The Utility of Surveillance Endomyocardial Biopsies in Detecting Cellular Rejection in Pediatric Heart Transplant Patients

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Background: Routine surveillance endomyocardial biopsies (EMBs) are commonly used to screen for cellular rejection in pediatric heart transplant recipients. With advances in immunosuppression, the benefit of EMB in asymptomatic pediatric heart transplant patients is unclear.

Methods: After orthotopic heart transplant (OHT), surveillance EMBs were routinely performed on all pediatric OHT patients with decreasing frequency. All biopsy specimens were reviewed by a cardiac pathologist, and graded according to International Society for Heart and Lung Transplantation (ISHLT) guidelines. A retrospective review of consecutive EMBs performed at our institution from January 1995 to September 2002 was conducted. The echocardiographic results, clinical history and treatment changes at the time of every biopsy were also recorded.

Results: Results of 866 EMBs from 91 patients were reviewed. Two hundred and thirty-seven EMBs (23.9%) were performed within thirty-days of OHT. 394 EMBs (45.5%) were performed one month after OHT and one year after OHT, 265 EMBs (30.6%) were performed more than one year after OHT. Of all EMBs, 1.00% were ISHLT grade 0, 3.58% were grade 1B, 19.4% were grade 1A, and 74.6% were grade 0. Six of the 265 EMBs were unable to be interpreted because of insufficient tissue. Of the twelve patients in whom the EMB was not grade 2 or higher, six were less than one month from OHT and asymptomatic. The other six patients with greater than 1B cellular rejection presented for biopsy because of symptoms and had abnormal function on echocardiogram. Of the 820 EMB performed in asymptomatic patients more than one month from OHT, there were no episodes of cellular rejection greater than 1B. There were 21 asymptomatic patient biopsies (2.56%) with grade 1B rejection. All grade 1B rejection detected by surveillance EMB resolved in both treated and untreated cases.

Conclusion: EMB should only be used to screen for cellular rejection in the first month after pediatric heart transplantation. For pediatric patients who are asymptomatic more than thirty days after OHT, EMB has failed to reveal significant episodes of cellular rejection in asymptomatic patients. The utility of surveillance EMB to detect humoral rejection was not assessed.

Noncultured Autologous Skeletal Muscle Cells Can Successfully Engraft in Ovine Myocardium

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Background: The concept of myogenic cell transplantation into the myocardium, known as cellular cardiomyoplasty (CCM), is based on the contribution of exogenous cells to replace lost or altered cardiomyocytes in order to restore functional performance of the heart. There is a large body of evidence showing that CCM performed with skeletal muscle cells can improve cardiac function in ischemic heart disease as well as dilated cardiomyopathy on numerous animal models. Most research teams have addressed autologous cell transplantation in vivo in a fresh cell or cell-seeded cardiac conduit or surgical or catheter based cell delivery. Considering the potential benefit of using non cultured muscle cells (little time, lower cost, reduced risk of contamination), we investigated the feasibility of grafting cells obtained recently after enzymatic dissociation of skeletal muscle biopsies in ovine myocardium. We hypothesized that non cultured skeletal muscle cells would massively engraft.

Methods: Autologous intramyocardial skeletal muscle cell implantation was carried out in 8 sheep. A skeletal muscle biopsy (about 10 g) was explanted from each animal. The sheep were left to recover over approximately three hours and reanesthetized when the cells were ready for the implantation. A left fifth intercostal thoracotomy was performed and 10 epical injections of the muscle preparation (between 10 and 20 million cells) were carried out. All sheep were euthanized 3 weeks after myocardial implantation. Immunohistochemistry was performed with monochonic antibodies to a fast skeletal isoform of myosin heavy chain. Results: Skeletal myo

Combination of Mesenchymal Stem Cell Transplantation and Angiogenic Gene Transfer for Myocardial Regeneration and Therapeutic Angiogenesis

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Bone marrow-derived stem cells including mesenchymal stem cells (MSCs) have attracted attention as potential platforms for the delivery of therapeutic genes. Lentiviral vectors are promising tools for the development of gene therapy since they can transduce both quiescent and dividing cells. We have previously demonstrated that MSCs can be differentiated into cardiomyocytes and restoration of blood flow is crucial for the fate of
transplanted MSCs after myocardial infarction. In the present study, we evaluated efficiency of lentiviral vector-mediated gene transfer into MSCs and investigated the myocardial regenerative and angiogenic effect of MSCs transplantation in combination with vascular endothelial growth factor (VEGF) gene transfer. Lentiviral vectors containing enhanced green fluorescent protein (EGFP) and lentiviral vectors containing IGF-1 plasmids and that were genetically modified to enhance survival (IGF) and promote vascularization (VEGF) were directly infected into the apex of normal heart, VEGF was secreted until 2 weeks after lentiviral vector-mediated gene transfer to myocardium and concentration gradient of VEGF was observed across the left ventricle (apex, 10.1±0.0; mid, 9.0±0.0; base, 3.3±0.2 ng/ml protein of LV). When compared to total concentration (14.0±2.1 ng/ml protein) of secreted VEGF at 1 week after lentiviral vector delivery, myocardial concentration (2.5±0.4 ng/ml protein) of VEGF was significantly greater at 2 weeks. These results demonstrate that MSCs transplantation with lentivirus-mediated ex vivo VEGF gene delivery might be applied in the treatment of ischemic heart disease because therapeutic angiogenesis can be mediated by this treatment strategy in addition to myocardial regeneration by MSCs transplantation which was already documented.

1185-87 Specific Dysregulation of Troponins and Inflammatory Marker Genes in Endomyocardial Biopsies From Heart Transplant Recipients

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BACKGROUND: Real-time reverse transcription polymerase chain reaction (RT-PCR) has been shown to be a sensitive method to detect gene expression in small samples, such as endomyocardial biopsy specimens. However, RT-PCR can be inefficient in the amplification of small amounts of cDNA. We hypothesized that monitoring gene expression in endomyocardial biopsies by microarray analysis may be a more sensitive method to detect changes in gene expression.

METHODS: We analyzed endomyocardial biopsy specimens from 14 patients with heart transplantation and 12 patients with ischemic heart disease, none of whom had undergone coronary artery bypass surgery. mRNA isolated from endomyocardial biopsy specimens was hybridized to cDNA microarrays containing genes that were differentially expressed in the transplanted myocardium compared to normal myocardium. Each sample was compared to control samples from normal myocardium.

RESULTS: There was a statistically significant increase in the expression of a number of genes associated with inflammatory reactions and immune responses in endomyocardial biopsy specimens from heart transplant recipients compared to ischemic heart disease patients. These included genes associated with monocyte/macrophage activation and chemokine production.

CONCLUSIONS: Microarray analysis of endomyocardial biopsy specimens from heart transplant recipients may be a more sensitive method to detect changes in gene expression compared to RT-PCR. This sensitivity may be important for detecting early signs of acute rejection.

855-2 Noninvasive Prediction of Coronary Stenoses in Heart Allografts Without Regional Wall Motion Disturbances and Normal Left Ventricular Ejection Fraction

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Background: Although most transplant centers perform annually routine coronary angiographies for screening, this surveillance often fails to detect coronary stenoses prior to a clinical event. After obtaining promising results for early transplant coronary artery disease (PTCAD) diagnosis, we assessed the hypothesis that both methods in a combined use could further improve allograft vasculopathy surveillance, especially in patients with apparently normal left ventricular (LV) function.

Methods: Throughout 18 months, 189 consecutive patients with post-transplant times over 1 year, normal LV ejection fraction, and without relevant regional wall motion disturbances underwent EBCT (coronary calcification detection) and PW-TDI (LV wall motion analysis) before coronary angiography. Coronary calcifications were quantified by the Agatston scoring system. With coronary artery diameter limited to the pericardial region of more than 70% of all diastolic filling phase, peak wall motion velocities (Sm and Em) and the systolic and diastolic times (Tm from onset of first heart sound to Sm and Em from onset of second heart sound to Em) were calculated. PW-TDI and EBCT data were tested for relationships with angiographic findings.

Results: The systolic peak velocity (Sm) and total calcification score (TCS) showed the highest predictive values. We found significant differences (p<0.0001) between patients with and without proximal stenoses of great epicardial coronary vessels for both TCS and Sm. Positive coronary stenoses (>50% obstruction) were absent in 99.2% of patients with TCS < 7 and in 99.0% of patients with Sm < 0.7 m/s.

Conclusions: Coronary calcification detected by EBCT and PW-TDI is associated with increased coronary artery stenoses in heart allografts without regional wall motion disturbances and normal LV function. This combined use of EBCT and PW-TDI may be of additional importance to coronary angiography in patients with apparently normal LV function.