206 **ASSESSMENT OF GAP JUNCTION COMMUNICATION BETWEEN HUMAN UMBILICAL ENDOTHELIAL CELLS AND MONOCYTES IN RESPONSE TO TUMOUR NECROSIS FACTOR (TNF-A)**

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Introduction Atherosclerosis is a chronic inflammatory disease characterised by accumulation of monocytic cells and lipids within the sub-endothelial space. This is facilitated by monocyte (MO) trans-endothelial (EC) migration early in atherosclerosis development and requires direct MO-to-EC contact. This is enhanced by adhesion molecules in the presence of pro-inflammatory stimuli such as tumour necrosis factor-α (TNF-α). Both cell types express connexin (Cx) 43 isoforms that permit formation of gap junctions (GJ); channels permeable to small molecules (<1KDa). We hypothesised that MOs and ECs communicate through formation of Cx43 GJs, which is enhanced by TNFα.

Materials and Methods Human umbilical vein endothelial cell (HUVEC) monolayers were maintained in endothelial growth medium whose functional integrity was assessed by measuring trans-endothelial electrical resistance (43.94±3.32Ω.cm2;n=6). A dye transfer assay examined functional GJ formation between MOs and ECs. HUVEC monolayers were loaded with 5mM calcein-AM dye: free calcein is membrane impermeable and only permeates through GJs. Suspensions of freshly isolated MOs from peripheral blood were added to HUVEC monolayers. Calcein transfer to MOs was measured by flow cytometry and normalised to positive control values obtained from MOs directly loaded with calcein. A negative control used a Cx43 deficient MO cell-line, THP-1 monocytes. Experiments were carried out in endothelial basal medium (EBM) in the absence and presence of 2ng/ml TNF-α. The efficiency of dye transfer to MOs through GJs was measured in: 1) the presence of GJ inhibitor, 2mM heptanol; 2) THP-1 monocytes; 3) HUVECs transfected with Cx43-siRNA. Data are means±SEM, compared by ANOVA, the null hypothesis was rejected at $p<0.05$.

Results Dye transfer to MOs, after incubation with calcein-loaded HUVECs monolayers for 30 minutes in EBM was recorded in 66.3 ±2.1% (n=3) of cells. This fraction was attenuated by heptanol (9.9±4.5%;n=3;P<0.001) and was comparable to the fraction of loaded THP-1 cells (8.33±4.66;n=3). For HUVECs transfected with CX43-siRNA, the fraction of calcein-loaded MOs also decreased compared to control,11.3±4.5% vs 74.1±2.1%, respectively (n=3; P<0.05). TNF-α enhanced the fraction of calcein-loaded MOs to 89.6±2.5% (n=3;P<0.001) in control monolayer. Such an effect was prevented in monolayer transfected with Cx43-siRNA (13.70 ±2.45%;n=3; P<0.001)

Summary and Conclusions These results show that calcein-dye transfer through GJs formed between ECs and MOs. Dye transfer was attenuated by lack of Cx43 in MO, knock down of Cx43 in HUVECS with Cx43-siRNA or application of GJ blockers. TNF-α increased calcein transfer to MOs and had no effects in HUVECs with Cx43-siRNA. This is the first study to report the important role of GJ communication between MOs and ECs as an underlying mechanism for early stages of atherosclerosis development.