Interleukin-18 Bioactivity and Dose: Data Interpretation at a Crossroads

In their recent paper, Ijima et al.[1] state that IL-18 induced retinal pigment epithelium (RPE) degeneration in mice. We would like to highlight some important and key facts that relate to the use of recombinant proinflammatory cytokines for ophthalmologic research. Firstly, we recently reported that IL-18 could induce retinal and RPE toxicity in mice when used at a sufficiently high dose. We established a titration curve for recombinant mouse IL-18, whereby we showed that a dose of 3 ng or higher of a highly purified mature recombinant IL-18 could induce toxicity in the retina and RPE of mice and not specifically in the RPE alone.[2] Here, the authors state that they injected 1 μg IL-18 either intravitreally or subretinally, and subsequently determined whether it impacted laser-induced choroidal neovascularization (CNV) or caused RPE cell death. It is entirely unclear why the authors chose this dose for their assay, as injecting 1 μg of any commercially available cytokine will likely cause the same effect. In their analysis of IL-18 levels in the blood of human subjects, the authors see maximal levels of approximately 80 pg/mL. What is the rationale for then injecting 1 μg IL-18 into the microenvironment of the mouse vitreous? The very fabric of research examining the role of inflammation as it pertains to disease is based on titration biology and simply choosing a random and extraordinarily high dose of a proinflammatory cytokine to prove toxicity is not appropriate.

In addition, we have previously compared the bioactivities of the murine IL-18 that we used in our recent study (GlaxoSmithKline generated material: SB-528775; King of Prussia, PA, USA) and the same commercially available material used in the study by Ijima et al.[1] (murine IL-18 from R&D Systems/MBL, Abingdon, UK). We found that mouse recombinant IL-18 from MBL is completely ineffective at activating Natural Killer (NK) cells, a key cell type used to assay for bioactivity of this cytokine in vitro (see Figs. 1D, 1E from our paper).[2] Therefore, the bioactivity of the two differently sourced cytokines is profound and this likely explains the lack of efficacy of IL-18 in preventing laser-induced CNV. Also, given the extensive retinal lysis and RPE cell death that would have accompanied injection of 1 μg IL-18 intravitreally, it is not clear how the authors accurately assessed CNV volumes, as the margins of the induced CNV would have been indistinguishable from the cell death induced (as is clear in Fig. 2B).

The concept that IL-18 can specifically induce apoptosis in RPE cells is not supported by the fact that it does not do this in vitro in either primary RPE cells or RPE cell lines at concentrations as high as 10 μg/mL and at a range of time points post stimulation.[2] Apoptosis is a cellular process that has evolved to protect the body from any potential inflammation that accompanies dying cells. The idea that proinflammatory, IL-18–mediated signaling can directly cause apoptosis runs contrary to the purpose of apoptosis, and the caspase-3 levels observed in the toxicity assays in Figure 4 are undoubtedly from lysed RPE cells, as the morphology of cells stained for ZO-1 in Figure 4C doesn't resemble apoptosis. Either way, the subretinal dose of 1 μg IL-18 in mice has no physiologically relevant interpretation that would allow any biologically important comment to be made.

The data presented in Figure 1 are intriguing and highlight the difficulties in data interpretation as it pertains to the NLRP3 inflammasome and AMD. While it is known that up to 30% of geographic atrophy (GA) patients may progress to develop CNV, as the authors state, they have included only patients with either GA or CNV for their comparisons. The authors then go on to state that IL-18 levels are higher in dry AMD patients compared with control and that there is no difference in IL-18 levels in wet AMD patients. The systemic IL-18 in patient samples is certainly not derived from the eye and, given that none of their dry AMD patients had evidence of CNV, it can also be stated from the data presented that "increased systemic IL-18 prevents CNV development," as the wet AMD cohort had no change in IL-18 levels. It should be noted that recombinant human IL-18, as a therapeutic agent being developed by GlaxoSmithKline, has now been injected systemically into human subjects at doses of up to 1000 μg/kg and no subject has reported symptoms associated with GA, even with dosing every 5 days with a 23-day rest period for up to 6 months, strongly implying that high systemic levels of IL-18 do not cause GA in humans. Data presented here suggest that high systemic levels of IL-18 in dry AMD patients may prevent the onset of CNV in this patient cohort.

Finally, the authors have failed to discuss two recent reports detailing the antiangiogenic role of IL-18 in the eye in both human aqueous samples and in a new murine model of atrophic/neovascular AMD,[3,4] in addition to previous reports associated with its role in neovascularization in the eye.[5–7]

These are exciting times to be involved in AMD research and it is clear that IL-18 has a role in AMD pathobiology. However, a more rational and collegiate approach to data interpretation is essential if we, as a community, are to avoid the dogma that has restricted other areas of research in the past.

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References

1. Ijima R, Kaneko H, Ye F, et al. Interleukin-18 induces retinal pigment epithelium degeneration in mice. *Invest Ophthalmol Vis Sci*. 2014;55:6673–6678.
2. Doyle SL, Ozaki E, Brennan K, et al. IL-18 attenuates experimental choroidal neovascularization as a potential therapy for wet age-related macular degeneration. *Sci Transl Med*. 2014;2:230ra44.
3. Shen J, Choy DF, Yoshida T, et al. Interleukin-18 has antipermeablity and antiangiogenic activities in the eye: reciprocal suppression with VEGF. *J Cell Physiol*. 2014; 229:974–983.
4. Marneros AG. NLRP3 inflammasome blockade inhibits VEGF-A-induced age-related macular degeneration. *Cell Rep*. 2013;4:945–958.
5. Cao R, Farnebo J, Kurimoto M, Cao Y. Interleukin-18 acts as an angiogenesis and tumor suppressor. *FASEB J*. 1999;13:2195–2202.
6. Qiao H, Sonoda KH, Ikeda Y, et al. Interleukin-18 regulates pathological intraocular neovascularization. *J Leukoc Biol*. 2007;81:1012–1021.
7. Qiao H, Sonoda KH, Sassa Y, et al. Abnormal retinal vascular development in IL-18 knockout mice. *Lab Invest*. 2004;84:973–980.

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