alanine scanning mutagenesis of the TILRR core protein, targeting conserved residues with predicted effects on secondary structure, demonstrated distinct control of inflammatory and anti-apoptotic intermediates. These results demonstrate that TILRR amplification involves selective control of NF-κB-regulated inflammatory and anti-apoptotic responses, and are consistent with induction of discrete conformational changes in the IL-1 receptor complex through TILRR association.

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BAS/BSCR6 α-KETOGLUTARATE: BIOLOGICAL EFFECTS OF A NOVEL BIOMARKER OF HEART FAILURE

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Heart failure is often associated with renal impairment (cardiorenal syndrome). Using a metabolomics approach, our group identified α-ketoglutarate as a novel metabolite that was significantly elevated in patients with heart failure.[1] It was as strongly associated with heart failure as the 'gold-standard' biomarker brain natriuretic peptide. α-Ketoglutarate is a citric acid cycle intermediate, central in cardiac energy production. It is the ligand of GPR99, a G-protein coupled receptor mostly expressed in the kidney.[2] In HEK 293 cells, GPR99 acted through a Gq-mediated pathway to increase intracellular calcium. Importantly, we found that the GPR99 receptor is present in neonatal rat cardiomyocytes (NRCM). We tested the expression of 84 genes previously known as regulators of angiogenesis in NRCM treated with 1 mM α-ketoglutarate. Using real-time PCR, we found significant increase in the expression of VEGF receptor-1 and placental growth factor, suggesting a possible effect of α-ketoglutarate in the regulation of angiogenesis and growth. These findings show that binding of α-ketoglutarate to the GPR99 receptor in the heart leads to activation of the Gq pathway and causes upregulation of VEGFR1 and placental growth factor. This might have a role in vascular adaptation to hypertrophy. In addition, in vivo animal studies have previously shown that protein kinase C has a role in renal dysfunction. This leads to the novel and testable hypothesis that α-ketoglutarate also contributes to the development of the cardiorenal syndrome.

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BAS/BSCR7 DEMONSTRATION OF GENE EXPRESSION WITHIN A THROMBUS: FURTHER REGULATION OF THE HAEMOSTATIC RESPONSE

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Vascular injury and plaque rupture activate the haemostatic response, resulting in the formation of a thrombus comprising platelets, red cells and leucocytes, incorporated into a mesh of plasma proteins. Whether cells within the thrombus act simply as structural and secretory components, or have a more active role involving gene expression is unclear. To investigate this, thrombi were produced at 37°C in a Chandler loop, using re-calcified citrated blood from healthy donors (n=6). The thrombi were removed after 2, 4 and 6 h of rotational incubation, and homogenised to extract total RNA. Following in vitro transcription, samples were hybridised to Illumina WG6 beadchips, and data normalised using Illumina Beadstudio. Differences in gene expression were assessed using a Student t test, applying fdr2d correction to eliminate false positives (R Bioconductor). Genes which demonstrated significant (>twofold) time-dependent increases included genes encoding proteins involved in chemotaxis (IL8, CCL2, CXCL1, CXCL2, CXCR4), cell adhesion (ITGAV, ITGA5, ITGB1, ALCAM), regulation of coagulation (THBD, PLAU, SERPINE1, ANXA5), wound healing (TGM2, ENDG, SPP1, LAMB3, PTGS2, TNFAIP6) and regulatory transcription factors (FOS, BMP6, IRAK2, KLRG1, PPARG). Whereas initiation of thrombosis is driven by plasma proteins and facilitated by the platelet surface, this study provides evidence that thrombus resolution may be driven by changes in gene expression within the thrombus that regulate the haemostatic response, thrombus growth and facilitate wound healing. This finding could have implications for individuals at risk of plaque rupture, where variation in gene expression may affect not just the formation of an occlusive thrombus but also the rate of resolution.

BAS/BSCR8 DOES MACROPHAGE FOAM CELL FORMATION PROMOTE EXTRACELLULAR MATRIX FORMATION OR DEGRADATION? A GENOMIC STUDY

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Foam cell macrophage (FCM) formation is an early event in atherosclerosis that precedes both fibrous cap development and subsequent cap rupture; FCM have been implicated in both events. To understand this further we compared the transcriptomes of FCM and non-foamy macrophages (NFM) produced in subcutaneous sponges implanted into fat-fed ApoE null or C57Bl6 mice, respectively (n=4 each). RNA samples of high quality by A260/280>2 were compared on Illumina bead chips. Differential expression was classified as significant ($p<0.05$ after Bonferroni correction for multiple testing) or suggestive ($p<0.001$ unadjusted). 62 genes were significantly upregulated and 59 downregulated in FCM compared with NFM. A total of 370 and 381 genes were upregulated and downregulated using the more relaxed criterion. Fold changes confirmed by quantitative RT-PCR (n=5—7) included upregulation of cathepsin C (15×), cathepsin E (19×), matrix metalloproteinase (MMP)-2 (18×) and MMP-23 (22×) but also upregulation of tissue inhibitor of matrix metalloproteinase (TIMP)-2 (4×) and TIMP-3 (8×) and downregulation of MMP-13 (5×). Surprisingly, several matrix proteins were significantly upregulated, including collagen Iα1 (55×) and VIα1 (31×), osteonectin (72×) and biglycan (19×), although thrombospondin declined (2×). Hence our genomic analysis demonstrated changes that could lead to both matrix degradation and deposition. Ingenuity pathway analysis implicated activation of LXR/RXR in FCM, in agreement with other literature, and highlighted responses to platelet-derived growth factor and transforming growth factor β. The hypothesis that interaction of these pathways accounts for the ambiguous behaviour of FCM in matrix remodelling deserves further investigation.

Contributors A C Thomas, J L Johnson and W J Eijgelaar are contributed equally.