

## **The ratio of pro- and anti-angiogenic cytokines produced by retinal pigment epithelial cells is shifted to support angiogenesis by complement**

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### **Purpose**

The complement system of age-related macular degeneration (AMD) patients is marginally but chronically over-activated. Retinal pigment epithelial (RPE) cells and photoreceptor cells undergo cell death during the development of this potentially blinding eye disease. In this study the balance between the pro-angiogenic vascular endothelial growth factor (VEGF) and the anti-angiogenic pigment epithelium-derived factor (PEDF) by RPE cells in response to complement serum was analysed.

### **Methods**

Increasing concentrations of complement competent human serum were incubated with human RPE cells. Controls with the addition of zymosan to activate the complement cascade, zymosan alone, and heat-treated serum with inoperative complement were included. The secretion of VEGF and PEDF was measured by sandwich ELISA. Immunocytochemistry was performed for the in situ detection of VEGF and PEDF. The experiments were supplemented by RT-PCR expression analysis and Western Blot detection of both antagonists.

### **Results**

Human complement competent serum stimulated the RPE cells to produce enhanced amounts of VEGF while unspecific stimuli showed no influence on the secretion of VEGF. The combination of complement competent serum and zymosan was revealed as the most effective treatment for an increased VEGF production. The PEDF-specific staining of RPE cells decreased with augmented concentrations of complement competent serum. PCR data showed an enhanced amount of VEGF-encoding transcripts and an unaltered or lower amount of PEDF-specific transcripts. Western Blots confirmed the shift in favour of VEGF when compared to PEDF after complement treatment of RPE cells.

### **Conclusions**

Activated complement may shift the balance between VEGF and PEDF produced by RPE cells towards the blood vessel chemoattractant VEGF. This finding may reveal a mechanism how enhanced complement activation might contribute to a pro-angiogenic retinal environment supporting neovascularisation during the late stage of exsudative AMD.

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