Monocytes from patients with Peripheral Artery Disease inhibit endothelial cell migration by producing anti-angiogenic $VEGF_{165}b$

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Peripheral artery disease (PAD) is a significant contributor to morbidity and mortality in patients with cardiovascular disease as a consequence of a combination of atherosclerosis and reduced vessel collateralisation. One of the key factors regulating blood vessel growth (angiogenesis) is Vascular Endothelial Growth Factor (VEGF), responsible for mediating endothelial cell migration, and proliferation. Paradoxically, higher expression levels of VEGF in cardiovascular patients are associated with impaired angiogenic capability, probably due to the expression of different mRNA splice isoforms of VEGF. Monocytes are actively involved in angiogenesis so we tested the hypothesis that in PAD patients monocytes secrete elevated level of anti-angiogenic isoform VEGF-$A_{165}b$ and reduced the effect of pro-angiogenic VEGF-$A_{165}a$ on endothelial migration. To test this, we used a fully humanised anti-VEGF-$A_{165}b$ antibody in an endothelial cell migration assay.
Blood was collected from four PAD patients (defined as Ankle:Brachial Pressure index <0.9) and monocytes were isolated with CD14 microbeads. $CD14^+$ labelled monocytes were seeded in a 24-well plate and primary endothelial cells (Human umbilical vein endothelial cells; HUVEC) were grown on 8µm inserts on the top of monocytes and endothelial cells stimulated to migrate across the pore by incubating the with pro-angiogenic VEGF-$A_{165}a$ at 40ng/ml. 24hr later we counted the number of cells migrated across a porous membrane in triplicate by staining them with DAPI (nuclear staining dye), treated and untreated with the anti-$VEGF_{165}b$ antibody (N=3 per patient). VEGF-$A_{165}a$ induced migration of endothelial cells (208±13% of control, $P<0.001$), which was blocked by co-incubation with monocytes from PAD patients (151±9% of control, $p<0.01$). The inhibition of migration induced by monocytes from patients with PAD was reversed by anti-$VEGF_{165}b$ antibody (225±22% of control, $P<0.001$ compared with monocytes and VEGF-$A_{165}a$ but not significantly different from VEGF-$A_{165}a$ alone).
Our findings support the concept that $VEGF_{165}b$ could be a new target to treat ischemia in PAD patients and is associated with impaired vascularisation. We identify that humanised anti-$VEGF_{165}b$ antibody could prove to be a potential therapeutic and could promote the angiogenic response by contribute to tissue revascularization in ischemic muscle of patients with PAD.