Method Male Wistar rats at 6 months (young), 12 months (adult) and 24 months (old) (n=5 per group) were killed by a Home Office approved. The SAN region of the heart was dissected and maintained in bicarbonate-buffered saline at 37°C. Intrinsic pacemaker activity was recorded under control conditions and in the presence of 3μMM cyclopiazonic acid (CPA) to inhibit SERCA. Western blot was used to assess expression of SERCA and the SR calcium-release channel, the ryanodine receptor RYR2, both shown expressed relative to levels in the young SAN. Data are shown as mean ± SEM.
Results Intrinsic SAN beating rate significantly decreased in old age (young 260±17bpm vs. old 216±15bpm; P=0.04). CPA caused slowing in both young and adult SAN by 26±9 and 49±10bpm respectively (P=0.001), but not in the old SAN. SERCA2a and RYR2 expression increased from young to adult (SERCA young 100±9.8% vs. adult 135±10.8%; P=0.002: RYR2 young 100±21.9% vs. 152±11.2%; p=0.0006), but declined substantially in old age to levels below the young (SERCA old 72±11.7%; RYR2 old 53±4.3%).
Conclusion The data show a diminished SR influence on pacemaking in the old SAN. In contrast developmentally the SR may increase its pacemaker role from young to adult animals.

18 TILRR FUNCTIONAL MUTANTS SELECTIVELY INHIBIT INFLAMMATORY AND ANTI-APOPTOTIC RESPONSES

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Toll-like and IL-1 receptors control inflammatory responses. TILRR (Toll-like IL-1 Receptor Regulator), is an IL-1 co-receptor which associates with the type I IL-1 receptor (IL-1RI) to amplify activation of NF-kappaB and inflammatory responses. Earlier studies have demonstrated increased levels of TILRR in the atherosclerotic plaque. Further, that injection of a polyclonal anti-TILRR antibody, which blocks TILRR/IL-1RI association and reduces inflammatory responses, causes a 25% decrease in plaque formation in ApoE-/- mice on a high fat diet. Alanine scanning mutagenesis identified two sites within the TILRR core protein, which allow distinct control of Il-1 activities. The R425A substitution blocks enhanced cell survival, but functions as wild-type in relation to inflammatory responses. In contrast, a D448A substitution reduces MyD88-dependent inflammatory responses, but has no impact on cell survival [1]. Current studies use peptides designed to block these distinct functional sites to further analyse consequences of selective inhibition on downstream events. Results show a successive reduction in inflammatory responses by the peptide designed to block D448 dependent interactions, with no effect of the peptide targeting anti-apoptotic signals or a non-specific control. Ongoing studies are testing the effect of the peptides on IL-1-induced cell survival.

1. Zhang, X., et al., JBC, 2012, doi:10.1074/jbc.C111.321711

19 THE GENERATION AND CHARACTERISATION OF LO1: A UNIQUE IGG MONOCLONAL NATURAL ANTIBODY AGAINST OXIDISED LDL (OXLDL)

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Background Although current evidence implicates IgG natural antibodies against oxLDL in atherogenesis, it has previously been difficult to isolate these as monoclonals. We set out to make IgG monoclonal natural antibodies from a LDLR-/- mouse for mechanistic studies and translational applications.
Methodology and Results One antibody we obtained is a unique IgG3k antibody designated mAb LO1. This reacts with copper-oxidised but not native LDL. Further characterisation revealed that LO1 reacts in vitro with malondialdehyde-conjugated LDL (MDA-LDL), a known LDL oxidation product implicated in plaque vulnerability. Sequencing of LO1 heavy and light chain variable regions showed it to be almost entirely germ-line, consistent with it being a natural antibody. Studies using a subsequently generated neutralising anti-idiotype single chain antibody (H3) against LO1 indicated that it is likely to react with a conformational epitope involving discrete ApoB peptide sequences. Immunocytochemical staining demonstrated that LO1 binds epitopes in mouse and human atherosclerotic lesions. In culprit human carotid endarterectomy tissue, LO1 but not IgG3k control stained intracellular deposits in macrophages and occasional extracellular deposits adjacent to the edge of the lipid necrotic core. This pattern is consistent with the distribution of heavily oxidised LDL in vulnerable plaque. LO1 was successfully labelled, and function retained, with a NIRF imaging agent. We are also molecularly expressing various LO1 constructs to be used in imaging studies.
Conclusions LO1 provides a promising tool for the analysis in detail of the function of an individual IgG autoantibody in relation to atherosclerosis and the targeting of oxidised LDL in vivo

20 IN VIVO TRACKING OF HUMAN PLURIPOTENT STEM CELL VASCULAR DERIVATIVES.

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There is compelling in vivo evidence that the inclusion of mural cells in addition to endothelial cells (ECs) can augment the formation of functional blood vessels in therapeutic angiogenesis. However, it remains unclear whether CD34+ vascular progenitor cells, which have the potential to differentiate into both ECs and vascular smooth muscle cells (VSMCs), are superior to fully differentiated VSMCs at supporting revascularisation. In addition, it would be of interest to determine whether VSMCs from different embryological origins have differential influences on vessel development. To address these questions, we have developed chemically-defined protocols to differentiate human embryonic stem cells (hESCs) to CD34+ progenitors, ECs and origin-specific SMCs. In order to overcome the challenge of tracking the fate of these cells in vivo, a stably transduced hESC line (C4) that expresses mStrawberry and luciferase has been created. This cell line retains its pluripotency and thus its ability to differentiate into the vascular derivatives, without compromising efficiency or expression of mStrawberry and luciferase. In an in vivo pilot study, C4 hESCs were successfully visualised by bioluminescence imaging up to 2 hours after subcutaneous injection into Rag2 mice. However, at 24 hours the cells were no longer detectable. Although Rag2 mice are immunocompromised, they retain natural killer cells, which may be responsible for the apparent cell death. Further in vivo optimisation is planned, using alternative immunodeficient mice, to establish a model in which the revascularisation potential of hESC-derived vascular progenitors and cells can be assessed in vivo.