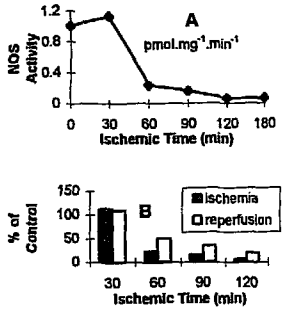
with complete loss of activity by 120 min (fig A). During reperfusion a partial two fold recovery of NOS activity was noted after 60 or 90 min of ischemia but not after more than 120 min of ischemia (fig B). During ischemic durations greater than 30 min intramyocardial pH fell asymptotic to a value of 5.5. When purified constitutive NOS was subjected to pH 5.5 enzyme activity was similarly lost and only partially restored with restoration of the pH to values of 7.4.



These data suggest that ischemia and reperfusion-induced pH changes could account for the loss of activity seen during ischemia and the partial return observed on reperfusion. The loss of NOS activity observed during ischemia may contribute to the loss of endothelial dependent vasodilation in the post-ischemic heart.

915-85 Expression of Cell Adhesion Molecules in Human Arteriosclerosis

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The interactions between cell adhesion molecules and components of the extracellular matrix are often discussed in relation to their ability to modulate the proliferative, migratory and apoptotic processes involved in plaque formation. Therefore, we assessed the expression of several integrin subunits and gpIV (thrombospondin receptor) by the analysis of coronary and peripheral arteriosclerotic tissue from 22 patients, using immunoperoxidase staining with monoclonal antibodies (Immunotech). Morphometric results are presented as means ± SD of positive/total cells found in ten different intimal areas/lesion. The data are as follows:

Cell adhesion protein	Positive cells/mm²	Total cells/mm²	Expression (%)
α_2 (CD49b)	9 ± 16	436 ± 324	2
α_3 (CD49c)	92 ± 89	431 ± 289	21
α_5 (CD49e)	29 ± 38	418 ± 309	7
α_6 (CD49f)	0	406 ± 318	0
α_v (CD51)	198 ± 237	393 ± 268	50
β_1 (CD29)	348 ± 259	391 ± 268	89
β_3 (CD61)	58 ± 87	349 ± 283	17
gpIV (CD36)	211 ± 244	387 ± 271	54

Smooth muscle cells are the predominant intimal cell type and frequently display distinct signals of α_3, α_v, β_1, β_3 and gpIV cell surface receptors. Only sparse immunoreaction was detected for the integrins α_2 and α_5, and none for α_6. Interestingly, positive correlations were found between the intimal cell density and the expression of α_3, α_v, β_1 subunits and of gpIV ($r > 0.70$; $p < 0.01$). Five lesions expressed high levels (> 80%) of α_3, α_v and β_1 integrins in adjacent medial areas.

In summary, our study demonstrates distinct expression patterns of specific integrin subunits and of gpIV in human arteriosclerotic lesions. Cell adhesion proteins may be attractive targets of cell-directed, therapeutic approaches, the ultimate goal being the mitigation of plaque growth, possibly by modulating cellular anchorage and inducing apoptosis.

915-86 Time Course of PECAM-1, ICAM-1, and E-Selectin Expression in Response to TNF-α Stimulation in Human Coronary Artery Endothelial Cells

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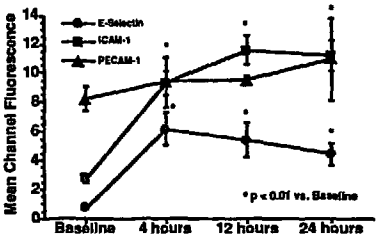
Leukocyte-endothelial cell adhesion molecules (CAMs) have been implicated in the pathogenesis of myocardial ischemia-reperfusion and coronary artery restenosis in animal models. Accordingly, we investigated the time course of TNF-α induced expression of ICAM-1, E-selectin, and PECAM-1 on human coronary artery endothelial cells (HCAECs). HCAECs were grown to confluence and treated with rhTNF-α (10 ng/ml) for 0, 4, 12, or 24 hrs (n = 6–8/group) and analyzed using indirect immunofluoresence techniques. Monoclonal antibodies against ICAM-1 (RR 1/1, Boehringer Ingelheim), E-selectin (CY1787, Cytel), and PECAM-1 (WM59, BioDesign) were utilized to determine the relative expression of these CAMs at the specified time intervals. These data demonstrate that ICAM-1 and E-selectin expression can be upregulated on HCAECs by rhTNF-α at 4, 12, and 24 hrs following cytokine stimulation. PECAM-1, however, is constitutively expressed at extremely high levels, and appears to be unaffected by rhTNF-α stimulation. We conclude that CAMs are expressed on human coronary artery endothelial cells, and the degree of expression is markedly increased by rhTNF-α stimulation.



Furthermore, these CAMs may play an important role in the pathogenesis of coronary artery disease in humans.

915-87 A Single Intracoronary Bolus of Basic Fibroblast Growth Factor Increases Myocardial Perfusion Bed in a Porcine Model of Chronic Ischaemia

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Basic fibroblast growth factor (bFGF) is a polypeptide that induces endothelial cell and smooth muscle cell proliferation. We studied its effects in reducing the ischaemic burden in a porcine coil stenosis model. *Methods* A stenotic lesion was created in the right coronary artery (RCA) in 9 juvenile Yorkshire pigs using a balloon delivered copper-gold coil. In 3 additional control pigs no coil was delivered. At 28 ± 5 days later, the RCA stenosis was confirmed by angiography and the RCA perfusion bed was quantified with contrast echocardiography (CE) using a selective injection of sonicated albumin into the RCA. Animals were then allocated to receive either a single bolus of 100 mcg of bFGF (n = 7) or NAP04 buffer vehicle (n = 5) delivered into the left coronary artery. Repeat CE was performed 14 days later. Total myocardial and RCA perfusion beds were obtained by blinded analysis of the short axis images. The RCA perfusion bed size was expressed as a percent of the total perfusion bed and the percent change between studies was calculated. *Results* The mean diameter stenosis at the coil was 83.3 ± 18.7% and was no different in the bFGF and vehicle pigs. In those animals with a stenosis who received bFGF the RCA perfusion bed increased by 13.2 ± 4.3% whereas in the animals who received vehicle it decreased by 7.5 ± 3.9% ($p = 0.014$). No increase in perfusion bed occurred in those pigs without a coil. *Conclusion* In the presence of a coronary stenosis, a single bolus of bFGF delivered into the contralateral artery improves perfusion in the stenotic vessel's territory.

915-88 Cell — Cell Interactions in the Development of Atherosclerosis: Macrophage Conditioned Media Stimulates Vascular Smooth Muscle Cell Proliferation and Matrix Production

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Matrix protein production and vascular smooth muscle cell proliferation are the hallmarks of atherosclerosis. Cell-cell interactions are important in the regulation of proliferation and protein synthesis. We tested the interaction between macrophages, a prominent cell in the injury-response of coronary arteries, and vascular smooth muscle cells (VSMC) obtained from porcine coronary arteries. The purpose of this study was to examine the effects of macrophage conditioned media on proliferation and the expression of osteopontin, an Arg-Gly-Asp-containing acidic phosphoprotein in vascular smooth muscle cells. Osteopontin is present in atherosclerotic plaques and is necessary for calcification associated with vascular disease. Macrophage conditioned media at 1:4 dilution significantly increased proliferation in VSMCs (571 ± 70%, $p < 0.001$). Osteopontin production was increased by 138 ± 0.03% ($p < 0.007$) in the presence of macrophage conditioned media (1:4) as compared to control media. Northern analysis with a porcine cDNA probe revealed expression of osteopontin mRNA after stimulation of the smooth

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