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Reviewer A

Comment 1: This manuscript has been aimed at summarizing inflammatory pathways in neovascularization taking place in the retina and the choroid during diabetic retinopathy and age-related macular degeneration, respectively. While the topic is interesting, the text remains rather superficial. The selection of inflammatory pathways should be justified more clearly. Why those and not e.g. NF-kB pathway? Moreover, despite similarities, there must be also differences between pathologies in retinal vs. choroidal neovascularization. Also within distinct pathways, analysis should be deepened and all respective articles considered. For example, the role of IL-18 in choroidal neovascularization has been studied a lot more than referred to in the present manuscript.
Reply 1: According to reviewer1's comment, we have revised our manuscript to justify the selection of inflammatory pathways, and empathize and clarify the differences between pathologies in retinal vs. choroidal neovascularization, and finally we added the role of IL-18 in retinal and choroidal NV in separate section.
Changes in the text:

(Old, line 35; New line, 35): "Interleukin-18" was added between inflammasome and programed cell death ligand-1.

(Old, line 71-75): In this review article, we aim to describe the pathological aspects of cells and inflammatory molecules associated in inner and outer BRB and retinal and choroidal NV. In addition, we discuss the current therapeutic research on inflammatory drug targets, to gain new insights for future therapeutic approaches regarding ocular vascular diseases.

➔ (New, Line 71-74) In this review article, we aim to describe and discuss the pathological aspects of cells associated in inner and outer BRB and retinal and choroidal NV, and inflammatory molecules *in the current advances of the therapeutic targets for retinal and choroidal angiogenic conditions*.

(Old line 181) We review inflammatory molecular pathways of the complement system

➔ (New line 192) We review the current advances in inflammatory molecular pathways

(Old line 184; New line 196) "interleukin 18 (IL-18)" added between (NLRP3) and programed cell death ligand-1(PD-L1).

(Old line 185) to gain insights into potential candidates to treat retinal and choroidal NV

➔ (New line 198,199) to gain insights into potential candidates of therapeutic targets to treat retinal and choroidal NV.

(Old line 248) the maturation of pro-interleukin (IL)-1beta

➔ (New line 272) the maturation of pro-interleukin (IL)-1beta and pro-IL-18

(New line 300-332) New section: "Inflammatory pathways in retinal and choroidal NV: Interleukin (IL)-18" added.

Proinflammatory IL-1beta and IL-18 are downstream cytokines of NLRP3 activation. Like NLRP3 upregulation in retinal and choroidal NVs as mentioned above, IL-1beta and IL-18 are also upregulated in aqueous and vitreous fluids of NPDR and PDR and are considered increased in CNV patients as well based on *in vitro* studies and *in vivo* animal studies. The upregulation of IL-1beta seems to be associated with oxidative stress, BRB permeability and tissue deterioration in DR and AMD. However, there are controversial results of IL-18 function in retinal and choroidal NVs, indicating the physiological complexity of IL-18 functions: Deficiency of IL-18 resulted in severe choroidal NV development and treatment of IL-18 attenuated choroidal NV formation via reducing VEGF in laser-induced choroidal NV model, and IL-18 deficiency in VEGF-A^{hyper} model increased choroidal NV compared to that in NLRP3 and IL-1beta deficiency in VEGF-A^{hyper}. More interestingly, NLRP3 and IL-1beta targeting resulted in inhibition of dry AMD pathology but IL-18 inhibition deteriorates AMD pathology in CEP-adducted serum albumin-immunized model. However, any deficiency of either IL-1beta, NLRP3 or IL-18 did not inhibit CNV formation in VEGF-A^{hyper} model. it was later argued that IL-18 does not exhibit either pro- or anti-angiogenic effects on laser-induced choroidal NV formation. Nevertheless, anti-VEGF treatment increases ocular IL-18 level and the increased IL-18 level is correlated with good visual outcome in patients with macular edema, and similar observation of the upregulated IL-18 by anti-VEGF treatment in ischemic retinopathy animal model, suggesting the anti-angiogenic activity of IL-18. It is noteworthy that the expression level of pro-IL-18 and IL-18 is known to be permanent in RPE cells, and not increased by NLRP3 inflammasome activity and it is well known that endothelium and epithelium cells are hardly activated by inflammation, thus the cytokine roles of resident and recruited immune cells will be important. Also, the certain level of IL-18 concentration may have a protective role in retinal and choroidal NV, whereas higher amount of IL-18 combined with other cytokines may be detrimental.

Reviewer B

Comment 1: The manuscript discussed various cells and inflammatory pathways involved in retinal/choroidal neovascularization and current advances in therapeutic targets for angiogenic retinal/choroidal conditions. The article is informative and will be of interests to vision scientists.

Below are my comments:

• The manuscript can be improved by better arranging the sections. The current arrangement in session titles under "Main text" does not highlight the main topic of the article, which include (1) cells involved in retinal/choroidal NV, and (2) inflammatory pathways involved in retinal/choroidal NV.

• The prevalence of diabetes mellitus (DM) affects over 415 million and continues to increase throughout the world (3). The number of DM is out of data and the reference is inaccurate.

Please see WHO 2020 report on DM prevalence (https://www.who.int/news-room/fact-sheets/detail/diabetes). Reference #3 was about the prevalence of DR in the USA in 2004.
• Page 3 line 85, the information on mural cell and EC ratio in other body regions will help readers to understand the uniqueness of retinal blood vessels.
• Retinal glial cells include macroglia (astrocytes and Muller cells) and microglia. Their functions are very different. Macroglia are mesenchymal cells that provide structural support to retinal vasculature and neurons; whereas microglia together with perivascular macrophages are innate immune cells that maintain retinal homeostasis. In my view, microglia should be separated from astrocytes and Muller cells and should be discussed along with other immune cells (e.g., macrophages).
• Page 8, first paragraph: Complement pathway in retinal/choroidal NV. The outcome of clinical trials on complement inhibitors in neovascular AMD should be discussed.
• In addition to IL18, the role of other IL1 family cytokines such as Il1β and IL-33 in retinal/choroidal NV should be discussed.
Reply 2: According to reviewer2' comments, 1) we have improved the arrangement: we numbered each section and added a new section of "cells in retinal and choroidal NV" before the description of each cell type. 2) The prevalence of DM was updated. 3) the information on mural cell and EC ratio in other body regions was added. 4) Microglia description was arranged from "glia" part to "microglia and macrophage". 5) We discussed the outcomes of the finished and ongoing clinical studies on complement targeted drugs. 6) We added IL-18 in separate section of Inflammatory pathways in retinal and choroidal NV.

Changes in the text:

(Old line 58) 415 million
➔ (New line 58) 442 million in 2014 (and the WTO web site was referenced).

(Old line between line 77 and 78; New Line 77-83, a section added)
2.1 *Cells in retinal and choroidal NV*
The final effector cells in angiogenesis are ECs, but the initiative main pathological cells are mural and RPE cells in retina and choroidal NVs, respectively. Mural and RPE cells are also essential part consisting of and maintaining inner and outer BRB. There are further many cell types associated with the pathological processes of retinal and choroidal NVs. We categorize them for the convenience of description: EC and mural cell, RPE, glial cells, and microglia and macrophages.

(Old line 82) which is quite uncommon in other body regions. Mural cells support
➔ (New line 89-90) which is quite uncommon in other body regions, varying ratio of mural cell to EC, 1:100 in skeletal muscle.

(Old line 127) Cells in retinal and choroidal NV: Retinal glial cells
➔ (New line 137) Cells in retinal and choroidal NV: Astrocytes and Muller glia

(Old line 129-130) as well as microglia, the retina resident immune cell type (Fig. 2, 3a), are associated in inflammation of retinal and choroidal NV.

➔ (New line 139-140) are mainly associated in inflammation of retinal and chroidal NVs (Fig. 2).

(Old line 139-145) The whole sentence was deleted and moved in the New manuscript (New line 152-159)

(Old line 147) Cells in retinal and choroidal NV: Macrophages

➔ (New line 150) Cells in retinal and choroidal NV: Microglia and Macrophages

(Old line between 147 and 148; New line 151-152) added: Microglia are the retina resident immune cell type (Fig. 2, 3a) and associated in the inflammation of retinal and choroidal NV.

(Old line 149) The recruited monocytes

➔ (New line 160) The resident microglia and recruited monocytes

(Old line 149, 150) M1 and M2 macrophage

➔ (New line 161, 162) M1and M2 microglia and macrophages.

(Old line 151) M1macrophages

➔ (New line 162, 163) M1 microglia and macrophages,

(Old line 218, 219) Current clinical trials attempt to target complement components for Wet form, as well as Dry AMD

➔ (New line 232-243) Clinical trials have often attempted to target complement components not only for Dry AMD but also for Wet AMD. It is of note that while CFD inhibitor Lampalizumab (NCT02247479; NCT02247531, Phase 3, Genentech), C3 inhibitor Comstatin (NCT01157065, Phase 2, Apellis) and C5 inhibitor Eculizumab (NCT00935883, Phase 2, Alexion pharmaceuticals) failed in clinical trials to treat Dry AMD, but c3 inhibitor APL-2 Pegcetacoplan (NCT03525600, NCT03525613, Phase 3, Apellis Pharmaceuticals) and c5 inhibitor (Zimura; NCT02686658; Phase 2/3, IVERIC bio) are currently in clinical trials and awaiting the results. IB1302 Bispecific antibody fusion protein targeting VEGF and complement cascade (NCT04820452, Innovent Biologics, Inc) just finished Phase 1 and waiting Phase 2 for Wet AMD. The estimated study start date is May first, 2021.

(Old line between 274,275; New line 299-330) A section added: Inflammatory pathways in retinal and choroidal NV: Interleukin (IL)-18:

Proinflammatory IL-1beta and IL-18 are downstream cytokines of NLRP3 activation. Like NLRP3 upregulation in retinal and choroidal NVs as mentioned above, IL-1beta and IL-18 are also upregulated in aqueous and vitreous fluids of NPDR and PDR and are considered increased

in CNV patients as well based on *in vitro* studies and *in vivo* animal studies. The upregulation of IL-1beta seems to be associated with oxidative stress, BRB permeability and tissue deterioration in DR and AMD. However, there are controversial results of IL-18 function in retinal and choroidal NVs, indicating the physiological complexity of IL-18 functions: Deficiency of IL-18 resulted in severe choroidal NV development and treatment of IL-18 attenuated choroidal NV formation via reducing VEGF in laser-induced choroidal NV model, and IL-18 deficiency in VEGF-A^{hyper} model increased choroidal NV compared to that in NLRP3 and IL-1beta deficiency in VEGF-A^{hyper}. More interestingly, NLRP3 and IL-1beta targeting resulted in inhibition of dry AMD pathology but IL-18 inhibition deteriorates AMD pathology in CEP-adducted serum albumin-immunized model. However, any deficiency of either IL-1beta, NLRP3 or IL-18 did not inhibit CNV formation in VEGF-A^{hyper} model. it was later argued that IL-18 does not exhibit either pro- or anti-angiogenic effects on laser-induced choroidal NV formation. Nevertheless, anti-VEGF treatment increases ocular IL-18 level and the increased IL-18 level is correlated with good visual outcome in patients with macular edema, and similar observation of the upregulated IL-18 by anti-VEGF treatment in ischemic retinopathy animal model, suggesting the anti-angiogenic activity of IL-18. It is noteworthy that the expression level of pro-IL-18 and IL-18 is known to be permanent in RPE cells, and not increased by NLRP3 inflammasome activity and it is well known that endothelium and epithelium cells are hardly activated by inflammation, thus the cytokine roles of resident and recruited immune cells will be important. Also, the certain level of IL-18 concentration may have a protective role in retinal and choroidal NV, whereas higher amount of IL-18 combined with other cytokines may be detrimental.

Additional changes:

(Old line 91; New line 99-101) add sentences: Thus, it is considered that the mural is mainly an initiative pathological cell type in retinal NV, whereas RPE is the key cell type in pathogenesis of choroidal NV.

(Old line 117) signaling pathways

➔ (New line 127) signaling pathways and the cell types

(Old line 148) NV recruits cells from peripheral blood stream and bone marrow

➔ (New line 159) The NVs recruit cells from peripheral blood stream and bone marrow as well

(Old line 253-255) The attenuation of NLRP3 inflammasome activation, as a result of treatment with IL-22, curcumin, cepharanthine and piperine, has mitigated diabetic nephropathy

➔ (New line 278-280) The attenuation of NLRP3 inflammasome activation and oxidative stress, as a result of treatment with IL-22, curcumin, cepharanthine, piperine or fenofibrate has mitigated diabetic nephropathy

(Old line 257-273) However, it appears that curcumin, cepharanthine and piperine function as anti-oxidants as well as a NLRP3 inhibitor. Future studies of NLRP3 specific

inhibitors, therefore, will detail the particular cell-type mechanisms and roles of NLRP3 in DR animal models and therapeutic approaches (Fig. 4b). Similarly, AMD patients and animal models have shown increased levels of NLRP3 inflammasome activation. Although there have been controversial results concerning NLRP3's involvement in choroidal NV, NLRP3 deficiency affects mouse choroidal NV formation in different ways, ranging from exacerbation to decline, or marginal effects. Deficiency of IL-18, a downstream cytokine of NLRP3 activation, resulted in severe choroidal NV development, while treatment with IL-18 attenuated choroidal NV formation. However, it was later concluded that IL-18 does not exhibit either pro- or anti-angiogenic effects on laser-induced choroidal NV formation. It is noteworthy that there is a report on the anti-angiogenic activity of IL-18 in ischemia-induced retinal neovascularization, and that laser-induced choroidal NV models do not fully represent the human Wet AMD pathobiology of choroid/RPE/retinal environments. Thus, further studies on NLRP3 mechanisms and validation of retinal and choroidal NV might need to be pursued.

- ➔ (New line 280-297) and recently, intravitreal injection of MCC950, a specific inhibitor of NLRP3 ameliorated retinal NV in an oxygen-induced ischemic retinopathy model. Similarly, AMD animal models of Dry and Wet forms have shown increased levels of NLRP3 inflammasome activation, and clinical aspects of AMD such as drusen and complement activation suggest inflammasome's association in pathophysiology conditions of AMD, and in advanced AMD, peripheral and resident immune cells are recruited. However, it is still unclear how NLRP3 is directly associated with choroidal NV. Laser-induced choroidal NV in NLRP3 genetic deficiency exhibited exacerbation, but VEGF-A^{hyper} choroidal NV model in NLRP3 genetic deficiency exhibited decline. When it is considered that laser-induced acute model does not fully represent clinical Wet AMD pathophysiology and VEGF-A^{hyper} is relatively chronic, studies in other animal models such as knockout of very-low-density lipoprotein receptor (VLDLR) will confirm further. When it is considered that inflammation itself is neutral like weapon, a gun sometimes kills enemy but self as well, NLRP3 inflammasome might take either protective or deleterious roles, depending on the stages and conditions of diseases, and the studies on the associated-downstream pathways and cell specific mechanisms will be important.