S16

Solvent Factors are Central to the Enhancement of Endothelial Progenitor Cells Function by Platelets

Dadush-Raz, Oshrat[1]; Leshem Lev, Dorit[2]; Issan, Yossi[3]; Perl, Leor[2]; Eisen, Alon[2]; Battler, Alexander[2]; Lev, Eli[2]

[1]Rabin Medical Center, Cardiology, Tel Aviv University, Sackler Faculty of Medicine, Petah Tikva, Israel; [2]Rabin Medical Center, Cardiology, Petah Tikva, Israel; [3]Tel Aviv University, Sackler Faculty of Medicine, Petah Tikva, Israel

Introduction: Recent evidence has suggested the importance of endothelial progenitor cells (EPCs) in the process of repair post vascular injury and that platelets mediate their recruitment to the site of injury, maturation and differentiation. Yet, the mediators of this reaction are unclear. Platelet activation releases microparticles (PMPs)/ and solvent factors which may mediate this reaction. Thus, the aim of our study is to investigate the main mediators improving EPC functional properties and to identify the specific factors involved in this reaction.
Methods: Human EPCs were isolated from donated buffy coats and cultured for 7 days on a traditional fibronectin matrix in one of the following conditions: 1. Alone (control) 2.Co-incubated with platelets 3. Co incubated with platelets activation products: PMPs/ supernatants 4. Co incubated with platelets and FGF/PDGF inhibitor. EPC functional properties were evaluated by their capacity to form colonies and by the expression of mature endothelial markers such as Tie-2 and DiI-Ac-LDL, using FACS analysis.
Results: After 7 days of culture, the capacity to form colonies and the expression of endothelial markers were higher in EPC co incubated with platelets, PMPs or supernatants compared to EPCs alone. Notably differentiation was higher when EPC were incubated with supernatants compared to PMPs or platelets alone. Furthermore, PDGF/FGF inhibitions significantly reduce Endothelial cell markers expression almost to baseline.
Conclusions: This preliminary study implies that platelets enhance EPC functional properties mainly through solvent factors and less by PMPS. Indeed, it seems that direct interaction is not essential for the platelets' effect on EPC functional properties. Two important mediators which enhance EPC's capacity for differentiation are PDGF and FGF. Further study is required to check additional aspects of EPC functional properties in response to platelet products and PDGF/FGF inhibition.

Adoptive Transfer of Regulatory T Cells Improves Cardiac Function and Reduces Infarct Size

Sharir, R.[1]; Entin – Meer, M.[2]; Maysel-Auslender, S.[2]; Hochhauser, E.[3]; Chepurko, Y.[4]; Keren, G.[1]; George, J.[5]

[1]Tel-Aviv Sourasky Medical Center, The Department of Cardiology, Tel Aviv University, Sackler Faculty of Medicine, Tel-Aviv, Israel; [2]Tel-Aviv Sourasky Medical Center, The Department of Cardiology, Tel-Aviv, Israel; [3]Tel Aviv University, Sackler Faculty of Medicine, Rabin Medical Center, Felsenstein Medical Research Center, Petah Tikva, Israel; [4]Rabin Medical Center, Felsenstein Medical Research Center, Petah Tikva, Israel; [5]Tel-Aviv Sourasky Medical Center, The Department of Cardiology, Kaplan Medical Center, Tel-Aviv, Israel

Background: Naturally occurring regulatory T cells (nTregs) comprise 5–10% of peripheral CD4+ T cells. Landmark studies have pointed to their essential role in tuning down pathogenic and autoreactive immune responses. We were able to demonstrate that nTregs are capable of reducing the size of atherosclerotic lesions. These findings motivated us to investigate the potential involvement of Treg in myocardial ischemia.
General aim: To test the HYPOTHESIS that regulatory T cells have a potentially beneficial effect in myocardial ischemia.
Methods: Splenocytes were stained with Tregs markers. Functional suppression assays were performed by coculturing T effectors (Teffs) with Tregs from control and MI mice. For adoptive transfer assays, mice were injected Tregs, Teffs or PBS.
Results: The data point out that the levels of CD4+CD25highFOXP3 in splenocyte cells in mice undergoing LAD ligation are higher than in Sham ($p<0.02$). However, there is no statistically significant effect of MI on the suppressive properties of Tregs. We have shown that the amelioration of cardiac damage with Treg cell transfer was accompanied by decreased infarct area.
In Conclusion: Tregs appear to play an active role in the remodeling process after experimental MI.

Low Level Laser Phototherapy Arrests Pre-Existing Aortic Aneurysm in Apolipoprotein E-Deficient Mice

Gertz, S. David[1]; Gavish, Leah Y.[1]; Beeri, Ronen[2]; Gilon, Dan[2]; Rubinstein, Chen[3]; Berlatzky, Yacov[3]; Bulut, Atilla[2]; Reissman, Petachia[4]; Gavish, Lilach[1]

[1]The Hebrew University, Hadassah Faculty of Medicine, Institute of Medical Research (IMRIC), Jerusalem, Israel; [2]Hadassah University Hospital, Department of Cardiology, Jerusalem, Israel; [3]Hadassah University Hospital, Department of Vascular Surgery, Jerusalem, Israel; [4]Shaarei Zedek Hospital, Surgery, Jerusalem, Israel

Background: Using high-frequency ultrasonography (HF-u/s), we showed that low level laser phototherapy (LLL) inhibits the formation of abdominal aortic aneurysms (AAA) in apolipoprotein-E-deficient (Apo-$E^{-/-}$) mice. This study tests the effect of LLL on the progression of pre-induced AAA.

Methods: AAA was induced in Apo-$E^{-/-}$ mice (age16-20 weeks [w]) by infusion of angiotensin-II using osmotic minipumps (1000ng/kg/min, 4w). HF-u/s (40MHz, 0.01mm resolution, Vevo-770, VisualSonics)(B- and M[motion]-modes) was used to measure the maximum cross-sectional diameter (MCD) of the suprarenal abdominal aorta and the anterior wall displacement (AWD). The aortas of mice that developed aneurysmal dilatation at 2w over baseline were then exposed retroperitoneally and treated with LLL (780nm, 2Joules/cm^2, 9min) or sham-operated. HF-u/s was repeated at 4w to determine the effect of LLL on the pre-induced aneurysms.

Results: The aortas that developed AAA >50% MCD at 2w continued to grow (on the average) as per HF-u/s measurements at 4w. However, LLL halted further dilatation (MCD, 2w vs 4w, non-treated [n=8]: 2.10±0.2 vs 2.33±0.28mm, p=0.04 by paired 2tt-test; LLL [n=10]: 2.24±0.32 vs 2.09±0.56mm, p=0.2). Direct comparison showed a 19% increase in control vs treated (ΔMCD[4w-2w]: 0.23±0.26 vs -0.15±0.33mm, $p<0.02$ by Mann-Whitney-u-test). Individually, of all mice with AAA ≥40% dilatation at 2w, 7(58%) of 12 non-treated, but none of 12 LLL, had an increase in MCD (>1SD) after 4w ($p<0.004$ by Fisher Exact). M-mode of the aortic wall at the site of MCD showed a marked decrease in AWD in control animals at 4w vs 2w reflecting a marked decrease in wall elasticity (ΔAWD= -0.03±0.04mm. $p<0.02$). This difference was not observed in LLL treated animals (0.0±0.04, $p>0.9$).

Conclusions: LLL prevents *de novo* development and, as shown here, arrests the progression of pre-existing aortic aneurysms and its associated deterioration in the biomechanical integrity of the aortic wall in Apo-$E^{-/-}$ mice.

Decrease in VEGF and in Inflammatory Markers is Associated with Diabetic Proliferative Retinopathy

Blum, Arnon[1]; Socea, Dorina[2]; Ben Shushan, Rotem[3]; Keinan Boker, Lital[4]; Tamir, Snait[3]
[1]Baruch Padeh Poria Hospital, Medicine, Tiberias, Israel; [2]Baruch Padeh Poria Hospital, Ophthalmology, Tiberias, Israel; [3]Laboratory of Human Health and Nutrition Sciences, MIGAL-Galilee Technology Center, Human Health and Nutrition Sciences, Kiryat Shmona, Israel; [4]Israel Center for Disease Control, Ministry of Health, Jerusalem, and School of Public Health, Haifa University, Haifa Israel, Epidemiology, Jerusalem, Israel

Diabetic retinopathy is the most severe complication of diabetes mellitus (DM), associated with microvascular damage.
Methods: 73 patients with DM type II (Group A: 25 patients [12 males], age 62.8±10.8 years, no diabetic retinopathy; Group B: 25 patients [19 males], age 61.9±9.4 years, non-proliferative retinopathy; and Group C: 23 patients [13 males], age 59.2±10.3 years, proliferative retinopathy) and 23 healthy subjects (14 males; age 44.3±11.6 years) served as controls. We studied levels of hs-CRP, sVCAM-1, and VEGF in different subgroups of patients with DM type II.
Results: Hs-CRP levels were high in patients (4391±4175, 4109±4533, 3005±3842 ng/ml, respectively) compared with controls (1659±1866 ng/ml); however, only patients in groups A ($p=0.01$) and B ($p=0.03$) had a significant change compared with controls. Similar findings were observed for sVCAM-1 levels (706±347, 746±328, 638±208 ng/ml, respectively) vs. controls (552±143ng/ml). A significant difference in sVCAM-1 levels were found between groups A ($p=0.05$) and B ($p=0.01$) and controls, but not for group C ($p=0.125$). Soluble VEGF levels (493±353ng/ml, 625±342ng/ml, 368±223ng/ml, respectively, vs. controls 392±355ng/ml) showed no significant difference ($p\geq0.05$) except for group B ($p=0.03$); however, a significant decrease was observed with disease progression ($p=0.006$).
Conclusions: All patients with DM type II had high inflammatory and angiogenic markers, but a decrease in these markers was observed in patients with progressive disease (diabetic proliferative retinopathy). Biomarkers of inflammation and angiogenesis may detect progression of diabetic vascular disease and may guide earlier interventions to prevent systemic complications.

Adhesion Molecule ICAM-1, VCAM-1, e-Selectin, VEGF Correlate with Age and Troponin in ACS Patients

Arnson, Yoav[1]; Amital, Howard[2]; Itzhaky, Dganit[3]; Barak, Vivian[4]; Mosseri, Morris[1]
[1]Meir Medical Center, Cardiology, Kfar Saba, Israel; [2]Tel Hashomer, Internal Medicine, Tel Aviv, Israel; [3]Meir Medical Center, Biochemistry and Immunology Labs, Kfar Saba, Israel; [4]Hadassah-Hebrew University Medical Center, Immunology Laboratory, Jerusalem, Israel

Introduction: The aim of this study is to examine the concentrations of endothelial and vascular adhesion molecules in patients with an acute coronary disease, and to examine their corolation with age and severity of the infarct.
Methods: Blood was drawn from 50 patients presenting with ACS (either STEMI or NSTEMI) upon the first day of their arrival. ELISA was used to measure sVCAM-1, sICAM-1, e-Selectin, VEGF, IL-6, IL-8 and TNF levels. Cardiac troponin T was used to estimate severity of the infarct. The correlations between adhesion molecule levels and ACS severity was done by comparing circulating molecule levels with patient age, troponin and CRP levels using person's correlation.
Results: The patient's age was significantly correlated with higher levels of sVCAM-1 (r=0.48, p=0.001), and with reduced levels of sICAM-1 (r=-0.356), e-Selectin (r=-0.376) and VEGF (r=-0.3). Higher Troponin levels were correlated with higher e-Selectin (r=0.4), and VEGF (r=0.44). Higher CRP levels were correlated with sICAM-1 (r=0.36), and IL-6 (r=0.47).
Discussion and Conclusion: An acute coronary syndrome raises a cascade of inflammation reactions. sVCAM-1 represents the severity of atherosclerotic burden and was found to increase with age. Increased age however was correlated with a reduction in many other cellular molecules such as sICAM-1, e-Selectin and VEGF. Higher troponin levels were correlated with higher levels of e-Selectin and VEGF. These finding strengthen the evidence that inflammatory process is critical in the pathogenesis of an acute cardiac ischemia, and suggest a diminished response related with older age. These observations may have further implication in clinical practice both for understanding better ways for risk stratification and for finding novel therapeutic pathways.

Myocardial Infarction Enhances Renal Tubular Damage in Rats with Chronic Renal Failure

Entin-Meer, Michal[1]; Maysel-Auslender, Sofia[1]; Ben-Shoshan, Jeremy[1]; Laron, Ido[1]; Klipper, Shelly[1]; Schwartz, Idit[2]; Goryainov, Pavel[1]; Keren, Gad[1]
[1]Tel-Aviv Sourasky Medical Center, Cardiology, Tel-Aviv, Israel; [2]Tel-Aviv Sourasky Medical Center, Internal Medicine & Nephrology, Tel-Aviv, Israel

Background: Cardiac events are the main cause of death among patients with end-stage renal failure (RF). To date even a mild renal disease is considered a major risk factor for cardiovascular complications after myocardial infarction. To improve our understanding of the potential additional renal damage introduced by subsequent cardiac events and to propose measures for early diagnostics of this grave condition, we have established a model for myocardial infarction in chronic RF (CRF)-inflicted animals.
Methods: We have utilized a rat model in which myocardial infarction was induced four weeks post establishment of subtotal nephrectomy. Changes in renal performance were then assayed using two platforms: histological and biochemical.
Results: The data demonstrate that even though creatinine (Cr)-clearance and sera BUN levels are not further deteriorated compared to CRF alone, the combined disease- cardiorenal syndrome enhances pathological fibrosis in the kidney's medulla. We further show that acute MI enhances moderate renal injury as determined by Ngal levels which remain stably elevated upon CRS.
Conclusions: Acute cardiac injury in the setting of chronic renal failure is associated with biochemical and histological changes in the renal tubular sections. The data suggest that elevated urine Ngal can serve as a non-invasive biomarker for an LV dysfunction-related renal injury, even in the setting of stable renal function.