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EDITORIAL

# Targeting myeloid cells in ischemic retinal vascular diseases

Christina Nürnberg[1,2] · Norbert Kociok[1] · Antonia M. Joussen[1]

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The discovery and clinical application of anti-VEGF-directed antibodies has revolutionized the treatment regime and clinical outcome in patients with wet age-related macular degeneration, diabetic retinopathy, retinal vein occlusion, and retinopathy of prematurity [1–3]. Still, the search for more specific cellular and molecular targets is ongoing.

Several landmark papers have characterized the major overlap between inflammatory mechanisms and angiogenesis in various ocular vascular disease models: In diabetic retinopathy, blockage of leukocyte adhesion and diapedesis through inhibition of CD18 and ICAM-1 prevents endothelial cell death leaving behind acellular capillaries [4, 5]. In a mouse model of choroidal neovascularization resembling the hallmark of the exudative form of age-related macular degeneration, macrophage depletion through systemic administration of clodronate liposomes significantly reduced macrophage infiltration, the size and leakage of laser-induced choroidal neovascularization, and expression of VEGF protein [6]. Eventually, Michael Davies and colleagues also showed that hematogenous macrophages are recruited to the site of neovascularization in the ischemic retina [7]. While several investigators focused on the characterization of the inflammatory cells detailing macrophages and microglia cells [8, 9], other approaches have concentrated on the mechanistic role of inflammatory cells in ischemic retinal vascular diseases. Knowing that these cells exert a dominant role in angiogenesis and vascular pathology of the eye, the hypothesis that these cells also resemble a major source of VEGF expression is appealing. Indeed, following studies on ischemic injury proved that macrophage numbers were significantly increased, that they were closely associated to the vitreoretinal interface and expressed VEGF [10]. Moreover, local [11] or systemic genetic [12] depletion of macrophages and residential microglia reduced pathological neovascularization in animal models of oxygen-induced retinopathy. Building up the hypothesis that VEGF secreted by inflammatory cells is a major player that promotes pathological retinal neovascularization, Christiana Ruhrberg and our group examined conditional knockout mice lacking VEGF and its upstream regulator hypoxia-inducible factor 1$\alpha$ in myeloid cells [13, 14]. Using this model, both groups independently showed that there was no difference between the VEGF knockouts in myeloid cells and wild types looking at the neovascular response by measuring VEGF expression levels and the avascular and neovascular area. Additionally, specifically focusing on residential microglia and residential macrophage numbers in the retina through flow cytometric analyses, we found an increase in the percentage of residential microglia following oxygen treatment, confirming that ischemia is a potent stimulator of microglia proliferation. Interestingly, relative numbers of infiltrating macrophages did not increase after oxygen treatment. Data provided by Christiana Ruhrberg complemented these findings by employing Tie2-Cre VEGF$^{fl/fl}$ mice in order to examine tissue-resident macrophages and microglia more specifically since it is known that Tie2-expressing macrophages promote angiogenesis [15].

In accordance with our results, the vascular response to ischemia in these knockout mice was not different from control animals confirming that VEGF expressed by residential microglia is not responsible for pathological neovascularization in the ischemic retina.

In retrospect, the reduced neovascular response following clodronate-mediated depletion of macrophages reported by Ishida was likely due to the missing induction of VEGF expression in cells other than macrophages themselves. While endothelial cells themselves are likely to be a major source of VEGF, it is not fully understood how and which different other cell types are involved in the pathogenesis of retinal vascular diseases and whether this involvement alters among

✉ Christina Nürnberg
christina.claudia.nuernberg@gmail.com

[1] Department of Ophthalmology, Charité - Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Augustenburger Platz 1, 13353 Berlin, Germany

[2] Department of Neurology, Heidelberg University Hospital, Im Neuenheimer Feld 400, 69120 Heidelberg, Germany

specific diseases. Retinal ganglion cells, retinal pigment epithelium cells, and Müller cells are widely known to be a great source of retinal VEGF [16–18]. In particular, it has been demonstrated that knockout of Müller cell-derived VEGF expression significantly reduced vascular leakage in pathological blood vessels in a mouse model of diabetes [19]. First in vitro data employing human MIO-M1 cells, a Müller cell line, and murine BV-2 cells, a microglia cell line, presented by us, reveal that macrophages activate Müller cell VEGF expression in a paracrine manner through a soluble factor [14]. Even though these findings will need to be supported by in vivo data, targeting cell-cell communications and interactions seems to be a promising novel therapeutic approach for retinal ischemic diseases.

When aiming at treating patients with vascular diseases over extended periods, it might be important to discover pathomechanisms that lead to over-excessive VEGF expression rather than aiming at full VEGF blockage in order to reduce side effects to the retinal function. Rather than completely disrupting the physiological relevant angiogenic VEGF-dependent pathway, targeted inhibition of macrophage activated VEGF secretion, e.g., in Müller cells might help sustaining ocular VEGF homeostasis. Eventually, combining anti-inflammatory treatments and VEGF inhibition might lead to an improvement in drug safety and long-term clinical outcome in patients suffering from vision loss.

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