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

We are gratified at the opportunity to clarify both some of our data as well as the interpretation of our findings as described in Doyle et al.2 One issue raised in Doyle et al.,5 was the choice of dose of recombinant IL-18. Based on the empirical intracellular clearance rate of similar-sized biologics in a rat eye3 as well as computational modeling of spherical diffusion in a mouse eye, we estimate that the half-life of recombinant IL-18 in the mouse eye is on the order of 1 hour. We administered a 1-μg bolus of IL-18, which based on previous reports of similar compounds, was rapidly cleared from the eye. Within 11 half-lives (approximately 11 hours), the concentration of IL-18 in the eye is below 80 pg/mL, which is the steady-state concentration in the serum we measured in humans with dry AMD. Note that the earliest time-point in which we observed a biological effect at this dose was 3 days, with retinal pigment epithelium (RPE) degeneration noted after 7 days. Therefore, from a pharmacokinetic standpoint, a 1-μg bolus of recombinant IL-18 would be ineffective at activating Natural Killer (NK) cells, a key cell type used to assay for bioactivity of this cytokine in vitro (see Figs. 1D, E from our paper5). Furthermore, as reported in Doyle et al.,5 lower doses of IL-18 also induce retinal degeneration, but we estimate that the fundamental conclusion of our studies. Although the findings of these studies draw similar conclusions, it is important to bear in mind some important differences in their experimental design. First, whereas the reported RPE defects in Doyle et al.,5 were observed 24 hours after administration of IL-18, we measured RPE degeneration 7 days after IL-18 delivery. Second, whereas Doyle et al.,5 injected into the vitreous humor, in the studies on RPE degeneration, we performed subretinal IL-18 administration. It is significant that despite these differences, both studies have drawn the same conclusion that IL-18 induces RPE toxicity.

We have addressed the bioactivity of R&D Systems/MBL sourced recombinant mouse IL-18, Doyle et al.2 states that “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, E from our paper5).” In reference to these data, Doyle et al.5 states, “both sources of mIL-18 upregulated the expression of CD69 and induced IFN-γ in NKp46+/NK1.1+CD3− splenocytes, in conjunction with IL-12; mIL-18 (GSK) was significantly more potent than IL-18 (R&D Systems; Figs. 1D, E).” Therefore Doyle et al.’s5 statement in JCI is contradicted by their own findings. Their claim that R&D Systems/MBL murine IL-18 is biologically inactive also is disproven by dozens of studies using this reagent.6-8

With respect to RPE toxicity, recombinant mouse IL-18 from R&D Systems has been previously demonstrated to induce RPE degeneration in wild-type mice and it does not do so in MyD88−/− mice,9 suggesting that this compound induces RPE toxicity via the established IL-18 signaling pathway. Indeed, Doyle et al.5 reports RPE and retinal degeneration as effects of this source of IL-18 in the eye. Doyle et al.2 states that “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.” The accuracy of this claim is unknown. However, given the wealth of literature on the biological effects and specificity of R&D Systems-sourced IL-18, is the burden to demonstrate a specific effect of IL-18 on laser CNV volumes not squarely on the GSK-produced source?

A cautious interpretation of the bioactivity of GSK sourced IL-18 is warranted given the curious pharmacokinetic responses reported in5 with respect to systemic administration. For example, it was reported that single dose of GSK IL-18 administered subcutaneously 14 days prior to laser injury was equally effective as daily administration for 7 consecutive days starting at the time of laser injury. The half-life of recombinant IL-18 in the mouse circulation is 11 hours,10 suggesting that GSK sourced IL-18 has an extraordinary 20 million-fold therapeutic window when administered subcutaneously. More confusingly, a single dose given at the time of laser injury did not significantly reduce laser CNV volume.

Indeed, given that five research groups have independently demonstrated that recombinant IL-18 as well as enforced expression of mature IL-18 do not reduce CNV,4 we wonder whether Doyle et al. have considered and tested the possibility that the antiangiogenic effects they report with GSK IL-18 might have been due to nonspecific or off-target effects unrelated to IL-18. Such circumspersion is all the more warranted given Doyle et al.’s earlier erroneous conclusion that IL-18 neutralization increases CNV,11 which was based on comparing the effects of an anti–IL-18 antibody (Abcam) with a “mock injection” (i.e., no injection of a vehicle or an isotype antibody). Indeed, five other groups, using rigorous biological and chemical controls, independently determined that it was the high levels of glyceral in the Abcam anti-IL-18 antibody preparation that was responsible for the proangiogenic effect that was erroneously attributed by Doyle et al.11 to the IL-18 antibody.

Doyle et al.5 question the accuracy of our CNV calculations, “as the margins of the induced CNV would have been indistinguishable from the cell death induced (as is clear in Fig. 2B).” As stated in our methods, CNV lesions were imaged using confocal microscopy to detect isolecitin B4-stained endothelial cells from the most anterior aspect of the neovascular lesion to the RPE layer. Retinal pigment epithelium death observed after IL-18 administration did not result in obliteration of the cell layer, the boundary between RPE and choriocapillaris is obvious. Because a small z-plane size was used (1 μm), fluorescence from choriocapillaris underlying degenerated RPE was not included in the analysis. It is clear in Figure 2B that there is no effect of recombinant mouse IL-18 on neovascularization of the choroid after laser photoocoagulation.

Doyle et al.2 states that “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 even at concentrations as high as 10 μg/mL and at a range of time points post stimulation.”5 The lack of apoptosis reported in Doyle et al.5 is entirely predictable given that, unlike RPE cells in the eye, cultured RPE cells lack FasL expression,12 which is a central, acknowledged downstream death signal of IL-18.4,9,13

Doyle et al.,2 states that “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 signalling (sic) can directly cause apoptosis runs contrary to the purpose of apoptosis…” We are surprised at this statement as it runs contrary to the immunology literature, which is replete with dozens of studies identifying multiple inflammatory stimuli (e.g., TNF-α) inducing apoptosis (interferon signaling in viral infection being a notable example), and numerous studies.
reporting apoptosis being a biological consequence of IL-18,\textsuperscript{18,19} including Doyle et al.'s own work.\textsuperscript{5} We would suggest that imputing anthropomorphic concepts such as 'purposes' to biological processes that unfold stochastically over an evolutionary time scale can be hazardous. Further, when a biological observation (that is consistent with dozens of similar published findings) does not reconcile with one's divining of the 'purpose' of a biological process, a re-examination of one's understanding is warranted. Doyle et al.,\textsuperscript{2} states that, ‘…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.’ This exercise in sophistry is a flagrant distortion of our observations. First, neither healthy control subjects nor dry AMD groups exhibited CNV; therefore, systemic IL-18 levels do not correlate (negatively or positively) with CNV. If we were to employ similar reasoning, since 32% to 58% of patients with CNV also develop geographic atrophy (GA),\textsuperscript{22} we could erroneously conclude that lower systemic IL-18, which we observed in CNV patients compared with GA patients, prevents GA development in the setting of CNV. Second, our study does not purport to (nor was it designed to) determine whether systemic IL-18 causes CNV in humans. We specifically excluded patients with mixed (neovascular and atrophic AMD) disease phenotypes in order to permit analyses in ‘purer’ populations. We have now measured IL-18 levels in patients with combined CNV and GA, and find that serum IL-18 is increased in these patients as well, and is not statistically different from serum IL-18 levels in patients with GA alone (P = 0.86), empirically demonstrating that Doyle et al.’s statement is ungrounded.

Doyle et al.,\textsuperscript{2} notes that clinical trials of systemic administration of GSK recombinant human IL-18 have not reported geographic atrophy as adverse events. We are unable to locate in the published literature any reports detailing retinal findings (e.g., fundus photography, optical coherence tomography, fundus autofluorescence, fluorescein angiography) in patients who have received this drug (PubMed & Google Scholar searches, October 14, 2014). Ophthalmologists are well aware that drugs with known propensity to cause RPE toxicity (e.g., hydroxychloroquine) do not result in patient-reported vision loss for years, and excellent vision (20/40 or better) is preserved in the majority of patients even after RPE pigmentation changes are observed by fundus examination.\textsuperscript{23} Another example is deferoxamine, which also is known to induce RPE toxicity. Even among only the deferoxamine-treated patients with visual symptoms (not to mention the many more who did not report visual symptoms), the majority have excellent vision (20/40 or better).\textsuperscript{24} Absence of evidence is not evidence of absence for, as the biomedical and public health communities are acutely aware, an astonishing number of adverse effects in clinical trials are not detected or reported.\textsuperscript{25–27} Moreover, the purported safety of GSK IL-18 in those clinical trials, which primarily studied nonelderly patients, remains to be determined in the much older CNV population that is at risk for geographic atrophy. Finally, we have not claimed that systemic administration of IL-18 would induce RPE toxicity. Indeed, there may be differences between systemic and intravitreal administration based on bioavailability and manner of presentation. We look forward to publication of the ocular phenotypes of patients treated with GSK IL-18.

Finally, Doyle et al.,\textsuperscript{2} mentions other studies on the influence of IL-18 on CNV and ocular angiogenesis. The report by Shen et al.,\textsuperscript{28} administered R&D Systems/MBL IL-18 (the very same reagent that Doyle et al.,\textsuperscript{2} earlier claimed lacks signaling activity), and found reduced laser CNV area. CNV area is not a robust metric for measuring laser CNV response and is not considered best practice, which is to measure the volume of the resulting neovascular lesion.\textsuperscript{29} Furthermore, the reported reduction achieved the barest of statistical significance (P = 0.0497). Also as with the recent report by Doyle et al.,\textsuperscript{3} this study was conducted using only a single dose of IL-18 administered into the eyes of wild-type mice. No dose response or loss-of-function studies were performed, which reduces the robustness of these findings. It should be noted that a Bayesian analysis indicates that this observation has a 26% probability of being spurious, given the reports by five other groups with the same IL-18 reagent showing no effect on CNV volume.

Doyle et al.,\textsuperscript{2} refers to another study in which the role of IL-18 is inferred in the development of CNV due to overexpression of VEGFA in mice.\textsuperscript{30} The reduction in VEGFA-induced CNV lesions by knockout of IL-18R1 did not reach statistical significance compared with the wild-type control despite a relatively large sample size (N > 7 mice). This suggests that either the magnitude of the effect of IL-18 in these mice is not great, or the variability of this model is high. Importantly, the readout for this study was the incidence of CNV lesions, the relevance of this parameter and its relationship to laser CNV volume and neovascular AMD is not known. Finally, with regards to other ocular models in which IL-18 is purported to modulate angiogenesis, our experiments and data interpretation do not speak to these, just as they do not speak to other studies that demonstrate a proangiogenic effect of IL-18.\textsuperscript{31–35}

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References


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