for therapeutic neovascularization. The present study was conducted to determine the effect of CD34+ progenitor cells on the formation of capillary tubes by human microvascular endothelial cells (hMVEC) in vitro. Using real time video microscopy we studied the process of 'homing' of progenitor cells to sites of tube formation in a 3D capillary tube assay. In this assay tubular structures were induced by the simultaneous presence of VEGF and TNFa. 24 h after induction of tube formation CD34+ progenitor cells were isolated from either human peripheral blood or cord blood and added to the culture. Movement of the CD34+ cells over the hMVEC monolayer was recorded by time-lapse video microscopy for 8 h. During analysis of the data the areas of tubular structures were marked and the number of cells entering and leaving the areas were counted. As control the same surface areas were projected to areas of the corresponding hMVEC culture but devoid of tubes. During movement over the endothelial monolayer the percentage of the CD34+ cells that migrated into the areas of tube formation and remained there was 80±1% of the total number of entering cells, while in the non-tubular areas this figure was 17-fold less (4.6±1.2%). Immunohistochemistry confirmed the viability of the migrated CD34+ cells. The fraction of cells that incorporated into the lining of the tubular structures was less than 1% after a 5 day period). Blocking antibodies against ICAM-1, VCAM-1, E-selectin, and P-selectin did not inhibit this process (accumulation 75± 1%). The influence of oxygen tension and possible role for SDF1 and CXCR4 are currently under investigation.

doi:10.1016/j.vph.2006.08.247

B12.08

Induction of ICAM-1 and VCAM-1 adhesion molecules in endothelial cells by TNF receptor 1 through the NF-kB1 pathway

Z. Zhou, D.J. MacEwan

University of East Anglia, Norwich, United Kingdom

Background: Tumor necrosis factor (TNF) is a pro-inflammatory cytokine, principally derived from mononuclear phagocytes, the primary targets of which include vascular endothelial cells (EC). The aims of this study are to elucidate the effect of TNF and its subtypes on activation of NF-kB family and downstream expression of adhesion molecules ICAM-1 and VCAM-1 in Endothelial cells.
Methods: Human Umbilical Vein Endothelial Cells (HUVECs) were used in this study and Western Blottings were carried out to detect the level of proteins of interests.
Results: TNF acting through the TNFR1 subtype could activate the processing of NF-kB1 p105 in around 4 h. TNF and TNFR1 stimulations also induce the expression of NF-kB2 p100 after 12 h, which is thought to be a transcriptional target of the NF-kB1 signalling pathway. By contrast, TNFR2 had no effect on either the processing NF-kB1 p105 of or induction of NF-kB2 p100. Adhesion molecules ICAM-1 and VCAM-1 were induced by TNF and TNFR1, and can be blocked by Emodin, the inhibitor of NF-kB. While the inhibitor of MAPKs including ERKs, p38 MAPK and JNK, had no effects on the expression of ICAM-1 and VCAM-1 after TNF stimulation.
Conclusion: TNFR1, but not TNFR2, is responsible for the activation of NF-kB1 pathway through degradation of IkB-a and processing of NF-kB1 p105, by which activate the expression of ICAM-1 and VCAM-1. Transcriptional induction of NF-kB2 p100 by TNFR1 may influence the amplitude and duration of its activation of NF-kB responses in HUVECs.

doi:10.1016/j.vph.2006.08.248

B12.09

Interaction of platelets with dendritic cells — Potential role for immunomodulatory processes in vascular remodelling

Harals Langer[1], Gregor Braun[1], Karin Daub[1], Andreas E. May[1], Peter Brossart[2], Martin Schaller[3], S. Wesselborg[4], Meinrad Gawaz[1]

[1] *Medizinische Klinik III, Universitätsklinikum Tübingen, Tübingen, Germany*
[2] *Medizinische Klinik II, Universitätsklinikum Tübingen, Tübingen, Germany*
[3] *Dermatology, Universitätsklinikum Tübingen, Tübingen, Germany*
[4] *Medizinische Klinik I, Universitätsklinikum Tübingen, Tübingen, Germany*

Introduction: Immunological processes and thrombotic events take place next to each other within atherosclerotic plaques. In this study we investigated the interaction of platelets with dendritic cells (DCs), specialized antigen-presenting cells.
Methods/results: DCs, which express the adhesion receptors VLA-4, MAC-1 and PSGL-1 showed no adhesion to immobilized collagen I. However adhesion of DCs was substantially mediated by platelets immobilized on collagen I under static and dynamic flow conditions. Prolonged coincubation of DCs with platelets for several days induced DC activation as verified by a mixed lymphocyte reaction assay. Phase contrast microscopy, electron microscopy and phagocytosis assays revealed a complete phagocytosis of platelets by DCs after 10 days. This resulted in programmed cell death of the DCs, as verified by apoptosis assays.
Conclusions: We conclude, that platelet mediated phagocytosis of platelets by DCs may play a critical role in immunomodulation or repair at sites of vascular lesions, revealing a possible new role of platelets in vascular remodelling.

doi:10.1016/j.vph.2006.08.249