Background: Bone marrow (BM) derived stem cells secrete multiple growth factors and have been shown to differentiate to cardiomyocytes and endothelial cells in mice. This study evaluated the effect of trans-epicardial BM cell transplantation in a porcine non-reperfused myocardial infarction model.
Methods: In 14 domestic pigs, myocardial infarction was created by occluding the first diagonal artery with coils. Twenty-eight days later, Iliac crest BM cells were aspirated, filtered, labeled with bromodeoxyuridine (BrdU) and cultured for 48 hrs. Injections of 0.2 ml (~1.5 million cells/ml) (BM, n=11 pigs and saline, n=3 pigs) were evenly distributed 1cm apart in the scar (8 injections) and around the scar (8 injections). Animals were sacrificed at 4 (n=3), 14 (n=4) and 28 days (n=7) for histology and immunohistochemistry analysis. Trans-epicardial echocardiography was performed at the time of injection and at sacrifice to assess regional contractility.
Results: Positive BrdU cells were identified in infarcted areas at 4, 14 and 28 days of BM transplanted animals. Muscle cells (α-actinin positive cells) in the scar tissue were 21.1±15.7 /mm2 in the BM group and 13.6±2.4 /mm2 in the saline group at 28 days (p=0.07). At 28 days the number of endothelial cells (factor VIII positive cells) was greater in the BM group than in the saline group (19.5±8.9 vs. 8.9±15.1 cells/mm^2 p=0.07). Capillaries >50μm at 28 days were 10.95±3.02 /mm^2 in the BM group and 4.8±0.4 /mm^2 in the saline group (p=0.01). Wall Motion Score Index was 2.0±0.1 at baseline and 2.3±0.12 at 28 days in the BM group (p=0.18) and 2.0±0.1 in the saline group at 28 days, (p=0.40 vs BM).
Conclusion: Bone marrow cell engraftment is feasible with viable transplanted cells at 28 days within scar tissue of non-reperfused myocardial infarction. Angiogenesis was improved after BM injection. However, higher muscle cell area and vascular density in the scar were not correlated with improvement of left ventricular function at 28 days.

1178-140 Caldesmon Regulates Apoptosis and Cell Cycle Progression in Capillary Endothelial Cells and Inhibits Angiogenesis In Vitro

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Capillary endothelial (CE) cells can be switched between growth and apoptosis by modulating their shape and cytoskeleton (CSK) using micropatterned adhesive islands (Science 1997; 276:1425-1428). To further examine how the CSK contributes to this switching mechanism, we used an adenoviral vector carrying caldesmon, which inhibits CSK tension generation antagonizing Rho activity and disassembles acto-myosin filaments when overexpressed. Associated changes in cell shape and CSK organization were visualized in living cells by expressing GFP-caldesmon under tight control of a Tet-off system. Increase in cellular GFP-caldesmon resulted in progressive loss of actin stress fibers, disassembly of focal adhesions and cell retraction; the most highly retracted cells covered about one-third the project cell area of controls. The apoptotic index measured by quantitating TUNEL staining increased in parallel as GFP-caldesmon levels were increased and cell retraction was promoted. The smallest cells exhibited levels of apoptosis similar to that observed during anoikis in fully detached cells (42 vs. 55% TUNEL staining, respectively). Conversely, cell cycle progression into S phase (monitored by nuclear incorporation of BrdU at 24 hr) decreased from 35 to 9% as cells were progressively rounded up under similar conditions. In chicken aortic ring assay, GFP-caldesmon transfection inhibited vascular sprouting up to 60% if compared with those of GFP alone or control (5.2mm vs. 10.5mm vs. 12.1mm diameter of sprouting area, respectively). These data confirm that the CSK mediates the mechanism by which extracellular matrix-dependent changes in cell shape influence CE cell growth and apoptosis during angiogenesis. The adenoviral vectors encoding GFP-caldesmon under Tet-off control also may prove useful in future studies analyzing the role of CSK tension and structure during control of capillary development in angiogenesis.

1178-141 Can Vascular Endothelial Growth Factor Improve Blood Flow in Diabetes?

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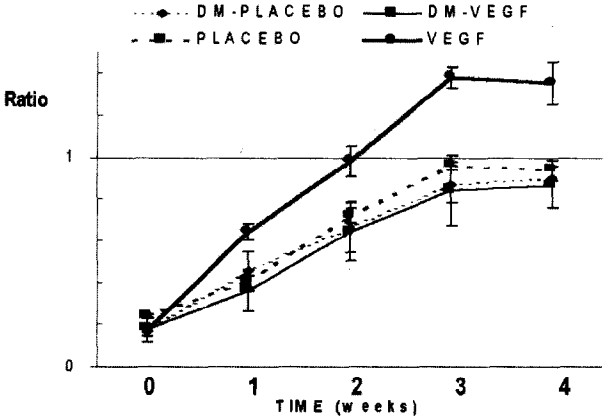
Diabetes Mellitus (DM) is associated with increased cardiovascular morbidity and mortality. Collaterals can protect from ischemia. Considerable debate exists concerning the size and extent of the collateral circulation in DM. Our aim was to evaluate the usefulness of continuous perimuscular infiltration of naked-DNA encoding VEGF, to augment collateral formation and tissue perfusion in a DM mouse unilateral ischemic hindlimb model.
Methods: DM was induced with Streptozotocin (80 mg/kg). An osmotic pump with saline or 500-microgram VEGF was implanted intra-abdominaly with an outlet-tube fenestrated and tunneled into the muscle. Ischemic/normal limb blood flow was measured using a laser Doppler blood flowmeter once a week; tissues were analyzed for smooth muscle actin and factor-8. DM mice were compared to normal mice.
Results: In normal mice, a faster restoration of blood flow was observed in the VEGF treated, however in the DM mice, there was no difference in the rate of flow restoration between the VEGF-treated or placebo-treated arm (graph). The blood flow was almost complete for the normal mice but reached a ratio of 0.8-0.9 for both DM arms and for normal placebo. The blood vessel density was higher for the both DM arms compared to the normal mice.



Conclusion: Our results demonstrate the rapid and successful restoration of blood flow using naked-DNA encoding VEGF in a normal mice but lack of benefit of VEGF administration in DM mice. This may have major implication on angiogenic treatment in patients with DM.

1178-142 IL-6 Is Produced by Splenocytes Derived From CMV-Infected Mice in Response to CMV Antigens, and Induces MCP-1 Production by Endothelial Cells: A New Mechanistic Paradigm for Infection-Induced Atherogenesis

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Background: Atherosclerosis is an inflammatory disease. One of the candidate inflammatory triggers is infection. To further characterize the interaction between infection, cytokine induction, and atherosclerosis, we tested the hypothesis that cytomegalovirus (CMV) infection induces the pro-inflammatory cytokine interleukin-6 (IL-6), which in turn induces "pro-atherosclerotic" changes in vascular endothelial cells (ECs).
Methods: ELISA was used to determine the levels of monocyte chemoattractant protein-1 (MCP-1) in the supernatant of mouse and human ECs incubated with IL-6, and IL-6 levels in supernatants of splenocytes, derived from CMV infected and uninfected mice, stimulated with mouse CMV antigens.
Results: IL-6 induced, in a dose response fashion, MCP-1 expression in human ECs: 0, 2, 10, and 50 pg/ml IL-6 increased MCP-1 levels in EC conditioned medium from 1120±65, to 1148±105, 1395±40, and 2119±130 pg/ml, respectively, (P trend < 0.001). IL-6 also induced MCP-1 expression in mouse ECs (p<0.002). Importantly, IL-6 concentration in the supernatants of splenocytes stimulated with CMV antigens rose from undetectable levels in uninfected mice to14.9±5 pg/ml in the infected mice (P<0.04).
Conclusions: These results suggest a previously unrecognized, but potentially important mechanism whereby CMV, and other pathogens, contribute to atherogenesis: T-lymphocytes, clonally expanded in response to antigens presented by CMV infection, home to sites of vascular injury and locally release IL-6 when presented with either pathogen antigens that may be present in the plaque, or when they cross-react with host peptides homologous to the relevant pathogen antigens; IL-6 then triggers ECs to release MCP-1, which recruits more monocytes and T-cells into the vessel wall and thereby exacerbates local inflammation, and thus atherogenesis.

1178-143 Angiogenesis Induced by Human Hepatocyte Growth Factor Gene Without Nitric Oxide Synthesis in a Rat Ischemic Hindlimb Model

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Background: Vascular endothelial growth factor has been reported that it has the effect of angiogenesis through the nitric oxide (NO). Hepatocyte growth factor (HGF) has also been reported to have the effect of angiogenesis. However, it has not been reported whether HGF induces angiogenesis through the NO. In this study, we examined the feasibility of gene therapy using the HGF gene to treat peripheral arterial disease model rat in chronically inhibition of NO synthesis.
Methods: L-NAME (NO synthesis inhibitor) was obtained by drinking water (1mg/ml) from before resection of left femoral artery throughout this experiment. Sprague-Dawley rats were divided into three groups, which were transfected Human HGF naked plasmid DNA vector (500μg) or control vector (500μg) with L-NAME administration, and control vector without L-NAME administration. The naked plasmid was transfected into an ischemic hindlimb by intramuscular injection at1 week after resection. At 4 weeks after transfection, angiogenesis were assessed by angiography and tissue capillary density.
Results: At 4 weeks after transfection, blood pressure was significantly increased in rats administrated with L-NAME, but HGF transfection by intramuscular injection was not effect on blood pressure. The human HGF vector transfected rats administrated with L-NAME was resulted in significant increase in peripheral blood flow assessed by angiography compared with control vector administrated with L-NAME. Consistent with the increase in blood flow, a significant increase in tissue capillary number could be detected