting endothelial homeostasis include high levels of modified low-density lipoproteins (LDL), shear stress, free radicals, and hypertension (175). Indeed, dysfunctional endothelium induces an inflammatory response that may progress to a vascular lesion in CVDs or pave the ground for cancer metastasis (176). As described in the section 3 of this review, infiltrating inflammatory cells secrete a wide array of proangiogenic cytokines that stimulate EC activation, proliferation, and migration. However, the role of angiogenesis in atherosclerosis and CVDs is still a controversial and unresolved issue. Although proangiogenic therapy is useful for the treatment of ischemic heart disease and to enhance endothelial protective functions, angiogenesis may contribute to the growth of atherosclerotic lesions, and is a key factor in plaque destabilization and rupture at every vascular scale (177). The vasa vasorum, a specialized microvasculature that originates primarily in the adventitia of large arteries, is activated during atherosclerosis (178) and may well be influenced by MDM and EC-monocyte interactions. Vasa vasorum provides oxygen and nutrients to the external layers of the arterial wall and its expansion arises preceding endothelial dysfunction, intimal thickening, or plaque formation (178). The presence of intraplaque vasa vasorum is a marker of plaque expansion, progression, hemorrhage, instability, and rupture (178). In this scenario, proangiogenic molecules released by monocytes and MDM promote the growth of vasa vasorum and intimal lesions in both early and late stages of the disease. Moreover, the interactions between monocytes or MDM and ECs within atherosclerotic lesions may also be influenced by the local EC subpopulation. Indeed, analysis of the different EC subpopulations in aorta has identified a lymphatic EC cluster and two other populations specialized in lipoprotein handling, angiogenesis, and ECM production (179). Therefore, different subpopulations of monocytes and MDM may interact with distinct EC populations with vascular disease-relevant functions to decide the fate of the vascular lesion.

#### 4.1.3 Monocytes and MDM in other vascular alterations

There are other scenarios of vascular remodeling where monocytes and MDM display essential regulatory roles such as in pulmonary hypertension, thrombosis disorders, and venous malformation. Data obtained with single-cell analysis has revealed that non-classical and intermediate monocytes are enriched among all the monocyte subsets associated with pulmonary hypertension, and these phenotypes were associated with a decrease in hypoxia-inducible transcription factor-1 $\alpha$  (HIF-1 $\alpha$ ) (180). Indeed, nonclassical (CD14<sup>+</sup>CD16<sup>+</sup>) monocytes sense hypoxia, modulate pulmonary

vascular remodeling, and induce pulmonary hypertension (181). Moreover, the presence of perivascular MDM has emerged as a key pathogenic driver of pulmonary hypertension *via* interstitial macrophage-dependent inflammation and vascular remodeling (182). In thrombosis, monocytes and MDM release tissue factor, which activates prothrombin and initiates a coagulation cascade resulting in fibrin formation and thrombus formation (183). Monocytes and MDM also participate in the pathogenesis of venous malformation and cavernous venous malformation stimulating angiogenesis *via* VEGF overexpression in monocytes and MDMs and up-regulation of VEGF receptors in ECs (184).

#### 4.1.4 Monocytes and MDM in the regulation of vascular smooth muscle cells

Monocytes and MDM also modulate vascular smooth muscle cells (VSMCs) functions during atherosclerosis (185). The regulation of VSMC phenotypes may influence plaque morphology (necrotic core size and fibrous cap thickness) and the deposition and distribution of milieu components (lipoprotein, ECM, and chemokines). In turn, VSMCs, also interact with ECs to orchestrate response to injury or control EC growth (186, 187). VSMCs are major contributors to modifications of vascular microenvironment in CVDs by producing ECM proteins (e.g., fibrin, fibronectin, collagen, and proteoglycans) and agents that regulate ECM formation (e.g., tissue inhibitors of metalloproteinases, tissue factor). Alterations in some of these subendothelial matrix components influence EC apoptosis, which plays pivotal roles in atherosclerosis (188). Moreover, these variations in the ECM composition alter mechanical and functional properties of the vascular wall, which influence the behavior of monocytes, MDM, and ECs (189, 190). Namely, the interaction of the substratum component chondroitin sulfate (CS) has demonstrated a wide array of regulatory functions on monocytes/MDM and ECs in the context of inflammation and atherogenesis (191). In advanced atherosclerosis, there is a decrease in CS, with a concomitant increase of dermatan sulfate in arterial walls (192). Exogenous administration of CS disrupts the release of leukocyte activators and chemokines from aortic ECs inflamed with TNF- $\alpha$  and interferes with the release of inflammatory cytokines in activated monocytes and macrophages, and with their migration (Figure 5B) (191). Indeed, oral administration of CS to ApoE knockout mice has demonstrated to reduce the area of atheroma plaques *via* interference with the release of monocyte attractants and the uptake and accumulation of Ox-LDL in macrophages and foam cells (193). These findings have motivated a growing interest in incorporating either CS or other immunomodulator drugs in the composition of new intravascular devices for the treatment of CVDs (194–196).

#### 4.1.5 Therapeutic strategies to modulate monocytes and MDM in atherosclerosis and CVDs

Monocytes and MDM have a significant impact on lesion progression at all stages of atherogenesis, and there has been great effort in design novel therapies to interfere with the monocyte-EC or macrophage-atheroma plaque interactions in atherosclerosis (Table 3) (2, 161, 210). Standard therapies for atherosclerotic vascular disease (e.g. angiotensin converting enzyme inhibitors,

TABLE 3 Novel therapies to modulate monocytes and MDM.

Treatment	Target cell	Disease	Treatment strategy	Reference
Chondroitin sulfate (CS)	ECs Monocytes and macrophages	Atherosclerosis	Disruption of the propagation of inflammation	(193)
Prostaglandins Anti-diabetic thiazolidinediones	Macrophages	Atherosclerosis	Stimulation of the M2-like macrophage phenotype	(197)
NPs with siRNA against LDL scavenger receptors	Macrophages	Atherosclerosis	Reduction of LDL accumulation	(198, 199)
NPs with Liver-x-receptor ligand	Macrophages	Atherosclerosis	Reduction of LDL accumulation	(198, 199)
NPs with siRNA against CCR2	Macrophages	Atherosclerosis	Reduction of monocyte recruitment	(200, 201)
NPs with siRNA against Ca <sup>2+</sup> /calmodulin-dependent protein kinase $\gamma$	Macrophages	Atherosclerosis	Enhancement of macrophage efferocytosis	(202)
PLGA-NPs with SYK pathway inhibitor	Macrophages	Steatohepatitis	Inhibition of inflammatory pathways	(203)
Liposomes loaded with dexamethasone	Macrophages	Acute and chronic liver diseases	Induction of anti-inflammation	(204)
Dendrimer-graphene nanostars with PPAR- $\gamma$ agonist GW1929	Macrophages	Hepatic fibrosis	Reduce Hepatic inflammation and fibrosis	(205)
Nanostructured lipid carriers containing curcumin	Macrophages	Hepatic fibrosis	Reduction of hepatic inflammation	(206)
Dendrimer-graphene nanostars containing cDNA MMP9	Macrophages	Hepatic fibrosis	Local digestion of collagen fibers	(207)
CXCR4-targeted lipid-coated PLGA NPs with sorafenib and AMD3100	TAM	Hepatocellular carcinoma	Reduction of M2-like macrophage polarization and TAMs infiltration	(208)
NPs with mRNA encoding BisCCL2/5i	TAM	Hepatocellular carcinoma	Induction of macrophages polarization to antitumoral M1-like subtype	(209)

aspirin, corticosteroids, etc.) induce general immune responses but not a specific macrophage targeting. Immunosuppressive therapies may display side effects in patients such as vulnerability to infection and cancer. A precise macrophage targeting seems essential for the treatment of atherosclerosis. Molecular signaling of peroxisome proliferator-activated receptors (PPARs) is of major relevance for the regulation of macrophage lipid metabolism and inflammatory responses. Natural ligands such as prostaglandins and anti-diabetic thiazolidinediones induce PPARs, which in turn stimulate the M2-like macrophage phenotype and a reduced progression of atherosclerosis (197). Liver X receptors are up-regulated in M2-like macrophages and, as occurs with PPARs, exert relevant antiatherosclerotic effects *via* regulation of cholesterol metabolism and M1-like macrophage inflammatory response (211). Statins are efficient cholesterol-reducing agents that also reduce immune responses *via* inhibition of macrophage inflammatory activity (212). However, these and other conventional pharmacological agents cannot selectively target macrophages. Numerous potential therapies based on nanoparticles (NPs) to target and treat macrophages have been used during the last decade to treat experimental atherosclerosis (Figure 5C). For example, a wide array of NPs functionalized with different targeting ligands such as mannose, hyaluronan, folate, DNA, peptides, antibodies, HDLs and LDLs have been employed to target intraplaque macrophages and to enhance selectivity and delivery of anti-inflammatory drugs to reduce atherosclerosis (213). Other strategies aim to reduce LDL

accumulation in macrophages either reducing the macrophage expression of LDL scavenger receptors (SRs) or delivering the liver-x-receptor ligand to increase the expression of cholesterol transporters (198, 199). Other researchers have developed NPs to inhibit the expression of the chemokine receptor 2 (CCR2) associated with monocyte recruitment as a potential treatment for atherosclerosis (200, 201). Another interesting strategy for the treatment of atherosclerosis is the enhancement of macrophage efferocytosis – the phagocytic clearance of dying cells and apoptotic and necrotic debris. Indeed, this process is diminished in atherosclerotic blood vessels. Interestingly, NPs delivering siRNA against Ca<sup>2+</sup>/calmodulin-dependent protein kinase  $\gamma$  (a known blocker of macrophage efferocytosis) have shown plaque stabilization in mice (202). Therefore, it seems clear that specific modulation of macrophage interactions and functions in vascular lesions using targeted nanoparticles may be of therapeutic interest for the treatment of atherosclerosis and CVDs in the future.

## 4.2 Modulation of monocytes and MDM in chronic liver diseases

### 4.2.1 Contribution of monocytes and MDM to the pathogenesis of chronic liver diseases

Chronic liver injury from different etiologies may lead to hepatic fibrosis, cirrhosis, and/or hepatocellular carcinoma (HCC). Cirrhosis



is among the most prevalent diseases in Western countries. The prognosis of these patients is grim, except for those who can benefit from liver transplantation. This is due to the multiple organic derangements, including renal failure, variceal bleeding, or bacterial peritonitis (214). These patients develop an important and progressively accentuated cardiocirculatory dysfunction, portal hypertension, arterial hypotension, high cardiac output, and diminished systemic vascular resistance. It is commonly assumed that increased endothelial production of NO is of major importance in the pathogenesis of the circulatory dysfunction occurring in cirrhosis (215), but the contribution of other endogenous systems has also been considered (216). In this scenario, MDM also contribute to vascular dysfunction in cirrhosis *via* release of vasodilators such as NO or adrenomedullin (217, 218). Hypoxia appears as a common phenomenon associated with the induction and release of NO from macrophages during liver cirrhosis (219). Another general activator of macrophages in liver cirrhosis is bacterial lipopolysaccharide (LPS). Intestinal permeability is increased in patients with advanced liver cirrhosis, and bacterial translocation may cause infection and spontaneous bacterial peritonitis that usually results in renal failure and death despite the efficacy of the antibiotic therapy (214). Macrophages isolated from cirrhotic patients with bacterial peritonitis have shown a high release of VEGF that results in increased vascular permeability in the peritoneal vessels of these patients (220). LPSs also suppress the expression of the cannabinoid receptor CB2 in circulating monocytes and peritoneal macrophages, impairing the defense response mechanisms in cirrhotic patients with liver disease (221). Macrophage CB2 activation has also been associated with reduced angiogenesis attributed to a lower monocyte infiltration during liver fibrosis and a lesser macrophage release of proangiogenic factors (222, 223).

#### 4.2.2 Role of monocytes and MDM on liver regeneration

MDM are critical players during all the stages of chronic liver injury: initiation, progression, resolution, and regeneration of the hepatic function (224). Indeed, the liver is a unique organ in its capacity to regenerate the entire organ mass after a liver insult or a hepatic resection (225). After liver resection, injured hepatocytes, liver progenitor cells and KCs from the portal space recruit circulating monocytes to injured liver *via* secretion of monocyte chemoattractant protein 1 (MCP-1) (226). Then, recruited monocytes trigger endothelial c-Met and Tie2 pathways by direct interaction (92–94), and activate the paracrine release of different cytokines and endothelial growth factors critical for liver regeneration, such as the family of molecules Notch and Wnt (91, 227). Indeed, either complete depletion of hepatic macrophages or interference of the canonical Wnt/ $\beta$ -catenin signaling pathway in macrophages reduces liver regeneration (228, 229). KCs elaborate a precise control role of sinusoidal endothelium releasing priming factors (e.g., IL-6 and TNF- $\alpha$ ) and induce hepatocytes to act in response to growth factors (e.g., HGF, TGF- $\beta$ , and EGF). Then, proliferation of hepatocytes sequentially advances from periportal to pericentral areas of the lobule, as a wave of mitosis under circadian control. In turn, hepatocyte proliferation needs to be perfectly coordinated with the expansion of the hepatic vascular network during liver

regeneration (230). A previous work has described how this sequence of phenomena is fine-tune regulated by monocytes and MDM in regeneration occurring after liver resection. It has been demonstrated that circulating monocytes are selectively recruited to sprouting spots in regenerating livers. This process starts in portal areas and expands to the rest of hepatic tissue coinciding with the waves of hepatocyte mitosis, the hepatic expression of iNOS and vasodilation to facilitate monocyte infiltration and endothelial migration as explained in section 3.1 of this review. These interactions between monocytes and hepatic ECs commensurate with phosphorylation and disruption of VE-cadherin connections, which is crucial for endothelial tip migration and elongation (231).

MDM display different roles in liver regeneration initiated after chronic injury and fibrosis. MDMs that infiltrate during the progression of liver fibrosis propagate inflammation and induce scarring *via* activation of myofibroblasts (232). The balance between stimuli from microenvironment and the presence of different subsets of macrophages is determinant towards liver disease or repair (233). Recent advances in flow cytometry and single-cell transcriptomics have allowed a broad understanding of the array of macrophage phenotypes within healthy and diseased liver (234). Nowadays, these technologies have allowed the determination of different subsets of mononuclear phagocytes in the liver fibrotic niche, which is composed of ten main clusters: scar-associated macrophages (SAMacs), KCs, tissue monocytes (TMs), conventional dendritic cells (cDCs) and each corresponding cycling (proliferating) cell subsets (234). SAMacs express the unique markers TREM2 and CD9 (234). These macrophages displayed a hybrid phenotype, between TMs and KCs and similar to MDM in mouse liver injury models. SAMacs have a pro-fibrogenic phenotype and accumulate within the fibrotic niche in cirrhotic liver. TREM2<sup>+</sup>CD9<sup>+</sup> SAMacs have showed a monocyte-like morphology and a distinctive topographical distribution and differentiation trajectory separated from KCs (234).

#### 4.2.3 Monocytes and MDM on liver cancer

Liver cancer is the third leading cause of cancer-related mortality worldwide and HCC accounts for nearly 90% of the incidence of all hepatic cancers (235). The tumor microenvironment (TME) is composed by a highly complex cellular composition including different populations of myeloid cells and lymphocytes. Indeed, the presence of myeloid cells in the TME is frequently associated to altered patient survival (236). In this scenario, TAM is one of the main members of the TME with a critical role on HCC occurrence and development *via* angiogenesis stimulation, interference with T cell anticancer activity, promotion of drug resistance, and cancer metastasis (148, 237). The state-of-the-art combination of two single-cell RNA sequencing technologies has recently allowed the analysis of all CD45<sup>+</sup> immune cells in HCC patients from different hepatic zones (tumor, adjacent liver, hepatic lymph node, blood, and tumor ascites) (238). In this study, TAMs were associated with poor prognosis and these macrophages highly expressed two marker genes, SLC40A1 and GPNMB, in HCC tumors. Namely, SLC40A1 encodes ferroportin, an iron exporter, and regulates TLR-stimulus-induced pro-inflammatory cytokines, including IL-6, IL-23, and IL-



1 $\beta$ , thus pointing out that iron metabolism is implicated in determining innate immunity in the TME.

#### 4.2.4 Therapeutic strategies to modulate monocytes and MDM in chronic liver diseases

Cirrhosis is a limiting factor for anticancer therapy and a major risk factor for the development of HCC. Indeed, cirrhosis may challenge surgical and interventional approaches to treat liver cancer, alter pharmacokinetics of anticancer drugs, enhance side effects of chemotherapeutics and susceptibility to hepatotoxicity. Therefore, conventional and future treatments designed to modulate monocytes and MDM in liver diseases need to consider the different molecular signals involved during liver cirrhosis (M1-like inflammatory macrophages and fibrogenic MDM signals) or liver cancer (anti-inflammatory M2-like TAM in the TME and immunosuppression). Various strategies have been developed to treat liver inflammation and to target hepatic macrophages (Table 3) (239). For example, polylactic-co-glycolic acid (PLGA) NPs with a Spleen Tyrosine kinase (SYK) pathway inhibitor have been used to target and treat macrophages in chronic liver injury induced by steatohepatitis, since SYK is a critical mediator in inflammatory pathways (203). Liposomes loaded with the anti-inflammatory drug dexamethasone have obtained anti-inflammatory and anti-fibrotic results in mouse models of acute and chronic liver disease (204). A PPAR- $\gamma$  agonist GW1929 targeted to MDM with dendrimer-graphene nanostars has been used to reduce hepatic inflammation and fibrosis (205). Phosphatidylserine (a component that mimics apoptotic cells recognized by macrophages) has been used to decorate nanostructured lipid carriers containing curcumin to reduce hepatic inflammation and fibrosis (206). Another interesting approach has been the delivery of a plasmid expressing the collagenase metalloproteinase 9 into inflammatory macrophages using dendrimer-graphene nanostars for local digestion of collagen fibers, reduction of hepatic injury, and hepatic regeneration (207). Different strategies have also been developed to target and treat selectively TAM in HCC (240). For example, CXCR4-targeted lipid-coated PLGA NPs with sorafenib and AMD3100 (a CXCR4 antagonist) revealed that blocking the interaction of TAM CXCR4 with SDF1 $\alpha$  reduced M2-like macrophage polarization and TAMs infiltration, and simultaneously tumor progression was delayed in a mouse model of HCC (208). CCL2 and CCL5 are two chemokines that attract TAMs infiltration and induce their polarization to the M2-like phenotype. A specific CCL2/CCL5 dual inhibitor (BisCCL2/5i) coating lipid NPs or a mRNA encoding BisCCL2/5i inhibited TAM infiltration and induced the polarization of M2-like macrophages to antitumoral M1-like subtype (209). Overall, further studies are still necessary to delineate how to combine conventional therapies against HCC cells with TAM-targeted nanotherapeutics to overcome the limitations of current pharmacological treatments in HCC.

## 5 Conclusions

This review has emphasized that interactions between monocytes and ECs or between transmigrated and differentiated

MDM and the milieu are critical to the orchestration of vascular and tissue remodeling. The interplay between monocytes and ECs determines the selection of sprouting points during angiogenesis and may be involved in vasculogenesis. Infiltrated MDM regulate the promotion of vascular and tissue growth *via* the release of different vascular growth factors, regulation of vascular permeability, and control over ECM turnover. Tumors take advantage of the physiological functions of proangiogenic monocytes and MDM to increase cancer cell irrigation and metastasis.

Blood monocytes and MDM play a critical role in the pathogenesis of myocardial infarctions, strokes, and CVDs overall. They actively contribute to new vessel formation inside the arterial wall and atherosclerotic plaques resulting in local ischemia and inflammation, and regulate the activity and phenotype of VSMC, thereby influencing plaque morphology and ECM deposition.

Chronic liver diseases are characterized by a vigorous activation of inflammatory subsets of monocytes and MDM that mediate hepatic angiogenesis, inflammation, and fibrosis. Monocytes and MDM display different roles during regeneration of healthy liver or after chronic injury and fibrosis. Monocyte-EC interactions orchestrate harmonized angiogenesis and synchronized liver mass growth after resection in healthy liver. In contrast, monocytes and MDM perpetuate angiogenesis and inflammation in chronic liver diseases and pave the ground for hepatic tumor growth.

Conventional and novel therapeutic strategies are being developed to selectively target monocytes and MDM to modulate the progression of chronic diseases such as CVD or liver diseases, and cancer. There is still an urgent need for more selective treatments and molecular insights on the different macrophage stripes involved in the zonation phenomena occurring during vascular and tissue remodeling, and cancer. It is mandatory to decipher surface markers and mechanisms involved in the control of specific subsets of monocytes and MDM to improve therapeutic interventions without distortion of the necessary physiological functions of these immune cells in pathogen detection and tissue regeneration.

## Author contributions

MM-B, BS-C, PM-L: Drafting of the manuscript and preparation of tables and figures. ERE and WJ: Revision of the manuscript. All authors contributed to the article and approved the submitted version.

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## Conflict of interest

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