The influence of peripheral circulating monocytes from AMD patients on endothelial cell migration

[1,2]Yizhuo. Gao, [1]Jyoti. Agrawal, [1]Jason. O. Amartey, [2]W. Amoaku, [1]D. O. Bates

[1]Tumour and Vascular Biology Laboratories, Division of Cancer and Stem Cells, BioDiscovery Institute; and [2]Dept of Ophthalmology, Division of Clinical Neurosciences; School of Medicine, University of Nottingham.

Purpose: Vascular endothelial growth factor (VEGF) is known to play an important role in both the onset and progression of age-related macular degeneration (AMD). VEGF-A pre-mRNA can be alternatively spliced at exon 8 to generate two families of isoforms: VEGF-A_{165}b and VEGF-A_{165}a and can be highly expressed by monocytes. We aimed to investigate the whether peripheral blood monocytes from AMD patients could influence endothelial cell angiogenic behaviours in vitro.

Method: We collected blood and isolated monocytes from 3 patients with AMD and 3 healthy controls using CD14 magnetic bead purification. We undertook an endothelial cell migration assay across a polycarbonate porous membrane (8μm pores) in the presence of 1nM recombinant human (rh) VEGF-A_{165}a with or without 50,000 monocytes and either human IgG or a neutralising antibody to VEGF-A_{165}b. Endothelial migration was assessed by counting cells on the abluminal side of the membrane after staining with DAPI.

Results: The migration rate of endothelial cells was significantly enhanced by rhVEGF-A_{165}a (167±13% of cells not treated with VEGF-A_{165}a). This was significantly reduced by incubation with monocytes from wet AMD patients (105±3.9%, $p<0.01$) but not by monocytes from healthy control patients (111±11%, $p=0.6$). The migration rate of endothelial cells in the AMD monocytes group was significantly enhanced by the anti-VEGF-A_{165}b antibody (162±10%, $p<0.01$ compared with untreated monocytes), but the effect of normal monocytes was not altered (114±14%, $p=0.9$).

Conclusion: Peripheral circulating monocytes from patients with AMD can inhibit angiogenic behavior in endothelial cells, which is due to expression of VEGF-A_{165}b.