2:15

## 409-2 Regulated Expression of Fas Ligand on Endothelium Modulates Leukocyte Infiltration of the Blood Vessel Wall

M. Sata, K. Walsh. *Elizabeth's Medical Center, Tufts University School of Medicine, Boston, MA, USA*

The vascular endothelial surface is in constant contact with circulating cellular constituents of the blood, but little is known about the mechanisms by which endothelial cells (ECs) actively prevent inflammatory cell infiltration of the subendothelial space. Fas ligand (FasL), a type II membrane protein, induces apoptotic cell death in Fas-bearing cells and the Fas-FasL system has been implicated in the regulation of physiological cell turnover, particularly in the immune system. Although the expression of FasL was originally considered restricted to activated T lymphocytes, FasL has been identified in immune-privileged tissues suggesting that expression of FasL contributes to their immune-privileged status by preventing the infiltration of inflammatory leukocytes. Here, we report the surprising finding that ECs express FasL, which can induce apoptosis in Jurkat cells. Tumor necrosis factor $\alpha$ (TNF$\alpha$) down-regulated FasL expression in ECs with an accompanying decrease in cytotoxic activity. Local administration of TNF$\alpha$ into the rabbit ear central artery down-regulated the FasL expression, and this correlated with robust mononuclear cell infiltration into the arterial wall. This TNF$\alpha$-induced mononuclear cell infiltration could be markedly attenuated by pre-infecting the endothelium with a replication-defective adenovirus that constitutively expresses FasL. These findings not only establish a novel role of the vascular endothelium, but also hold a clue to the understanding of the pathogenesis of the atherosclerosis.

2:30

## 409-3 Non-viral Gene Transfer in Porcine Myocardium Enhanced by Transmyocardial Laser Revascularization

U. Sayeed-Shah, M.J. Mann, S. Grachev, R. Reul, S.J. Ferzoco, R. Laurence, V.J. Dzau, L.H. Cohn. *Brigham & Women's Hospital, Boston, MA, USA*

Transmyocardial laser revascularization (TMR) may be augmented by pro-angiogenic factors. We hypothesize that non-viral direct gene transfer is feasible at the site of TMR and that thermal injury may enhance gene uptake and expression. 10 yorkshire pigs had 3 equidistant 100 $\mu$l intramyocardial injections within 4 mm of 60 TMR sites sampled. Two vehicles (fusigenic HVJ-liposomes, and naked plasmid) encoding the gene for $\beta$-galactosidase ($\beta$-gal) were used for transfection. Similar injections were placed in 20 sites in nonTMR myocardium. $\beta$-gal protein levels were measured in tissue homogenates by ELISA with subtraction of background from TMR myocardium transfected with firefly luciferase gene.

*Results.* $\beta$-gal expression was detected in 56 of 60 TMR-transfected samples (93%), and 10 of 20 nonTMR transfected sites (50%), $p < 0.01$ by two tailed Fisher's Exact Test. The level of transgene expression did not vary significantly among the doses tested (992 ± 146 and 1211 ± 238 for 5 and 15 $\mu$g HVJ-liposomes); (1099 ± 322 and 761 ± 179, for 100 and 200 $\mu$g naked plasmid). However, the level of transgene expression in positive sites of TMR-transfected myocardium was 2.4 times higher than in nonTMR-transfected myocardium with positive expression (987 ± 114 vs. 408 ± 74 pg $\beta$-gal/mg protein), $p < 0.05$ by two-tailed Student's T-Test.

*Conclusion:* Non-viral gene transfer at sites of TMR is feasible, and that thermal injury associated with TMR enhances both the degree and efficiency of myocardial transgene expression.

2:45

## 409-4 Tumor Necrosis Factor-$\alpha$ and Insulin Induce Plasminogen Activator Inhibitor Type-1 in Adipocytes

T. Sakamoto, J. Woodcock-Mitchell, K. Marutsuka, J.J. Mitchell, B.E. Sobel, S. Fujii. *Department of Medicine, University of Vermont College of Medicine, Burlington, VT, USA*

Obesity is associated with hyperinsulinemia and elevated concentrations of tumor necrosis factor (TNF)-$\alpha$ in adipose tissue, and TNF-$\alpha$ has been implicated in the induction of synthesis of plasminogen activator inhibitor-1 (PAI-1), the primary physiologic inhibitor of fibrinolysis, by cultured adipocytes. To define mechanism(s) by which TNF-$\alpha$ induces PAI-1 production, 3T3-L1 preadipocytes were differentiated into adipocytes and exposed to TNF-$\alpha$ (1–10 ng/ml) for 24 h. The conditioned media were assayed for PAI-1 by Western blotting. TNF-$\alpha$ (10 ng/ml) selectively increased the synthesis of PAI-1 (2.6 ± 0.2 fold) (mean ± SE) (n = 6) without increasing activity of plasminogen activators. PAI-1 accumulation was inhibited by cycloheximide (25 $\mu$g/ml, n = 9) implying a requirement for protein synthesis. Superoxide (generated by xanthine oxidase 10 mU/ml plus hypoxanthine 0.6 mM) and hydrogen peroxide (100 $\mu$M) were shown to be potent inducers of PAI-1 (n = 6) as well. Furthermore, the hydroxyl radical scavenger tetramethylthiourea (20 mM) completely abolished the TNF-$\alpha$ induced increased in PAI-1 (n = 6). Exposure of adipocytes to TNF-$\alpha$ or insulin alone over 5 days increased PAI-1 production. The effect of TNF-$\alpha$ (5 ng/ml) was synergistically amplified by the concomitant presence of insulin plus TNF-$\alpha$. These results suggest that TNF-$\alpha$ stimulates PAI-1 production in adipocytes, an effect potentiated by insulin. Adipocyte generation of reactive oxygen centered radicals mediates the induction of PAI-1 production by TNF-$\alpha$. The induction was potentiated synergistically by insulin and that appears likely to contribute to the fibrinolytic system dysfunction known to exist in obese, hyperinsulinemic patients.

3:00

## 409-5 Apoptosis is Markedly Increased in the Aortic Valves from Rabbits on Hypercholesterolemic Diets

N.M. Rajamannan, G.M. Sangiorgi, K. Arnold, T.G. Mohacsi, P.J. Carlson, A.J. Tajik, R.S. Schwartz. *Mayo Clinic, Mayo Foundation Rochester MN, USA*

*Background:* Recent studies evaluating the risk factors leading to the development of degenerative aortic valvular disease (davd) have found similar inciting factors as implicated in vascular atherosclerosis. Despite a well established clinical background, the cellular causes of davd are still unknown. Vascular apoptosis, programmed cell death, has been induced in vitro and in vivo by experimental hypercholesterolemia. The aim of this study was to evaluate the effect of hypercholesterolemic diet on apoptosis in rabbit aortic valves.

*Methods:* New Zealand rabbits (n = 5) were fed with a 1% cholesterol diet for 8 weeks. Five other animals were fed a normal chow diet and used as controls. After sacrifice aortic valve sections were cut and stained by TUNEL, and PCNA. Morphologic features of apoptosis were evaluated by transmission electron microscopy. Number of apoptotic and proliferating cells measured by computerized morphometry.

*Results:* Multiple stages of apoptosis were evident by transmission electron microscopy and TUNEL staining in hypercholesterolemic tissue. ($p < 0.001$)

| | Apoptosis (cell/mm$^2$) | PCNA (cell/mm$^2$) |
|---|---|---|
| Normal Diet | 0 | 4.4 ± 14.6 |
| High cholesterol diet | 129.3 ± 74.9 | 1172.3 ± 299.1 |

*Conclusions:* Apoptosis and proliferation are increased after high cholesterol diet. These data suggest that hyperlipidemia may play an important role in the early development of aortic valvular degeneration.

# 410 Young Investigators Awards Competition – Clinical Cardiology

Monday, March 30, 1998, 4:00 p.m.–5:30 p.m.
Georgia World Congress Center, Room 364W

4:00

## 410-1 New Real-time Interactive Cardiac Magnetic Resonance Imaging System

P. Yang, A. Kerr, A. Liu, D.H. Liang, C. Hardy, C. Meyer, A. Macovski, J. Pauly, B. Hu. *Cardiology and Electrical Engineering, Stanford University, Stanford, CA, USA*

We conducted an initial clinical trial of our newly developed cardiac magnetic resonance imaging (CMRI) system. Our CMRI system allows continuous dynamic acquisition and display of any scan plane at 16 images/second without the need for cardiac gating or breath-holding. We evaluated LV function in 74 patients to compare the clinical utility of CMRI to echocardiography (ECHO), the current gold standard.

*Method:* A conventional 1.5T GE Signa MRI scanner was modified only by an interactive workstation and a bus adapter. The first group consisted of 31 patients with acceptable ECHO image quality. The second group consisted of 43 patients with suboptimal ECHO image quality. Two independent observers scored wall motion and image quality using the standard 16-segment model and rank-order analysis.

*Results:* CMRI evaluation was complete in less than 15 minutes. In the first group, no significant difference was found between ECHO and CMRI studies ($p$ = NS). In the second group, adequate visualization of wall segments was obtained 38% of the time using ECHO and 97% of the time with CMRI ($p < 0.0001$). When grouped into coronary segments, inadequate visualization of