1054-138 Myeloperoxidase Containing Macrophages and Neutrophils and Stainable Intracellular Iron in Plaque Progression to Rupture and Organize

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Background. MPO-containing macrophages have been described at sites of plaque rupture. The specificity of this marker for plaque rupture is unknown, and the presence of neutrophils or iron-containing macrophages at plaque rupture sites has not been described.
Methods. We studied 16 acute ruptures, 10 organizing ruptures, 29 thin cap fibroatheromas, and 14 fibroatheromas from sudden coronary death victims by immunohistochemical and histochemical techniques. Inflammatory cells were typed with anti-CD68 (macrophages), anti-BP-30 (neutrophil bactericidal glycoprotein), and anti-MPO. Iron was localized by Mallory's Prussian blue stain.
Results. MPO positive cells were present in the majority of ruptured caps, but only few non-ruptured caps (table). Iron containing foam cells were present in the caps of 93% of acute ruptures, of 84% of organizing ruptures, 32% of thin cap atheromas, and 10% of fibroatheromas. In addition, there were deeper areas of hemosiderin deposition in 50% of acute and organizing ruptures, 14% of fissures, 36% of thin cap atheromas, and 27% of fibrous cap atheromas.
Conclusion. Although MPO positive macrophages and neutrophils are present in the majority of acute ruptures, neutrophils appear to be a transitory infiltrate. Iron-containing macrophages are present at acute ruptures as well as healed ruptures with organization and may represent a means of detecting sites of acute events in the coronary segments.

Cap type	% with neutrophils	Neuts./ mm2	MPO+ cells/mm2	% MPO + cells = neuts.	Iron macrophages/ mm2
Fibrous	0	0	0	0	1.2
Thin cap	12	2 ± 2	13 ± 5	22 ± 14	20 ± 13
Rupture	89	90 ± 32	127 ± 29	65 ± 23	88 ± 44
Organized rupture	11	5 ± 5	47 ± 20	11 ± 11	90 ± 41

1054-139 Importance of Methodology in Chlamydia Pneumoniae Serology

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Background: An association between Chlamydia pneumoniae seropositive status and coronary heart disease remains controversial. We examined the concordance between 4 commonly used enzyme immuno asssays and the microimmunofluorescence test, the gold standard method, and investigated whether the choice of assay influenced antibody seroprevalence in patients with coronary atherosclerosis and healthy individuals.
Methods: MRL Chlamydia MIF IgG test (MIF), Labsystems Chlamydia pneumoniae IgG EIA (LS), R-Biopharm Elegance Chlamydia pneumoniae IgG EIA (RB), Medac Chlamydia pneumoniae IgG sELISA (MCp) and Medac Chlamydia IgG rELISA (MC) were tested on sera from 112 healthy men. Male patients with angiographically defined coronary artery disease were also investigated using the LS and MCp test.
Results: The agreement between LS (73/112, 65 %) or MC (55/112, 49 %) and MIF (89/112, 79 %) was moderate to fair (kappa = 0.583; kappa = 0.235). MCp (kappa = 0.679) and RB (kappa = 0.665) showed good agreement with MIF, with 90/112 (80 %) and 87/112 (78 %) of controls reacting positive. Furthermore, a significant difference in seroprevalence between patients and healthy subjects was observed with the LS assay, but not with the MCp test .
Conclusion: The concordance between MIF and other commonly used serologic assays for Chlamydia pneumoniae antibody detection is good to fair. The choice of serological assay is important when evaluating whether Chlamydia pneumoniae seropositivity is related to coronary artery disease.

test	Seropositive controls (%)	Seropositive patients (%)	Seropositive patients (%)	p value
LS	71/112 (63.4)	68/82 (82.9)	patients with diffuse atherosclerosis, 1, 2 or 3 vessel disease	0.003
MCp	90/112 (80.4)	87/98 (88.8)	patients with diffuse atherosclerosis, 1, 2 or 3 vessel disease	0.094
LS	71/112 (63.4)	61/73 (83.6)	patients with 1,2 or 3 vessel disease	0.003
MCp	90/112 (80.4)	79/89 (88.8)	patients with 1,2 or 3 vessel disease	0.106

1054-140 Reactive Oxygen Species Contribute to Inflammation-Induced Endothelial Dysfunction

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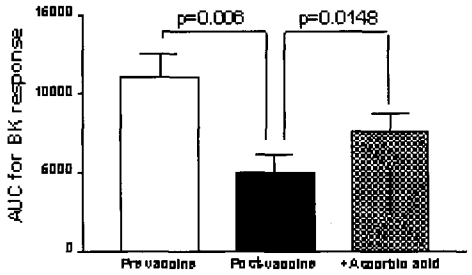
Background: Infection/inflammation might increase the risk of vascular events by inducing endothelial dysfunction. We tested the hypothesis that acute inflammation impairs the release or action of atheroprotective nitric oxide (NO) from the endothelium by limiting the availability of substrate or enhancing the generation of reactive oxygen species.
Methods: Forearm blood flow was measured using venous plethysmography in 18 healthy volunteers (age 28±6 years). Dose-response curves to intra-arterial L-NMMA (1-4 umol/min), noradrenaline (NA; 60-240 pmol/min), bradykinin (BK; 20-80 pmol/min) and glyceryl trinitrate (GTN; 8-32 nmol/min) were constructed before and 8 hours after administration of typhoid (Typhim Vi) vaccine to generate an inflammatory response. In others responses to BK and GTN were repeated after intra-arterial infusion of L-arginine (substrate for NO; 50umol/min n=5) or anti-oxidant vitamin C (25mg/min n=8).
Results: Vaccination induced a cytokine response and reduced dilatation to endothelium-dependent agonists BK (p=0.006) and L-NMMA (p<0.0001) without affecting the response to GTN (p=0.36) or NA (p=0.62). Post vaccine, blood flow responses to BK (but not GTN) were partially reversed by vitamin C (fig) but unaffected by L-arginine.
Conclusion: Inflammation reduces vascular NO bioavailability, an effect that is partially reversible with local anti-oxidants. These findings suggest a role for reactive oxygen species in inflammation-induced endothelial dysfunction.



1054-141 Differential Proliferative Index of Resident Smooth Muscle Cells and Infiltrating Leukocytes After Balloon Angioplasty and Stenting of Swine Coronary Arteries

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Background. Inflammation plays a central role in vascular lesion development after balloon angioplasty and stenting. Quantitative assessments of leukocyte infiltration and proliferation in injured vessels is lacking. To evaluate proliferation of resident smooth muscle cells (SMCs) and infiltrating leukocytes, we conducted a flow cytometry-based analysis of injured vessels. ***Methods.*** Coronary arteries of 5 juvenile swine were stented (RCA), balloon overstretched (LAD), or left intact (LCX). Naive arteries were used as controls. Animals were injected with 60 mg/kg BrdU 24 hours before sacrifice, which was performed 3 days after the intervention. After enzymatic dissociation, cell suspensions were immunofluorescently-labeled for CD45, BrdU and α-SM actin. Selected samples were stained for DNA content using 7-AAD. Stained samples were analyzed on a FACSCalibur flow cytometer. ***Results.*** The number of cells in stented and ballooned vessels was not significantly different from control arteries (naive: $1.56\pm0.34\times10^4$, LCX: $1.98\pm0.34\times10^4$, LAD: $1.75\pm0.25\times10^4$, RCA: $1.62\pm0.29\times10^4$ cells/mg tissue, means±SEM). A remarkable inflammatory infiltrate was detected 3 days after injury in ballooned and stented vessels (naive: 5.8±1.9%; LCX: 7.9±1.2%; LAD: 24.0±3.5%; RCA: 33.1±3.3%, p<0.001). The proliferative index of CD45+ cells in uninjured and injured vessels was similar but significantly higher than that in peripheral blood leukocytes (PBL) (naive: 15.9±1.5%; LCX: 15.6±4.9%; LAD: 15.3±1.5%, RCA: 14.5±1.3%, PBL: 3.9±0.3%, p<0.05). Unlike CD45+ cells, the proportion of proliferating SMCs in ballooned and stented vessels was significantly higher when compared to uninjured and naive arteries (naive: 3.63±1.0; LCX: 4.3±1.6; LAD: 33.2±4.5; RCA: 45.0±3.7, p<0.05) ***Conclusions.*** Our results document with precision the inflammatory cell involvement in the early response to balloon and stent injury. The differential rates of proliferation detected by flow cytometry demonstrate its sensitivity, and suggest that activated leukocytes may mediate the proliferative response of resident SMCs.

1054-142 Prostaglandin E1 Induces Vascular Endothelial Growth Factor-A in Human Adult Cardiac Myocytes and Coronary Artery Smooth Muscle Cells

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Background: Vascular Endothelial Growth Factor-A (VEGF-A) induces proliferation, migration and NO-synthesis in endothelial cells and is able to stimulate neoangiogenesis in ischaemic organs. A significant increase of VEGF-A serum levels was shown after myocardial infarction. These data suggest the importance of the VEGF system during repair and neovascularization. Recently we showed that Prostaglandin E1 (PGE1) induced angiogenesis in hearts of patients with ischaemic heart disease (Mehrabi et al. Cardiovasc. Res. 2002). In this study we aimed to investigate whether PGE1 affects the expression of VEGF-A in cultured human adult cardiac myocytes (HACM) or human cor-