• Review •

Diverse roles of macrophages in intraocular neovascular diseases: a review

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Abstract

• Macrophages are involved in angiogenesis, and might also contribute to the pathogenesis of intraocular neovascular diseases. Recent studies indicated that macrophages exert different functions in the process of intraocular neovascularization, and the polarization of M1 and M2 phenotypes plays extremely essential roles in the diverse functions of macrophages. Moreover, a large number of cytokines released by macrophages not only participate in macrophage polarization, but also associate with retinal and choroidal neovascular diseases. Therefore, macrophage might be considered as a novel therapeutic target to the treatment of pathological neovascularization in the eye. This review mainly summarizes diverse roles of macrophages and discusses the possible mechanisms in retinal and choroidal neovascularization.

• KEYWORDS: macrophage; retinal neovascularization; choroidal neovascularization; proliferative diabetic retinopathy; retinopathy of prematurity; age-related macular degeneration

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INTRODUCTION

Intraocular neovascularization is a major complication that leads to vision loss[1-2], which comprises retinal neovascularization (RNV) and choroidal neovascularization (CNV). RNV contributes to numerous retinal diseases, such as proliferative diabetic retinopathy (PDR), retinopathy of prematurity (ROP) and retinal vein occlusions while CNV is a major complication of age-related macular degeneration (AMD)[1-3]. The pathological neovascularization could lead to leakage and hemorrhage, followed by fibrous proliferation, which may result in severe vision loss[4-6].

The main clinical treatments of intraocular neovascular diseases include laser photocoagulation, vitrectomy, drug delivery by intraocular injection, *etc*. In particular, drug delivery of anti-vascular endothelial growth factor (VEGF) agents have been proved to be effective in inhibiting neovascularization and are widely used for clinical applications in a group of ocular disorders[7-11]. However, laser photocoagulation and vitrectomy cannot fundamentally block neovascularization, while patients receiving long-term medication therapy of anti-VEGF treatment might develop resistance to those drugs with decreasing sensitivity to the therapy, and part of the patients may result in varying degrees of complications[7,12]. Thus, in addition to anti-VEGF drugs, other novel therapeutic targets with high efficiency and safety are needed.

Macrophages, as essential angiogenic effector cells, play dual roles in tumor growth and angiogenesis[13]. Those key cells that control tumor angiogenesis are called tumor-associated macrophages (TAM), which programmed by several factors, such as macrophage colony-stimulating factor (M-CSF), VEGF-A and monocyte chemoattractant protein 1 (MCP-1)[14-16].

In the present review, diverse roles of macrophages in intraocular neovascular diseases are described from basic researches to clinical investigations. Possible mechanisms of macrophages in intraocular angiogenesis are also discussed.

M1 AND M2: POLARIZATIONS OF MACROPHAGES

Macrophages can be divided into at least two major phenotypes with diverse functions: pro-inflammatory M1 and anti-inflammatory M2 macrophages[17-19]. The division of M1 and M2 macrophages are named reflect from Th1 and Th2 activation[20]. The M1 phenotype is known as classically activated and pro-inflammatory macrophages, which has been reported to play crucial roles in destroying foreign organisms and inhibiting tumor cells. On the other hand, M2 phenotype is known as alternatively activated or immunosuppressive

macrophages, which are understood to be important in debris scavenging, wound healing, chronic infections, tumorigenesis and angiogenesis[17,21-26]. M1 phenotype can be polarized by lipopolysacchatide (LPS) and interferon (IFN)-γ, while other cytokines like interleukin (IL)-4, IL-10 and IL-13 can induce M2 polarization[18,23]. It has been reported that M2, rather than M1 macrophages, enhanced angiogenesis *in vivo*, and M2 macrophages highly expressed basic fibroblast growth factor (bFGF), insulin-like growth factor-1 (IGF1), placental growth factor (PGF) and MCP-1[17]. TAM phenotype has been skewed toward M2-polarized cells, while M1 macrophages were also mixed in tumor angiogenesis[14,27]. The dual roles of macrophages in angiogenesis have been becoming an issue of great concern in many medical aspects, thus an increasing number of studies concentrated in the polarization of macrophages. Nevertheless, the functions of different macrophage phenotypes in intraocular neovascularization still remain unclear.

MACROPHAGES IN CHOROIDAL NEOVASCULARIZATION

AMD is a leading disease that causes blindness in aged population[28], and severe visual loss of AMD patients is mostly caused by CNV (which is also called subretinal neovascularization). Macrophages were found to be attracted to Bruch's membrane in the patients of AMD[29], the presence of extracellular deposits is related to macrophage recruitment to Bruch's membrane, as well as the phenotype of resident subretinal macrophages[29]. In CNV, retinal pigment epithelium (RPE) cells express MCP-1, a cytokine that involved in macrophage recruitment[30].

Besides, although M1 and M2 chemokines are both increased in AMD maculae, it has higher transcript ratio of M1 chemokine CXCL11 to M2 chemokine CXCL22 in advanced AMD maculae compared to the control[31]. Thus, macrophage polarization might contribute to the pathogenesis of AMD[31].

Laser-induced CNV is an experimental mouse model to investigate the mechanisms of the development of CNV, which also accompany with increased macrophages[32]. The size of laser-induced CNV was significantly reduced by the depletion of macrophages using clodronate liposomes, which indicated that macrophages might be the key mediators in the formation and pathogenesis of CNV[33-34]. Interestingly, however, another study showed that intravitreal injection of macrophages inhibited CNV in the same model[35]. In that study, bone marrow-derived macrophages were stimulated by granulocyte macrophage colony-stimulating factor (GM-CSF), which might lead to the bias toward M1 phenotype[18], meaning that M1 macrophages may inhibit CNV rather than promote it. Another study reported that loss of Smad3 inhibited the development of laser-induced CNV by suppression of infiltrated macrophages, which also demonstrated that macrophages are vital for the pathogenesis of CNV[36]. Blockade of VEGF receptor significantly decreased retinal microglia and macrophages that infiltrated into laser-induced CNV[37], indicating that VEGF receptors might be involved in the recruitment of macrophages.

Zandi *et al*[23] reported that both M1 and M2 macrophages were remarkably increased in the posterior segment of the eyes with laser-treatment of CNV. The study showed that M1 macrophages inhibited CNV while M2 macrophages enhanced it, and selective Rho-associated kinase (ROCK)-2 inhibition decreased CNV by regulation of macrophage polarization. Tahiri *et al*[38] indicated that lymphocyte-derived microparticles modulated macrophage polarization towards M1 phenotype and also suppressed laser-induced CNV.

Hagbi-Levi *et al*[39] reported that delivery of M1-polarized macrophages from neovascular-AMD patients (but not unaffected controls) enhanced CNV, while M2-polarized macrophages from both neovascular-AMD patients and controls promoted CNV in a rat model. These results demonstrated that the pathogenesis of AMD might interfere the proangiogenic functions of macrophages. Yang *et al*[40] demonstrated different dynamic patterns of M1 and M2 macrophages in both experimental CNV mouse model and clinical patients. We recently showed the different distributions of M1 and M2 macrophages in the CNV model. During the first week after laser-treatment, M1 macrophages tended to concentrate around the laser areas and the outer layer of the retinas, and M2 macrophages were mainly recognized in the inner layer of the retinas in CNV model[41]. In addition, infiltration of M1 macrophages of the outer retina precedes damage in another AMD mouse model[42]. Thus M1 macrophages may have more direct roles in early stage of inflammatory, while M2 macrophages may be essential in advanced stage of CNV pathogenesis.

MACROPHAGES IN RETINAL NEOVASCULARIZATION

Clinical Investigations in Proliferative Diabetic Retinopathy Patients Macrophages were predominantly found in surgically removed membranes of PDR patients[43]. Although the authors tried several markers to show macrophages from different stages, the definition of polarization has not been clearly defined at that time. In another study, VEGF was localized around macrophages in neovascular membrane of PDR patients[44]. Recently it has been reported that CD163, a marker of M2 macrophages, overexpressed in vitreous and fibrovascular membranes of patients with PDR, while the M1 marker CD80 was below the level of detection in the same samples of those patients[45]. Interestingly, in the study, there was a low correlation between M2 macrophages and VEGF, but a higher correlation between M2 macrophages and a matricelluar protein periostin[45]. M2 macrophage-related proteins M-CSF and IL-13 were remarkably higher expressed

in the vitreous of patients with PDR, which supported that M2 macrophages might be involved in the pathogenesis of RNV[46].

Clinical Investigations in Retinopathy of Prematurity Patients Cytokines including MCP-1, macrophage inflammatory protein 1 alpha (MIP-1α) and macrophage inflammatory protein 1 beta (MIP-1β) increased significantly in the serum of ROP patients compared to the health controls[47]. Preponderance of M1 over M2 macrophages was recognized in retrolental fibrous membranes of advanced ROP patients[48]. A possible reason may be that severe inflammation occurred in those ROP infants, and pro-inflammatory M1 macrophages were recruited in response of the pathogenesis in inhibiting such pathological neovascularization.

For the above disorders, the role of macrophages is still controversial. Macrophages might be recruited by pathological neovascularization after a long-term pathogenesis, but they may also promote the pathological neovascularization, which could be regarded as a positive feedback regulation. Thus, *in vivo* studies are required for further investigations.

Oxygen-induced Retinopathy Mouse Model Oxygen-induced retinopathy (OIR) is a commonly used mouse model for investigating RNV[49]. Unlike laser-induced CNV model, the pathogenesis of OIR is more complicated, as it contains two types of retinal angiogenesis: pathological neovascularization and physiological revascularization. The pathological one sprouted with abnormal vessels from the inner retina into the vitreous, and the other is against avascular areas with healthy vascular regeneration[49-50]. In an earlier time, most of the researchers concentrated on the pathological neovascularization only, but recently, more scientists are paying attention to the physiological revascularization. It is intriguing that the above two types are usually inversely correlated: if the physiological revascularization increased, the pathological neovascularization often reduced by the same time[51].

A study showed that leukocytes mediate retinal vascular remodeling in a similar rat model of ischemia-induced retinopathy[52]. In OIR mouse model, the number of macrophages and microglia increased in the retinas at P14 and P17, and the expression of CCL2 (MCP-1) also enhanced in response to the ischemia-treatment[53]. By intravitreal injection of clodronate liposomes, the macrophages were depleted, by the same time, both avascular areas and neovascular tufts reduced significantly[54]. Another study also showed that macrophages promoted vasculogenesis of RNV in the OIR model[55]. The vitreal macrophages express VEGF in response to OIR treatment[56], and the expression of VEGF significantly decreased after depletion of macrophages[55]. However, though myeloid cells accumulate in both CNV and OIR models,

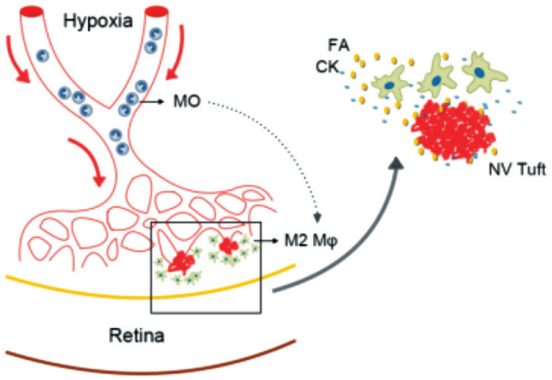

Figure 1 Hypothesis of the mechanisms of M2 macrophages in hypoxia-induced retinal neovascularization MO: Monocytes; Mφ: Macrophages; FA: Growth factors; CK: Cytokines.

they are not the major source of VEGFA, suggested that the angiogenic effects of macrophages might be based on other factors or cytokines[57]. M2 macrophages, rather than M1 phenotype, enhanced pathological neovascularization in the OIR model[50]. The possible mechanism(s) of M2 macrophages in RNV was summarized (Figure 1). The promotion of angiogenesis by M2 macrophages might be mediated by several kinds of cytokines and molecules. On the other hand, Zhu *et al*[58] showed that diverse polarized macrophages played active roles in contributing to different stages of RNV in the OIR model.

It has been reported that MCP-1 could be one of the essential monocyte attractants[59]. As MCP-1 is also involved in the induction of RNV[60], it is possible that MCP-1 plays a role in attracting or modulating macrophages in the hypoxia-induced RNV. M-CSF has been used for stimulating macrophages from bone-marrow derived cells in several previous studies[23,50,61]. Davies *et al*[53] reported that M-CSF was constitutively expressed at all times in OIR mice, while GM-CSF was not present. The vitreous concentrations of M-CSF were significantly higher in PDR patients, and there was a strong positive correlation between the concentrations of M-CSF and CD163 (an M2 macrophage marker)[46]. M-CSF might be a possible factor in inducing and recruiting macrophages after hypoxia.

To sum up, in the hypoxic microenvironment, monocytes were recruited to the vitreous and retina, probably attracted by MCP-1. Several cytokines polarized and activated these monocytes to M2-phenotype macrophages, and the cells concentrated around the neovascular tufts, promoted the development and pathogenesis of RNV.

MACROPHAGE-RELATED CYTOKINES

M1 macrophages are deemed to attenuate ocular neovascularization, while M2 macrophages tend to enhance the angiogenesis in the eye. Macrophages produce a group of cytokines, and macrophage polarization could be induced by many cytokines[18]. In ocular diseases, cytokines are also considered as key factors in regulating angiogenesis. It has been proved

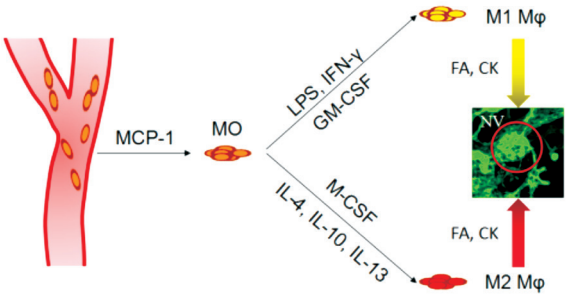

Figure 2 Cytokines participate in macrophage attraction and polarization in intraocular neovascularization MO: Monocytes; Mφ: Macrophages; FA: Growth factors; CK: Cytokines.

that cytokines are involved in the pathogenesis, in recruitment of monocytes and polarization of macrophages, as well as in the effect of angiogenesis (Figure 2)[62].

High levels of IL-12, IL-18 and IL-23, and low levels of IL-10 were released by M1 macrophages[63-64]. However, on the other hand, M2 macrophages produced more IL-10 but less IL-12 and IL-23[65-66]. Th1 cytokines, such as IL-12 and IFN-γ, inhibited pathological angiogenesis in cornea, retina and choroid[23,67-69]. IFN-γ probably is a mediator of the inhibitory effects of IL-12 in Th1 response, and the downstream chemokines CXCL9 and CXCL10 may play some roles in the pathogenesis[67]. IL-18 negatively regulates pathological RNV by regressing the blood vessels in the OIR mouse model[70]. However, the role played by IL-18 in laser-induced CNV remains controversial[71-73]. It has been reported that a typical Th2 cytokine IL-10 promoted pathological neovascularization in both CNV and OIR models[35,63], while pretreatment of low-dose LPS suppressed CNV which possibly is regulated by the induction of IL-10[74]. Besides, Yang *et al*[75] reported that IL-10 suppressed experimental subretinal fibrosis formation, a following complication of CNV. All these studies provided controversial ideas of IL-10 in intraocular neovascularization. In contrast, another Th2 cytokine IL-4 attenuated laser-induced CNV, and this may resulted by different polarization and variable effects of subtypes in M2 macrophages (such as M2a, M2b and M2c)[76].

It is interesting that IL-23, as well as IL-17a neutralization inhibited ocular neovascularization[77-78]. The reason might be that IL-23 and IL-17 are involved in the Th17 pathway, which shows totally different effects in angiogenesis from Th1 pathway. IL-27 is involved in both induction of Th1 differentiation and inhibition of Th17 differentiation[79]. Hasegawa *et al*[80] demonstrated that IL-27 suppressed CNV formation *via* inhibition of VEGF. The immunological system of cytokines is very complicated, thus further studies are necessary to show the role played by Th17-related cytokines in macrophage polarization as well as intraocular neovascularization.

Takeuchi *et al*[81] reported that Th2 and Th17-related immune responses could be involved in the pathological progress of PDR. Another study by Suzuki *et al*[82] demonstrated that intravitreal injection of bevacizumab affects the expressions of both pro- and anti-inflammatory cytokines, which indicated that anti-VEGF treatment might also modulate immune response through the networks of such cytokines. Thus, macrophages might include more phenotypes and/or subgroups that are related to different types of cytokines, which might also be involved in the intraocular neovascularization.

CONCLUSION

Increasing evidences proved that macrophages, as well as its polarization, should play an important role in developing and/or inhibiting RNV and CNV. Recent studies demonstrated that M2 polarization of macrophages is thought to be more essential in promoting intraocular neovascularization. Moreover, a group of growth factors and cytokines are considered to participate in the pathogenesis caused by macrophages in the intraocular neovascular diseases. Therefore, specific molecular target associate with macrophages could be considered as a potential therapeutic treatment for the future clinical applications.

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