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The complex TIE between macrophages and angiogenesis

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Abstract

Macrophages are primarily known as phagocytic immune cells, but they also play a role in diverse processes, such as morphogenesis, homeostasis and regeneration. In this review, we discuss the influence of macrophages on angiogenesis, the process of new blood vessel formation from the pre-existing vasculature. Macrophages play crucial roles at each step of the angiogenic cascade, starting from new blood vessel sprouting to the remodelling of the vascular plexus and vessel maturation. Macrophages form promising targets for both pro- and anti-angiogenic treatments. However, to target macrophages, we will first need to understand the mechanisms that control the functional plasticity of macrophages during each of the steps of the angiogenic cascade. Here, we review recent insights in this topic. Special attention will be given to the TIE2-expressing macrophage (TEM), which is a subtype of highly angiogenic macrophages that is able to influence angiogenesis via the angiopoietin–TIE pathway.

KEYWORDS

angiogenesis, ANGPT-TIE pathway, macrophages, TIE2-signalling

1 | INTRODUCTION

Macrophages are important regulators of tissue homeostasis, growth, morphogenesis and repair. An accumulating body of research indicates that macrophages are extremely plastic cells that play an active role in development and tissue regeneration. Here, we review recent insights in the role of macrophages in angiogenesis, that is the growth and remodelling of new blood vessels from the existing vasculature.

2 | MACROPHAGES, A DIVERSE GROUP OF CELLS

Macrophages are phagocytic immune cells and are part of the so-called mononuclear phagocytic system, together with dendritic cells and monocytes. Recent data from ontogenetic studies show that macrophages consist of an extremely diverse group of cells with distinct functional phenotypes (Bonnardel & Guilliams, 2018; Hoeffel & Ginhoux, 2018). Macrophages are frequently categorized in tissue-resident macrophages or bone marrow-derived macrophages. Tissue-resident macrophages are long-lived cells which have dedicated functions and phenotypes depending on the tissue in which they reside (Bonnardel & Guilliams, 2018; Hoeffel & Ginhoux, 2018). These macrophages are mainly recruited during embryogenesis from the fetal yolk sac or liver and are either directly derived from hematopoietic progenitor cells or pass through an intermediate monocyte stage (Bonnardel & Guilliams, 2018). Bone marrow-derived macrophages are mainly derived from patrolling monocytes which invade tissues during inflammation. However, the ontogenetic division between tissue-resident and bone marrow-derived macrophages is not clear cut. In the brain, tissue-resident macrophages are almost exclusively derived from the early embryonic yolk sac-derived macrophages and maintained by self-renewal, whereas tissue-resident macrophages in the gut and dermis are initially derived from early embryonic populations, but are subsequently replenished by monocyte-derived macrophages from the bone marrow (Bonnardel & Guilliams, 2018).

Macrophages are highly plastic cells that can change their polarization status based on pro- or anti-inflammatory stimuli present

in the tissue. Pro-inflammatory conditions give rise to so-called classically activated macrophages (M1), whereas anti-inflammatory conditions give rise to alternatively activated macrophages (M2). However, this strict binomial classification does not cover the broad range of polarization states, as it is currently believed that the M1 and M2 macrophages represent the extremes of a wide polarization spectrum (Murray, 2017). Moreover, the activation states of macrophages are not definitive as macrophages can change their polarization depending on the environmental conditions (Davies, Rice, Mcvicar, & Weiss, 2019; Davies & Taylor, 2015). Because of their anti-inflammatory, pro-resolution phenotype, M2-type macrophages are mostly associated with angiogenesis, tissue growth and morphogenesis (Moore & West, 2019).

3 | MACROPHAGES INVOLVED IN ANGIOGENESIS

Angiogenesis is an extremely complex process which involves the growth of new capillaries from pre-existing blood vessels and their subsequent remodelling to a mature and functional vascular plexus (De Spiegelaere et al., 2012; Potente, Gerhardt, & Carmeliet, 2011). Angiogenesis establishes a vascular network to ascertain the transport of oxygen, nutrients and waste products to and from the growing tissue. Although angiogenesis is crucial for normal development, growth, regeneration and inflammation, this process is frequently associated with pathological situations (Potente et al., 2011). The formation of new blood vessels via angiogenesis is also a large contributor to inflammation. In response to inflammatory stimuli, the vasculature will become more leaky and prone to form new blood vessels. The higher permeability of the blood vessels also facilitates the recruitment of immune cells to the tissue. However, an excess of newly formed blood vessels is associated with chronic inflammation, resulting in numerous leaky and dysfunctional capillaries which can aggravate the problem (Eklund, Kangas, & Saharinen, 2017).

Angiogenesis is a multistep process, called the angiogenic cascade, which starts with the activation of the initially quiescent endothelial cells (ECs) and the partial breakdown of the basement membrane and extracellular matrix (De Spiegelaere et al., 2012). Subsequently, new capillaries arise through endothelial sprouts which migrate towards angiogenic stimuli. To ensure an efficient blood circulation, these vascular sprouts need to fuse with other sprouts or capillaries, a process known as anastomosis. Finally, the new capillaries mature to stable blood vessels and superfluous vessels are removed. In addition to endothelial sprouting, new blood vessels can also arise through the splitting of pre-existing vessels in a process called intussusceptive angiogenesis (De Spiegelaere et al., 2012; Potente et al., 2011). Interestingly, two studies have reported that mononuclear cells are involved in stabilizing intraluminal pillars, which are the hallmark of intussusceptive angiogenesis (Dimova et al., 2013, 2019). Future studies should resolve whether these cells are monocyte/macrophages or other immune cells.

Macrophages have since long been recognized as paracrine regulators of blood vessels sprouting. The first direct demonstration of the regulatory role of macrophages during angiogenesis was reported in 1977. This study demonstrated that activated macrophages induced neovascularization in the guinea pig cornea (Polverini, Cotran, Gimbrone, & Unanue, 1977). Subsequent studies revealed that macrophages secrete growth factors, cytokines and enzymes which attract new blood vessels and modify the extracellular matrix to facilitate neovascularization (Corliss, Azimi, Munson, Peirce, & Murfee, 2016; Cursiefen et al., 2004; Deshmane, Kremlev, Amini, & Sawaya, 2009; Leibovich et al., 1987). Interestingly, accumulating data indicate that macrophages play a role in each of the steps of the angiogenic cascade.

3.1 | Matrix remodelling

Macrophages can secrete proteases that cleave the extracellular matrix and start matrix remodelling to pave the way for endothelial sprout migration and vascular remodelling (Figure 1). As an example, matrix metallopeptidase 9 (MMP9) is secreted by macrophages. Macrophage-derived MMP9 was found to be important for vascular remodelling in the human decidua (Hazan et al., 2010). In vitro experiments on the chicken choriallantoic membrane (CAM) model revealed that a strong angiogenic response was observed when M2-polarized macrophages were embedded in an avascular collagen scaffold on the CAM (Zajac et al., 2012). This angiogenic response coincided with a strong expression of MMP9 in M2 macrophages, but not in M1-polarized macrophages which had a limited effect on angiogenesis (Zajac et al., 2012). Furthermore, monocytes/macrophages have been shown to secrete matrix metallopeptidase 2 (MMP2), which is another protein involved in matrix remodelling and angiogenesis (Corliss et al., 2016; Detry et al., 2012; Webster & Crowe, 2006).

3.2 | Endothelial activation and capillary sprout formation

Macrophages can secrete a number of pro-angiogenic growth factors, such as vascular endothelial growth factor A (VEGFA) (Eubank, Galloway, Montague, Waldman, & Marsh, 2003; Lucas et al., 2010), tumour necrosis factor (TNF) (Leibovich et al., 1987), fibroblast growth factor 2 (FGF2) (Jetten et al., 2014), transforming growth factor beta 1 (TGFB1) (Ferrari, Cook, Terushkin, Pintucci, & Mignatti, 2009; Lucas et al., 2010) and many more (Corliss et al., 2016) (Figure 1).

VEGFA is known as the most potent pro-angiogenic growth factor. Numerous studies have shown that it can be expressed by macrophages (Eubank et al., 2003; Guo et al., 2018; Lucas et al., 2010). VEGFA induces endothelial tip cell formation, the subsequent capillary sprout formation and migration of these capillary sprouts towards the VEGFA-secreting source (Potente et al., 2011).

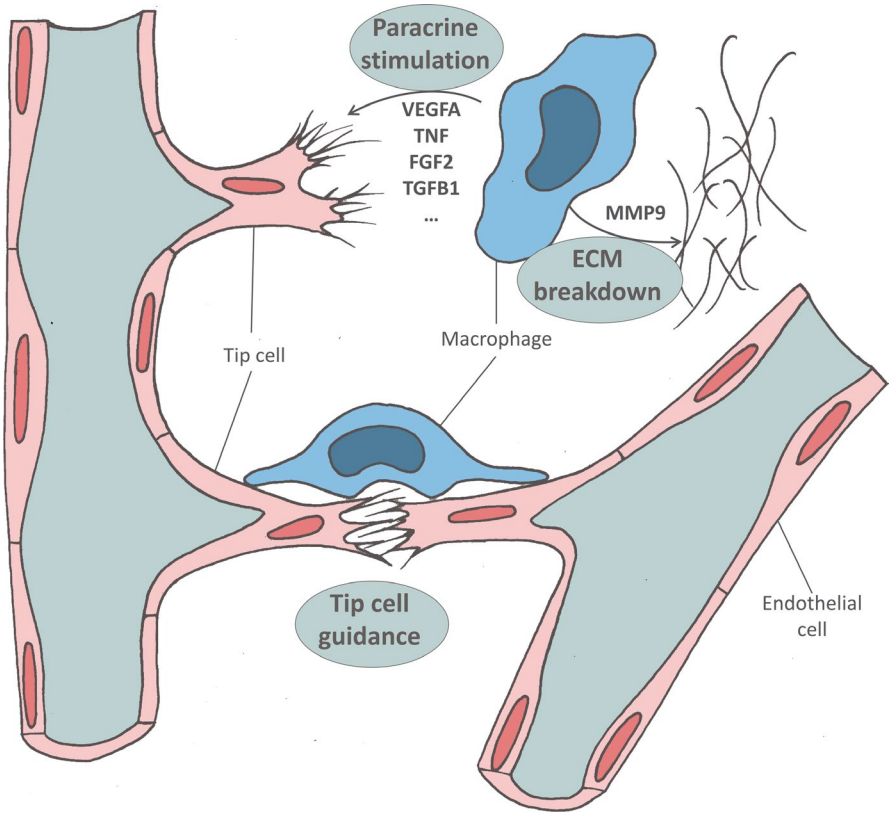

FIGURE 1 Macrophage functions during matrix remodelling and capillary sprouting. Secretion of matrix modulating enzymes enables macrophages to break down and remodel the extracellular matrix. By secreting growth factors and chemokines, macrophages can stimulate endothelial activation and migration of vascular sprouts. By directly interacting with these sprouts, macrophages facilitate anastomosis formation

Experiments in mouse wound healing models demonstrated that inflammatory macrophages are the main source of VEGFA expression during wound healing (Lucas et al., 2010; Willenborg et al., 2012). In these studies, the VEGFA expressing macrophages were CCR2+/LY6C+, had high expression levels of the pro-inflammatory (M1) markers IL6 and iNOS (NOS2) and also expressed the TIE2 receptor (Willenborg et al., 2012). Two recent studies on zebrafish wound healing and development indeed showed that macrophages are essential during angiogenesis and vessel repair (Gerri et al., 2017; Gurevich et al., 2018). These macrophages expressed high levels of VEGFA and TNF, an M1 marker (Gurevich et al., 2018). Interestingly, Guo and colleagues recently described that uptake of haemoglobin by $CD163^{+}$ macrophages (M2) induces a pro-angiogenic phenotype in macrophages associated to atherosclerotic plaques through enhanced hypoxia-inducible factor 1 subunit alpha (HIF1A) and VEGFA expression (Guo et al., 2018).

Using their extracellular matrix remodelling properties, macrophages may also form initial networks which are subsequently invaded by ECs. Macrophages can establish a capillary-like network in the ischemic myocardium of mice and in matrigel implants (Anghelina, Krishnan, Moldovan, & Moldovan, 2004; Moldovan, Goldschmidt-Clermont, Parker-Thornburg, Shapiro, & Kolattukudy, 2000; Schmeisser et al., 2001). A more recent study indicated that the formation of these micro-tunnels is regulated by hypoxia, through HIF1A (Barnett et al., 2016). The macrophages lining the micro-tunnels also express endothelial markers such as CD31 (Barnett et al., 2016), von Willebrand factor, vascular endothelial (VE)-cadherin, and endothelial nitric oxide synthase (eNOS or NOS3) (Schmeisser et al., 2001). This led to the hypothesis that macrophages lining micro-tunnels may transdifferentiate to ECs (Bailey et al., 2006; Schmeisser et al., 2001). However, this hypothesis remains an issue of debate as lineage tracing experiments have failed to find any contribution of bone marrow-derived cells to the endothelium (Corliss et al., 2016).

3.3 | Macrophages as chaperones in anastomosis of vascular sprouts

In addition to their paracrine role in guiding endothelial sprouts, macrophages can also act as cellular chaperones that physically interact with endothelial tip cells and facilitate the guidance and anastomosis of vascular sprouts (Figure 1) (Fantin et al., 2010). Seminal work by Fantin et al. (2010) revealed that a subset of macrophages directly interacts with endothelial tip cells and mediates fusion of vascular sprouts during angiogenesis in the developing mouse hindbrain, the retina and in the developing zebrafish trunk. These macrophages were characterized by expression of TIE2 (tyrosine kinase with immunoglobulin-like and EGF-like domains, also known as TEK) and neuropilin 1 (NRP1) suggesting that these cells are similar to M2 type tumour-associated macrophages (De Palma, Murdoch, Venneri, Naldin, & Lewis, 2007; Fantin et al., 2010). However, recent work on zebrafish wound healing revealed that the majority of macrophages localized near endothelial tip cells express TNF indicating an M1 phenotype of the tip cell guiding macrophages (Gurevich et al., 2018). The somewhat contradicting results in these studies are likely

contributed to their different experimental set-up. Fantin et al. (2010) looked at the presence of the cell surface proteins TIE2 and NRP1 via immunofluorescence while Gurevich et al. (2018) monitored the homing of macrophages to the wound bed in transgenic fish (Tg(tnfα:GFP)). It would be interesting to compare the expression patterns of the cell populations from these two studies to see to which extend these cell populations differ at gene expression level.

There is only limiting information on how macrophages are able to interact with ECs. However, some new insights were recently gained into possible mechanisms. A study on macrophage-assisted blood vessel regeneration in the zebrafish brain after laser ablation revealed that the macrophage–EC interaction was mediated by physical adhesion via the adhesion molecules Cdh5 and Pecam1 rather than by factor secretion (Liu et al., 2016).

3.4 | Vascular remodelling (pruning)

The initial process of angiogenesis usually results in high numbers of new capillaries that invade the previously avascular tissue. In order to form a functional and stable capillary network, superfluous capillaries have to be removed and the remaining capillaries need to mature and be remodelled to a hierarchical vascular network.

Macrophages are required for the pruning of superfluous blood vessels (Figure 2a). Experiments in the developing mouse testis revealed an abnormal and poorly organized vasculature in the testes of mice in which macrophages were depleted using *Cx3cr1-Cre* mice (Defalco, Bhattacharya, Williams, Sams, & Capel, 2014). However, the total number of ECs was unaffected. This, together with observations that macrophages engulfed apoptotic ECs, indicates a role for the macrophage in vascular remodelling and pruning, but not in initiation of angiogenesis in the developing mouse testes (Defalco et al., 2014). The macrophages that were involved in remodelling stained positive for NRP1 and TIE2, but only expressed minute amounts of the endothelial growth factors VEGFA and angiopoietin 1 (ANGPT1). Angiopoietin 2 (ANPGT2) was not detected. In addition, the macrophages were mostly of the M2 type as flow cytometry indicated they stained positive for CD206 (also known as MRC1) and Maf and negative for CD86 and MHCII. Furthermore, qPCR revealed low levels of interleukin 12 (IL12) and high levels of arginase 1 expression (Defalco et al., 2014). Similar results were recently observed in a zebrafish wound healing model, in which TNF-negative macrophages (M2) were important for vascular remodelling by inducing endothelial apoptosis (Gurevich et al., 2018).

Macrophages also seem to play a role in the survival of blood vessels. West, Sefton, and Sefton (2019) compared the angiogenic effect of macrophages and mesenchymal stromal cells (MSC) embedded in collagen implants in mice. Although no difference was observed in the initial angiogenic response, the vessel densities persisted much longer in implants with macrophages compared to implants with MSC, indicating a role of macrophages in the survival of new blood vessels (West et al., 2019).

3.5 | Maturation

New blood vessels need to undergo maturation to become stable and functional. This process involves recruitment of pericytes and other perivascular cells, strengthening of the interendothelial cell junctions and stabilization of the extracellular matrix.

Macrophages have been involved in the recruitment of pericytes around new blood vessels (Figure 2b). Spiller et al. (2014) observed that M2a-polarized macrophages (MRC1+/CD206+) express high levels of platelet-derived growth factor B (PDGFB), which is known to recruit pericytes to blood vessels (Spiller et al., 2014). Macrophages may also directly contribute to vascular stabilization as perivascular macrophages are highly abundant around blood vessels (He et al., 2016). Interestingly, embryonic macrophages have been observed to transdifferentiate into pericytes in the developing mouse brain, indicating an additional direct contribution of macrophages to vascular maturation (Yamamoto et al., 2017). Other studies reported that macrophages can differentiate to mural vascular smooth muscle cells and hereby increase the structural integrity of new blood vessels (Figure 2b) (Kumar et al., 2013; Metharom, Kumar, Weiss, & Caplice, 2010).

An important part of vascular maturation is the stabilization of the interendothelial cell junctions to limit the permeability of the endothelial monolayer. Although pro-angiogenic macrophages increase microvascular permeability through the expression of pro-angiogenic factors such as VEGFA (Guo et al., 2018), a number of studies have also demonstrated a function of macrophages in limiting vascular permeability. Non-contact coculture of primary bovine or human brain capillary ECs with human blood-derived non-polarized macrophages decreased the paracellular permeability in ECs (Zenker, Begley, Bratzke, Rubsarrien-Waigmann, & Von Briesen, 2003). Zhang et al. (2012) showed that perivascular resident macrophage-like melanocytes, a hybrid cell type similar to resident macrophages and melanocytes, decrease vascular permeability. This is mediated through the secretion of pigment epithelial-derived factor, which has a direct effect on the expression of several tight junctions-associated proteins including occludin, Vascular Endothelial (VE)-cadherin and Zonula Occludin (ZO)-1 (Zhang et al., 2012). VE-cadherin is an important adhesion molecule located specifically at interendothelial adherens junctions (Vestweber, 2008). Zonula occludin proteins form an important component of tight junctions (Itoh, Nagafuchi, Moroi, & Tsukita, 1997). He et al. (2016) showed enhanced permeability in macrophage depleted mice. This phenotype could be rescued by injecting M2-like but not M1-like macrophages. Further in vitro experiments indicated that M2-like macrophages control vascular permeability by regulating phosphorylation of VE-cadherin (He et al., 2016). Using single-cell sequencing, Chakarov et al. (2019) recently described a subset of tissue-resident macrophages that

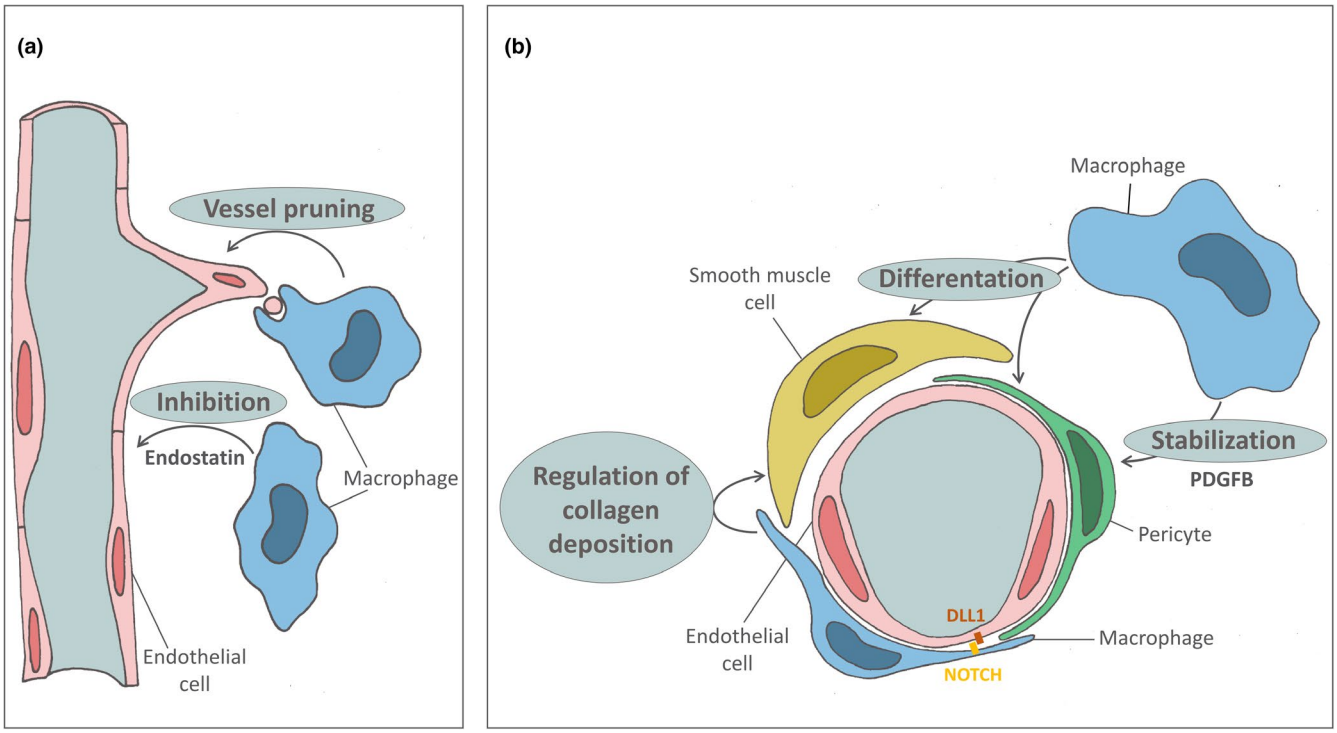

FIGURE 2 Macrophage functions in vascular anti-angiogenesis (a) and in maturation (b). (a): Macrophages can facilitate vessel pruning, by phagocytising endothelial apoptotic bodies from regressing vascular branches. Macrophages can also block angiogenesis by paracrine secretion of anti-angiogenic growth factors. (b) Macrophages secrete growth factors that enhance recruitment of pericytes and smooth muscle cells to the vasculature. Macrophages can also directly differentiate to pericytes or perivascular smooth muscle cells. Perivascular macrophages interact with smooth muscle cells to regulate the amount of collagen that is deposited by the perivascular smooth muscle cells. Endothelial cells directly communicate with perivascular macrophages by binding of the DLL1 to the macrophage NOTCH resulting in a pro-arteriogenic phenotype of the perivascular macrophages

are located near blood vessels. These macrophages, which were characterized as Lyve1^{hi}MHCII^{lo}Cx3cr1^{lo}, reduced vascular permeability as selective depletion of this macrophages subset in vivo exacerbated vascular leakage and immune cell infiltration (Figure 2b) (Chakarov et al., 2019).

3.6 | Arteriogenesis

Macrophages have been found to be important during the process of arteriogenesis. Using a mouse hindlimb ischaemia model, Hamm et al. (2013) and Patel et al. (2013) observed that TIE2-expressing macrophages are indispensable for arteriogenesis, but not for initial angiogenesis in the ischemic tissue. Specific depletion of TIE2-expressing macrophages led to a reduced collateral vessel formation and aggravated ischaemia, leading to increased hypoxia. The effect of the depletion was not due to a lower number of microvessels, indicating that the TIE2-expressing macrophages with an M2-like phenotype (MRC1+) were required for vascular maturation rather than for the formation of new blood vessels. Interestingly, the TIE2 agonist, ANGPT1 was required to induce the pro-arteriogenic phenotype on the macrophages (Hamm et al., 2013; Patel et al., 2013). Further studies have shown that macrophages are regulated by on-site stimuli to functionally change to pro-arteriogenic macrophages with an anti-inflammatory phenotype (Avraham-Davidi et al., 2013; Krishnasamy et al., 2017). Indeed, Seaman, Cao, Campbell, and Peirce (2016) reported that M2-type macrophages (CD68^{+}MRC1^{+}) are required for reperfusion of ischemic fat pads after arterial ligation in mice. In this model, reperfusion was not caused by enhanced angiogenesis, but by the enlargement of collateral vessels, indicating a role for M2 type macrophages in arteriogenesis (Seaman et al., 2016). Interestingly, ECs can directly regulate macrophage maturation to pro-arteriogenic macrophages in a process involving canonical NOTCH signalling through binding of the endothelial DLL1 on the macrophage NOTCH receptor (Krishnasamy et al., 2017). A recent study by Lim et al. (2018) showed that Lyve1^{+} macrophages in the arterial wall maintain vessel homeostasis and arterial tone by regulating the collagen deposition of the vascular smooth muscle cells. This regulation requires a direct interaction between the macrophages and the smooth muscle cells through engagement of Lyve1 to the hyaluronan pericellular matrix of the smooth muscle cells (Lim et al., 2018).

3.7 | Anti-angiogenesis

Apart from stimulating microvascular growth and maturation, macrophages can also inhibit angiogenesis. Macrophages can secrete anti-angiogenic growth factors, inhibiting angiogenesis in a paracrine way (Corliss et al., 2016) (Figure 2a). In addition,

macrophages are able to actively phagocytize apoptotic ECs (Defalco et al., 2014; Gurevich et al., 2018). But it remains an issue of debate whether this is a common mechanism for EC apoptosis (Kochhan et al., 2013).

Schif-Zuck et al. (2011) characterized a subset of pro-resolving macrophages that express neither iNOS nor Arg1 (M2 markers), but low levels of the M1 enzymes, COX2 (also known as MT-CO2) and MMP9 in mice. These CD11b^{low} (CD11b is also known as Itgam) macrophages secrete neither pro-inflammatory cytokines nor IL10, but high levels of TGFB1. These macrophages were polarized after reaching a threshold of apoptotic body engulfment, termed satiated efferocytosis (Schif-Zuck et al., 2011). Further studies revealed that in vitro generated CD11b^{low} macrophages inhibit angiogenesis by releasing endostatin and by expressing lower levels of VEGFA compared to control macrophages (Michaeli et al., 2018). A recent study by Ganta, Choi, Farber, and Annex (2019) in a mouse hindlimb ischaemia model showed that macrophages can adopt an anti-angiogenic phenotype after VEGF$_{165}$b binding on the macrophage VEGF receptor 1 (VEGFR1 or Flt1), which inhibited ischemic muscle neovascularization in a paracrine manner (Ganta et al., 2019). In this study, the macrophages were classified as M1 macrophages (Cd80^{high} Arg1^{low}).

4 | ANGPT-TIE SIGNALLING IN MACROPHAGES

A large fraction of angiogenic macrophages is characterized by the expression of the TIE2 receptor (also called TEK or CD202B) (Coffelt et al., 2010). The TIE2 receptor forms a major part of the so-called angiopoietin–TIE system, which is an important pathway during angiogenesis, vessel homeostasis and tissue repair. Dysregulation of the ANGPT-TIE system is frequently found in pathological conditions, such as tumorigenesis and inflammation (Saharinen, Eklund, & Alitalo, 2017). To this date, the ANGPT-TIE system has mainly been studies in ECs as TIE receptors were originally believed to be specific to ECs. However, TIE receptors are also expressed on hematopoietic cells such as monocytes and macrophages, indicating that in these cells the ANGPT-TIE system is also active (De Palma & Naldini, 2011).

4.1 | The ANGPT-TIE pathway in endothelial cells

The ANGPT-TIE system is composed of two receptors (TIE2 and TIE1) and three ligands of the angiopoietin family (ANGPT1, ANGPT2 and ANGPT4). TIE2 activation is mediated by ANGPT1 and leads to vascular stabilization and cell survival. Angiopoietin 2 (ANGPT2) acts as a context-dependent antagonist of ANGPT1 and induces endothelial activation (Garcia et al., 2014). ANGPT4, the human orthologue of mouse ANGPT3, is far less studied than ANGPT1 and ANGPT2. Although the function of ANGPT4 remains to be elucidated, recent in vitro characterization experiments reveal that ANGPT4 is able to exert similar functions as ANGPT1 (Elamaa et al., 2018).

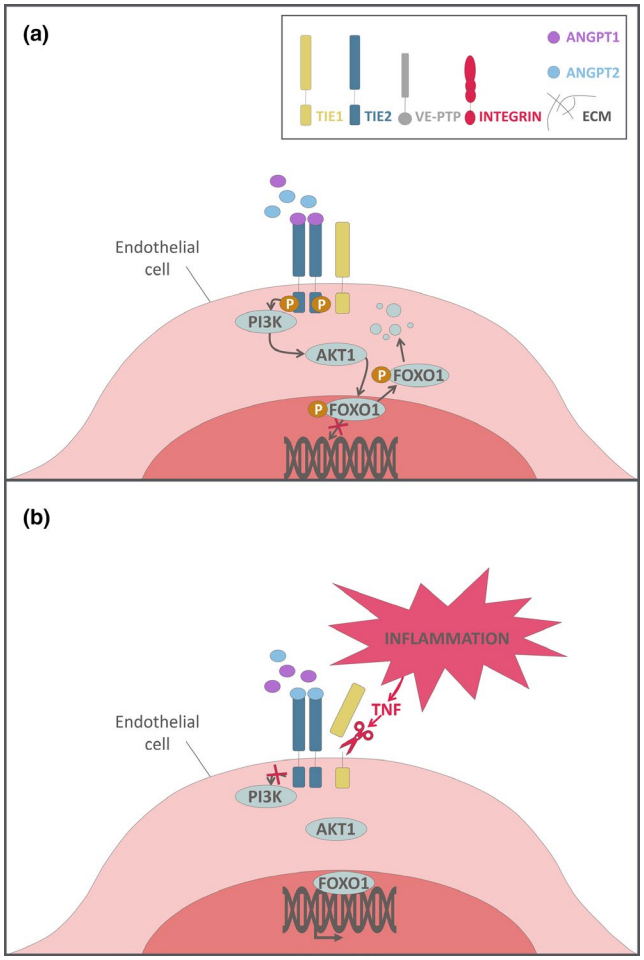

FIGURE 3 Angiopoietin–TIE signalling in endothelial cells in homeostasis (a) versus inflammation (b). (a) In homeostasis binding of ANGPT1 to the TIE2 receptor results in the activation of the AKT1 pathway via PI3K (also known as PIK3CB). This pathway will phosphorylate FOXO1, which triggers the nuclear exclusion and subsequent degradation of this transcription factor. (b) In inflammation, when TNF is released, the ectodomain of the TIE1 receptor is cleaved. As a result, ANGPT2 will act as an antagonist instead of an agonist. As the antagonistic activity of ANGPT2 dominates the agonist activity of ANGPT1, the TIE2 receptor will not be phosphorylated. Therefore, the AKT1 pathway is not activated and FOXO1 is not phosphorylated. Because of this, FOXO1 can perform his function as transcription factor resulting in the expression of genes associated with blood vessel destabilization and apoptosis

TIE1 is considered to be an orphan receptor as no ligands have been identified yet (Mueller & Kontos, 2016). However, recent data indicate that TIE1 is an important mediator of the agonist/antagonist action of ANGPT2 (Eklund et al., 2017).

4.1.1 | ANGPT-TIE signalling during homeostasis versus inflammation

During vascular homeostasis, ANGPT1-mediated TIE2 activation induces vascular quiescence and vessel maturation through inhibition

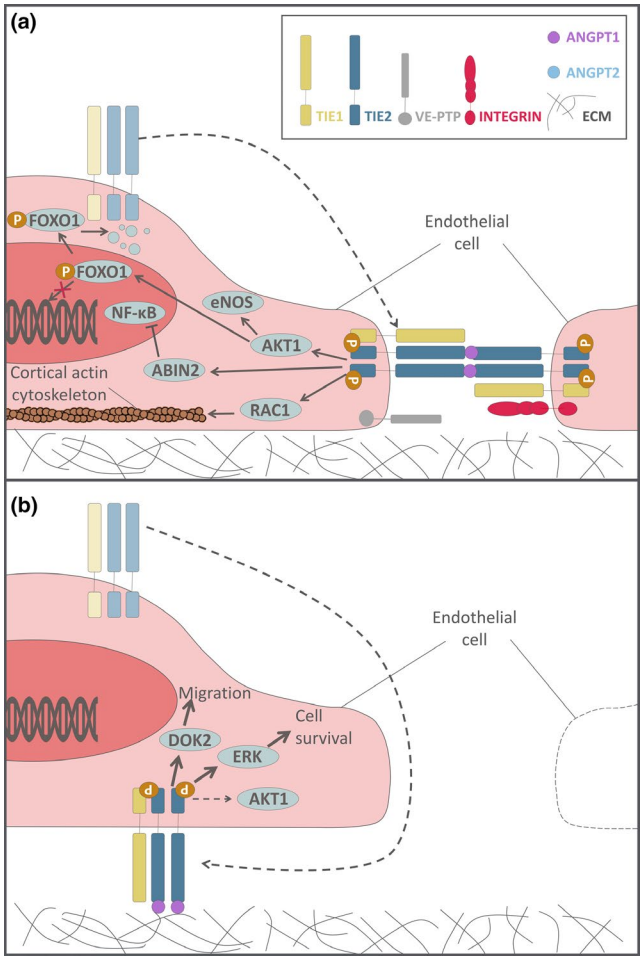

FIGURE 4 Angiopoietin–TIE signalling in cell–cell contacts (a) versus cell-matrix contacts (b). (a) When cell–cell contacts are present, binding of ANGPT1 to the TIE2 receptor will cause translocation of the receptor complex to the cell–cell junction. This complex depends on α5β1 integrin and consists of TIE1 and TIE2 receptors from opposing cells that associate with each other *in trans*. ANGPT1-TIE2 can also recruit the enzyme VE-PTP, which is able to dephosphorylate the TIE2 receptor resulting in a higher vascular permeability. Upon TIE2 phosphorylation, AKT1 will be activated causing the degradation of FOXO1 and the production of eNOS (also called NOS3). Besides the AKT1 pathway, other pathways promoting vessel stabilization will also be activated such as the ABIN2 pathway and RAC1 pathway. The ABIN2 pathway is able to inhibit the function of NF-κB, a regulator of endothelial inflammatory responses. The RAC1 pathway on the other hand, promotes stabilization of the cortical actin cytoskeleton. (b) In the absence of cell–cell contact, the TIE receptors will form a complex with extracellular matrix-bound ANGPT1 at cell-matrix contacts. These TIE receptor complexes *in cis* favour other pathways than the TIE receptor complexes *in trans*. For example, the AKT1 pathway becomes less important in *in cis* complexes, while on the other hand the ERK pathway (involved in cell survival) is triggered more. Also, the DOK2 pathway, involved in cell migration, is typically activated in the absence of cell–cell contacts

of the FOXO1 pathway (Figure 3a) (Daly et al., 2004). FOXO1 is a transcription factor that modulates the expression of genes associated with blood vessel destabilization and apoptosis (Daly et al., 2004). In the presence of TIE1, ANGPT2 will also activate TIE2 and has a similar agonist role as ANGPT1 stimulation (Korhonen et al., 2016). However, when TNF is released during inflammation, the TIE1 receptor is cleaved, decreasing ANGPT2-mediated TIE2 activation. In this context, ANGPT2 will act as an antagonist dominating the agonistic activity of ANGPT1 which will lead to vascular destabilization (Figure 3b) (Kim et al., 2016).

4.1.2 | ANGPT-TIE signalling in quiescent versus migrating endothelial cells

Cell–cell contacts are very important in defining the outcome of TIE2 signalling. In the quiescent vasculature where ECs are in close contact with each other, ANGPT1 stimulates the TIE2 receptor to translocate to endothelial cell–cell junctions and form TIE receptor signalling complexes *in trans* leading to vascular stabilization (Figure 4a) (Eklund et al., 2017; Korhonen et al., 2016; Saharinen et al., 2008). This is in contrast with the situation where ECs are not tightly surrounded by other ECs, for example in sparsely seeded EC cultures used as a model for migratory endothelial cells (Figure 4b). In these cultures, ANGPT1 induces a migratory cell phenotype and the TIE2 receptor translocates to the free cell margin in the cell rear. In the cell rear, TIE2 would be important for endothelial cell polarization as response to a migration-promoting growth factor (Saharinen et al., 2008). This hypothesis was based on the finding that $TIE2^{-/-}$ endothelial cancer cells suffered from an impaired cell rear polarization. In the same study, it was also found that ANGPT1 bound to extracellular matrix will cause translocation of TIE2 to the cell–matrix contacts *in a cis* association, which further supports endothelial migration (Figure 4b) (Korhonen et al., 2016; Saharinen et al., 2008).

TIE receptor signalling complexes *in trans* consist of TIE2 and TIE1, but also include VE-PTP (or PTPRB) and depend on α5β1 integrin (Korhonen et al., 2016; Saharinen et al., 2008). Binding of ANGPT1 to TIE2 in *trans* complexes will induces a downstream signalling pathway via the serine kinase AKT1, which will lead to an inhibition of FOXO1 (Daly et al., 2004). Moreover, AKT1 also activates eNOS (or NOS3), known for his vascular protective functions (Saharinen et al., 2017). Other important pathways that become activated by phosphorylated TIE2 are the RAC1 and the ABIN2 pathway (also called TNIP2 pathway). RAC1 signalling will lead to stabilization of the endothelial cortical actin cytoskeleton. ABIN2 activation inhibits the function of the transcription factor NF-κB, an important regulator of inflammatory responses in ECs (Figure 4a) (Saharinen et al., 2017).

In signalling complexes *in cis*, different pathways become dominant when TIE2 is activated. The pathway initiated by the phosphorylation of AKT1 becomes less important while the ERK pathway (also known as MAPK1 pathway), which is involved in cell survival, is triggered more strongly (Fukuhara et al., 2008). Additionally, the DOK2 pathway, which is responsible for the regulation of cell shape and migration, becomes activated as well (Figure 4b) (Master et al.,

2001). This difference in gene expression profiles between vascular ECs in the presence or absence of cell–cell contacts implies that downstream signalling is defined by the spatial localization of the TIE2 receptor. This could partially explain the versatile functions of angiopoietins during vessel quiescence and remodelling (Eklund et al., 2017).

4.2 | TIE2 expressing macrophages

TIE2 expressing monocytes/macrophages (TEMs) were first described in mouse tumour models where they were found to promote angiogenesis (De Palma et al., 2005; De Palma, Venneri, Roca, & Naldini, 2003). Since then, TEMs have been extensively studied in cancer-related research. However, there is also increasing evidence of TEMs being important mediators in wound healing as well as non-pathological angiogenesis by interacting with ECs (Baer, Squadrito, Iruela-Arispe, & De Palma, 2013).

4.2.1 | Angiogenic capacities of TEMs

Multiple in vitro and in vivo studies have revealed that TEMs are strongly pro-angiogenic. The macrophages facilitating tip cell anastomosis as described earlier were identified as TEMs (Fantin et al., 2010). TEMs also accelerate collateral vessel formation and revascularization when delivered into ischemic hindlimbs of mice (Patel et al., 2013). Furthermore, in vitro angiogenic assays with TIE2- monocytes reveal increased sprouting and endothelial tubule formation (Coffelt et al., 2010). Gene expression analysis also supports the angiogenic phenotype of TEMs. Quantitative real-time PCR revealed that non-polarized TEMs express higher levels of pro-angiogenic genes such as MMP9, VEGFA, COX2 (or MT-CO2) and WNT5A than $TIE2^-$ monocytes (Coffelt et al., 2010). Interestingly, the same study revealed that expression of the pro-angiogenic enzymes thymidine phosphorylase and cathepsin B was enhanced when TEMs were exposed to ANGPT2, which triggers the question whether stimulation of TIE2 with ANGPTs could alter macrophage polarization. However, flow cytometry on in vitro differentiated macrophages originated from human donor blood revealed that exposure to either ANGPT1 or ANGPT2 did not influence the expression of CD16 (or FCGR3A), CD64 (or FCGR1A), CD163 or CD200R1. This opposed to classical polarizing cytokines such as IL10, IL4 and IFNG (Garcia et al., 2014).

4.2.2 | TEMs along the angiogenic cascade

As described earlier, macrophages are an extremely plastic group of cells which perform diverse functions during the angiogenic cascade. This raises the question if these phenotypes are also linked to TIE2 expression and if TEMs are involved in all the steps of the angiogenic cascade.

Initially, TEMs were described in tumour models where they play an essential role in neovascularization as experimental TIE2 knockout completely prevented human glioma neovascularization in the mouse brain (De Palma et al., 2005). The recruitment of TEMs to the tumours is probably due to the expression of ANGPT2 by the activated EC. In vitro migration assays revealed that TEMs isolated from human blood migrated towards ANPGT2, suggesting a homing mechanism for TEMs to tumours (Venneri et al., 2007). Pucci et al., (2009) found that the TEMs recruited to tumours in an experimental mouse model had an enhanced expression of scavenger receptors (MRC1 and CD163) and a downregulation of inflammatory mediators (IL1B, TNF, CXCL10 and IL12A) indicative for an M2 phenotype (Pucci et al., 2009). These initial data from cancer research indicated that TEMs are primarily M2 type macrophages and that they mainly play a role in the initiation of angiogenesis.

TEMs have also been found to play a role in arteriogenesis in studies investigating vascular stenosis (Hamm et al., 2013) and critical limb ischaemia (Patel et al., 2013). These arteriogenic TEMs are also believed to have an M2 phenotype as they express MRC1 (Hamm et al., 2013; Patel et al., 2013). For example, depletion of TEMs in a mouse hindlimb ischaemia model reduced the formation of collateral vessels and therefore aggravates the ischaemia (Hamm et al., 2013). Interestingly, expression of the TIE2 receptor is not restricted to M2 macrophages as flow cytometry revealed that pro-inflammatory M1 macrophages generated by in vitro polarization with IFNG also express TIE2 (Garcia et al., 2014).

4.2.3 | TIE2 signalling in TEMs

Only few studies investigated which pathways could be triggered when TIE2 is activated in TEMs. In vitro experiments by García et al. (2014) revealed that binding of ANGPT1 or ANGPT2 leads to TIE2-induced activation of the JAK-STAT pathway in human macrophages differentiated in the presence of IFNG or IL10. This in turn triggered enhanced production of pro-inflammatory cytokines such as CXCL3, CXCL5, CXCL8, IL6 and IL12B, especially when co-stimulated with TNF. Activation of STAT3 and STAT5 has been described in ECs downstream of TIE2 phosphorylation, although this activation is rather weak (Korpelainen, Karkkainen, Gunji, Vikkula, & Alitalo, 1999).

In another study performed on THP-1 cells (a human monocyte cell line), Western blot analysis revealed a stronger and faster phosphorylation of p38 (also known as MAPK14) and ERK after ANGPT1 stimulation compared to ANGPT2 stimulation (Seok et al., 2013). Interestingly, in contrast with the situation in ECs, ANGPT1 nor ANGPT2 stimulation managed to activate the AKT1 pathway in these THP-1 cells (Seok et al., 2013). The results of this study are in contrast with the study performed by Chen and colleagues, which describes AKT1 pathway activation by stimulating the TIE2 transfected RAW264.7 cells (mouse macrophages cell line) with ANGPT2 but not the ERK pathway (Chen et al., 2016).

4.2.4 | TIE receptors in EC versus TEMs

Because of the presence of TIE2 on both EC and macrophages, it is tempting to extrapolate the knowledge obtained from research in EC to macrophages. For example, the fact that ECs are able to form ANGPT-TIE complexes at interendothelial junctions raises the question if similar complexes can be established between TEMs and ECs. It has already been shown that ANGPT2 secreted by ECs may act as a chemoattractant to recruit TEMs to malignant tumours and sites of inflammation (Murdoch, Tazzyman, Webster, & Lewis, 2007). CXCL12/CXCR4 signalling may also be involved in mediating interactions between TEMs and ECs (Grunewald et al., 2006). Moreover, TIE1 can also be expressed by macrophages (Garcia et al., 2014), which raises the question whether this receptor is present in a sufficient amount to influence the outcome of ANGPT2 binding to TIE2 as seen in ECs. If TIE complexes can be formed between TEMs and ECs, it would be very interesting to investigate the downstream signalling cascades.

5 | CONCLUSION AND FUTURE PERSPECTIVES

Macrophages form an interesting target for therapies that aim to enhance, block or normalize angiogenesis. Considering the diverse and sometimes opposite roles of these cells on blood vessel formation and stabilization, it is clear that macrophages are hyperplastic cells. It will be crucial to link these different roles with specific macrophage phenotypes. Considering the discussed research, M1-like macrophages seem to be involved primarily in the formation of new blood vessels and vessel degeneration while vascular stabilization and maturation is facilitated by M2-like macrophages. However, this simple model does not hold in all cases, as some reports indicate that M2-like macrophages are also important in inducing the formation of new blood vessels (Ganta et al., 2017). This is also observed in cancer, where the TIE2 expressing subset of tumour-associated macrophages is essential for sprouting angiogenesis to the tumour (De Palma, Biziato, & Petrova, 2017). Of course, the simple binary M1–M2 division of macrophages is clearly not sufficient to describe the diverse phenotypes that macrophages can adopt. Moreover, different studies use different sets of markers to identify the phenotype of macrophages and sometimes there is information lacking about how the macrophages were cultured and polarized (Murray, 2017). Using novel methods, such as single-cell sequencing, future research will enable a more comprehensible characterization of the macrophage phenotypes (Chakarov et al., 2019).

As mentioned before, little is known about ANGPT-TIE signalling in TEMs. Future studies should be conducted to investigate the downstream signalling pathways that follow TIE2 activation. Studying the interactions between ECs and TEMs, will probably also lead to very interesting insights.

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