

Chapter 2

Regulation of Angiogenesis by Macrophages

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Abstract Abnormal angiogenesis is a cardinal feature in the pathophysiology of several diseases of the retina including retinopathy of prematurity, diabetic retinopathy and choroidal neovascularization associated with age-related macular degeneration. Recent evidence has implicated macrophages as components of the innate immune system that play a key role in regulating angiogenesis in the retina and choroid. This review will focus on the role of macrophages in regulating ocular angiogenesis.

Immune vascular interactions are important role in regulating angiogenesis during development and in disorders of aging such as cancers, atheromatous heart disease and blinding eye disease such as age-related macular degeneration (AMD) (Apte et al. 2006; Espinosa-Heidmann et al. 2002; Hansson 2005; Kelly et al. 2007; Nakao et al. 2005; Taylor et al. 2005). AMD is the leading cause of blindness in North America in people over 50 years of age and blindness in AMD occurs largely from the exudative (wet) form of the disease that is characterized by the development of abnormal blood vessels underneath the retina i.e. CNV (Klein et al. 2004; van Leeuwen et al. 2003). Strong evidence has implicated macrophages in this process. Macrophages have been demonstrated to be necessary and sufficient in order to induce regression of lens vasculature during development and in inhibiting the growth of abnormal blood vessels in the eye in AMD (Ambati et al. 2003; Apte et al. 2006; Dace et al. 2008; Lobov et al. 2005). On the other hand, alternative lines of evidence have also implicated macrophages in promoting abnormal blood vessel growth in AMD (Espinosa-Heidmann et al. 2003; Sakurai et al. 2003).

Recent studies have demonstrated that cytokines can influence macrophage polarization and their regulation of angiogenesis. Mice that lack interleukin-10 (IL-10^{-/-}) are significantly impaired in their ability to generate CNV after laser-induced tissue injury (Apte et al. 2006; Kelly et al. 2007). In the eye, IL-10 promotes

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angiogenesis by altering macrophage function. Polarization of macrophages can play a pivotal role in determining the ultimate effector function of these cells (Mosser 2003). Macrophages stimulated in presence of IFN- γ , LPS or GM-CSF produce high levels of cytokines such as IL-12, IL-23, IL-6, TNF- α and iNOS2 with low levels of IL-10, TGF- β and arginase 1(Arg-1). This signature defines a classically activated, anti-angiogenic macrophage (M1) that is also important in anti-bacterial and inflammatory functions. Our laboratory has previously demonstrated that GM-CSF cultured macrophages polarize to an M1 phenotype and can inhibit laser-induced CNV upon injection in to the eyes of host mice (Apte et al. 2006; Kelly et al. 2007). In presence of IL-10, IL-4, or IL-13, macrophages become alternatively activated and polarized to a pro-angiogenic phenotype (M2) characterized by high levels of IL-10, TGF- β and Arg-1 and low levels of pro-inflammatory cytokines such IL-6 and TNF- α . This is especially relevant as aging in mice leads to a drift in the macrophage population to a pro-angiogenic phenotype (Kelly et al. 2007). Although classification of macrophages in to two distinct compartments such as M1 and M2 might be too simplistic, there is clearly a spectrum of macrophage phenotype as it pertains to angiogenic function.

It is important to elucidate factors that regulate macrophage-mediated regulation of developmental and post-developmental angiogenesis especially since abnormal angiogenesis in the aged eye in AMD and in the young eye in ROP leads to blindness. VEGF-A is an important factor in inducing CNV in patients with AMD. Currently, a number of treatments directed at exudative AMD and neovascularization from diabetic retinopathy are focused on neutralization of VEGF-A activity in the eye (Brown et al. 2006). These treatments have offered physicians an ability to intervene in this difficult disease and significantly reduced the risk of severe vision loss from complications of unbridled angiogenesis. They have also enhanced the likelihood of improving vision in patients affected by the disease. Unfortunately, VEGF inhibitors have to be delivered directly in to the eye with multiple, frequent intraocular injections, a process that is highly unpalatable to the patient and one that may be associated with serious ocular adverse events such as infections, hemorrhage and retinal detachments (Brown et al. 2006; Rosenfeld et al. 2006). It has been shown that anti-VEGF agents delivered in to the eye can reach the systemic circulation and that chronic systemic inhibition of VEGF may be associated with significant cardiovascular and cerebrovascular risk. In addition, anti-VEGF therapy, although relatively effective in treating neovascular processes in the eye has not been proven efficacious as a) prophylactic therapy to reduce disease progression and the possibility of developing CNV in AMD or b) curative in successfully inhibiting CNV without sustained therapy. Although complement factor H polymorphisms are also associated with AMD, there is no data that suggests a role for complement in macrophage function and its regulation of angiogenesis (Edwards et al. 2005).

We are now in an era where identifying signaling pathways within macrophages that stimulate abnormal angiogenesis is critical in advancing the gains achieved with anti-VEGF therapy and in order to develop preventive therapies. Also, it is crucial to identify safe and effective modalities of targeted delivery of potential therapeutic

agents to the site of disease processes in order to maximize sustained treatment benefit and minimize local or systemic adverse events.

2.1 Macrophage Polarization and Its Role in Angiogenesis

Polarization of macrophages can play a pivotal role in determining the ultimate effector functions of these cells (Mosser 2003). Macrophages stimulated in presence of IFN- γ , LPS or GM-CSF undergo classical activation and produce high levels of IL-12, IL-23, IL-6, TNF- α , iNOS2 and low levels of IL-10, TGF- β and Arg-1. This signature highlights an M1 macrophage that has important anti-bacterial and inflammatory functions and has also been shown to be anti-angiogenic. Our laboratory has previously demonstrated that GM-CSF cultured macrophages can inhibit CNV upon injection in to the eyes of host mice at the time of tissue injury (Apte et al. 2006). In presence of IL-10 or IL-4, macrophages undergo alternative activation and become polarized to a pro-angiogenic phenotype characterized by high levels of IL-10, TGF- β and Arg-1 and low levels of M1 cytokines. This alternatively activated macrophage has also been called an M2 macrophage. We have also demonstrated that senescence and IL-10 can influence the macrophage phenotype. Aging and high levels of IL-10 in the micromilieu (as seen with senescence) can independently influence macrophages to become pro-angiogenic.

In non-ocular tumors, it has been shown that the tumor micromilieu can influence the polarization and activation of the tumor associated macrophages (TAM) to a pro-angiogenic M2 phenotype. The M2 TAM has been shown to promote the growth and proliferation of several non-ocular tumors while the M1 TAM has pro-inflammatory and anti-angiogenic properties. It is clear that the combined effect of the cytokine profile is what drives the angiogenic phenotype of the macrophage e.g. although TNF- α has been shown to be pro-angiogenic in cancers, it is also secreted at functionally significant levels by the anti-angiogenic M1 macrophages (Sethi et al. 2008). Although we have shown that the level of FasL on the surface of the macrophage can be regulated by aging (and IL-10) and may represent a membrane-bound marker for defining macrophage polarization, it is unclear what other factors, especially signaling intermediates, play a role in the drifting macrophage phenotype and its downstream effects on immune surveillance function. This is especially important in the light of published data that FasL on the macrophage and the RPE are critical in regulating angiogenesis (Apte et al. 2006; Kaplan et al. 1999).

Immune privilege can be characterized as a subversion of the effector immune response that is unique to the eye, the central nervous system and the fetal-maternal interface in the uterus (Nieder Korn 2006). Evolutionarily, it may have evolved to protect delicate tissues in the eye from innocent bystander damage and necrotic cell death that are part of a vigorous cell-mediated immune response to antigen. Immune privilege in the eye is orchestrated by membrane-bound molecules such as FasL and TRAIL as well as soluble factors such as TGF- β , VIP, α -MSH and MIF among others (Apte et al. 1997; Apte and Nieder Korn 1996; Nieder Korn 2006). The

deviant immune response in privileged settings such as the eye is highly complex and is ultimately characterized by the suppression of delayed type hypersensitivity (DTH). The F4/80+ macrophage is essential for induction of immune privilege (Lin et al. 2005). Although there is apparent loss of privilege in retinal degenerate [rd] mice as they age, the effect of normal aging on immune privilege and immune deviation is not reported (Welge-Lussen et al. 1999). Formation of blood vessels in the eye after birth i.e. post-developmental angiogenesis is always pathologic and is associated with diseases of the eye such as ROP, DR and AMD. Teleologically, the anti-angiogenic function of young, healthy ocular macrophages can potentially be perceived as an extension of immune privilege in order to inhibit the destructive effects of abnormal blood vessel growth on visual function. We have demonstrated that aging, IL-10 and hypoxia unmasks the pro-angiogenic phenotype in a macrophage.

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