Late-Breaking Basic Science Abstracts

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Abstract Topics Include:

• Signaling in the Heart and Vasculature
• Genetics
• Stem Cells
• Vascular Medicine/Biology
Interferon-Beta Signaling Is Enhanced in Patients With Insufficient Coronary Collateral Development and Inhibits Arteriogenesis in Mice

Stephan H Schirmer, Academic Med Cntr, Amsterdam, The Netherlands; Imo E Hoefer, Univ Med Cntr, Utrecht, The Netherlands; Joost O Flederus, Perry D Moerland, Pieter T Bot, Jan Baan, Jr., Jose S Henriques, Renée J van der Schaaf, Maritje M Vis, Arton J Horrevoets, Jan J Piek, Niels van Royen; Academic Med Cntr, Amsterdam, The Netherlands

Several growth factors stimulate collateral artery growth in experimental models, but attempts to enhance arteriogenesis in the clinical setting were hitherto unsuccessful. Circulating cells, especially monocytes, orchestrate arteriogenesis. We hypothesized that patients with differing coronary collateral development differ in gene expression of circulating cells, thereby disclosing novel targets for the stimulation of arteriogenesis. Overall flow index (CFI) was measured in 45 non-diabetic Caucasian patients with single-vessel coronary artery disease undergoing elective PCI. Patients were grouped into collateral responders (CFI > 0.21) and non-responders (CFI < 0.21). Baseline patient characteristics showed no differences. CDF4+ stem cells and monocytes were isolated from peripheral blood. Monocytes were stimulated with LPS or cultured to macrophage-like cells to mimic the cellular phenotype during arteriogenesis. Whole genome gene expression analysis revealed strongly increased expression of interferon (IFN)-beta and several IFN-related genes in stimulated monocytes from non-responders. Microarray data were verified using RT-PCR and ELISA of cell culture supernatants. These data suggest an inhibitory effect of IFN-beta on arteriogenesis. Therefore, we analyzed the effect of the IFN-pathway on arteriogenesis in a well-established murine hindlimb model of arteriogenesis. Hindlimb perfusion was measured 7 days after unilateral femoral artery ligation using fluorescent microsphere infusion. Perfusion restoration was attenuated in mice treated systemically with IFNbeta compared with controls (31.46 ± 4.12% vs. 41.88 ± 8.2%, p < 0.001) and increased in mice lacking the IFNalpha/beta receptor (34.29 ± 6.55%, p < 0.001 compared to control). In conclusion, circulating monocytes of patients with differing arteriogenic response display differential transcriptomes upon stimulation/culture. Non-responders show increased gene expression of IFN-beta and its downstream targets. CD4+ T cells may modulate IFN-beta expression and arteriogenesis.

Novel Role of the Histone Lysine Specific Demethylase 1 (LSD1) in the Enhanced Pro-Atherogenic and Inflammatory Responses in Vascular Smooth Muscle Cells of Diabetic Mice

Marpadga A Reddy, Louisa Villeneuve, Wang Mei, Linda Lanting, Rama Natarajan; Beckman Research Institute of City of Hope, Duarte, CA

Insulin resistance and type 2 diabetes (T2D) are major risk factors for the development of atherosclerosis. We recently showed that cultured Vascular Smooth Muscle Cells (VSMCs) derived from db/db mice, an established mouse model of T2D and obesity, exhibited enhanced histone demethylation of lysine 36 in H3K4me2, a key epigenetic marker of gene activation, and the recently discovered histone demethylase, Lysine Specific Demethylase 1 (LSD1) that inhibits H3K4 methylation, in mouse VSMC (MVCm) derived from diabetic (db/db) and control (db/+ ) mice. Chromatin immunoprecipitation (ChIP) assays showed that levels of H3K4me2 were significantly elevated and conversely the recruitment of LSD1 was significantly reduced on the promoters of MCP-1 and IL-6 genes in db/db VSMC relative to db/+ cells. Immunoblotting of cell lysates demonstrated that LSD1 protein levels were also significantly decreased in db/db VSMC. Moreover, TNF-α - induced inflammatory gene expression and levels of H3K4me2 at these gene promoters were enhanced in db/db cells. In addition, LSD1 gene silencing using siRNAs in db/+ VSMC significantly increased both basal and TNF-α induced inflammatory genes. In contrast, overexpression of LSD1 in db/db VSMC inhibited the enhanced inflammatory gene expression in these diabetic cells. Moreover, transfection of VSMC with LSD1 siRNAs led to greater binding of monocytes to VSMC relative to control scrambled siRNAs, demonstrating functional relevance. These new results demonstrate for the first time a novel role for LSD1 in the regulation of inflammatory gene expression in VSMC and monocyte-VSMC interactions in diabetes. Importantly, they also provide the first functional role for the recently discovered chromatin histone lysine demethylases in the sustained vascular complications associated with diabetes.

Chromatin Remodeling via PP2A and Class Ila HDAC Activation: Identification of a Novel Epigenetic Role for Nitric Oxide in Gene Expression

Barbara Illi, Istituto Cardioologico Monzino, Milano, Italy; Claudio Dello Russo, Istituto di Ricerche Biomolecolari “P. Angeletti”, Roma, Italy; Claudia Colucci, Istituto Dermopatico dell’Immacolata, Roma, Italy; Michele Palliaino, Università degli Studi di Siena, Siena, Italy; Francesco Spallotta, Jessica Rossati, Fabio Martelli, Antonello Mai, Istituto Dermoatogico dell’Immacolata, Roma, Italy; Luca Bini, Università degli Studi di Siena, Siena, Italy; Paolo Bigi, Istituto Cardioologico Monzino, Milano, Italy; Christian Steinikhuner, Istituto di Ricerche Biomolecolari “P. Angeletti”, Pomezia, Italy; Paola Gallinari, Istituto di Ricerche Biomolecolari “P. Angeletti”, Pomezia, Italy; Maurizio C Capogrossi, Carlo Gaetano; Istituto Dermoatogico dell’Immacolata, Roma, Italy

Introduction and Background: Nitric oxide (NO) is a potent regulator of vascular cell function. It exerts its action at multiple level including transcription. Although some evidences suggest that in human endothelial cells (ECs) NO may act as a potent negative regulator of gene expression the mechanism by which this effect is achieved remains elusive. Recently, the activation of histone deacetylases (HDACs) gained consideration as an important mechanism for the negative control of gene expression. Hence, wishing to investigate how NO regulates transcription, we asked whether HDAC function could be regarded as a NO target. Methods and Results: Immunofluorescence and western blot analyses revealed that, in presence of shear stress or NO donors, class IIa histone deacetylase HDAC4 and HDAC2 localized to the nucleus. This process was abrogated by NO inhibitors, which consistently increased histone H3 acetylation and decreased the total nuclear HDAC activity in ECs. NO donors, in fact, abrogated serum-induced histone H3 acetylation, which was restored by treatment with the class II HDAC specific inhibitor MC1568. In the same experiment the class I HDAC specific inhibitor MS-27-275 had no effect suggesting that NO could be selective for class II HDAC function. Phosphorylated class II HDAC members are predominantly cytosolic and bound to 14–3–3 chaperones, becoming nuclear upon de-phosphorylation. Attempting to dissect the molecular mechanism underlying the effect of NO on class II HDAC localization, HEV-2C were stably engineered with the polyoma virus small T antigen (smt-HEVEC) which functional inactivates PP2A phosphatases. In this context, NO failed to stimulate HDAC4 and 5 nuclear localization compared to mock-transfected cells. Moreover, in cells expressing a HDAC4-Flag fusion protein, treatment induced loading of macrophage class II HDACs containing HDAC4, 5 and an active PP2A phosphatase as determined by antibody column purification and mass spectrometry. Conclusions: This work provides new insights about a novel mechanism by which NO may have an impact on the global chromatin landscape contributing to gene expression regulation at an epigenetic level.
pathology, male RAP +/- (n=17) and -/- mice (n=13) were fed a normal diet and infused with AngI (1,000 ng/kg/min by osmotic minipump) for 28 days. AngI increased systolic blood pressure similarly in RAP +/- and -/- mice (pre-infusion: 139 ± 4, post-infusion 168 ± 4; -/-; pre-infusion: 133 ± 3; post-infusion: 169 ± 3 mmHg, respectively). AngI-infusion decreased plasma renin concentrations equivalently in RAP +/- and -/- mice. RAP genotype or AngII infusions had no significant effect on total plasma cholesterol concentrations. RAP deficiency increased diameter of abdominal aortas from 1.07 ± 0.04 to 1.65 ± 0.16 mm (p<0.05) and incidence of AAAAs from 0 to 62% (p<0.001). In addition to increased AAAAs, RAP/-/- mice infused with AngII had intra-medial hemorrhages in the arch, thorax, and suprarenal aorta. Tissue sections demonstrated that hemorrhages were characterized by the accumulation of erythrocytes between intact elastin layers with a greater predominance on the adventitial side.

Conclusion: RAP deficiency markedly increased AngII-induced vascular pathology that included the development of AAAAs and intra-medial hemorrhage.

Increased Levels of Activated C-jnk N-terminal Kinase Have Been Associated With the Downregulation of Cardiac Ion Channel Expression During In Vivo Aging

Matthew K Lancaster, Univ of Leeds, Leeds, United Kingdom; Sandra A Jones; Univ of Hull, Hull, United Kingdom

The size of the elderly population is continually increasing and it has been predicted by 2035 >25% of the Western population will be elderly. Sinoatrial (SA) node dysfunction observed clinically as rhythm disturbances, sinus pauses and arrhythmias, is at its highest incidence in the elderly population and without clinical intervention, patients are at high risk of sudden death. The activation of stress-association signaling pathways within the heart progressively increases during aging and may contribute to arrhythmia generation. Our hypothesis investigated if stress activated c-jun N-terminal kinase (JNK) was responsible for the age-dependent decrease in the expression of protein gap junction connexin43 (Cx43) and Cx40. The right atrium was examined from rodents between birth and ~38 months (n=5 per age group). Protein analysis by immunofluorescence and Western blot showed a substantial decline in Cx43 and Cx41,2 channel within the SA node during aging. These changes were associated with slowing of the action potential conduction velocity to 0.90±0.01m/s at 38 months from 0.38±0.01m/s at 1 month (ANOVA, p<0.01). The oldest animal possessed mostly phosphorylated Cx43 (P-Cx43) at the intercalated disks (0.99±0.03, P-Cx43 total Cx43) compared with the newborn (0.48±0.02). Associated with the phosphorylation of Cx43 protein is activated-JNK (which is phosphorylated JNK, expressed as a ratio to non-phosphorylated JNK), increased from 0.14±0.01 at birth to 0.68±0.05 at 38 months (ANOVA p<0.01). The decline of both Cx43 and Cx41,2 channels correlated with increased levels of activated JNK during progressive aging (ANOVA p<0.01; Linear regression r=1.0305X-0.3484; R2=0.96). This is the first evidence that shows the stress-induced JNK signalling pathway is a signalling mechanism associated with the age-dependent uncoupling of myocytes in aged tissue. Increased levels of activated JNK were concurrent with substantial losses of both Cx43 and Cx41,2 channel with the SA node, resulting in diminished electrical communication. With manipulation of this pathway, it may prove possible to limit the progression of cardiac dysfunction in the elderly.

Atrial Fibrillation and Conduction Blockade in a Mouse Model of Dilated Cardiomyopathy With Reduced Phosphoinositide 3-Kinase Activity

Xiao-Jun Du, Elizabeth A Woodcock, Helen Kiriazis, Lynette Pretorius, Ziqiu Ming, Baker Heart and Stroke Institute, Melbourne, Australia; Junichi Sadoshima, New Jersey Med Sch, Newark, NJ; Tetsuo Shioi, BIDMC, Harvard Med Sch, Boston, MA; Mago Izumo, Novartis Institutes for BioMed Resrch, Cambridge, MA; Anthony M D'Urso, Garry L Jennings, Julie R McMullen; Baker Heart and Stroke Institute, Melbourne, Australia

Background: atrial fibrillation (AF) is the most common sustained arrhythmia presenting at cardiological departments worldwide. AF is difficult to treat and experimental studies have been restricted by a lack of small animal models. Objective: To assess whether a newly developed mouse model with features known to contribute to the development of AF (i.e. atrial dilatation and hypertrophy, and electrotcardiogram (ECG) abnormalities) in particular AF. Methods: The mouse model was generated by genetically crossing cardiac-specific transgenic mice overexpressing the stress-activated protein kinase, mammalian sterile 20-like kinase 1 (Mst1, model of dilated cardiomyopathy (DCM) without reported ECG abnormalities) with cardiac-specific transgenic mice expressing a dominant negative mutant of phosphoinositide 3-kinase (pi3K) (p110δi110δ). Double transgenic mice (DTg: Mst1-dpi3Kδi110δ), single transgenics and non-transgenic littermate controls (NTg) were subjected to morphological, functional and ECG analyses at 4–4.5 months of age. AF was considered if P-waves were absent during periods with overtly irregular R-R intervals. Results: The DCM phenotype of DTg was more severe than Mst1 alone and lifespan was reduced (~5 months compared with 8 months). DTg mice had depressed cardiac function (fractional shortening: NTg: 54±3: 3, DTg: 24±4: p<0.05, N=6), atrial enlargement (NTg: 6.7±0.5: DTg: 28.7±0.8 mm, p<0.05, N=6) and fibrosis. All DTg mice displayed signs of atrial-ventricular conduction blockade and amplitudes of P- and R-waves were suppressed (P-amplitude: NTg: 0.15±0.01, DTg: 0.06±0.01 mm, R amplitude: NTg: 1.45±0.07 mV, DTg: 0.34±0.06 mV, p<0.05, N=6). Sudden onset of AF was detected in 40% of DTg mice (6/16) but not single transgenics or NTg. As ECGs were recorded for only 5–10 min, the incidence of AF was most likely underestimated in DTg. Conclusions: We have developed a new mouse model of AF. The relatively long lifespan provides time to follow the progression of disease and the model should be a useful tool to test...
Overexpression of A1 Adenosine Receptor Causes Decreased Cardiac Contractility and Abnormal Calcium Handling by Selectively Perturbing Caveolin 3-Associated T-Tubule Structure

Tung Chan, Paul H Chung, Hajime Funakoshi, Elina Chesek, Jianliang Song, David E Herrmann, Jean-Francois Jasmin, Michael P Lisanti, Joseph Y Cheung, Arthur M Feldman; Thomas Jefferson Univ, Philadelphia, PA

Background: Mice overexpressing the A1-adenosine receptor (A1-AR) develop a dilated cardiomyopathy and impaired myocyte calcium homeostasis (low systolic [Ca2+]i and decreased contraction amplitudes); however, the mechanisms responsible for these changes remains unclear. We hypothesized that overexpression of A1-AR might alter coupling between the receptor and membrane invaginations (caveolae) containing the integral protein, caveolin, that are found along the T-tubule. Results: Mice overexpressing the A1-AR demonstrated a marked decrease in the expression of caveolin 3 (Cav3) but not of Cav2 or Cav1 as measured by western blot analysis (p < 0.05) but did not demonstrate a change in sodium–calcium exchanger (NCX). When isolated cardiac myocytes were evaluated using immunofluorescent microscopy, Cav3 demonstrated a punctate-striated staining pattern that co-localized with the T-tubule marker, NCX. However, this punctate-striated pattern was lost in myocytes that overexpressed A1-AR. To demonstrate that the A1-AR directly couples with Cav3, we transiently overexpressed Cav3 alone or in combination with the A1-AR in the rat embryonic heart derived cell line, H9C2. Overexpression of Cav3 alone resulted in the formation of evenly punctuated structures that were identifiable by immunofluorescent microscopy as Cav3. However, co-overexpression of A1-AR and Cav3 resulted in the co-aggregation of A1-AR and Cav3 into elongated structures consistent with loss of the normal T-tubule structure. Importantly, the changes in Cav3 at the protein level as well as morphologic alterations of T-tubule structure were not seen in myocytes isolated from mice with cardiac-restricted overexpression of the A2A-adenosine receptor. Furthermore, abnormal localization of Cav3 into elongated structures consistent with loss of the normal T-tubule structure. These results suggest that the coupling of select G-protein-coupled receptors and caveolins might be a novel target in the treatment of heart failure.
Novel Associations of the Wnk1 Gene With Risk for Essential Hypertension

Stephen J Newhouse, Chris Wallace, Mirmoza Hadi, Richard J Dobson, Barts and the London, Queen Mary’s Sch of Med and Dentistry, London, United Kingdom; Mark Farrall, Univ of Oxford, Oxford, United Kingdom; Morris Brown, Univ of Cambridge, Cambridge, United Kingdom; Nilesh J Samani, Univ of Leicester, Leicester, United Kingdom; Anna Dominiczak, John M Conneil, Univ of Glasgow, Glasgow, United Kingdom; John Webster, Aberdeen Royal Infirmary, Aberdeen, United Kingdom; Martin Tobin, Univ of Leicester, Leicester, United Kingdom; Cother Hajat, Paul Burton, Univ of Leicester, Leicester, United Kingdom; Carola B Marcano, Barts and The London, Queen Mary’s School of Medicine and Dentistry, London, United Kingdom; Martin Farrall, Univ of Oxford, Oxford, United Kingdom; Morris Brown, Univ of Cambridge, Cambridge, United Kingdom; Nilesh J Samani, Univ of Leicester, Leicester, United Kingdom; Anna Dominiczak, John M Conneil, Univ of Glasgow, Glasgow, United Kingdom; John Webster, Aberdeen Royal Infirmary, Aberdeen, United Kingdom; Martin Tobin, Univ of Leicester, Leicester, United Kingdom; Cother Hajat, Paul Burton, Univ of Leicester, Leicester, United Kingdom; Carola B Marcano, Barts and The London, Queen Mary’s School of Medicine and Dentistry, London, United Kingdom.

We have previously reported association between WNK1 and BP variation in families from the British Genetics of Hypertension (BRIGHT) Study and association has been reported with 24-hour ambulatory BP in the GRAPHIC study. We set out to replicate and extend our findings with BP and essential hypertension (EH) and to test if phenotypes related to WNK1 function (serum Na, Cl, Ca and urinary Na and K) were also associated with WNK1. Twenty eight tag SNPs (SNPs) that capture 100% of HaMap II were genotyped in 1700 cases and 1700 controls from the BRIGHT resource. Logistic regression was used to test for association with EH and linear models for quantitative analyses. Haplotype associations were explored using haplo.stats. All analyses were adjusted for age, sex, BMI and geography to adjust for population stratification. Multiple SNPs were found to be significantly associated with both BP and EH from the BFIR.ME-CHR.GEO.GWAS.0001 and DBP (mp = 0.0025). Haploype analysis revealed striking associations with SBP (global p = 1.15x10^-10), DBP (p = 1.2x10^-10) and EH (p = 1.2x10^-10). Notably, SNPs spanning the length of the gene were also significantly associated with ionised serum Ca (mp = 0.0014), Na (mp = 0.0053) and Cl (mp = 0.0009) and urine K (mp = 0.0011) and Na (mp = 0.0009).Our analysis confirms association of WNK1 with BP, and now provides novel evidence for association with EH and electrolyte homeostasis. These new data provide compelling evidence to initiate further genetic and functional studies to identify causative variants and explore the role of WNK1 in BP regulation and EH.

Discovery of Novel Genes for Serum Urate and Low-Density Lipoprotein Cholesterol—Two Biomarkers of Cardiovascular Disease

Chris Wallace, Stephen J Newhouse, Barts and The London, Queen Mary’s Sch of Medicine and Dentistry, London, United Kingdom; Peter Braund, Univ of Leicester, Leicester, United Kingdom; Feng Zhang, King’s College London Sch of Medicine, London, United Kingdom; Martin Tobin, Univ of Leicester, Leicester, United Kingdom; Marie Falchi, Koorosh Ahmadi, King’s College London Sch of Medicine; Richard J Doughty, Ana Carolina B Marcano, Barts and The London, Queen Mary’s Sch of Medicine and Dentistry, London, United Kingdom; Cother Hajat, Paul Burton, Univ of Leicester, Leicester, United Kingdom; Panagiotis Deloukas, The Sanger Ctrn, Cambridge, United Kingdom; Morris Brown, Univ of Cambridge, Cambridge, United Kingdom; Martin Farrall, Univ of Oxford, Oxford, United Kingdom; Tim Spector, King’s College London Sch of Medicine, London, United Kingdom; Richard J Doughty, Ana Carolina B Marcano, Barts and The London, Queen Mary’s Sch of Medicine and Dentistry, London, United Kingdom.

Serum and urine biochemistry measurements are used routinely in daily clinical practice to define co-morbid traits such as dyslipidaemia, or as biomarkers of target organ damage (e.g. urea, creatinine and renal function). Many of these traits are under tighter genetic control than their related diseases. Our hypothesis is that genetic variation may influence the inheritance of such commonly measured biochemical traits and these in turn may serve as risk factors for common diseases, or associated complications. As part of the Wellcome Trust Case Control Consortium, 500,000 SNPs genomewide were genotyped in 1955 UK hypertensive individuals characterized for 25 serum and urine biochemical traits. For each trait we assessed association with individual SNPs, adjusting for age, sex and BMI. Lipid measurements were further examined in a meta-analysis of genomewide data from a type 2 diabetes scan. Our analysis discovered two highly significant novel associations. The first was between serum urate and SLCA28, a glucose transporter (p = 3 x 10^-15), in which a common allele was associated with a 0.2mmol/L increase in serum urate. We were able to confirm this finding in two epidemiological collections reflecting the normal range of blood pressure variation in the UK. We did not find association between the associated SNP and BP. This suggests the correlation often observed between serum urate and increased BP and coronary artery disease, may be consequential rather than causative or that greater numbers of individuals are required to detect this association. The second association was between LDL levels and SNPs close to genes encoding PSRC1 and CELSR2 (p = 1 x 10^-7). The common allele was associated with a 6% increase in non-fasting serum LDL. This region showed increased association in the meta-analysis (r^2 = 4 x 10^-4) and has recently been associated with coronary disease. The association with LDL provides a potential biological mechanism which could explain the association with coronary disease.

Mesenchymal Stem Cell TherapyRestores Cardiac Stem Cell Niches After Acute Myocardial Infarction

Konstantinos E Hatzistergos, Henry Quevedo, Behzad N Oskouei, Qinghua Hu, Ramesh Kong, Hong Kong; Valeriy Timofeyez, Ning Li, Nipavan Chiamvimonvat, Univ of California, Davis, Sacramento, CA; Song-Yan Liao, Wing-Hon Lai, Univ of Hong Kong, Hong Kong, Hong Kong; Chung-Wah Siu, Jennifer C Moore, Deborah K Lieu, Univ of California, Davis, Sacramento, CA; Song-Yan Liao, Wing-Hon Lai, Univ of Hong Kong, Hong Kong, Hong Kong; Valery Timofeyez, Ning Li, Nipavan Chiamvimonvat, Univ of California, Davis, Sacramento, CA; Ronald A Li; Univ of California, Davis, Sacramento, CA.

Loss of non-regenerative, terminally differentiated cardiomyocytes (CMs) is irreversible; myocardiual repair is hampered by a severe shortage of donor cells and organs. Self-renewing, pluripotent embryonic stem cells (ESCs) can provide an unlimited cell source. However, our cellular electrical recordings revealed that their cardiac derivatives exhibit a number of immature and pro-arrhythmic properties (e.g., spontaneous firing activity, phase 4-like depolarization and depolarized resting membrane potentials; p < 0.05) that are similar to those of failing CMs. When transplanted in a large (porcine) and small (ginea pig) animal models, such immature ESC-derived CMs (ESC-CMs) indeed caused ventricular tachycardia (VT) (ablation) (90% of 6 transplanted animals but none in sham, saline-injected animals). These detrimental effects were modeled in rodent models probably because of their high intrinsic heart rates (>400-500bpm) relative to the transplanted cells. Using a combination of in silico simulations and somatic gene transfer techniques, we identified the absence of the Kir2-encoded inwardly rectifying K+ channels (Kir2.1) in ESC-CMs as the missing molecular component behind the pro-arrhythmic phenotype. After adenovirus-mediated over-expression of Kir2.1 channels, Ba+2-sensitive Kir2.1 became robustly expressed in both ESC- and hESC-CMs; the percentage of quiescent derivatives substantially increased to ~100% from ~50%. Furthermore, the REMPs became hyperpolarized to the adult cell level (p < 0.05). Upon excitation, normal APs without the arrhythmogenic “phase 4-like” depolarization could be elicited. Most importantly, Kir2.1-silenced ESC-CMs completely eliminated post-transplantation VT/VF (6 of 6 animals tested). These results provide proof-of-concept support that driven maturation can greatly facilitate hESC-based heart therapies by enhancing their post-transplantation safety.

Driven Maturation of Embryonic Stem Cell–Derived Cardiomyocytes Completely Abolites Post-Transplantation Arrhythmias

J-Dong Fu, Univ of California, Davis, Sacramento, CA; Hung-fat Tse, Univ of Hong Kong, Hong Kong, Hong Kong; Chung-Wah Siu, Jennifer C Moore, Deborah K Lieu, Univ of California, Davis, Sacramento, CA; Song-Yan Liao, Wing-Hon Lai, Univ of Hong Kong, Hong Kong, Hong Kong; Valery Timofeyez, Ning Li, Nipavan Chiamvimonvat, Univ of California, Davis, Sacramento, CA; Ronald A Li; Univ of California, Davis, Sacramento, CA.

The mechanism(s) underlying successful cell-based cardiac repair remain elusive, yet preclinical and early clinical trials support the efficacy of this therapeutic approach. Three mechanisms are propose—differentiation, paracrine signaling, and fusion. Here we tested the hypothesis of a fourth mechanism, that of reconstitution and proliferation of endogenous stem cell (SC) niches. Methods and Results: We tested this hypothesis in a model previously shown to exhibit major cardiac repair after MI. Three days post MI, female Yorkshire pigs (n = 6) underwent catheter (Stiletto, Boston Scientific) intramyocardial injections of GFP tagged syngeneic MSCs (n = 3) or placebo (n = 3). Two weeks later these pigs were evaluated for the presence of GFP+ cells, c-KIT, GATA-4, α-smooth muscle actin and connexin-43. The numbers of c-kit+ cells/mm² were quantified in the infarct (IZ), border and remote zones. There was a dramatic increase in MSC vs placebo group (0.84 ± 0.23 vs. 0.65 ± 0.02, p = 0.005). The increase in c-KIT+ cells in MSC group, greatest in the IZ with sequential declines in BZ and RZ (p = 0.01), was still greater in all zones relative to placebo (IZ: 1.92 ± 0.23 vs. 0.11 ± 0.04, p = 0.001, BZ: 0.57 ± 0.13 vs. 0.02 ± 0.006, p= 0.017, RZ: 0.09 ± 0.017 vs. 0.01 ± 0.005, p=0.016). The majority of c-KIT+ cells were GFP+. Importantly, the c-KIT+ cells formed clusters, resembling SC niches, and formed Ca (43 ± 141 connections) with the inactivated phenotype. In placebo group c-KIT+ cells were isolated and did not form clusters. Additionally, some c-KIT+ cells expressed GATA-4 indicative of cardiomyocyte (CM) differentiation. Rare immature and mature GFP cells co-stained with a-actinin indicating capacity of MSCs for CM differentiation as well. Conclusions: Together these data show the capacity for MSCs to stimulate cell based cardiac repair programs that involve endogenous c-KIT+ cells as well as participation of the MSCs themselves. Immature MSCs form connections via Cx-43 with endogenous cell populations in vivo producing a functional cardiac niche.
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Secretion of Pro-Survival and Pro-Angiogenic Growth Factors In Vitro and In Vivo by Cardiac Progenitor Cells From Human Biopsies  

Isotta Chimenti, Unv. “La Sapienza”, Roma, Italy; Rachel Rucksedel Schmid, Michelle K Leppo, Gary Gerstenblith, Johns Hopkins Univ, Baltimore, MD; Alessandro Giacomello, Elisa Messina, Unv. “La Sapienza”, Roma, Italy; Eduardo Marban, Johns Hopkins Univ, Baltimore, MD  

Human cardiосapient-derived cells (CDCs) improve cardiac function and promote regeneration in a mouse infarct model. The release of growth factors (GFs) might be in part responsible for their beneficial effects. CDCs were obtained from endomyocardial biopsies from 15 patients. Conditioned media (CM) with low to no serum was collected after 48 hours from CDCs and normal human dental fibroblasts (HDFs) as control. Concentrations of VEGF, HGF and IGF1 were measured in the CM by ELISA. CDCs released significant amounts of VEGF, HGF and IGF1, which were released in low serum-CM (1.8 ± 0.4 pg of cells lysate, n = 6), but no IGF1, while HGF was detected only in serum-free, glucose-free CM (2.0 ± 0.9 pg/g, n = 3). Expression of these three GFs mRNAs was detectable by RT-PCR. No GFs were detected in HDF-CM, although corresponding mRNAs were expressed. Neonatal ventricular myocytes were subjected to 72 hours of hypoxia in the presence of serum-free CM and viability was assessed by Annexin V and 7-AAD. CDC-CM secreted significant amounts of pro-survival and pro-angiogenic GFs in vitro and in vivo, and this may be a key mechanism contributing to their therapeutic effects in the post-infarction period.

4018  
Dynamic Evaluation of Cardiac Stem Cell (CSCs) Outgrowth From Human Primary Explants  

Federica D’Aurizio, Patrizia Marcon, Natascha Bergamín, Stefania Martini, Elisa Puppato, Barbara Toffoletto, Nicoletta Finato, Univ of Udine, Udine, Italy; Ugolino Livi, Azienda Ospedaliero-Universitaria Di Udine, Udine, Italy; Alessandra Poz, Daniela Cessellii, Antonio Paolo Beltrami, Carlo Alberto Beltrami, Univ of Udine, Udine, Italy  

Several reports demonstrated that human hearts host a population of CSCs, able to generate, in vitro and in vivo, all cardiac cell lineages. Moreover, we recently generated in culture, from human hearts, a population of multipotent adult stem cells (MASCs), expressing Oct-4 and Nanog and able to differentiate into derivatives of all the germ layers. Aim To determine whether human MASCs are an in vitro phenomenon due to extensive manipulation or if they have an in vivo counterpart.

Methods and Results: 1mm² atria fragments (n=85) obtained both from donors (n=5) and recipients (n=5), were placed in MASC medium. Outgrown cells were daily fixed while tissue fragments were transferred into new dishes. At day 3 (T3), 6 (T6) and 9 (T9) tissue fragments were cut. Half of the slices were transferred into new dishes and half formalin fixed. MASC cell lines (n=4) were obtained at T3 and T6. Cells outgrown from tissue fragments followed a bi-kinetic pattern, as defined by number of outgrown cells per tissue volume, peaking at T3 and T6 in donors, T6 and T9 in recipients. Although outgrown cells expressed oct-4, nanog and c-kit since beginning, the fraction of cells expressing these markers in recipients (being 90 ± 16, 64 ± 16 and 82 ± 3%, respectively) and recipients (being 80 ± 19, 61 ± 18 and 58 ± 3%, respectively). When expanded, outgrown cells assumed MASC characteristics, including clonogenicity, multipotency and lack of c-kit expression. The study of paraffin-embedded tissue fragments showed that oct-4, c-kit and c-kit+ cells were present both in donor and recipient uncultured fragments (being 51 ± 1, 12 ± 3 and 1.2 ± 0.8 cells/mm², respectively), and that their cell density increased with time (2 ± 0.1, 4.8 ± 1.3 and 2 ± 0.4 folds at T6 with respect to T0). Furthermore, clusters of the aforementioned cells migrating towards the culture dish, through the intestinal tissue were observed. Conclusion: The presence of oc-t4+ and nanog+ cells in uncultured tissue fragments, as well as in extensively cultured cells, from both donors and recipients, suggests that MASCs shouldn’t be considered simply an in vitro phenomenon. Furthermore, MASC plasticity seems to involve also c-KIT expression, which is down regulated following cell migration and outgrowth.

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Autologous Cardiac-Derived Stem Cells Decrease Infarct Size Compared to Placebo in a Porcine Model of Ischemic Cardiomyopathy  

Peter Johnston, Tetsuo Sasano, Kevin Mills, Richard Lange, Eduardo Marban; Johns Hopkins Univ, Baltimore, MD  

Introduction: Prior study has shown cardiac stem cells may be isolated from human endomyocardial biopsy specimens and grown to large numbers in vitro. These cardiосapient derived cells (CDCs) enhance myocardial regeneration and improve systolic function in an immunodеficient mouse model of infarction. Here we present the effects of autologous CDCs in a pre-clinical model of ischemic cardiomyopathy. Methods: Twelve mature pigs had myocardial infarction induced by 2.25 hr balloon inflation in the left anterior descending artery. Immediately afterwards endomyocardial biopsies were taken from the right ventricular septum. Tissue was processed and CDCs were isolated and expanded by techniques previously described. Four weeks later pigs underwent cardiac MRI with measurement of infarct size by delayed gadolinium enhancement. Several days later repeat cardiac catheterization was performed with therapeutic coronary injection of autologous CDCs (300,000 cells/kg); the remaining 6 received placebo infusion (carrier solution alone). Eight weeks later repeat cardiac MRI was performed, followed by cardiac catheterization. Results: Animals receiving CDCs had a significant decrease in relative infarct size in the 8 weeks after infusion (20.9% to 15.8% of LV mass infarcted, p = 0.03 for change) while those receiving placebo did not (17.7% to 15.3%, p = 0.25 for change). The difference in relative infarct size was driven by a greater increase in LV mass in pigs receiving CDCs (69.0% to 73.6%, p = 0.05 for change) as compared to placebo (60.0% to 72.9%, p = 0.06 for change), neither group had a significant change in absolute infarct size. At final catheterization pigs receiving greater 3PiDx mass to placebo (1988.38 mmHg/sec vs 2100.06 vs 1528.49 mmHg/sec vs 1272.19), through this difference did not reach statistical significance (p = 0.11). Conclusions: Intra-coronary infusion of CDCs results in a significant decrease in relative infarct size, driven by a significant increase in LV mass, an effect that may be due to myocardial regeneration. Hemodynamic results suggest this effect has positive functional benefits.

4020  
Torcetrapib-Induced Blood Pressure Elevation Is Independent of Cholesteryl Ester Transfer Protein Inhibition and Is Accompanied by an Increase in Circulating Aldosterone Levels  


Inhibition of cholesteryl ester transfer protein (CETP) with torcetrapib (TOR) in humans increases plasma high density lipoprotein cholesterol levels but is associated with a modest increase in blood pressure (BP). In a phase 3 study, evaluating the effects of TOR in atherosclerosis, there was an excess of deaths and adverse cardiovascular events in patients taking TOR.

Methods and Results - The CETP inhibitors TOR and anacetrapib (ANA) were evaluated for effects on hemodynamics in a variety of pre-clinical animal species. TOR was shown to evoke an acute increase in BP in all species evaluated whereas an increase was not observed with ANA. The pressor effect of TOR was not diminished in the presence of alpha adrenergic, angiotensin II or endothelin receptor antagonists. TOR did not have a direct contractile effect on isolated vascular smooth muscle preparations suggesting its in vivo effects are via the release of a secondary autacoid or mediator. In this regard, torcetrapib was associated with an acute increase in plasma levels of aldosterone and was shown to release aldosterone from primary adrenocortical cells in vitro. Such an increase in aldosterone levels was not observed with ANA, TOR did not increase BP in acutely adenalecetomized rats. However, acute administration of exogenous aldosterone to adrenal intact rats did not increase BP. Conclusions - TOR evokes an acute increase in BP and an acute increase in plasma aldosterone in a variety of animal species via a mechanism independent from CETP inhibition. The pressor response to torcetrapib is absent in acutely adenalecetomized animals suggesting the adrenal glands may be contributing to this effect.

4021  
Mechanistic Studies of Hemodynamics With a Series of Cholesterol Ester Transfer Protein Inhibitors  

Michael DePasquale, Delvin Knight, Will Loging, Lee Morehouse, Steven Winter, Pfizer Global R&D, Groton, CT; Eileen Blasi, Pfizer Global R&D, LaJolla, CA; Joan Keiser; Pfizer Global R&D, Ann Arbor, MI  

Background: ILLUMINATE, the Phase 3 clinical trial of morbidity and mortality (M&M) with the cholesterol ester transfer protein inhibitor (CETP), torcetrapib (T, CP-529,414), combined with atorvastatin was terminated in Dec, 2008 due to an imbalance in all cause mortality. The underlying cause of the M&M remains undetermined. Results of ILLUMINATE are being presented at a late breaking clinical trials session during the meeting. At T, produced dose related increases in blood pressure (BP) in clinical trials the mechanism of the increase in BP has been an area of intense investigation. A series of CETPIs including T and structurally related compounds were studied surveying pathways involved in regulation of BP after CETPI treatment. Methods: Studies were conducted in rats, rabbits and non-human primates treated with CETPIs (CP-529,414, CP-524,515, CP-532,623) and their inactive enantiomers. In addition, CP-532,623 was studied in Phase 1 clinical trials. Changes in BP and heart rate were determined in conjunction with pharmacologic blockade of numerous pressor agents/pathways in preclinical species. In studies with pharmacologic blockers appropriate positive controls were utilized. Results: T and/or structural analogs increased BP in rabbits following IV.
administration and in rats and cynomolgus monkeys after IV or oral administration. CP-532,623 also produced dose related increases in BP in human subjects after single dose oral administration. Changes in pressure were rapid in onset and were correlated with parent drug levels in all species; although rats and rabbits required higher exposures to elicit a change in pressure. Structural analogs devoid of CETPI activity increased BP as well or better than potent CETPIs. Increases in pressure were also observed in rats, a species devoid of CETPI. Blockade of aldosterone, angiotensin, endothelin, rho kinase, PAF, vasopressin, and cyclooxygenase pathways among others failed to attenuate the acute pressor response to CETPIs in vivo.

Conclusions: Preclinical to clinical translational studies to date suggest that the BP effects of T are not related to CETPI. These results are important as we attempt to understand the cause of the increased M&M associated with T treatment in human subjects.

**TABLE II: RETICULATED PLATELET % AND PLATELET ACTIVATION OF LARGE AND SMALL PLATELETS**

<table>
<thead>
<tr>
<th>Small Platelets</th>
<th>Large Platelets</th>
<th>All Platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td>(lower 20%)</td>
<td>(upper 20%)</td>
<td>All</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Platelet Function Parameter</th>
<th>Lower Tertile</th>
<th>Middle Tertile</th>
<th>Upper Tertile</th>
<th>P-value (ANOVA between groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet Function Parameter</td>
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<td>Middle Tertile</td>
<td>Upper Tertile</td>
<td>P-value (ANOVA between groups)</td>
</tr>
<tr>
<td>RPs</td>
<td>3.04 ± 0.4</td>
<td>2.95 ± 0.4</td>
<td>4.85 ± 0.3</td>
<td>0.001</td>
</tr>
<tr>
<td>PAC-1 (Mean Fluorescence intensity)</td>
<td>6.11 ± 0.7</td>
<td>5.36 ± 0.6</td>
<td>6.18 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>P-selectin (Mean Fluorescence intensity)</td>
<td>30.8 ± 6.3</td>
<td>36.8 ± 6.1</td>
<td>49.3 ± 2.9</td>
<td>0.005</td>
</tr>
<tr>
<td>1.5 mM AA LTA (%)</td>
<td>10.4 ± 1.4</td>
<td>13.1 ± 1.5</td>
<td>26.5 ± 3.2</td>
<td>0.001</td>
</tr>
<tr>
<td>1.0 μg/mg Collagen LTA (%)</td>
<td>11.0 ± 1.6</td>
<td>13.5 ± 1.8</td>
<td>19.4 ± 3.0</td>
<td>0.002</td>
</tr>
<tr>
<td>Low response to Aspirin (%)</td>
<td>11.1 ± 1.1</td>
<td>51.9 ± 0.01</td>
<td>1.0 ± 0.01</td>
<td>0.001</td>
</tr>
<tr>
<td>Low response to Clopidogrel (%)</td>
<td>7.7 ± 2.2</td>
<td>48.2 ± 4.8</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>Low response to Both Aspirin and Clopidogrel (%)</td>
<td>0.0 ± 3.7</td>
<td>37.0 ± 3.0</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

**Role of Reticulated Platelets and Platelet Size in Modifying Anti-Platelet Effects of Aspirin and Clopidogrel in Patients With Stable Coronary Artery Disease**

Carlos L Alvar, Muthiah Vaduganathan, Armando Telesz, Karthik Rammohan, Bahrul Nure, Timothy Delao, Mehrim Arik, Juan F Granada, Eli I Leiv, Neal S Kleiman, Sasidhar Guthikonda; The Methodist Rsch Institute, Houston, TX

Objective: Reticulated platelets (RPs) are young platelets, which reflect platelet turnover are larger and possibly more active than non-RPs. We evaluated the relationship between platelet size, number of RPs and platelet function in patients with stable coronary artery disease (CAD) taking aspirin and clopidogrel. Methods: Ninety patients with stable CAD taking aspirin (75 mg/day) and clopidogrel (250 mg/day) were included. Platelet aggregation was assessed with light transmission as an index of aggregation (LTA) in response to 5 μM ADP, 1.5 mM arachidonic acid (AA) and 1 μg/mg collagen. RPs were measured using thiazole orange. Platelet size was determined by flow cytometry. Low response to aspirin was defined as AA LTA >20%, low response to clopidogrel and RPs >50%. Dual drug low response was defined as patients meeting both criteria. Patients were stratified into tertiles based on percentage of RPs. Platelet size gates were established with flow cytometry to define the upper 20% and lower 20% of platelet size; expression of RPs, PAC-1 and P-selectin expression (Table II). Conclusions: Platelet turnover and platelet size strongly determine the response to antiplatelet therapy in patients with stable coronary artery disease both aspirin and clopidogrel.
(MCPIP). In the present study we report that in the white adipose tissue of high fat diet-fed mice, MCPIP was induced when body mass and fasting glucose levels were elevated, beginning at about 8 weeks on the diet. Six MCPIP-induced novel genes, with no previously known function, htlz, lq1, creld2, umr1, cd12 and cd19, were induced in the white adipose tissue, starting at three months of high fat feeding. The increases in fasting glucose level, MCPIP transcript level and the transcript levels of the novel genes showed further progressive increases during 5 months on the high fat diet. Differentiation of NIH 3T3-L1 cells into adipocytes in culture induced by Insulin, Dexamethasone and 3-isobutyl-1-methylxanthine was accompanied by induction of MCPIP and the six novel genes induced by MCPIP. Under these conditions MCP-1 production was induced reaching a peak level at 2 days, while in WT and creld2 reached maximal levels at 4, whereas in cd19 reached maximal levels at 1–2 days, and cd12 and cd19 reached maximal levels in one day. Involvement of MCPIP in the differentiation process was indicated by the finding that transfection of 3T3 cells with MCPIP expression plasmid caused differentiation of these cells into adipocytes in the absence of any differentiation inducing agents. The readily detected differentiation was accompanied by induction of the six novel MCPIP induced genes. Knockdown of MCPIP with specific siRNA suppressed induction of the six MCPIP-induced genes and differentiation of 3T3 cells into adipocytes. The differentiation of 3T3-L1 cells into adipocytes induced by MCPIP expression was further confirmed by the expression of a set of adipogenic markers PPARγ, adiponectin, aP2, LDLR, cEBPβ and cEBPδ during the transcript and protein levels. These results indicate that MCP-1 induces adipogenesis and that this differentiation is mediated via the newly discovered transcription factor, MCPIP. Key words: MCPIP, 3T3-L1, Differentiation, Novel Genes, Adipogenesis.

4027 Differential Make-Up of Atrial and Ventricular KATP: Atrial KATP Channels Are Encoded by SUR1

Thomas P Flagg, Harley T Kurata, Washington Univ, Saint Louis, MO; William A Coetzee, NYU Sch of Medicine, New York, NY; David J Lefer, Albert Einstein College of Medicine, New York, NY; Mark A Magnuson, Vanderbilt Univ Sch of Medicine, Nashville, TN; Colin G Nicholas, Washington Univ, Saint Louis, MO

The isoform-specific molecular structure of the ATP-sensitive potassium channel (KATP) endows different activation patterns with important implications when considering sulfonylurea role of diazoxide in pre-conditioning. Consistent with this observation, total KATP conductance induced by metabolic inhibition is not different between ventricular and atrial myocytes, however, SUR1 is not detected, indicating that SUR1 is not expressed. SUR1-/- atrial myocytes showed a diazoxide-activated KATP current of 0.47 vs. 3.14 nS/pF, 7.73 pA in 4,6). We also considered the possibility that SUR1 is a principal subunit of KATP channel in cardiac ventriculuc myocytes. Using a novel antibody directed against the COOH-terminus of SUR1 (AbC082, we now show that SUR1 is also expressed in the mouse heart, raising the possibility that it also contributes to sarcolemmal KATP structure and function. SUR1 is specifically detected in heart, detailed analyses of total heart proteins isolated from wild type but not SUR1-/- animals. When proteins are purified from isolated WT ventricular myocytes, however, SUR1 is not detected, indicating that SUR1 is not expressed. Consistent with this observation, total KATP conductance induced by metabolic inhibition is not different between ventricular and atrial myocytes.

4028 Determination of Cardiac Metabolism In Vivo With Hyperpolarized Carbon-13 Magnetic Resonance

Marie A Schroeder, Lowri E Cochlin, George K Radda, Kieran Clarke, Damian J Tyler; Univ of New York, NY; Mark A Magnuson, Vanderbilt Univ Sch of Medicine, Nashville, TN; Colin G Nicholas, Washington Univ, Saint Louis, MO

Determination of Cardiac Metabolism In Vivo With Hyperpolarized 1–13C-Pyruvate as a metabolic tracer to detect changes in cardiac metabolism caused by dietary manipulation. Two groups of 3 Wistar rats were each fed a high fat diet for 2 and 4 weeks on the high fat diet (p < 0.05). No difference was observed between 2 and 4 weeks. Thus, for the first time, hyperpolarized pyruvate revealed changes in cardiac PDH activity in vivo. These results suggest an unprecedented potential for hyperpolarized MR to detect metabolic dysfunction in clinical heart disease.

4029 Angiotensin II Reduces the Suppressive Function of Human CD4+ CD25+ Regulatory T Lymphocytes

Luigina Guastti, Franca Marino, Emanuela Rasini, Marco Cosentino, Laura Schembrì, Massimiliano Legnaro, Marco Ferrari, Sergio Locchini, Achille Venco; Dept of Clinical Medicine, Univ of Insubria, Varese, Italy

Background. CD4+CD25+ regulatory T cells (Treg) are specialized lymphocytes which play a key role in the suppression of immune responses, thus being pivotal cells for the suppression of detrimental Th1 responses. Since the proinflammatory peptide Angiotensin II (Ang II) is involved in the atherosclerotic process acting through the activation of its type 1 receptors (AT1Rs), we sought to investigate the potential relationship between Ang II and Treg function. Methods. AT1R expression on human CD4+ T lymphocyte subsets (venous blood of 6 male healthy donors) was investigated by means of a cytofluorimetric method and measured as mean fluorescence intensity (MFI) and the AT1R mRNA expression was measured by real-time PCR (expressed as 2^-ΔΔCT). In addition, the effect of Ang II on the ability of Treg to suppress mitogen-induced effector T cell (Thf) proliferation was examined (measurement of BrdU incorporated during DNA synthesis). Results. Two-color flow cytometric analysis showed that AT-Rs were extensively expressed on the membrane surface of the CD4+ T lymphocytes. Expression on Treg was significantly higher than in Tef (7.48±0.90 MFI vs. 4.38±0.71 MFI, P<0.01) and Treg showed a significant effect of Treg to an even higher extent (11.16±1.15 MFI, P<0.01 vs both Tef and Treg). Higher expression of AT-Rs in the Treg subset was confirmed even at the mRNA level: values in Tef were 0.46±0.09 2^-ΔΔCT while in Treg they were 1.28±0.28 2^-ΔΔCT; P=0.05 vs Tef. Forthy-eight-hour incubation of Treg with the mitogen phytohaemagglutinin (PHA) 10 μg/mL induced a strong proliferative response which was not affected by Ang II 50 μM Co-culture of Tef with Treg 1:1 resulted in the suppression of PHA-induced proliferation of Tef by 90.5±6.7% (P<0.01 vs Tef alone). Ang II 50 μM significantly reduced the suppressive effect of Treg, down to 32.8±6.3% (P<0.05 vs co-culture of Treg and Tef without Ang II). Conclusions. The present results show the first evidence of preferential expression of AT-Rs on human Treg. Moreover we show for the first time that Ang II is able to reduce the suppressive activity of these cells on Tef function, therefore highlighting a novel immune-mediated mechanism contributing to the proatherogenic effects of Ang II.