

FC4 THE ALTERNATIVE PATHWAY IS CRITICAL FOR PATHOGENIC COMPLEMENT ACTIVATION IN DIET-INDUCED AND ENDOTOXIN-INDUCED ATHEROSCLEROSIS IN LOW-DENSITY LIPOPROTEIN RECEPTOR-DEFICIENT MICE

doi:10.1136/hrt.2010.205781.10

¹T H Malik, ¹A Cortini, ¹D Carassiti, ²V W Y Leung, ¹M J Lewis, ²D O Haskard, ¹M Botto. ¹Rheumatology Section, Imperial College, London, UK; ²BHF Cardiovascular Medicine Unit, Imperial College, London, UK

Our previous studies have shown that the early components of the classical complement pathway protect low-density lipoprotein receptor deficient mice (Ldlr^{-/-}) from atherogenesis when fed a low-fat diet. However, the role of the alternative pathway remains unknown. To investigate this, we crossed mice lacking the alternative pathway activator Factor B (Bf^{-/-}) with Ldlr^{-/-} mice. Lipid profiles after 12 weeks on a high-fat diet showed significantly reduced levels of total cholesterol (25.2%±0.9 mmol/l, n=14, vs 40.5%±1.8 mmol/l, n=17, p<0.0001) and lipoproteins (~1.5-fold lower VLDL and LDL) in the Bf^{-/-}.Ldlr^{-/-} mice compared with the Ldlr^{-/-} animals. Consistent with this, high-fat fed Bf^{-/-}.Ldlr^{-/-} mice had decreased cross-sectional aortic root lesion fraction area (median 12.2%, IQR 8.92% to 15.82% vs 16.3%, 14.76% to 19.74%, p=0.0016) and reduced lesion complexity. These changes were associated with reduced complement activation in the circulation and in atherosclerotic plaques. There was no difference in lipid profiles between Bf^{-/-}.Ldlr^{-/-} and Ldlr^{-/-} mice fed a low-fat diet, but in these groups administration of lipopolysaccharide (LPS) led to significant increase in atherosclerosis only in Ldlr^{-/-} and not in Bf^{-/-}.Ldlr^{-/-} (aortic root lesion fraction: Bf^{-/-}.Ldlr^{-/-}: 7.85%, range 4.93 to 15.24%, n=9; Ldlr^{-/-}: 22.53%, range 17.36 to 25.25%, n=10, p=0.0009), indicating that the alternative pathway also has a key role in endotoxin-mediated exacerbation of disease. These data indicate that amplification of complement activation by the alternative pathway in response to diet or infection may convert the complement system from a protective to a more atherogenic role.

FC5 REAL TIME IMAGING OF LYMPHOCYTES IN APOE^{-/-} MOUSE AORTIC TERTIARY LYMPHOID ORGANS

doi:10.1136/hrt.2010.205781.11

¹G Grassia, ¹G J Dever, ¹P Gordon, ¹I B McInnes, ²R Spanbroek, ²A J R Habenicht, ¹J M Brewer, ¹P Garside, ¹P Maffia. ¹Division of Immunology, Infection & Inflammation, University of Glasgow, UK; ²Institute for Vascular Medicine, Friedrich Schiller University of Jena, Germany

Rationale for the study Recent developments in imaging technologies such as multiphoton laser-scanning microscopy (MPLSM) have allowed real time in vivo imaging and analysis of immune cells interactions in the context of the immune system, providing important insight into the dynamics of a developing immune response. However, a number of technical limitations have precluded real time imaging of individual immune cells within intact atherosclerotic vessels. Here we provide a feasible protocol for MPLSM with real time imaging and analysis, at a cellular resolution, of adoptively transferred lymphocytes, in the adventitia of intact ApoE^{-/-} mouse arteries and the associated aortic tertiary lymphoid organs (ATLOs) which have recently been implicated in the late stages of atherosclerosis (*JEM* 2009;206:233–48).

Methodology Cell suspensions were prepared from peripheral and mesenteric lymph nodes of C57BL/6 mice and labelled with Cell Tracker™ Red CMTPX (Molecular Probes). Lymphocytes were injected IV into aged ApoE^{-/-} (80–87 weeks) recipients. Twenty four hours after transfer, excised intact mouse abdominal aortas were imaged by MPLSM and analysed by Volocity 5 software (Improvision).

Results and conclusions The use of this system enabled real time imaging of immune cells in atherosclerotic vessels at a cellular resolution. At 24 h after transfer several infiltrated lymphocytes were detectable in the ATLO in aged ApoE^{-/-} mice. Cells showed a great motility with a mean velocity of 0.45 µm/s and a peak velocity of 1.16 µm/s. Consequently, this system could be a powerful tool to study the atherosclerotic immune responses.

Acknowledgements This work is supported by a BHF grant (PG/06/083/21198).

BAS/BSCR poster abstract

BAS/BSCR1 VACCINATION AGAINST INFLUENZA PROMOTES STABLE ATHEROSCLEROTIC PLAQUES IN APOE^{-/-} MICE

doi:10.1136/hrt.2010.205781.12

A Bermudez-Fajardo, E Oviedo-Orta. Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, UK

Current evidence suggests a relationship between seasonal influenza viral infection and cardiovascular disease (CVD) complications. Experimentally, animals inoculated with influenza A virus develop thrombotic complications similar to those seen in humans. Conversely, several clinical trials have suggested that influenza vaccination may have a protective effect on CVD. However, the potential mechanisms remain unstudied. In order to study this, we vaccinated six groups of male ApoE^{-/-} mice with 0.36, 1.8, 9 and 45 µg/ml of Vaxigrip (influenza vaccine, Sanofi Aventis), 45 µg/ml of Pneumo23 (Pneumococcus vaccine, Sanofi Aventis) and phosphate-buffered saline (PBS). Animals received one injection and two boosters 3 weeks apart before starting on a high-fat diet for 12 weeks. Plasma was collected pre-immunisation and at termination for profile analysis of antibodies, lipids and cytokines. Analysis of atherosclerosis development was carried out using paraffin sections of the brachiocephalic artery. We observed an increase in anti-influenza IgG1 but not in IgG2a, IgG2b, IgA or IgM in any of the groups. Animals that received Pneumo23 vaccine only showed increased levels of vaccine-specific IgM. No significant changes in plasma levels of lipids were noted. Animals vaccinated with 45 µg/ml Vaxigrip developed smaller atherosclerotic lesions with lower lipid content but were richer in inflammatory cells and collagen than control animals. Other groups showed no significant differences in plaque area, lumen and tunica media area. Animals vaccinated with 45 µg/ml Vaxigrip showed lower levels of interleukin (IL) 2, interferon γ and IL-17 and higher levels of IL-4. Our results suggest that vaccination against influenza may protect against CVD by inducing stable and smaller atherosclerotic plaques.

BAS/BSCR2 ANALYSIS OF MICROVESSELS WITHIN CAROTID PLAQUES: COMPARISON OF SYMPTOMATIC AND ASYMPTOMATIC PATIENTS TO IDENTIFY THOSE AT RISK FROM PLAQUE RUPTURE

doi:10.1136/hrt.2010.205781.13

¹E J R Hill, ¹M Chowdhury, ²F Serracino-Inglott, ²J V Smyth, ³M Slevin, ¹M Y Alexander. ¹Cardiovascular Research Group, The University of Manchester, Core Technology Facility, UK; ²Department of Vascular Surgery, Manchester Royal Infirmary, UK; ³School of Biology Chemistry and Health Science, Manchester Metropolitan University, UK

Introduction Stroke is the third most common cause of death world wide. Most cases of stroke are caused by atherosclerotic plaque rupture. A challenge facing clinicians is identifying asymptomatic patients at risk of plaque instability. Locally released angiogenic growth factors may contribute to microvessel instability within

plaques. Growth factors and inflammatory cytokines are potential serum biomarkers to identify patients at risk of stroke.

Materials and methods Immunohistochemistry and quantitative PCR (Q-PCR) were used to establish localisation and expression of angiogenic growth factors within carotid endarterectomy specimens from symptomatic and asymptomatic patients. Stable or unstable microvessels were distinguished by CD31 and CD105 staining. Systemic levels of circulating angiogenic growth factors and inflammatory cytokines were measured in venous blood using Bio-Plex arrays.

Results Hepatocyte growth factor (HGF) and its receptor c-Met were detected in CD31-positive endothelia, and α -SMA-positive cells, respectively. Q-PCR demonstrated upregulation of the angiogenic factors CD105, HGF ($p < 0.001$) and c-Met ($p = 0.011$) in symptomatic versus asymptomatic plaques. A significantly greater neovessel density was detected in symptomatic plaques ($p = 0.042$), associated with elevated expression of HGF and c-Met. Suspension arrays demonstrated elevated HGF ($p = 0.002$) and decreased platelet-derived growth factor (PDGF; $p = 0.036$) serum levels in symptomatic versus asymptomatic patients. Twenty-seven cytokines were examined; seven endarterectomy patients demonstrated significantly increased levels in comparison with controls. No differences were observed between preoperative and postoperative serum.

Discussion Plaque instability may be mediated by HGF-induced formation of microvessels, and decreased PDGF. We will investigate the effects of inflammatory cytokines with a view to comparing symptomatic versus asymptomatic patients. Targeting surgery to those who will benefit would eliminate unnecessary risk.

**BAS/
BSCR3** **PARTIAL RECONSTRUCTION OF MYOCARDIAL METABOLIC PATHWAYS FOLLOWING ANALYSIS OF PERIPHERAL SERUM USING METABOLOMICS IN EARLY CARDIAC ISCHAEMIA**

doi:10.1136/hrt.2010.205781.14

¹M A Mamas, ²W B Dunn, ²D Broadhurst, ¹S Chacko, ³M Brown, ¹M El-Omar, ¹F Fath-Ordoubadi, ²R Goodacre, ^{2,3}D B Kell, ¹L Neyses. ¹Biomedical Research Centre, Central Manchester NHS Foundation Trust, UK; ²Manchester Centre for Integrative Systems Biology, University of Manchester, UK; ³Bioanalytical Sciences Group, Manchester Interdisciplinary Biocentre, University of Manchester, UK

Cardiac metabolism and cardiac function are inextricably linked, with changes in cardiac metabolism during cardiac ischaemia contributing to the development of cardiac arrhythmias. Using metabolomics, we aim to identify metabolite changes occurring during cardiac ischaemia through analysis of peripheral serum to reconstruct myocardial metabolic pathways that contribute to the development of cardiac arrhythmias. Peripheral venous samples from 25 patients (and 20 patients in a validation cohort) were analysed in an untargeted fashion using LC-MS following cardiac ischaemia induced by transient coronary artery occlusion during PCI at baseline, 1 and 5 min. Following validation, 99 and 126 metabolite peaks were significantly different at 1 min and 5 min after coronary occlusion compared with baseline ($p < 0.05$). Predominantly metabolic pathways involving lipids were perturbed with changes in diacylglycerols (DG), lysophosphatidylcholines (LPC), phosphatidylcholine (PC) and free fatty acids (FFA). Myocardial metabolic pathways involving the synthesis of PC from DG and their subsequent breakdown by phospholipase A2 into LPC and FFA such as arachidonic acid (AA) stimulating the oxidation of adrenaline to form the arrhythmogenic metabolite adrenochrome were reconstructed. We are able to reconstruct metabolic pathways involving lipid metabolism within the myocardium during cardiac ischaemia through analysis of the peripheral serum using metabolomics. Our unbiased approach has identified metabolic pathways involved in the production and release of metabolites with pro-arrhythmic properties (AA, LPC and adrenochrome) and metabolites with anti-

arrhythmic properties (omega-3 fatty acids: eicosapentaenoic and docosahexanoic acid). This suggests that arrhythmogenesis may be a delicate balance between the endogenous formation of pro-arrhythmic and anti-arrhythmic metabolites.

**BAS/
BSCR4** **THE ROLE OF RECEPTOR ACTIVATOR OF NUCLEAR FACTOR κ -B LIGAND AND ITS DECOY RECEPTOR, OSTEOPROTEGERIN IN VASCULAR CALCIFICATION**

doi:10.1136/hrt.2010.205781.15

¹A S Williams, ^{1,2}A Ndip, ²E Jude, ²A J M Boulton, ¹M Y Alexander. ¹Cardiovascular Research Group, Manchester Diabetes Centre, Faculty of Medicine & Human Science, School of Biomedicine, University of Manchester, UK; ²Manchester Royal Infirmary, University Department of Medicine, Oxford Road, Manchester, UK

Charcot neuroarthropathy (CN) is characterised by pathological foot fractures and osteopenia in patients with diabetes, often resulting in debilitating deformity. Paradoxically, these patients show evidence of medial vascular calcification. Recently, accentuated signalling of the receptor activator of nuclear factor κ -B ligand (RANKL) and its decoy receptor, osteoprotegerin (OPG) have been implicated in the development of diabetic CN. This study aims to investigate the role of RANKL and OPG signalling in vascular calcification in patients with diabetes and CN, compared with healthy controls. RANKL and OPG serum levels were measured using ELISA in 12 patients with CN, 10 diabetic patients and five healthy controls. Serum RANKL and OPG levels were elevated in acute CN and in diabetic patients compared with healthy controls ($p < 0.05$). Immunohistochemistry identifies upregulation of RANKL in calcified tibial arterial sections versus non-calcified controls. Human vascular smooth muscle cells (hVSMC) were grown in osteogenic conditions, as our in vitro model of calcification. When hVSMCs were treated with serum from patients with diabetes and CN, we demonstrated (i) accelerated mineralisation of hVSMC, confirmed by Alizarin red staining, and elevated alkaline phosphatase activity compared with control cells and (ii) reduced mineralisation when co-incubated with OPG. These findings demonstrate that RANKL/OPG signalling is modulated in diabetic and CN patients. Furthermore, serum from these patients accelerates vascular calcification in vitro, an effect attenuated by OPG treatment. These are the first human data implicating RANKL/OPG in diabetic vascular calcification and suggest that OPG/anti-RANKL therapy may be a potential target in combating disease progression.

**BAS/
BSCR5** **TILRR POTENTIATES INTERLEUKIN-1-INDUCED ANTI-APOPTOSIS**

doi:10.1136/hrt.2010.205781.16

Gemma Montagut Pino, Xiao Zhang, Eva E Qvarnstrom. *Cell Biology, Vascular Science, University of Sheffield, UK*

The pathogenesis of atherosclerosis is determined, in part, by inflammatory responses, induced through members of the Toll-like and interleukin (IL)-1 receptor family and controlled by NF- κ B. We have identified a novel IL-1RI co-receptor, TILRR, which enhances the IL-1-induced activation of NF- κ B by increasing receptor-expression enhanced recruitment of the MyD88 adaptor during activation.¹ Here we investigate the role of TILRR on the anti-apoptotic effects controlled by NF- κ B. The results showed that TILRR reduces caspase-3 activity and enhances IL-1-induced phosphorylation of AKT. Alanine scanning mutagenesis of the IL-1 receptor TIR domain demonstrated that TILRR amplifies inflammatory responses through the membrane proximal part of the cytoplasmic portion, the so-called box 1, while the anti-apoptotic response is regulated through the central portion of the TIR domain, the so-called box 2. Similarly,