Shutting down the inflammasome pathway in diabetic retinopathy

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Purpose: Connexin43 hemichannel mediated adenosine triphosphate (ATP) release is an activator of the NLRP3 inflammasome pathway which underlies chronic inflammatory diseases including diabetic retinopathy. Using an in vitro model and a connexin43 hemichannel blocker, Peptide5, we sought to evaluate the role of connexin hemichannels in diabetic retinopathy and to determine the mechanism by hemichannel mediated ATP release amplifies and perpetuates the disease.

Methods: Retinal pigmented epithelial (ARPE-19) cells were exposed to high glucose, 10 ng/mL pro-inflammatory cytokines IL-1β and TNF-α, or a combination of both. Quantitative Cytometric Bead Array analysis was used to measure the release of the inflammatory cytokines IL-6 (pro-inflammatory cytokine), IL-8 (neutrophil chemotactic factor), MCP1 (Monocyte chemoattractant) and ICAM-1 (leukocyte-endothelial cell adhesion factor), as well as VEGF and ATP. Peptide5 was used to block connexin43 hemichannels.

Results: Co-application of high glucose and pro-inflammatory cytokines significantly increased the secretion of IL-6, IL-8, MCP-1, sICAM-1 and VEGF compared to the cytokines alone. High glucose alone had no effect. Hemichannel block using Peptide5 prevented the increase in cytokine release. Peptide5 also prevented ATP release following application of glucose and inflammatory cytokines. Adding exogenous ATP, however, negated Peptide5 protection against cytokine release.

Conclusion: Connexin43 hemichannels perpetuate inflammation and may underlie the onset of neovascularisation in chronic retinal disease by mediating an ATP autocrine feedback loop in the inflammasome pathway. Targeting connexin43 hemichannels offers potential to break the inflammatory cycle in diabetic retinopathy, including VEGF release. The results have implications for other chronic retinal disease indications and elsewhere.