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Lipid Mediators, M2 Macrophages, and Pathological Neovascularization

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Abstract

Sasaki and colleagues [1] (*JCI Insight* 2018;3,e96902) identified the leukocyte inflammatory lipid mediator leukotriene B4 (LTB4)/LTB4 receptor 1 receptor-signaling axis in M2 macrophages as a causal pathway for the vascular endothelial growth factor-dependent pathological neovascularization in a mouse model that mimics wet age-related macular degeneration. This observation provides a novel mechanism by which an eicosanoid lipid mediator drives retinal vascular pathology and suggests a novel therapeutic target for proliferative retinal vascular diseases.

Age-related macular degeneration (AMD) is the most prevalent retinal disease that ultimately leads to blindness in the elderly population [2]. AMD is divided into dry (non-exudative) and wet (neoangiogenic/exudative) clinical presentations. In contrast to the dry form, wet AMD has been linked to exaggerated expression of vascular endothelial growth factor (VEGF), causing vascular leak and endothelial proliferation that leads to abnormal vascular lesions in the retina and visual impairment. Current therapeutic approaches for wet AMD focus on VEGF-targeted drugs, which have shown efficacy in multiple studies [3]. However, long-term intravitreal injections of anti-VEGF protein therapeutics have a high cost burden, pose a significant risk of infection, and ultimately lead to unresponsiveness in significant fractions of patients. Thus, alternative cost-effective oral therapeutic approaches are warranted for wet AMD.

A well-established animal model for AMD uses a pulse of a laser beam to disrupt Bruch's membrane, the membrane that separates retinal pigment epithelium from the choroidal blood vessels of the retina. This laser-induced tissue damage leads to inflammatory and repair processes, upregulation of VEGF expression, and excessive choroidal neovascularization (CNV) that invades and disrupts the retinal macula, thus mimicking the pathology of wet AMD [4]. Macrophage infiltration and the presence of M2-polarized macrophages in damaged retinal tissues were identified as the process that exacerbates tissue injury [5].

LTB4, a proinflammatory lipid mediator derived from arachidonic acid via the 5-lipoxygenase pathway, is secreted by myeloid cells in response to multiple stimuli including tissue injury [6]. LTB4 is a potent stimulator of leukocyte chemotaxis by activating the

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LTB4 receptor 1 receptor (BLT1) G protein-coupled receptor. Moreover, the LTB4/BLT1 axis leads to activation of reactive oxygen species, release of lysosomal tissue-degrading enzymes, and is associated with multiple inflammatory diseases [7]. While the role of this axis is well established in several cell types including neutrophils and eosinophils, the potential role of LTB4/BLT1 is less clear in macrophages.

Drawing together these two avenues of research, Sasaki and colleagues performed a detailed analysis of the retina after laser-induced disruption and provided strong evidence for LTB4-dependent signaling via the BLT1 receptor as the causal mechanism for M2 macrophage-dependent VEGF production and the subsequent exacerbation of invasive neovascularization [1]. Using liquid chromatography–mass spectrometry analysis for eicosanoids, they showed that the arachidonic acid metabolites LTB4 and 5-hydroxyeicosatetraenoic acid were induced in response to laser disruption in the early phase of the inflammatory response. In mice deficient for expression of the BLT1 receptor (BLT1-KO), they observed a threefold decrease in vascular lesion volume compared to wild-type (WT) controls, suggesting a central role for this pathway in disease pathogenesis. They further demonstrated a 50% decrease in vascular lesion volume after transplantation of BLT1-null bone marrow into irradiation-ablated WT recipients. Notably, gene-profiling data of retina from BLT1-KO mice revealed a threefold decreased expression of multiple macrophage-associated, inflammation-related cytokines including VEGF. Intriguingly, they observed an age dependency that is reminiscent of wet AMD in humans.

A time course analyzing leukocyte infiltration of injured retina revealed an immediate neutrophil influx into the retina post laser induction that was followed by a subsequent wave of macrophage infiltration 3 days later. Sasaki and colleagues created M1-polarized and M2-polarized bone marrow-derived macrophages *in vitro* and found that the BLT1 receptor is induced on M2 macrophages in response to interleukin-10. Functional analysis revealed that in contrast to BLT-null M2-macrophages, $BLT1^{+}$ M2-macrophages mobilize calcium, migrate, and have a threefold increase in VEGF expression in response to LTB4 stimulation. Based on a previous study that demonstrated that adoptive transfer of M2-polarized macrophages into recipient hosts increases pathological neovascularization in the mouse model for retinopathy of prematurity [8], the authors observed that while infusion of WT M2 macrophages caused a fourfold increase in CNV lesion volume after laser injury, BLT1-null M2 macrophages do not exacerbate the vascular lesion size. These studies provide definitive proof of LTB4 action on macrophage BLT1 receptor as a causal signal in VEGF expression and vascular pathology in a mouse model of wet AMD.

Sasaki and colleagues also applied these findings with pharmacological inhibition of either BLT1, using an experimental receptor antagonist, or inhibitors of LTB4 synthesis, namely, 5-lipoxygenase, 5-lipoxygenase-activating protein, and leukotriene A4 synthase. It is noteworthy that 5-lipoxygenase inhibitor zileuton is already approved by the FDA for asthma therapy. All four drugs cause a threefold decrease in vascular lesion volume, and one drug, CP105696, decreased the number of infiltrating M2 macrophages by approximately 50%, thus confirming that targeting this LTB4/BLT1 pathway is a potential viable therapeutic approach to treating wet AMD. Further work is needed to choose an optimal therapy targeted against this pathway in the retinal tissue so as to enhance efficacy while

reducing system adverse effects. In addition, further research is needed on whether this approach can complement or replace anti-VEGF therapy as well as be useful in patients who are refractory to anti-VEGF treatments.

The notion that an LTB4-dependent BLT1 induction of VEGF expression in M2 macrophages can exacerbate neovascularization is intriguing and suggests that orally available, small molecule inhibitors of this pathway have the potential to treat other retinal vascular diseases. For example, retinopathy of prematurity, a syndrome induced in response to oxygen therapy and a leading cause of blindness in premature infants, may have a strong M2 macrophage component that induces retina-disrupting hypervascularization [8]. In addition, proliferative diabetic retinopathy is a leading cause of adult blindness and is characterized by macula edema and invasive growth of vasculature in the retina and vitreous humor [9].

Dietary omega-3 polyunsaturated fatty acids such as eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6) are important in retinal development and physiology. Indeed, dietary omega-3 fatty acid supplementation has been suggested as a treatment in wet AMD [10]. It is known that omega-3 fatty acids compete with arachidonic acid (20:4) in the sn2 position of phospholipids. In addition, omega-3 fatty acids compete with arachidonic acid in the lipoxygenase pathway, thus potentially reducing LTB4 levels. Whether the omega-3 protective effect involves the BLT1 signaling pathway in M2 macrophages need further investigation. In addition, recent work has implicated another lipid mediator signaling pathway involving epoxide hydro-lase metabolism of docosahexaenoic acid in diabetic retinopathy [11]. The observations of Sasaki and colleagues should stimulate further research into the potential role the LTB4-BLT1 axis in these eye diseases in the context of other lipid mediator mechanisms and angiogenesis of the retinal vasculature.

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