Role of Proangiogenic Factors in Immunopathogenesis of Multiple Sclerosis: A Systematic Review

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ABSTRACT

Angiogenesis is a complex and balanced process in which new blood vessels form from preexisting ones by sprouting, splitting, growth and remodeling. This phenomenon plays a vital role in many physiological and pathological processes.

However, the disturbance in physiological process can play a role in pathogenesis of some chronic inflammatory diseases, including multiple sclerosis (MS) in human and its animal model. Although the relation between abnormal blood vessels and MS lesions was established in previous studies, but the role of pathological angiogenesis remains unclear.

In this study, the link between proangiogenic factors and multiple sclerosis pathogenesis was examined by conducting a systematic review. Thus we searched the English medical literature via PubMed, ISI web of knowledge, Medline and virtual health library (VHL) databases. In this review, we describe direct and indirect roles of some proangiogenic factors in MS pathogenesis and report the association of these factors with pathological and inflammatory angiogenesis.

Keywords: Angiogenesis inducing agent; Blood-brain barrier; Experimental autoimmune encephalomyelitis; Endothelial cells; Extracellular matrix; Matrix metalloproteinase; Multiple sclerosis; Vascular endothelial growth factor A

INTRODUCTION

Angiogenesis is a complex and finely balanced process that consists of the formation of new blood vessels from the pre-existing ones such as capillaries and post-capillary venules. Angiogenesis plays a pivotal role during embryonic development and later; in adult life; in several physiological and pathological conditions.[1]

Under physiological conditions, angiogenesis depends on the tight balance of pro-angiogenic and anti-angiogenic factors.[2] Moreover, in normal tissues, vascular inactivity is maintained by the dominant influence of endogenous anti-angiogenic over pro-angiogenic stimuli.[3] However, disturbance of the

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mechanisms of physiological angiogenesis can play a role in pathogenesis of some diseases as a result of over proliferation of blood vessels as in cancers, arthritis, asthma, atherosclerosis, etc., or impaired angiogenesis as in diseases such as heart and brain ischemia, neurodegeneration, hypertension etc.[4] On the other hand, the newly formed blood vessels contribute to the perpetuation of inflammation by supporting the migration of inflammatory cells to the site of inflammation.[5] Angiogenesis is commonly found in chronic inflammatory diseases such as multiple sclerosis (MS), although the relation between abnormal blood vessels and MS lesions was established in previous studies, but its role remains unclear.[6] In addition, evidence from collecting data indicates that angiogenesis may have an effect in the pathophysiology of MS and its animal model of experimental autoimmune encephalomyelitis (EAE), similar to that observed in chronic inflammatory diseases of peripheral organs.[7]

MS and EAE are inflammatory demyelinating diseases of the central nervous system (CNS), associated with axonal and oligodendrocytes damage. It was suggested that both environmental and multiple genetic factors interplay results in the lesions characteristic of MS.[8] In both MS and EAE, lesions contain T cells, macrophages and activated glia, which can produce proangiogenic factors; these factors can play a potential role in pathological and inflammatory angiogenesis which will favor exacerbation of MS and EAE. Recognition of the important contribution of angiogenesis in MS progression has led us to focus on proangiogenic factors. This review describes the role of proangiogenic factors in immunopathogenesis of MS with emphasis on some parameters such as hypoxia [Hypoxia-inducible Factors-1α (HIFs)], immune cells (Macrophages), growth factors [vascular endothelial growth factor (VEGF)], proteinases [Matrix metalloproteases (MMPs)] and proangiogenic cytokines [Interleukin (IL)-1 and -8]. This review also attempts to associate these factors with pathological and/or inflammatory conditions that exacerbate MS suggesting that these factors could be a potential therapeutic target in both prevention and treatment of MS.

METHODS

To conduct this systematic review, the English medical literature in PubMed, ISI web of knowledge, Medline and virtual health library (VHL) databases was searched, with no restriction regarding year of publication. The following terms; Multiple Sclerosis, angiogenesis, angiogenic factors, VEGF, MMPs, proangiogenic cytokines, immune cells, pathological angiogenesis or inflammatory angiogenesis were used. We also reviewed bibliographies, searched the Science Citation Index Expanded database and searched for studies on the link between proangiogenic factors and MS pathogenesis. Relevant studies were identified, selected and combined to find the link or association between some proangiogenic factors and MS pathogenesis.
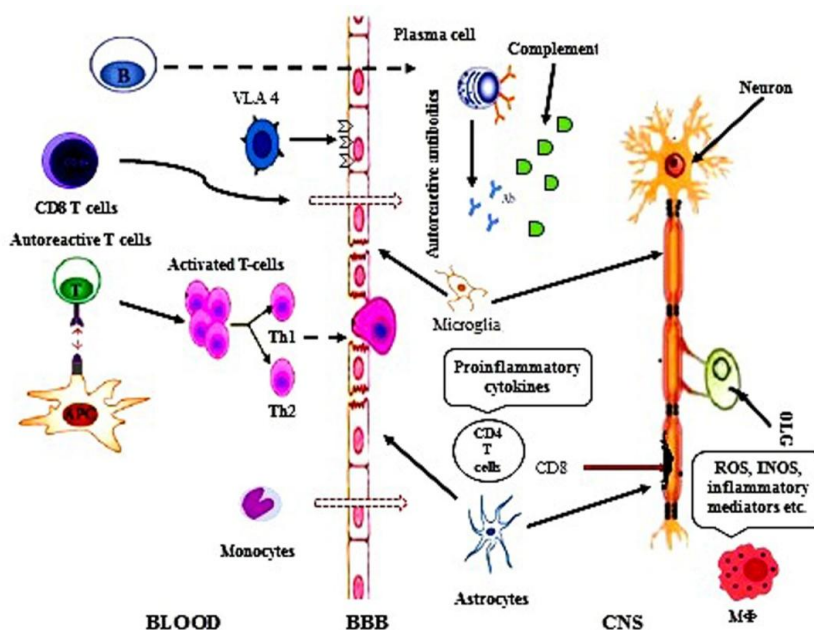
MS Pathogenesis

The pathological features of MS plaques are blood-brain barrier (BBB) leakage, destruction of myelin sheaths, oligodendrocyte damage and cell death, axonal damage, glial scar formation and the presence of inflammatory infiltrates that mainly consist of lymphocytes and macrophages. The inflammatory lesions are characterized by high infiltration of various populations of cellular and soluble mediators of the immune system, such as T cells, B cells, macrophages and microglia, as well as a broad range of cytokines, chemokines, antibodies, complement and toxic substances[9] (Figure 1). Moreover, MS lesions often develop along the blood vessels and alterations in BBB structure and function, in addition to changes in the basement membrane, are regarded as pathological features.[10] However, despite significant progress in the existing knowledge on the pathogenesis of MS, exact details of the inflammatory cascade remain unclear.[11]

At blood; infectious or chemotactic agents stimulate the activation of autoreactive T and B cells. The activated cells proliferate and differentiate before binding to BBB through the expression of very late antigen 4 (VLA4).

At BBB; the binding of autoreactive cells to BBB leads to disruption of the barrier, allowing the cells to infiltrate into the CNS.

At CNS; the autoreactive cells in CNS recognize myelin sheath as non-self as such mounting both humoral and cellular immune responses which may lead to the destruction of the axon and oligodendrocytes, thus affecting the neuronal function. CD4$^+$ T cells secrete proinflammatory cytokines to activate astrocytes and microglia, thus exacerbating inflammatory condition. CD8$^+$ T cells directly attack

Figure 1: the contribution of various cells in MS immunopathogenesis.

the axon which may lead to axonal damage and loss. Autoreactive antibodies attack the myelin sheath and activate complement. Other cells, such as astrocytes secrete MMPs and proinflammatory mediators to further degrade the barrier and attract more immune cells thus attacking the myelin sheath. Microglial secretion also attack barrier and the myelin sheath.

Macrophages become activated to secrete ROS, inducible nitric oxide synthase (iNOS) and inflammatory mediators for further destruction of the myelin sheath and oligodendrocytes. These actions lead to inflammation and demyelination in the CNS.

Angiogenesis and an Overview on Its Mechanism

Angiogenesis is a complex multistep process which results in stimulation, proliferation and migration of endothelial cells (ECs).[2] Vessel formation and growth are highly orchestrated processes involving numerous growth factors, chemokines, proteases, and inflammatory cells that play different roles in promoting and refining t.[12] Angiogenesis consists of three stages: the selection of "tip cells" to begin angiogenic expanding. The "tip cells" are EC found inside blood capillaries[4] and react specifically to the angiogenic factor VEGF-A. The VEGF-A empowers the cells for invasion and migration. The interactions between VEGF-A and ECs activate the expression of

transmembrane ligands called delta like ligands (DLL4) and their heterodimeric notch family receptor proteins[13] which control the selection of the "tip cells".[14] The second stage is mediated by interaction of VEGF-A and vascular endothelial growth factor receptor 2 (VEGFR-2), which consists of migration, proliferation of EC and tube formation.[15] The last stage is the maturation of newly formed vessels, inhibition of endothelial proliferation, migration of new capillaries and fusion of the newly formed vessels with others, in addition, pericytes and vascular smooth muscle cells also have an impact on this ligation[16] (Figure 2). Moreover, the transmembrane protein, platelet-derived growth factor β (PDGFB) and its receptor, platelet-derived growth factor receptor (PDGFR-B) mediate the role of the pericytes in the formation of walls of newly formed vessels.[17] Similar to VEGFR molecules, PDGFRs in their intracellular region contain a tyrosine kinase domain, but in contrast to VEGFR receptors, their extracellular region does not consist of seven passes.[18]

Proangiogenic Factors

Angiogenesis is mediated by the balance and interplay between numerous "pro- and anti-angiogenic" factors[19] within the perivascular and vascular microenvironment and requires the functional activities of a number of molecules, including growth factors and

their receptors, extracellular matrix proteins, adhesion molecules and proteolytic enzymes.[3] Although VEGF is a main angiogenic growth factor, numerous other proangiogenic factors exist, such as fibroblast growth factor (FGF), angiopoietin, tumor necrosis factor (TNF), and transforming growth factor (TGF). Angiogenic factors such as MMPs and VEGF breakdown vascular basement membrane and BBB, this action permits the immune cells to infiltrate into the CNS parenchyma in EAE and MS[20] (Figure 2). Both innate and adaptive immune cells are involved in the mechanisms of EC proliferation, migration and activation, through the production and release of a large spectrum of pro-angiogenic mediators. These cells may create the specific microenvironment that favors an increased rate of tissue vascularization.[21] Moreover, Angiopoietin-2 (Ang-2) increases in neurons, glia and inflammatory cells during EAE.[22, 23]

Hypoxia via activation of HIFs induces various proangiogenic factors to enhance the angiogenesis sequence. Endothelial progenitor cells (EPCs) derived from bone marrow serve as precursors of ECs. Proangiogenic growth factors such as VEGF activate receptors on ECs present in peripheral circulation and pre-existing blood vessels (local ECs). VEGF induces proliferation and migration of the ECs. The activated ECs begin to release proteases such as MMPs and COX. The MMPs degrade basement membrane and COX is associated with the release of inflammatory cytokines, chemokines, etc. The ECs then proliferate into the surrounding matrix and form solid sprouts connecting neighboring vessels. As sprouts extend toward the source of the angiogenic stimulus, ECs migrate in tandem, using adhesion molecules called integrins, thus increasing the rate of sprout elongation which enables new vessels to grow across gaps in the vasculature. Integrins are expressed by ECs, facilitating their adhesion to the ECM and their migration for tube formation. Immune cells are involved in secretion and expression of proangiogenic factors such as growth factors, proteases, cytokines and soluble mediators to activate angiogenesis. Cytokines such TNF-α and IL-1 and -8 are involved in modulation, induction, upregulation of proangiogenic and inflammatory mediators. Ang-1 and TGF-β cause vessel stabilization, although TGF-β shows opposite effect in some contexts.

Proangiogenic Factors in MS Immunopathogenesis

The pathological angiogenesis is often induced to certain extent by inflammation because macrophages, platelets, mast cells and other leukocytes are
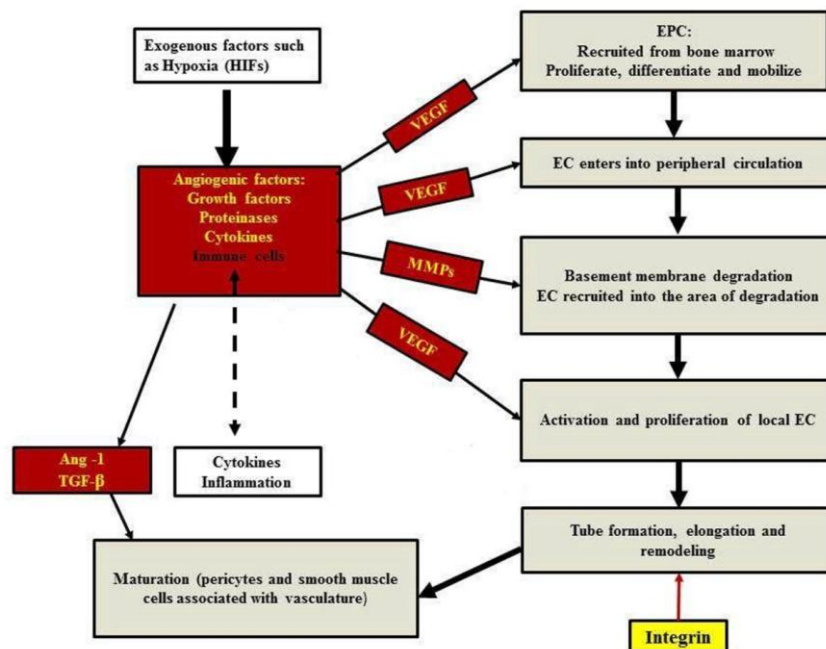

Figure 2. Stepwise mechanism of angiogenesis

'chemoattracted' to the sites of inflammation partly by proangiogenic factors, that in turn, attract endothelial and smooth muscle cells, fibroblasts, leukocytes or platelets.[24] The factors involved in the pathogenesis of MS have been shown to act either directly or indirectly to support angiogenesis. MMP -1, -2, -3 and -9, intercellular cell adhesion molecule (ICAM) -1, vascular cell adhesion molecule (VCAM) -1 and E-selectin are implicated to act in MS pathogenesis by facilitating the entry of mononuclear cells through the BBB in the MS.[8] Proinflammatory cytokines such as Interferon (IFN) -γ and TNF-α /-β are capable of improving angiogenesis in MS, while IFN-α and –β are anti-angiogenic.[25] A study reported an increased levels of Nitric oxide (NO) in MS patients and this correlated well with clinical and MRI markers of disease progression.[26] Furthermore, increased NO contributes both directly and indirectly to angiogenesis in inflammatory and vascular diseases.[27] Also endothelin-1 (ET-1), a signal peptide, that induces angiogenesis in cultured ECs and stimulates neovascularization in concert with VEGF, has been reported to be significantly raised in MS patients[28] and an ET-1 receptor antagonist was shown to improve acute EAE.[29] Additionally, Pertussis toxin injection was also reported to be associated with exacerbation of EAE, possibly due to the elevation of angiogenic factors that lead to BBB breakdown.[30] A study by Holley and colleagues demonstrated an increase in blood vessel density in MS lesions compared to normal controls[10] and increased proliferation of ECs within these blood vessels. Together, these data suggest that angiogenesis is occurring in EAE and MS.[31] In MS lesions and surrounding normal appearing white matter and grey matter, an angiogenic response is associated with disease progression[32] or otherwise in remission after relapses.[33] Moreover, several studies reported increased angiogenesis, severe inflammation and activated VEGF signalling in inflamed lesions.[6,34] In EAE, histological examination has demonstrated an increased density of blood vessels in areas of inflammation.[35] Moreover, VEGF also enhances at inflammatory sites during EAE and MS and infusion of VEGF worsens clinical scores during EAE.[36] Indeed, an injection of VEGF alone into the CNS of naive rats could induce inflammation and angiogenesis.[37] There was also an increase in serum VEGF in MS patients in relapse compared to healthy controls or MS patients in remission.[31]

Direct and Indirect Roles of Proangiogenic Factors in MS

Hypoxia

In response to hypoxia, the transcription factor HIF-1α activates hundreds of genes[38] including those of VEGF. On the other hand, HIF-1β, HIF-1α and HIF-2α induce the expression of the following mediators: VEGF, VEGFR1, VEGFR2, neuropilin-1, angiopoietin-2 (Ang2), nitric oxide synthase, TGFβ-1, PDGF-β, ET-1, IL- 8, insulin-like growth factor (IGF-II), Tie1 and cyclooxygenase-2.[39]

Furthermore, HIF-1α upregulates MMP-2 ,thus mediate the migration and activation of ECs, which leads to the degradation of extracellular matrix (ECM).[40] It also enhances the formation of endothelial tubes, in vitro; this effect is due to EC expression of HIF-1α.[41] In EAE, HIF-1α increases with other genes relevant in cell migration across the BBB[34,] thus leading to increased angiogenic response. Hypoxia modulates notch signaling via HIF-1α direct binding to the notch intracellular domain (NICD) increasing its transcriptional activity and regulating vessel branching[42] (Table 1). In addition, small mothers against decapentaplegic homolog 3 (Smad3) and HIF-1α cooperate with TGF-β to induce VEGF transcription in humans[43], thus supporting angiogenic responses.

Immune Cells

Immune cells synthesize and secrete proangiogenic factors during inflammatory responses to support neovascularization[44]. Leukocytes require proteolytic mechanisms for migration across basement membrane and through the CNS; this action is mediated by MMPs, which are upregulated under inflammatory conditions [44], (Table 1).

Neutrophils produce various soluble mediators which serve as activators of angiogenesis. These mediators include VEGF, hepatocyte growth factor (HGF), MMPs, IL-8 and TNF-α.[45] Neutrophils produce and release high levels of MMP-9. In contrast, neutrophils secrete little, if any, MMP-2.[46] Eosinophils produce many proangiogenic cytokines, angiogenin[47] and growth factors, and are regarded as pro-angiogenic. The effect of basophils in angiogenesis and inflammation is associated with their ability to express mRNA of several isoforms of VEGF, such as three isoforms of VEGF-A (121, 165 and 189) and two isoforms of VEGF-B (167 and 186).[48] Basophils also express VEGFR-2 and neuropilin-1. The neuropilin-1

acts as co-receptor for VEGFR-2 and increases VEGFR-2-induced responses.[49] In addition, basophils in peripheral blood infiltrate the sites of chronic inflammation containing VEGF-A in their secretory granules.[21] Besides, basophils release histamine, which displays angiogenic activity in several in vitro and in vivo settings.[49]

Macrophages play a key role in inflammation and angiogenesis. The angiogenic activity of macrophages is known to be associated with their secretory properties. Macrophages produce several pro-angiogenic cytokines as well as ECM-degrading enzymes.[50] Macrophages and T cells produce pro-angiogenic cytokines such as TNF, IL-8, TGF-β [51] and growth factors, such as VEGF, [52] PDGF, and fibroblast growth factor (FGF-2). These cells also express a wide range of angiogenesis-modulating enzymes, including MMP-2, -7, -9, -12, and cycloxygenase-2 (COX-2).[53] Macrophages produce high levels of many MMPs after interacting with matrix components, and their production is further enhanced after cellular contact with activated T cells[43] in Table 1. In EAE, MMP-7 was localized predominantly in invading macrophages, and treatment of Lewis rats with various inhibitors of MMP activity reduced the severity of both active and passive EAE.[43] Activated macrophages are reported to synthesize and release iNOS resulting in increased blood flow and angiogenesis.[54]

Table 1. Summary of the possible role of proangiogenic factors in MS pathogenesis

Group	Factors	Possible role in MS pathogenesis	Inference
Hypoxia	HIF-1α	• Activates several genes that induce expression of pro-angiogenic factors, e.g. genes of VEGF • Upregulates MMPs • Binds to NICD to increase its transcriptional activity	Indirect role
Immune cells	Neutrophils	• Produce VEGF, HGF, MMPs-9, IL-8 and TNF-α (soluble mediators)	Direct role
	Eosinophils	• Produce TNF-α, IL-8, angiogenin, VEGF, FGF-2, GM-CSF, and NGF	Direct role
	Basophils	• Express mRNA of several forms of VEGF isomers, VEGF-2, and neuropilin • Release histamine	Direct role
	Macrophages	• Secrete proangiogenic cytokines TNF, IL-8, proIL-1β, and TGF • Secrete ECM degrading enzymes MMPs, and COX-2 • Secrete growth factors PDGF, VEGF, and FGF2 • Synthesize and release iNOS (increase of blood flow)	Direct role
	Dendritic cells	• Express VEGFR1 and VEGFR2, and neuropilin	Direct role
	Microglia	• Express proangiogenic cytokines that favor MMP-9 expression	Indirect role
Growth factors	VEGF	• Induce proliferation and migration of ECs	Indirect role
Proteinases	MMPs	• Break down vascular basement membrane	Direct role
	COX	• Associated with the release of many inflammatory cytokines, chemotactic factors, prostanoids, leukotrienes and phospholipase	Direct role
Proangiogenic cytokines	IL-1α	• Modulate the expression of VEGF in inflammatory cells	Indirect role
	IL-1β	• Induce activation of COX-2 gene • Induce gene expression that favors vascular permeability, especially for HIF-VEGF axis	Indirect role
	IL-8	• Binds CXCR1 and CXCR2 to increase endothelial permeability • Upregulates MMP-2 and MMP-9 production and mRNA expression	Indirect role

Abbreviations: Vascular endothelial growth factor (VEGF), Matrix metalloproteinases (MMPs), Hepatocyte growth factor (HGF), Notch intracellular domain (NICD), Interleukin (IL), Tumor necrosis factor (TNF), Fibroblast growth factor (FGF), Granulocytes-macrophage colony stimulating factor (GM-CSF), Nerve growth factor (NGF), Transforming growth factor (TGF), Extracellular matrix (ECM), Cyclooxygenase (COX), Platelet derived growth factor (PDGF), Vascular endothelial growth factor receptor (VEGFR), Endothelial cells (ECs), Chemokine receptor (CXCR), Inducible nitric oxide synthase (iNOS), Hypoxia-inducible factor (HIF)

The angiogenic factors secreted by macrophages stimulate migration of other accessory cells that potentiate angiogenesis; in particular mast cells.[55] The presence of proangiogenic cells and soluble factors in EAE and MS lesions indicate the possibility of angiogenesis in MS. [56]

Dendritic cells (DCs) express both pro- and anti-angiogenic mediators when exposed to different combinations of cytokines and microbial stimuli and both positive and negative mediators of the angiogenic factors which can affect the biology of DCs. DCs express both VEGFR-1 and VEGFR-2.[57] Furthermore, expression of the VEGF co-receptor neuropilin-1 is induced during in vitro differentiation of monocytes into DCs.[58] Microglia in active MS lesions expresses a range of inflammatory cytokines implicated in the MMP-9 expression by inflammatory cells in vitro.[59]

Growth Factors

Throughout the process of angiogenesis, local growth factor plays role in cell activation, cell migration and cell proliferation, however these roles depend on the growth factor concentrations and gradients.[60] VEGF induces vascular proliferation as well as vascular permeability changes,[61] while FGF and PDGF induce oligodendroglial progenitor cell growth[62] and also contribute to angiogenesis.[3] Besides, FGF-2 and PDGF-AA are potent modulators of oligodendrocytes, the main responsible cells for remyelination.[63]

VEGF has been shown to be a very potent stimulator of angiogenesis which is significantly up-regulated in autoimmune diseases, including MS and EAE.[64] VEGF induces the proliferation and migration of ECs in angiogenesis[65] (Table 1). This action is regulated mainly through its two primary receptors, VEGFR1 and VEGFR2, where VEGFR2 is primarily expressed on CNS vascular endothelial cells that makes up the BBB.[66] Moreover, treatment with SU5416 compound as an effective inhibitor of VEGFR2 receptors significantly decreased the clinical signs of the disease in acute EAE.[67] VEGF expression is regulated by cytokines and growth factors such as PDGF, TNF-α, IL-1, and IL-6.[68] An increased expression of VEGF is associated with demyelinated lesions in both MS and EAE, implicating changes in vasculature as a potential component of CNS plaque formation.[35] In addition, VEGF upregulation in MS correlates with findings on MRI examination.[69] Furthermore, studies in rats have shown that an intracerebral infusion of VEGF in an acute model of EAE ,induced an inflammatory response in the brain suggesting that neuroinflammatory disease may be exacerbated by the over-expression of VEGF.[37] Similarly, VEGF expression was shown to be elevated during the onset of disease in guinea pigs, followed by increased Factor VIII staining, indicating neovascularization, and the number of blood vessels in the spinal cords correlated with pathological infiltration and demyelination.[11] In EAE, VEGF is expressed by astrocytes, monocytes and activated Th1 lymphocytes, all contributing to a BBB breakdown.[70] However, in EAE, astrocytes seem to constitute the major source of VEGF and its expression is induced upon IL-1β stimulation.[71] Therefore, during relapses the release of VEGF increases following the IL-1β-dependentactivation of astrocytes immune cells and neurons.[6] Similarly, circulating levels of VEGF augment in human MS patients during relapses associated with stress.[72] In addition, the final endpoint of the IL-1ß-VEGF axis favors a significant elevation of BBB permeability; with serum protein deposition in CNS tissue and edema.[73] Noteworthy, a study of serum and cerebrospinal fluid (CSF) PDGF-AA and FGF-2 in RRMS patients showed that CSF PDGF-AA was related to disease duration. On the other hand, the serum and CSF levels of these factors were weak indicators of disease severity, consistent with the previous findings.[63]

Proteinases

MMPs are endopeptidases secreted by activated T cells, macrophages[74] and ECs.[61] They break down the vascular basement membrane and allow the invasion of the surrounding stroma by ECs in the direction of the pro-angiogenic stimulus[61] (Table 1), thus serve as effectors of cell migration, cytotoxicity, inflammation and tissue remodeling via degradation of ECM components.[75] Moreover, MMPs have been reported to degrade the basal lamina of the blood vessels and likely attack myelin sheath during acute inflammatory phase of MS.[76] In addition, MMPs also modulate immune cell activation and migration across the BBB endothelium by regulating the activation of important modulators of cell transmigration such as chemokines, cytokines and cell adhesion molecules

(CAMs).[77] MMP-2, MMP-3, MMP-7 and MMP-14 mRNAs are reported to be elevated in RRMS.[78] Similarly, excessive proteolytic activity has also been detected in the blood and CSF in patients with acute MS.[76] MMP-9 was shown to increase in the brains of MS patients, which was suggested to play role in the breakdown of the BBB.[79] Moreover, increased levels of MMP-8 and MMP-9 have been shown in seraof MS patients [80]. In EAE, it has been shown that the expression of active MMP-2 and -9 by T cells, monocytes and DCs are required for their migration across the BBB and their subsequent invasion of the CNS compartment.[80]Active expression of these and other MMPs are known to mediate BBB disruption by degrading junctional complex proteins.[81] Extracellular MMP inducer (EMMPRIN) is a factor expressed by peripheral blood mononuclear cells (PBMCs) as a membrane-bound or as a soluble form, with both forms inducing MMP production.[82] EMMPRIN is expressed by infiltrating leukocytes and CNS resident cells in MS lesions. Similarly, in EAE, there is a high proportion of EMMPRIN-positive lymphocytes and monocytes/macrophages, colonizing in the areas of MMP-9 expression and MMP-2/-9 activity.[73]

Cyclooxygenase (COX) isoforms COX1 and COX2 catalyze the production of prostanoids from arachidonic acid.[83] It is worthy to note that COX2-induced production of prostanoids is associated with the release of many inflammatory cytokines and chemotactic factors, prostanoids, leukotrienes and phospholipases implicated in many inflammatory diseases.[84] Moreover, enhanced COX2- induced synthesis of prostaglandins thus stimulates and promotes angiogenesis.[85] Many factors such as cytokines, hormones, growth factors, and chemical stimuli up-regulate expression of COX2 in various cell types including EC.[86] In addition, inflammatory cytokines such as IL-1β and TNF-α increase the expression of COX2 mRNA and protein in different cell types in humans[87] (Table 1). IL-1β specifically stimulates COX2 expression and/or PGE2 production in vascular endothelial cells,[88] monocytes/macrophages and many other cells.[89]

Proangiogenic Cytokines

IL-1α is a proinflammatory cytokine secreted by a variety of activated immune cells. It has a strong angiogenic effect in vivo assay used for measuring angiogenesis; nevertheless the direct mechanism of its effect is yet to be found. However, IL-1α fails to stimulate angiogenesis in vitro and this may be due to lack of some necessary cells, accessories required and not found present in the in vitro model.[90] However, it was hypothesized that IL-1α can stimulate angiogenesis in vivo by modulating the expression of VEGF in inflammatory cells, a pivotal promoter of physiological and pathological angiogenesis.[90] In human astrocytes, IL-1β induced a pattern of gene expression to favor vascular permeability involving the HIF-VEGF axis.[91] IL-1β-induced activation of the COX2 gene is modulated by various transcription factors such as nuclear factor kappa B cells (NF-κB), IL-6 and cAMP response element (CRE)[88] (Table 1).

Another major inducer of permeability is IL-8/ CXCL8,[92] a chemokine that was initially characterized as a neutrophil chemoattractant, but thus far recognized as a mediator of permeability and angiogenesis.[93] It increases endothelial permeability during early stages of angiogenesis[94] by binding to CXCR1 and CXCR2, followed by their activation. These receptors are expressed in different cell types[95] (Table1). Also, studies in rodents, where only CXCR2 is functional, have shown a dependence of IL-8–induced permeability on CXCR2.[96] Incubation of ECs with IL-8 up-regulated MMP-2 and MMP-9 production and mRNA expression. Thus, it was suggested that IL-8 directly enhanced EC proliferation, survival, and MMP expression in CXCR1- and CXCR2-expressing ECs and regulated angiogenesis. Furthermore, treatment of ECs with IL-8 significantly enhanced production of MMPs and capillary tube organization.[95]

CONCLUSION

Angiogenesis as a process for the formation of new blood vessels serves as an important physiological phenomenon involved in a normal part of development, reproduction, and wound healing. However, the imbalance between pro- and anti- angiogenic factors results in pathological angiogenesis that is shown to be involved in many diseases, such as MS. HIF-1α upregulates the expression of different genes involved in various steps of angiogenesis such as VEGF, FGF and angiopoitin-2. VEGF and MMPs play a crucial role in the degradation of the vascular basement membrane and the breakdown of the BBB, so that, this action allows the immune cells to infiltrate into the

CNS parenchyma in EAE and MS. The immune cells synthesize and secrete proangiogenic factors during inflammatory responses to support neovascularization and this action contributes to the perpetuation of inflammation by supporting the migration of inflammatory cells to the site of inflammation. IL-1 and IL-8 exert their role in all proangiogenesis mechanisms. These denote that proangiogenic factors exert their roles either directly or indirectly in the immunopathogenesis of MS. Moreover, it could be suggested that these factors could play a possible role as a therapeutic target in the prevention and treatment of MS particularly during pathological or inflammatory angiogenesis.

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