T Cell Secreted Interferon-Gamma Mediates the Repair Response to Arterial Injury: Studies on Immune-Deficient Rag-1 KO Mice


Background: Arterial injury initiates an immune-modulated response but several levels of interferon-γ (IFN-γ) after injury has not been characterized. We tested the effect of adoptive transfer of T cells from wild type mice or administration of exogenous IFN-γ on the responses of immune-deficient Rag-1 KO mice.

Methods: Cardiac artery cuff injury was induced in Rag-1 KO and wild type mice. Serum IFN-γ levels were quantified by ELISA. T cell enriched splenocytes from wild type mice were injected i.v. (2-4x10^6 cells) into Rag-1 KO mice 48 hours prior to injury. Another group of Rag-1 KO mice was injected with murine IFN-γ (20, 00 units, every other day) starting on the day of injury. Neointimal area was measured 21 days after injury.

Results: Basal serum IFN-γ in wild type mice was 298.8±123.4 pg/ml (n=4), and undetectable in 3 of 4 mice tested 24 hours after injury. IFN-γ levels remained to baseline levels 3 days after injury (272+195.5 pg/ml; n=3). Rag-1 KO mice had low levels of IFN-γ (4.4±3.8 pg/ml; n=3), which was increased after adoptive T cell transfer (464.5±404.2 pg/ml; n=4) indicating that the T cells were viable.

Neointimal area in mice sq:

<table>
<thead>
<tr>
<th>Group</th>
<th>Rag-1 KO</th>
<th>Rag-1 KO+T</th>
<th>Rag-1 KO+IFN</th>
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<tbody>
<tr>
<td>Neointimal area</td>
<td>22 ± 8.1</td>
<td>9.2 ± 5.8</td>
<td>4.3 ± 1.6</td>
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</tbody>
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*p<0.05 vs. Rag-1 KO

Conclusion: These results suggest that adoptive T cell transfer in Rag-1 KO mice leads to increased basal IFN-γ and reduces neointimal formation in response to injury. Exogenous IFN-γ could be a useful adjunctive measure in modulating the repair response to arterial injury.

A Potential Protective Role of Heat Shock Protein 70 in Atherosclerosis


Background: Heat shock proteins (HSPs) constitute a large family of ubiquitous molecules, highly conserved across species that aid in a cell's response to stress. Despite their important biological functions, one important question is how do these molecules, HSP70 in particular, serve as a target for autoimmune mechanisms involved in atherosclerosis and thereby may contribute to atherosclerosis. Experimental evidence suggests a cardioprotective role of another member of HSPs, HSP70, in several examples of acute myocardial stress. We therefore examined whether HSP70 is associated with coronary artery disease (CAD). Methods: Blood samples from 421 patients (62% men, mean age 57 years) evaluated for CAD by coronary angiography, were tested for human HSP70 antigen (ELISA). Results: Serum HSP70 was detectable in 87% of study subjects. Unlike HSP70, the levels of HSP70 positively correlated with the prevalence of CAD (P<0.05). On multivariate logistical regression analysis, individuals with HSP70 levels above the medium (0.5ng/ml) had half the risk of CAD than individuals with levels below the medium, and that the association between elevated HSP70 levels and low CAD risk was independent of cardiovascular risk factors (P=0.01). A similar negative association between HSP70 levels and disease severity (number of diseased vessels) was also found (P=0.011). Interestingly, HSP70 also negatively correlated with CMV infection, even after adjustment for CAD risk factors and seropositivity to other infectious pathogens. (P<0.05). In conclusion, HSP70 serum levels inversely correlated with cardiovascular and infectious diseases severity.

Conclusion: These data provide the first evidence that human HSP70 is a potent marker for lowered CAD susceptibility, presumably through its multiple protective effects on a cell's response to stress. These studies raise the possibility that increased expression of HSP70 in cells of the vessel wall may have protective effects against the development of atherosclerosis without, as opposed to HSP60, serving as a target for autoimmune responses that attenuate beneficial effects and that may even exacerbate disease development.

Oncostatin M, a Member of IL-6 Family Cytokines, Induces MMP-9 Expression and Activation in Vascular Smooth Muscle Cells via ERK-Mediated Pathway

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Background: Oncostatin M (Osm) is a member of IL-6 family cytokines, which is produced by inflammatory cells, and regulates cell proliferation and/or the extracellular matrix metabolism of vascular smooth muscle cells (VSMCs). In atherosclerotic media, resident cardiac fibroblasts and adventitial fibroblasts apparently produce Osm. Osm induces cell proliferation, collagen synthesis and production of extracellular matrix. In particular, Osm induces extracellular matrix degradation.

Aim of this study is to investigate the effects of Osm on vascular smooth muscle cells (VSMCs). Thus, we examined the Osm-mediated intracellular signaling system and the role of Osm in cultured VSMCs from rats.

Methods: Activation of ERK1/ERK2 or Stat3 was determined on the basis of immunoblotting analysis. MMP-9 mRNA expression was evaluated by the Real-time RT-PCR analysis. MMP-9 Activity was examined by gelatin zymography.

Results: Osm transiently induced ERK1/ERK2 phosphorylations after 2 min with a peak at 15 min, returning to baseline by 60 min. Stat3 was also transiently phosphorylated by Osm in the similar time course. Effects of Osm on ERK1/ERK2 and Stat3 were dose-dependent of Osm (0.1-30 ng/ml). Real-time RT-PCR analysis showed that MMP-9 mRNA expression was transiently upregulated by Osm with a peak at 4 hrs. Furthermore, gelatin zymography revealed MMP-9 activity was increased in the conditioned medium obtained from the Osm-treated VSMCs. An ERK kinase inhibitor, PD98059, not only blocked the Osm-induced ERK1/ERK2 phosphorylations but also abolished the Osm-induced MMP-9 induction and activation. In contrast, overexpression of a dominant negative mutant of Stat3 using an adenovirus vector showed no effect on Osm-induced MMP-9 mRNA induction. Osm had no significant effects on the serum-induced VSMC proliferation.

Conclusion: Osm stimulated MMP-9 expression and activation through the ERK-mediated signaling cascade in cultured VSMCs. On the other hand, the Stat3 pathway may be involved in the observed MMP-9 related mechanism is involved in the progression of the atherosclerosis and the plaque rupture by regulating the extracellular metabolism.

Matrix Metalloproteinase Inhibition Reduces Axial Tensile Strength in the Arterial Wall

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Background: Emerging data suggests that P-selectin blockade may be important in limiting the response to vascular injury. P-selectin has been shown to mediate leukocyte-endothelium and leukocyte-platelet interactions. These interactions are mediated through binding of P-selectin to P-selectin glycoprotein ligand-1 (PSGL-1) located on the surface of leukocytes, endothelium and leukocyte-platelet interactions. PSGL-1 inhibition using a unique blocking monoclonal antibody would result in reduced neointima formation following carotid denudation injury in apolipoprotein E deficient mice (apoE-/-) mice fed a Western diet (WD).

Methods: Female ApoE-/- mice (n=26) were fed a WD for one week prior to wire denudation of the left common carotid artery followed by four weeks of WD. Three hours prior to injury each mouse was given a single bolus of 100mg of rat antimuscle blocking monoclonal antibody to PSGL-1, 4RA10, (n=12) or isotype control (n=16) via IP injection. Four weeks after injury, carotid arteries were removed, paraffin embedded and sectioned for histomorphometry. Results: Neointima formation at 28 days was significantly reduced (58%) in the 100mg PSGL-1 antibody treated groups versus isotype controls (13000mm2 ± 3000 vs. 10000mm2± 4000, p<0.01). Intra-media to media (IM) ratios were also reduced (0.24 vs. 0.01, 0.05 < p < 0.01).

Conclusions: Inhibition of leukocyte-endothelium and leukocyte-platelet interactions by PSGL-1 inhibition using a unique blocking monoclonal antibody, 4RA10, significantly limits the neointima formation following carotid denudation injury at 28 days in the cholesterol-fed, apoE-/- mouse.

Enhancement of Calcium Sensitization Mediated by a Rho Associated Protein Kinase in Resistance Arteries With Congestive Heart Failure

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Background: Although many studies suggest that enhanced contractile response of resistance arteries in congestive heart failure (CHF) is due to dysfunction of endothelium, there is very little study that a Rho associated protein kinase play a role of this enhancement of contractile response of resistance arteries in CHF. The aim of present study was to examine whether Ca2+ sensitivity is increase and to examine the role of Rho association of protein kinase in active Ca2+ sensitivity in CHF. Methods: Carotid arteries were isolated from rats subjected to 2 weeks of CHF. Conclusion: CHF increased the change in the response of protein kinase C (PKC) inhibitor arteries in CHF compared with control. Methods and Results: Heart failure rat induced by ligation of the left coronary artery. Sham operated rats were controls. Femoral arteries(100 micro-m)