and could contribute to EC activation at atherosclerosis prone sites of the vasculature.

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A1.31

The effect of subtoxic doses of verocytotoxin on endothelial cells

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Verocytotoxin (VT)-producing *E. coli* (VTEC) O157:H7 is the most important pathogen in D+Hemolytic Uremic Syndrome (HUS). VT, in a toxic dose, inhibits the protein synthesis and causes cell destruction of the renal endothelium. However, reports of the effects of the influence of a subtoxic dose are conflicting. Therefore, the effect of a subtoxic dose on protein synthesis in the endothelial cell was tested. This possibly is a better approach of the in vivo situation. For this purpose HUVEC was incubated during 6, 12, 24, 48 and 72 h with LPS (1 μg/ml), VT1 or VT2 (both 10 nM). MRNA of ICAM-1 and IL-8 was measured. The expression of adhesion molecules (ICAM-1, VCAM and E-selectin) was determined by cell-ELISA and FACS. Also Western blotting of ICAM-1 was performed. Both mRNA levels for ICAM-1 and IL-8 are massively elevated in 4 donors HUVEC after 24 h of stimulation with VT1 (ICAM-1 respectively 2263%, 767%, 2141% and 18,611%; IL-8 2156%, 2343%, 21,231% and 14,301%). An earlier, but almost equal up regulation, was observed after only 4 h of incubation with LPS. Neither VT1 nor VT2 increased the surface expression of adhesion molecules after 24 h. But after 48 h a small increase of ICAM-1 was measured with FACS-analysis. Western blot of ICAM-1 did also show a slight up regulation of ICAM-1 after 48 h of stimulation with VT1. In contrast, a distinct up regulation by LPS was evident already after 6 h. We can conclude that mRNA for ICAM-1 was abundantly up regulated by a subtoxic dose of VT1. It is not yet clear why abundantly increased mRNA levels are not reflected by highly increased expression of the protein in and on the surface of the cell. These results can possibly fit into the ribotoxic stress model, which is under current investigation as a model for the mechanism of toxicity of VT.

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A1.32

Rapid alterations in endothelial focal contacts during polymorphonuclear leukocyte transverse migration

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Once polymorphonuclear leukocytes (PMNs) have transmigrated across an endothelial monolayer, they usually undergo subendothelial transverse migration before penetrating into inner tissue layers. Although cell migration in general is accompanied with intracellular calcium signaling and dissolution/reconstruction of focal contacts, whether and how endothelial cells respond to the PMN transverse migration underneath is unknown. We have constructed a tissue flow chamber to allow direct observation of PMN transverse migration and its associated endothelial responses using either endothelial monolayer cultured on a collagen gel or vascular tissues. Our results indicated that transverse migrating PMNs were directly in contact with the basolateral side of endothelial cells. Moreover, paxillin, but not focal adhesion kinase or proteins with phosphorylated tyrosine, was transiently disturbed during PMN transverse migration. Paxillin disappeared from focal contacts at the PMN's leading edge and rejoined them at the PMN's rear end in less than 20 s. Unlike cell migration, this process was not accompanied with detectable endothelial calcium signaling. Taken together, endothelial cells showed rapid and reversible focal contact alterations in response to the transmigrating PMN underneath.

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Repetitive exposure to apoptotic cells induces an acceleration of atherosclerosis in apolipoprotein E knockout mice

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Atherosclerosis is an immuno-inflammatory disease of the arterial wall. Apoptotic cells accumulate within the lesions as plaques progress towards the advanced stages. Because theses cells bear oxidation-specific epitopes with immunogenic and pro-inflammatory properties, we hypothesized that repetitive exposure to apoptotic cells could participate to the development of atherosclerosis.
Methods and results: Twelve-week-old male ApoE KO received intravenous injection of saline (control, $n=8$), 1×106 fresh

bone marrow cells (BM, $n=7$), 7.5×105 apoptotic BM cells (BMaptv, $n=8$), or 7.5×105 apoptotic neonatal neuronal cells (Napt group, $n=6$). Animals received 6 successive injections every other week. Total cholesterol levels were not different between groups. The percentage of Annexin5 positive cells injected in each groups was significantly higher in BMapt and Napt (67±5%, 70±9% respectively) compared to BM (28±3%). We found that BM cells treatment had no effect on atherosclerosis development (246,658±24,513 μm2 in control vs. 284,026±25,577 μm2 in BM). Interestingly, stimulated purified CD4+T cells from these mice showed a marked reduction of interferon-gamma (INT-g) and IL-4 production, and an increase of IL-10 production, indicating a switch of the immune response toward a regulatory anti-inflammatory phenotype. In mice treated with BMapt and Napt cells, the development of atherosclerosis in the aortic sinus was significantly increased by 36% and 50% compared with control mice (334,542± 30,073 μm2 in BMapt and 369,445±21,748 in Napt, $P<0.05$) and the macrophage content increased significantly only in the Napt group (+49%, $P<0.05$), whereas collagen and smooth muscle cell content was not significantly modified. Compared to BM group, stimulated purified CD4+T cells from BMapt and Napt produced similar INT-g and IL-4 levels but increased IL-10 production. Mice treated with Napt developed anti ssDNA antibodies (+99%, $P<0.01$) compared to BM group, suggesting an autoimmunity response.

Conclusions: These findings indicate that repetitive exposure to low doses of apoptotic cells did not accelerate atherosclerosis, possibly due to the development of a regulatory immune response. High doses of apoptotic cells induced a marked acceleration of atherosclerosis associated with an auto-immune response.

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A1.34

Donor dopamine pretreatment influences leukocyte infiltration and cytokine expression in the brown Norway to Lewis kidney transplantation model

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Donor catecholamine usage improves initial renal function after transplantation, reduces the incidence of acute allograft rejection and improves transplantation outcome. Previously we have demonstrated in the Fischer to Lewis chronic rejection model that treatment of Fischer donors with dopamine (DA) also improved initial renal function and reduced the number of ED1 and MHC class II positive infiltrating cells after transplantation. In the present study we used the Brown-Norway (BN) to Lewis model as a model for acute rejection, it is a more severe rejection model, to test if DA treatment of BN donors also reduces the amount of infiltrating cell quantitatively and qualitatively. BN rats were pretreated via osmotic mini-pumps for 24 h with DA (5 μg/kg/min) or NaCl 0.9% as control. Hereafter the kidneys were flushed and stored at 4 °C for 24 h in UW solution. The renal allografts were orthotopically transplanted into Lewis recipients. As additional control, a syngeneic transplantation Lewis to Lewis was performed. A daily injection of cyclosporine A (2.5 mg/kg) was administered until the recipient rats were sacrificed. Animals were sacrificed at various time points (1, 3, 5, 10 day) postoperatively and the allografts were analyzed by light microscopy, immunohistochemistry (CD3, MHC class II and ED1) and by Rnase protection assay for cytokine mRNA expression. By light microscopy, evidence was found for severe tubilitis and vasculitis 5 days and 10 days after transplantation, no difference was found between DA and NaCl treated animals. In immunohistochemistry, the number of MHC class II+cells and CD3+cells was significantly decreased. No difference was found in the number of ED1+cells. The expression Ltα, TNF, IL-1 ß and IL-2 increased over time and was clearly less increased in DA treated animals.

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A1.35

Inhibitory effect of statins on IL6 mRNA expression in THP-1 cells subjected to an inflammatory stimulus

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Statins are used clinically for lipid-lowering but their pleiotropic effects, including an anti-inflammatory role, are being increasingly reported. We have investigated the effect of two commonly prescribed statins, pravastatin and atorvastatin, on the endogenous and stimulated IL6 mRNA expression in the macrophage-like differentiated THP-1 cell line THP-1 cells were differentiated into macrophage-like cells using phorbol myristate acetate (PMA) for 72 h. Differentiated cells were then treated with or without an increasing dose of statins in the presence or absence of interferon gamma (IFNγ), a known activator of macrophages, for 24 h. Quantitative RT-PCR (QPCR) was used to quantitate the level of IL6 mRNA relative to a known housekeeping gene. Whilst no significant difference was observed between the effects pravastatin or atorvastatin on the endogenous IL6 mRNA levels in differentiated THP-1 cells, we showed that both pravastatin and atorvastatin resulted in a dose-dependent reduction in IL6 mRNA induction by IFNγ in differentiated THP-1 cells (see Table).