16:50 - 18:20 S11 - Jan J Kellerman Young Investigator in Cardiology Awards Hall A

Chairs: **J. Leor**
J. Shemesh

16:50 **Heparanase Accelerates Atherosclerosis In-vivo: New Insights from Genetically Altered Mice Models**

D. Planer [1], S. Metzger [1], E. Zcharia [1], I. Vlodavsky [1], J. George [2], T. Chajek-Shaul [1]
[1] Jerusalem, [2] Tel Aviv

17:05 **A Combined Gene and Cell Therapy Approach for Conduction System Repair**

A. Hofshi, G. Arbel, L. Gepstein
Haifa

17:20 **Macrophages are Essential for Infarct Repair With and Without Stem Cell Therapy**

T. Ben-Mordechai, R. Holbova, A. Zuloff-Shani, M.S. Feinberg, Z. Silman, D. Danon, J. Leor
Tel Aviv

17:35 **Intracellular Survival Signaling Pathways and the Role of Raloxifene in Experimental Reversible Aortic Valve Calcification**

M. Shuvy, S. Abedat, R. Beeri, M. Valitsky, S. Daher, M. Kott-Gutkowski, A. Gal-Moscovici, C. Lotan
Jerusalem

17:50 **Myocardial Toll-Like Receptor 4 (TLR4) mediates dysfunction in septic shock and myocardial ischemia (MI)**

R. Fallach [1,2], A. Shainberg [1], M. Fainblut [2], Y. Chepurko [2], E. Porat [2], E. Hochhauser [2]
[1] Ramat Gan, [2] Petach Tikva

18:05 **A Functional Role for Eotaxin-2 in the Initiation and Progression of Experimental Atheroma**

M. Entin-Meer [1], S. Maysel-Auslander, A. Afek, G. Luboshits, G. Keren, J. George
[1] Tel Aviv, [2] Ramat Gan

Heparanase Accelerates Atherosclerosis In-vivo: New Insights from Genetically Altered Mice Models

D Planer [1], S Metzger [2], E Zcharia [3], I Vlodavsky [3], J George [4], T Chajek-Shaul [2]

[1] Heart Institute, [2] Internal Medicine, [3] Department of Oncology, Hadassah, Hebrew University Medical Center, Jerusalem, [4] The Department of Cardiology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel

Background: The role of Heparanase, Heparan-sulfate degrading enzyme, in atherosclerosis development and lipid metabolism was evaluated *in vivo.*

Methods: Three different models were used:

- Ubiquitously over expressed heparanase transgenic mice.
- Heparanase knock-out mice on the background of ApoE-/-.
- Heparanase over-expression limited to the hematopoietic system using bone-marrow transplantation model.

Atherosclerosis was assessed quantitatively in all models; lipid profile and metabolism were studied.

Results: Heparanase was proved to be pro-atherogenic in all 3 models: Heparanase over-expressing mice had increased fatty streaks formation compared to control (23984 vs. 4189μm^2, $p<0.001$); heparanase deficient mice (on ApoE-/- background) were relatively resistant to atherosclerosis compared to ApoE-/- mice (40462 vs 84660μm^2, $p=0.035$); and ApoE-/- mice transplanted with bone marrow from heparanase over-expressors had increased atherosclerotic plaque area compared to ApoE-/- mice transplanted with C57BL/6 marrow (30415 vs. 11346μm^2, $p=0.004$).

While in fasting state heparanase over-expression induced only slight elevation in triglycerides and cholesterol level, the difference became striking after oral fat load, while a mirror effect was documented in the heparanase deficient mice. Hepatic uptake of radiolabeled retinol was decreased in transgenic mice while plasma levels were higher - indicating reduced hepatic clearance of remnant lipoproteins, with an opposite effect demonstrated in heparanase deficient mice. No change in lipoprotein profile was demonstrated in the bone marrow transplantation model.

Conclusions: These three complementary models demonstrate, for the first time in-vivo, the pro-atherogenic effect of heparanase. The main mechanism involves reduced hepatic uptake of remnant lipoproteins with increased plasma availability of these atherogenic particles.

A Combined Gene and Cell Therapy Approach for Conduction System Repair

Anat Hofshi, Gil Arbel, Lior Gepstein

The Sohnis Laboratory for Cardiac Electrophysiology and Regenerative Medicine, and the Cardiology Department, Rambam Medical Center, The Bruce Rappaport Faculty of Medicine, Technion - Israel Institute of Technology, Haifa, Israel

Introduction: Impaired myocardial conduction may underlie both bradyarrhythmias and reentrant tachyarrhythmias. In the current study, we introduce a novel strategy for conduction system repair utilizing genetically engineered cells designed to form biological "conductive cables".

Methods and Results: An *in vitro* model of conduction block was established using spatially-separated, spontaneously contracting, non-synchronized, human embryonic stem cell-derived cardiomyocyte clusters. We next examined the hypothesis that HEK293 cells transfected with the $Na_v1.5$ voltage-gated sodium channel can couple with the cultured cardiomyocytes and synchronize the electrical activity of these spatially separated clusters. Cx43 immunostaining and Calcein-dye transfer experiments in co-culture studies confirmed formation of functional gap junctions between the engineered cells and neighboring cardiomyocytes. We next assessed the ability of the engineered cells to synchronize contractions between the separate clusters using a microelectrode array mapping (MEA) system. Synchronization was defined by the establishment of fixed local activation time differences (ΔLATs) between the two separate clusters and convergence of their spontaneous activation cycle lengths. Nontransfected control cells were able to induce synchronization between cardiomyocyte clusters separated by distances up to 200 μm. In contrast, the engineered cells synchronized contractions between cardiomyocyte clusters separated by up to 1000 μm, the longest distance studied. Finally, engineered cells expressing K^+ (Kv1.3) channels failed to induce any synchronization.

Conclusions: Genetically engineered cells, transfected to express Na^+ channels, can form biological conduits bridging and coupling excitable cells, allowing synchronization of contractions between distinct, widely separated cardiac cell clusters.

Macrophages are Essential for Infarct Repair With and Without Stem Cell Therapy

Tammy Ben-Mordechai [1], Radka Holbova [1], Adi Zuloff-Shani [2], Micha S Feinberg [1], Zmira Silman [1], David Danon [2], Jonathan Leor [1]

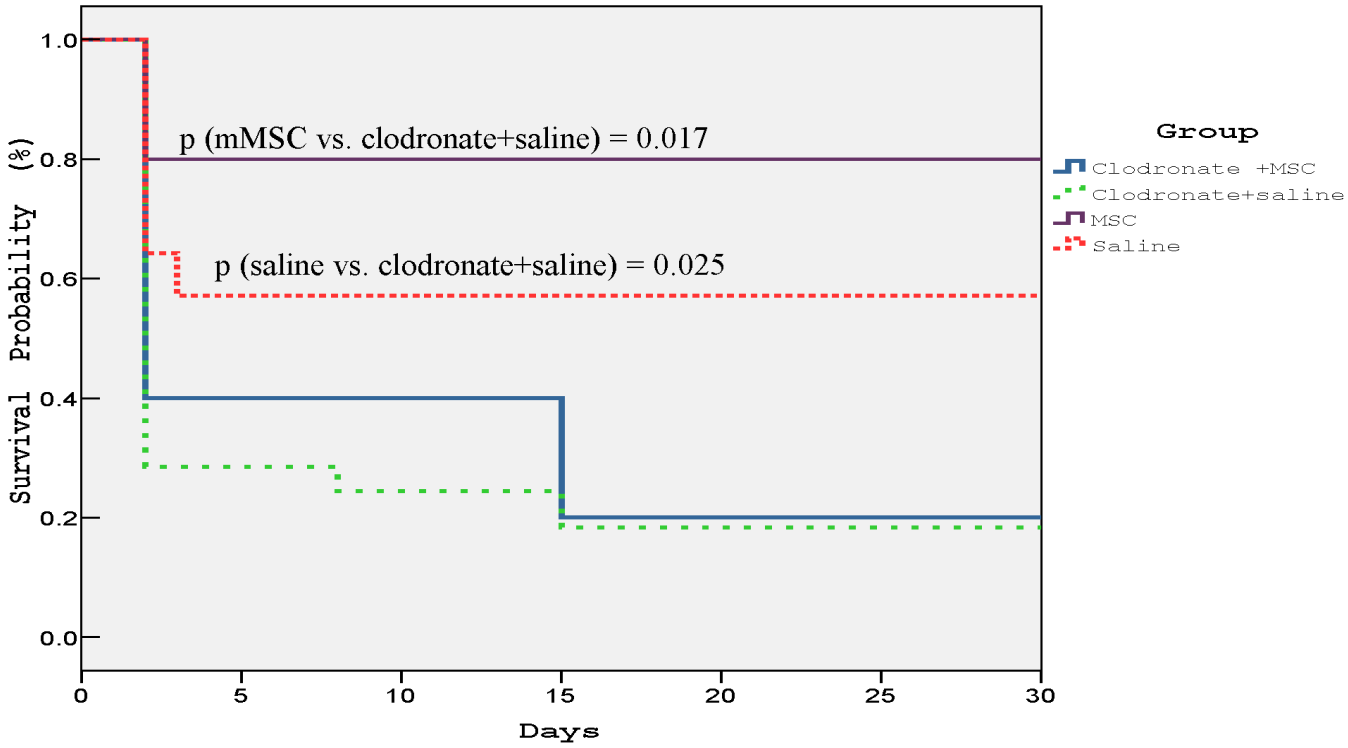
[1] Neufeld Cardiac Research Institute, Tel Aviv University, Tel Aviv, [2] Research and Development Unit, Blood Service Center, Magen David Adom, Israel

Myocardial healing is impaired in elderly and sick people partially because of defective inflammatory response. The present study aimed to determine the significance of macrophage (MΦ) activity in infarct repair and to test the hypothesis that young MΦ can improve infarct repair in aged mouse.

Methods and Results:

MΦ depletion was induced by clodronate injection (IV or IP) to Balb/C mice. Animals (n=57) with and without MΦ depletion were subjected to MI and randomized to injection of mesenchymal stem cells (mMSC; n=15) into the infarct or saline (n=42). Mortality after MI was significantly higher in MΦ depleted mice, with and without stem cell therapy, compared with controls (Figure1; p=0.02).

Figure 1: Survival curves of the four study groups.



In the next experiment, MΦ were isolated from the peritoneum of young (12w) or aged (8m) Balb/c mouse. The young or old MΦs (50,000) were injected into the infarcted myocardium of aged (8 months) mice (n=9, n=8; respectively) immediately after MI. The control group (n=9) was treated with saline injection. Serial echocardiography studies were performed 1 day and 4 weeks after MI. After 4 weeks, aged animals treated with saline or old MΦ experienced significant increase in infarct thinning and LV dilatation ($p<0.02$), while this variables of adverse remodeling were attenuated in animals treated with young MΦ.

Conclusions:

Macrophages are essential for infarct repair with and without stem cell therapy. The administration of young macrophages to repair MI could be important to sick and elderly people in whom the availability of autologous, functional stem cells is limited.

Intracellular Survival Signaling Pathways and the Role of Raloxifene in Experimental Reversible Aortic Valve Calcification

Mony Shuvy [1], Suzan Abedat [1], Ronen Beeri [1], Michael Valitsky [1], Sameh Daher [1], Miriam Kott-Gutkowski [2], Anca Gal-Moscovici [3], Chaim Lotan [1]

[1] Cardiovascular Research Center, Heart Institute, [2] Silberman Institute of Life Sciences, [3] Nephrology and Hypertension Services, Hadassah-Hebrew University Medical Center, Jerusalem, Israel

Background- Aortic valve calcification (AVC) is an inflammatory active process. Using our animal model of uremia-induced reversible AVC, we assessed the role of apoptosis in AVC. We also explored the effects of raloxifene- an estrogen receptor modulator on AVC.

Methods and Results - Gene array analysis was performed in aortic valves obtained from 3 groups (n=7 each): calcified valves- from rats fed with the uremic diet, valves after calcification resolution following diet cessation and controls.

Additional aortic valves were obtained from four groups of rats (n=10 each): control, calcified valves, valves after calcification resolution, and valves from rats fed with the same diet who also received raloxifen. Analysis included multislice computed tomography (MSCT), histology, antigen and gene expression.

Gene array results suggested that most apoptosis- related genes were changed in a pro-apoptotic direction in calcified valves. Apoptosis was confirmed in calcified valves. Protein analyses showed a significant decrease in Growth arrest 6 (Gas6), ERK and Akt survival pathways.

Resolution of AVC was accompanied by decreased apoptotic features and up-regulation of these pathways. As observed by MSCT and histology, raloxifene significantly decreased AVC. Its effect was associated with apoptosis inhibition and up-regulation of Gas6, ERK and Akt pathways.

Conclusions- We showed that AVC is involved in apoptosis, and in down regulation of several intracellular survival pathways which are restored after AVC resolution. The beneficial effect of raloxifene in AVC was related to activation of anti apoptotic pathways. This novel observation is important in developing efficient remedies for AVC.

Myocardial Toll-Like Receptor 4 (TLR4) mediates dysfunction in septic shock and myocardial ischemia (MI)

R Fallach [1,2], A Shainberg [1], M Fainblut [2], Y Chepurko [2], E Porat [2], E Hochhauser [2]

[1] Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, [2] The Cardiac Research Laboratory of the Department of Cardiothoracic Surgery, Felsenstein Medical Research Center, Tel Aviv University, Petach Tikva, Israel

TLR4 expressed in myeloid cells plays important role in regulating innate immune responses. TLR4 is also expressed in cardiomyocytes, questioning the relative contribution of both cell types to cardiac dysfunction during septic shock and MI.

To test whether cardiomyocyte TLR4 contributes to cardiac dysfunction, C57Bl and TLR4-deficient (TLR4-def) mice were studied *in vivo* in a septic shock model induced by LPS (i.p.) and MI induced by left anterior descending coronary artery ligation at 4, 24, 72 hours post-treatment and *ex vivo* (Langendorff isolated heart preparation). All C57Bl hearts (n=5, in each time point) displayed reduced left ventricular systolic pressure (Millar pressure transducer), along with increased myocardial levels of IL-1β, TNF-α (ELISA) and the up regulation of mRNA encoding TLR4 (quantitative RT-PCR). TLR4-def mice cardiac function was less affected vs. C57Bl mice post-MI, were unaffected by LPS and did not display significant elevation in heart cytokines. These data indicate that TLR4 plays an important role in myocardial dysfunction following septic shock and MI. Deterioration in cardiac function in wild type hearts and sustained function of TLR4-def hearts under *ex vivo* conditions of asanguineous perfusion, suggest that myocardial TLR4 is a direct mechanism of cardiomyocytes function suppression. TLR4 may therefore constitute a novel target in the treatment of the ischemic and septic heart.

	LPS at 4h			MI at 4h		
	LVP mmHg	IL-1β pg/mg protein	TNF-α pg/mg protein	LVP mmHg	IL-1β pg/mg protein	TNF-α pg/mg protein
C57Bl	67±8	606±167	139.4±12	74±6	691.9±109	193.8±35
TLR4-def	130±15	139.88±55	22.3±3	86±6	160.7±42	43.8±4

A Functional Role for Eotaxin-2 in the Initiation and Progression of Experimental Atheroma

Michal Entin-Meer [1], Sofia Maysel-Auslander, Arnon Afek, Galia Luboshits, Gad Keren, Jacob George

[1] Department of Cardiology, Tel Aviv Sourasky Medical Center, Tel Aviv, [2] Institute of Pathology, Sheba Medical Center, Tel Hashomer, Ramat Gan, Israel

The chemokine eotaxin-2 is a potent chemoattractant for inflammatory cells, the predominant of which are eosinophils. Eotaxin-2 binds to the eosinophil receptor CCL24, also named CCR3, and possesses a potent chemotactic activity for eosinophils. Human and murine atherosclerotic plaques are known to exhibit inflammatory phenotypes where a complex interaction of cytokined and chemokines play a role. We tested the hypothesis that eotaxin-2 plays a causative role in the initiation and progression of atherosclerosis.

Employing reverse-transcriptase PCR analysis, we have shown that eotaxin-2 is abundantly expressed in plaque from apoE knockout (KO) mice. Administration of polyclonal blocking antibodies to eotaxin-2 resulted in a robust reduction of early atherosclerotic plaques in apoE KO mice whereas prolonged treatment of mice with advanced plaques led to atheroma stabilization. A neutralizing monoclonal antibody (1D8) against eotaxin-2, produced in our laboratory, significantly attenuated adhesion of lymphocytes and monocytes as well as heart-derived H5V cells to fibronectin and successfully inhibited their migration towards VEGF. Furthermore, we have shown that 1D8 interferes with binding of eptaxin-2 to the chemokine-recognition site on CCR3. Similar to the polyclonal antibodies, 1D8 significantly reduced atherosclerotic plaques in apoE KO mice, pointing out to the promising therapeutic potential of this monoclonal antibody.

Conclusion. Eotaxin-2 represents a novel target in human atheroma and its blockade by neutralizing antibodies is associated with reduced fatty streak accumulation and plaque stabilization in mice.