transmembrane helices leading to an extracellular N-terminus and an extracellular loop between helices two and three. The C242T polymorphism causes a change of His72 to Tyr72. This change results in significant morphological changes of the extracellular loop of the p22phox, which is in the putative interactive region of the p22phox with the catalytic subunit (Nox2). This may interfere with their association, and subsequently result in a reduced cytochrome b function and a reduced ROS production by NADPH oxidase. These results give us insight into the molecular mechanism of this polymorphism in reducing vascular oxidative stress and may explain how this polymorphism is linked with reduced incidence of CHD.

BAS/BSCR49 HEPATOCYTE GROWTH FACTOR/C-MET SIGNALLING ACTIVATES NOTCH TRANSLOCATION AND IS ASSOCIATED WITH SMOOTH MUSCLE CELL MINERALISATION IN VITRO

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Vascular calcification is an established pathological process that contributes to several forms of cardiovascular morbidity—notably, atherosclerosis. The molecular mechanisms involved continue to be explored. Hepatocyte growth factor (HGF) signalling, via its receptor c-MET, has been identified in association with atherosclerotic plaque development. We have demonstrated that overexpression of HGF in human smooth muscle cells (hSMC) accelerates their mineralisation. Reports demonstrating upregulation of the notch ligand δ, and the presence of a feedback loop linked to the c-MET pathway, raise the possibility that the effects of HGF on mineralisation may be mediated via notch signalling. We aim to test the hypothesis that notch signalling is involved in Ad-HGF-induced in vitro mineralisation of hSMCs. We demonstrate accelerated mineralisation in response to adenoviral-mediated HGF overexpression, confirmed by alizarin red staining, calcium incorporation and increased alkaline phosphatase activity. In addition, we show upregulation and phosphorylation of c-MET and reduction of the mineralisation inhibitor osteopontin. We identify upregulation of the notch-3 intracellular domain via western blot analysis and, using immunocytochemistry, show an altered distribution of notch-3 in Ad-HGF-infected cells. Finally, we show (i) an attenuation of mineralisation in hSMCs following overexpression of NK4, the c-MET antagonist and (ii) that treatment of hSMCs with DAPT, the notch inhibitor, also decreased the rate of mineralisation compared with Ad-HGF infected cells and controls. These findings suggest a link with the notch pathway as a possible downstream effector of HGF and further elucidate a novel mechanism underpinning vascular calcification.

BAS/BSCR50 EFFECT OF IMMUNISATION WITH CHLAMYDIA PNEUMONIAE RECOMBINANT MAJOR OUTER MEMBRANE PROTEIN ON ATHEROSCLEROSIS DEVELOPMENT

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The major outer membrane protein (MOMP) of *Chlamydia pneumoniae* is a 40 kDa porin and represents approximately 60% of the outer membrane protein pool. Previous studies have shown that MOMP can dampen down T-cell-mediated immune responses. We decided to assess if this effect could have an impact on atherosclerotic plaque development. We used recombinant *Mycobacterium vaccae* (*M vaccae*) vectors expressing MOMP (with and without signal sequence) to vaccinate ApoE−/− mice intranasally. Animals received one initial dose and two booster doses 3 weeks apart and continued with a standard Chow diet for another 4 weeks. Control mice received phosphate-buffered saline or were left untreated. Plasma collected before immunisation and at termination was used to measure levels of interferon γ, interleukin (IL)-4 and IL-10 and also IgG1 and IgG2b. Atherosclerotic plaque development was assessed using paraffin sections of the brachiocephalic artery. Our results show that intranasal administration of *M vaccae* vectors expressing MOMP, with or without signal sequence, promotes anti-atherogenic T-cell responses and halts the development and progression of atherosclerotic plaques in ApoE−/− mice.

BAS/BSCR51 CONTROL OF VASCULAR CELL INFLAMMATORY RESPONSES THROUGH TILRR, AN INTERLEUKIN 1 CO-RECEPTOR

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Inflammatory responses are induced by members of the Toll-Like and interleukin (IL)-1 receptor family and controlled by NF-κB.

We have identified an IL-1RI co-receptor, TILRR, which potentiates activation of NF-κB and inflammatory responses. We show that induction of amplification depends on formation of a TILRR-containing receptor complex, which imparts enhanced recruitment of the MyD88 adaptor to the signalling receptor IL-1RI, and induction of Ras-dependent amplification of NF-κB.[1]

We have confirmed expression of TILRR in vascular cells and have demonstrated a correlation of the level of TILRR expression with the level of NF-κB activity and inflammatory responses, induced by IL-1 stimulation and by mechanotransduction.

Our recent studies have demonstrated expression of TILRR in vascular endothelial cells using immunohistochemistry. Sections of perfusion-fixed, paraffin-embedded vascular tissue were stained using a specific rabbit polyclonal anti-TILRR antibody, followed by incubation with a biotinylated goat anti-rabbit antibody. Ongoing studies using wild type and ApoE−/− mice are designed to assess the impact of diet on TILRR expression and on its association with the signalling partner IL-1RI.

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REFERENCE

1. **Zhang X,** Shephard F, Kim HB, *et al*. TILRR—A novel a novel IL-1RI- co-receptor potentiates MyD88-recruitment to control Ras-dependent amplification of NF-κB. *J Biol Chem* 2010;**285**:7222—32.

BAS/BSCR52 INVESTIGATING THE IMPORTANCE OF HEPARAN SULPHATE IN DETERMINING ENDOTHELIAL PROGENITOR CELL FUNCTION

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The increased risk of cardiovascular disease with age may, in part, be due to a decline in the function of endothelial progenitor cells (EPCs). Cell surface heparan sulphate proteoglycans (HSPGs) can

bind a plethora of factors that are essential for EPC function. However, these interactions are dependent upon specific structures of HS. We aim to establish whether structural changes of HS on EPCs underlie the age-associated functional deterioration of these cells. The number and function of EPCs in patients with systemic lupus erythematosus (SLE), a disease associated with accelerated vascular ageing, was compared with age-matched healthy controls (52 patients and 30 controls; mean age 52 and 50 years, respectively). To enumerate EPCs, mononuclear cells were labelled with CD133 and CD34 and analysed by flow cytometry. The formation of colony-forming units (CFUs) after 7 days in culture was a measure of EPC function. Cell surface HS structure was analysed using high performance liquid chromatography. While EPC levels did not significantly differ, impairment in EPC function with vascular ageing was evident from the significantly reduced mean number of CFU (7 (SD=5) vs 17 (SD=18), p=0.01), with fewer large CFU (17% vs 40%; p<0.05) in patients than in controls. Preliminary data suggest decreased 2-O-sulphation of HS in association with vascular age. Ongoing studies are investigating if this affects EPC migration, proliferation and integration into vascular structures. Proving our hypothesis will improve our understanding of age-associated endothelial dysfunction and ascertain whether EPCs and/or HS oligosaccharides have therapeutic potential to attenuate age-associated vascular pathologies.

BAS/BSCR53 RELATIONSHIPS BETWEEN SLEEP DURATION AND VON WILLEBRAND FACTOR, FIBRINOGEN AND FACTOR VII: WHITEHALL II STUDY

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Rationale Sleep is an emerging risk factor for cardiovascular disease (CVD). We examined the relationship between sleep duration and haemostatic factors important in CVD development, in a well-characterised occupational cohort.

Methodology The relationship between self-reported sleep duration and von Willebrand factor (vWF), fibrinogen and factor VII in 6400 individuals from the Whitehall II study was examined.

Results Owing to significant gender interactions (p<0.001), the analysis was stratified by gender. After multiple adjustments, vWF levels were significantly higher in men with both short (<6 h per night; 1.05 (95% CI 1.01 to 1.08)) and long (>8 h per night; 1.05 (95% CI 1.02 to 1.08)) duration of sleep compared with those who slept 7 h (p<0.05 for both). In women, levels of vWF were significantly higher in individuals who slept >8 h (1.11 (95% CI 1.06 to 1.16)) than in those who slept for 7 h (p<0.05). This difference was observed in premenopausal and postmenopausal women (p<0.05 for each). The association seen in women appears to be non-linear (p=0.02), but not in men (p=0.09). No statistically significant associations between sleep duration and fibrinogen or factor VII were seen.

Conclusions Men sleeping for both short and long periods had higher vWF levels than those who slept for 7 h. In women, there was a significant non-linear association with the highest levels mainly seen in long sleepers, irrespective of menopausal status. No major associations between sleep and either factor VII or fibrinogen were observed. Further longitudinal studies are required to fully investigate possible temporal relationships between sleep and vWF and the possible associated risk of CVD.

BAS/BSCR54 THE HYPOLIPIDAEMIC ACTIVITY OF NOVEL INDOLE-2-CARBOXAMIDES IN TRITON WR-1339-INDUCED HYPERLIPIDAEMIC RATS: A COMPARISON WITH BEZAFIBRATE

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Using Triton WR-1339-induced hyperlipidaemic rats as an experimental model, we investigated whether compound 1 (N-(3-benzoylphenyl)-1H-indole-2-carboxamide) and 2 (N-(4-benzoylphenyl)-1H-indole-2-carboxamide) novel anti-hyperlipidaemic agents have any effect on plasma triglyceride, total cholesterol (TC) and high-density lipoprotein cholesterol levels (HDL-C) levels. Hyperlipidaemia was developed by intraperitoneal injection of Triton WR-1339 (200 mg/kg body weight). At a dose of 15 mg/kg body weight, compounds 1, 2 and bezafibrate (BF) significantly reduced the elevated plasma triglyceride levels after 7 and 24 h. Furthermore, HDL-C levels were remarkably increased in all treated groups after 7 and 24 h compared with the hyperlipidaemic control group. However, only compounds 1 and 2-treated groups clearly showed a significant reduction in plasma TC levels after 24 h. It is therefore reasonable to assume that compounds 1 and 2 may have a promising potential in the treatment of hyperlipidaemia and coronary heart diseases.

BAS/BSCR55 DIVERSE BACTERIA PROMOTE MACROPHAGE FOAM CELL FORMATION: POTENTIAL ROLE OF TOLL-LIKE RECEPTOR SIGNALLING PATHWAYS

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We and others have shown that the molecular signatures of diverse bacterial species accumulate in human atheromatous lesions. Here, we aimed to determine the effect of non-viable bacteria on foam cell formation in vitro. Using human monocyte-derived macrophages and the murine J774 macrophage cell line, we found that any of a diverse panel of heat-killed Gram-positive or Gram-negative bacteria shown previously to accumulate in human atherosclerotic lesions promoted marked induction of foam cell formation in macrophages, as assessed by light-microscopy of Oil-red-O stained cells and Nile-red-based flow cytometric quantification of cellular lipid accumulation. As Toll-like receptors (TLRs) have a central role in the induction of inflammatory signalling by bacteria, we next examined if specific TLR-ligands could also promote foam cell formation in the absence of intact bacteria. Remarkably, stimulation of macrophages with purified ligands specific for any of the TLRs (including lipopeptide, polyI:C, LPS, flagellin, ssRNA, loxoribine and CpG DNA) led to significant lipid accumulation. This process was not dependent on oxidation of low-density lipoprotein (LDL) as neither antioxidants nor the scavenger receptor blocker polyinosinic acid reduced foam cell formation. Moreover, the presence of LDL was not required for TLR-mediated foam cell formation. Specific inhibitors of TLR signalling prevented foam cell formation induced by TLR ligands or bacteria. We conclude that although the bacterial signatures present in human atheroma are likely to reflect non-viable, killed organisms, it remains possible that molecules derived from these organisms may promote the differentiation of macrophages to lipid-laden foam cells via mechanisms that are likely to include stimulation of TLRs.