ASRM Concurrent Scientific Paper Presentations: Complex Reconstruction/CTA/VCA

January 17, 2016 – 7:15 AM to 8:15 AM

7:15 AM - 7:20 AM

Enzyme Activated Drug Eluting Hydrogels Delay Rejection in a Novel Orthotopic Model of Swine Forelimb Vascularized Composite Allotransplantatation

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Introduction

Vascularized Composite Allotransplantation (VCA) can restore form and function in previously unreconstructable injuries. The utility of VCA is restricted by systemic immunosuppression that confers morbidity and mortality while supporting a graft that is not lifesaving but improves quality of life.

Antirejection therapies that target the allograft can potentially reduce such chronic systemic toxicity. Here we investigate, in a novel swine orthotopic forelimb VCA model, a “smart” drug delivery system that releases immunosuppressive drugs only in the presence of an immune response secondary to acute rejection (AR). This study is the first of its kind in a stringent, unique large animal model of VCA with clinically translatable opportunities to study immunosurveillance and functional recovery including neural regeneration and bone healing.

Methods

This protocol was approved by the relevant Institutional Animal Care and Use Committee.

Experimental groups were as follows –

Controls

(no immunosuppression)
Intervention 6

(tacrolimus via hydrogel
delivery system)

Tacrolimus eluting hydrogels, responsive to matrix metallopeptidases 2 and 9 released by activated macrophages during AR, were implanted subcutaneously in transplanted forelimbs after surgery. Total combined initial dose of tacrolimus was 49mg. Whole blood trough levels and tissue levels of tacrolimus were measured by liquid chromatography – mass spectrometry (LC-MS) and limbs were evaluated clinically and histopathologically for AR. End point was Grade 4 rejection (according to the Banff scale, evaluated by a blinded and independent veterinary pathologist) or 30 days.

Results

Control limbs underwent Banff grade 4 AR by POD 6. 5 of 6 intervention group limbs showed no signs of clinical or histopathologic signs of AR at 4 weeks. One animal showed signs of Grade 4 AR by 30 days Animals mobilized freely while ambulating immediately postoperatively on transplanted limbs, allowing them to access food and water. At end point, systemic trough levels of tacrolimus were negligible (mean 1.2ng/ml).

Conclusions

Targeted local application of tacrolimus, using enzyme responsive hydrogel delivery, significantly delays the onset of acute rejection of VCA grafts in a clinically translatable orthotopic forelimb model, despite systemic levels of tacrolimus being clinically negligible.

This novel model is uniquely powerful by enabling the study of VCA immunology not only in terms of immune rejection, but also functional recovery and nerve regeneration.

On-going protocols are evaluating any longer-term toxicity and optimizing dosing for potential translation of these results to clinical research.

7:20 AM - 7:25 AM

Pedicled colon segment interposition as a salvage procedure when flaps fail in the reconstruction of cervical esophagus

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BACKGROUND: Free flaps have been widely used for reconstruction of cervical esophagus following cancer ablation in the neck, such as cancers in the pharynx, larynx, or thyroid cancer with posterior invasion to pharyngo-larynx . The most commonly used flaps are skin flaps

(forearm flap, anterolateral thigh flap, etc.) as well as jejunal flaps. With proper designing and technique, the free flaps can usually achieve uneventful healing. However, in few cases the flaps may fail and the neck is exposed to an environment of infection. The saliva leaked from the mouth to the neck wound also will cause high risk to the denuded carotid artery. In such situations a pedicled colon segment (instead of any other free flaps) is a reasonable option for prompt salvage.

METHODS: From 1983 to 2014 a total of 268 cases of reconstruction for cervical esophagus had been done with 7 cases of failure. Another 14 cases of failure were referred to our hospital for salvage. In the 21 cases colon segment interposition had been performed after failure of a free skin or jejunal flap. It was carried out two days after debridement of the necrotic tissue. Preoperatively colon preparation was given as other colon surgery. The transverse and descending colon are usually used with the middle colic vessels as the vascular pedicle. The colon segment passed in the substernal tunnel to reach the pharynx. If the tissue in the pharynx is fragile from previous infection, minimal sutures or even pull-through technique was employed for fixation of the pharyngeal end of the colon segment. The upper end of the thoracic esophagus was closed.

RESULTS: All cases were successful for restitution of the continuity of the esophagus. There was no infection in the neck , mediastinum or abdomen. Only one patient required another skin graft for the neck wound. The patients resumed oral intake at one month after surgery for the reason of safety to prevent leakage. Adjuvant chemotherapy/radiotherapy were provided for tumor control as required.

CONCLUSION: Comparing with left ascending colic vessels, the middle colic vessels provides a better arterial blood supply as well as venous drainage for the colon segment. It can be longer and thus has no tension at the pharyngeal end. It is a safe procedure for effective salvage when the free flaps for cervical esophagus has failed with the risk of saliva soaking around the denuded carotid artery.

7:25 AM - 7:30 AM

Whole Eye Transplantation: From Experimental Model to Clinical Application

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Purpose: Approximately 39 million people worldwide suffer from blindness. Whole eye transplantation gives the opportunity to provide viable retinal ganglion cells and an entire optical system to recipients with irreversible vision loss. A key obstacle to whole eye transplantation is the poor regenerative ability of the optic nerve. Recently, several groups have demonstrated optic

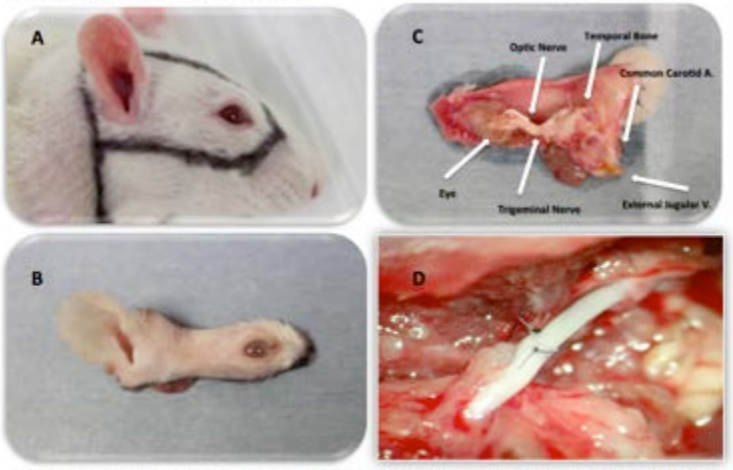
nerve regeneration with therapeutic intervention, showing promise for eye transplantation. There has been difficulty in establishing a consistent small animal model for basic science research. We previously established and published a functional face transplant model in the rat, and have expanded our model to include the whole eye, optic nerve and its blood supply. The purpose of our study is to evaluate gross morphology, viability and structural integrity in our orthotopic whole eye transplant model.

Methods: Syngeneic transplants were performed in Lewis (RT1l) rats. Donor flaps are composed of ocular tissue anterior to the optic chiasm, the skin of the eyelid and external ear. Recipient sites are prepared by removing a similar region of skin and ocular tissue with the optic nerve cut at the base of the globe. Grafts are transplanted to the recipient and vascular anastomoses are performed, as are nerve appositions between donor and recipient optic nerves. Slit lamp examination, Optical Coherence Tomography (OCT) and histological analyses were performed to evaluate the viability and structural integrity of the transplanted eye.

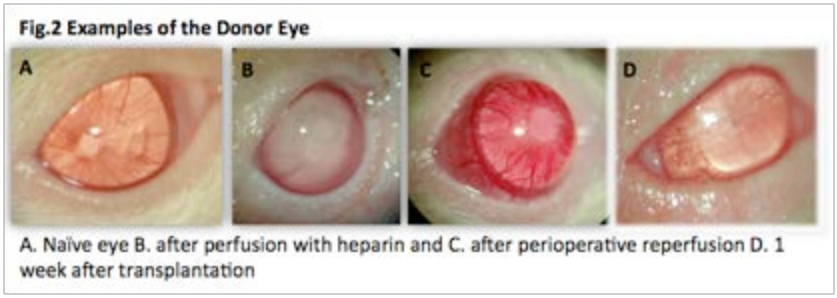
Results: 15 of 20 rats survived the surgical procedure with the maintenance of visual transparency of the anterior eye as evidenced by slit lamp examination. A variable degree of peripheral corneal neovascularization was seen in the transplanted eyes of the 15 surviving rats. OCT confirmed transparency of the anterior chamber and retinal blood flow. Histology confirmed corneal neovascularization and the relative preservation of the retinal layers with the exception of a degree of retinal nerve fiber layer and ganglion cell layer thinning.

Conclusions: We have established a viable orthotopic model for vascularized whole eye transplantation in the rat. Relatively preserved structural integrity and retinal blood flow were observed. The model is excellent for studying viability, functional return and immunology in whole eye transplantation.

Fig.1 Whole Eye Transplant Model



A. The hemi-face flap to be transplanted is marked and B. dissected free from the donor animal C. The posterior side of the flap D. Optic nerve apposition



7:30 AM - 7:33 AM
Discussion

7:33 AM - 7:38 AM
Combined modified Charles' procedure and lymph node flap transfer for advanced lymphedema of the lower limb with severe fibrosis
China Medical University Hospital , Taichung, Taiwan
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BACKGROUND: In advanced lymphedema of the lower limb there is severe fibrosis which is usually not reversible. This is different from the finding of upper limb lymphedema in which lipogenesis is more dominant which can be treated with compression and suction lipectomy. Therefore we use combined modified Charles' procdure as resection therapy to decrease the lymphatic load, and microsurgical lymph node flap (LNF) transfer to the ankle for improvement of lymhatic circulation of the foot. LNF transfer was needed because the foot sole was indispensible and could not be excised.

Method: From 1995 to May 2014 a series of 86 cases with advanced lymphedema of the lower limbs were included in this study. The inclusion criteria was tonicity below the value of 50 in our tonicity measurement. They were treated with combined modified Charles' procedure and LNF transfer. Pre- and postoperatively the patients were evaluated with circumference

measurement, tonicity measurement, scanning lymphangiogram, and MRI, as well as recordings of cellulitis attacks and subjective complaints. At 6 weeks after surgery they started to use compression garment for stabilization of the skin graft. The minimal follow-up time was one year.

RESULTS: There was dramatic decrease in circumference and cellulitis attack after surgery. The scanning lymphangiogram showed remarkable decrease in stasis of the injected Tc-99 colloid. The CT scan showed viability and even hypertrophy of the transferred lymph nodes. The collection of lymph to the transferred lymph nodes with subsequent antegrade drainage had been found. The scar was minimized with steroid injection.

CONCLUSION: For advanced lymphedema of the lower limb this is a good option of treatment with satisfactory result. Ten steps to modify the original Charles' procedure has been proved to successfully decrease the lymphatic load in these patients and to avoid most complications. The pitfalls of the combined method and technical details will be demonstrated.

7:38 AM - 7:43 AM

Prolonged Cold Ischemia Time Impacts Immune Response in a Murine Orthotopic Hindlimb Transplant Model

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Purpose: Prolonged cold ischemia time followed by reperfusion in transplanted tissues triggers innate immune activation leading to tissue damage. Sequelae can include acute and chronic rejection, and graft loss. Despite critical clinical significance, little is known about the role and mechanisms by which Ischemia-Reperfusion Injury (IRI) affects Vascularized Composite Allotransplantation (VCA) outcomes. This study investigates the effect of cold ischemia time on immune response, chimerism and allograft survival in VCAs with a vascularized bone marrow component.

Methods: A model of IRI in VCA was developed based on an established orthotopic hindlimb transplantation model in mice using the cuff technique. Twenty syngeneic (C57BL6 to C57BL6) and allogeneic (Balb/c to C57BL6) transplants were performed comparing cold ischemia times of 1 and 6 hours. Complementary full-thickness skin transplants were performed separately for comparison. The grafts were monitored for clinical signs of rejection. Skin, muscle and vessel biopsies were collected at postoperative days 1, 3 and 7 to assess cellular infiltration and cytokine expression by histology and qRT-PCR. Donor-specific bone marrow chimerism was assessed using flow cytometry.

Results: All grafts were maintained without overt clinical signs of rejection until the predetermined timepoints. Histology confirmed a significant progression of inflammatory cell infiltration by postoperative day 7 in both the 1 and 6 hour ischemia groups. After prolonged ischemia, bone marrow isolated from donor tibia demonstrated recipient myeloid and lymphoid

chimerism, however donor chimerism was not observed in the contralateral hindlimb marrow or spleen at postoperative day 7.

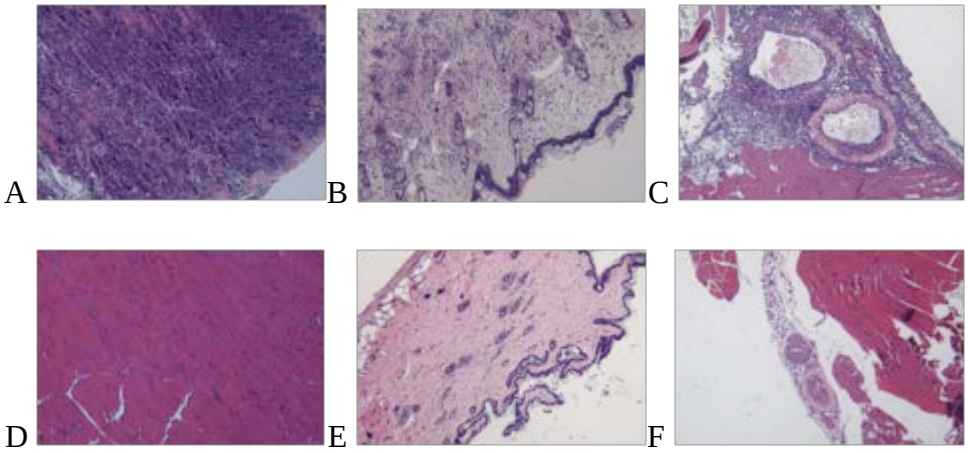

Figure 1. Hematoxylin and eosin sections of allogeneic transplant with 6-hr cold ischemia time at postoperative day 7. Hindlimb muscle from transplanted limb (A) compared to control limb (B), skin from transplanted limb (C) compared to control limb (D) and femoral vessels from transplanted limb (E) compared to control limb (F).

Conclusions: Prolonged cold ischemia times contribute to a vigorous histopathological immune response in VCA that may not be readily apparent on gross clinical inspection. By comparing syngeneic transplants to allogeneic transplants, confounding effects of the host allogeneic rejection response can be accounted for. This model is well-suited to study the mechanisms by which innate immune activation influences VCA outcomes.

7:43 AM - 7:48 AM

Management of the Salivary Glands in Face Transplantation

Cleveland Clinic, Cleveland, OH, USA
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Background: Since the first face transplant in 2005, 35 cases have been performed worldwide with acceptable graft survival and satisfactory return of function and appearance. With increased experience, it is emerging that the salivary glands can contribute to the challenges encountered in the peri-operative period. Inclusion of the glands has been associated with undesirable facial bulk, the need for facial nerve grafts, and salivary fluid collections. Additionally, acute bacterial sialadenitis in an immune compromised patient is potentially life-threatening and may mimic allograft rejection.

Methods: A comprehensive review of peer reviewed literature, meeting proceedings, media reports, and intra-institutional cases regarding management of the parotid and submandibular glands and facial nerve in facial transplantation was performed. Data gathered included: inclusion or exclusion of submandibular and parotid glands in the recipient and allograft, extent

of mucosal inclusion in the allograft, salivary complications and treatment, level and method of facial nerve repair, and motor nerve outcomes.

Results: Information on salivary gland management was available for 24 cases. Explicit mention of undesirable salivary events was documented in 11 cases, representing a reported incidence of 46%. The source of complications was the parotid in 4 cases (36%), a combination of the parotid and submandibular glands in 3 (27%), and minor salivary glands in 4 (36%). Post-operative botulinum toxin injections were reported in four cases, with resolution of the salivary collections post-injection. Facial nerve continuity was restored at the level of the trunk/primary divisions (66%) or the terminal branches (34%). Inclusion of the whole parotid dictated a trunk repair, whereas exclusion of the parotid was associated with a terminal branch repair. Functionally, on average motor reinnervation appeared around 3-4 months for direct terminal branch repairs, and around 5-6 months for direct trunk/primary division repairs, with increased recovery time when interpositional nerve grafts were utilized.

Conclusions: The salivary glands warrant increased attention in the surgical planning and postoperative care. Exclusion of the salivary glands from the facial allograft with repair of the terminal branches of the facial nerve appears to be preferable, where appropriate. Additionally if the salivary glands are included, botulinum toxin should be considered for prophylaxis and treatment of salivary collections.

7:48 AM - 7:51 AM
Discussion

7:51 AM - 7:56 AM
Host-derived angiogenesis with short term immunosuppression increases bone remodeling in a porcine VCA model
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Introduction

Rejection in vascularized composite tissue allotransplantation (VCA) is caused by cytotoxic T-cell allo-recognition, unless prevented by immunosuppressive therapy. The toxicity and complication profile of long-term immunosuppression in VCA prevents their widespread for non-life-saving procedures such as bone segment reconstruction. This study presents a novel method of bone VCA without the need of long-term immunosuppression using host-derived angiogenesis to maintain circulation.

Material and Methods

Segmental tibial bone VCAs were microsurgically allotransplanted in major mismatched Yucatan mini-pigs, placed orthotopically. To allow development of a host derived circulation, an arteriovenous bundle (AV-bundle) of the recipient pig was additionally implanted into the tibial

bone segment. Two weeks of immunosuppression was used to maintain perfusion of the VCA trough the pedicle until a new autogenous circulation had been established within the allotransplanted bone. There were 4 allotransplants in Group 1 with a patent AV-bundle within the bone VCA. The control-Group 2 consisted of 4 allotransplants with a ligated AV-bundle. At 16 weeks bone healing at the proximal and distal host/allotransplant junctions as well as of the allotransplant itself, new bone remodeling and bone VCA neoangiogenesis was quantified with micro-CT and histomorphometric analysis.

Results

The extent of neoangiogenesis within the segmental tibial bone allotransplants depended on the patency of the implanted AV-bundle. The micro-CT vessel volume in Group 1 with a patent AV-bundle ($0.11\pm0.04mm^3$) was higher than in Group 2 ($0.01\pm0.01mm^3$) (p=0.0286). Allotransplants with a host derived angiogenesis in Group 1 showed increased bone remodeling (bone formation rate to bone surface ratio: $8.2\pm3.1um^3/um^2/day$ versus $2.6 \pm2.9um^3/um^2/day$, p=0.03) than allotransplants without an additional angiogenesis in Group 2. Micro-CT analysis of newly formed bone volume at the proximal and distal host/allotransplant junction showed significant differences between Group1 and Group2 (p=0.044) with lower measures in Group 2 with a ligated AV-bundle (14.6±2.6ccm) as compared to Group 1 with a patent AV-bundle (18.8±1.9ccm). The allotransplant volume varied significantly between the two groups (p=0.015) with values of 7.3±1.7ccm in the allotransplant group with a ligated AV bundle and 11.3±1.7ccm in the allotransplant group with a patent AV-bundle.

Conclusion

In the porcine model, segmental tibial bone VCA in combination with a 2 week immunosuppresion and AV-bundle implantation created an autogenous neoangiogenic circulation, permitting long-term allotransplant survival with improved bone healing properties and increased bone formation rates. The method of host derived neoangiogenesis may allow future composite-tissue allotransplantation of bone without the risks associated with long-term immunosupression or tolerance induction.

7:56 AM - 8:01 AM

Evaluation of Viability, Structural Integrity, Aqueous Humor Dynamics and Functional Return after Whole Eye Transplantation

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Purpose: Approximately 39 million people worldwide suffer from blindness. Whole eye transplantation offers the opportunity to provide viable retinal ganglion cells and an entire optical

system to recipients with irreversible vision loss. The purpose of this study is to evaluate the viability, structural integrity and function of our orthotopic whole eye transplant model by assessing aqueous humor dynamics using gadolinium (Gd)-enhanced MRI, optic nerve structural integrity with diffusion tensor MRI (DTI) and functional return via electroretinography (ERG).

Methods: Syngeneic transplants were performed in 5 Lewis (RT1l) rats. Intraocular pressure (IOP) measurements were made using a TonoLab rebound tonometer. ***MRI Protocols***: Rats were intraperitoneally injected with 0.3mmol/kg Gd-DTPA (Magnevist). Four animals were scanned at 3 weeks and 1 animal was scanned at 10 weeks after transplantation using a 9.4-Tesla/31-cm Varian/Agilent scanner. The initial rate of Gd increase, peak % Gd signal enhancement and time-to-peak were calculated. Fractional anisotropy (FA), axial diffusivity (λ//) and radial diffusivity (λ⊥) DTI parametric maps were computed using DTIStudio. ***ERG Protocol***: Rats were housed in a dark box overnight. Diagnosys electrodes and subdermal needle electrodes were placed and light stimuli were delivered and retinal responses recorded.

Results: IOPs of the naive and transplanted eye were 15.9±3.1 mmHg and 16.5±3.2 mmHg, respectively. At 3 weeks after transplantation, the right AC had a similar time to peak but a significantly lower peak intensity and lower initial increase rate than the left AC. At 10 weeks, the right AC had comparable peak intensity to the left AC. Limited Gd enhancement was observed in the vitreous with no significant difference between left and right eyes (two-tailed paired t-tests, $p>0.05$). T2-weighted images showed the donor optic nerve had comparable morphology with the uninjured intraorbital optic nerve 3 weeks after transplantation, whereas in the prechiasmatic optic nerves, DTI quantitation of the right injured optic nerve showed significantly lower FA and λ// by 54±6.1% and 24.9±5.7%, respectively, and a significant increase in λ⊥ by 83±29.5% compared to the left uninjured optic nerve (two-tailed paired t-tests, $p<0.05$). ERG revealed the lack of an electrical response in the transplanted eye to light stimuli.

Conclusion: Our whole eye transplant model revealed the presence of aqueous humor dynamics and preserved integrity of blood-ocular barriers after transplantation. Future DTI and ERG studies will examine approaches for regaining neuronal structure and function of our whole eye transplant model.

Fig. 1 One month after whole eye transplantation

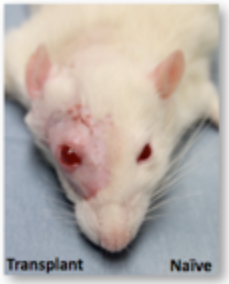
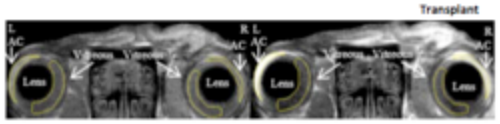
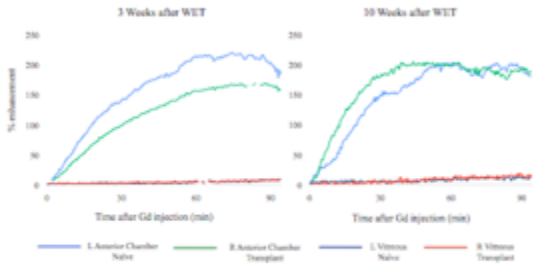


Fig. 2 Gd-enhanced MRI allows for evaluation of ocular anatomy, aqueous humor dynamics and blood-ocular and aqueous-vitreous barrier integrity



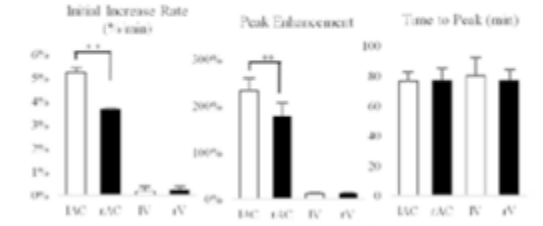
T1 weighted images at 0-10 min (left) and 60-70 min (right) after systemic Gd administration. Comparable Gd enhancement is seen in the anterior chamber of the naïve and transplanted eyes. Naïve and transplanted eyes have comparable intraocular pressures of 15.9±3.1 mmHg and 16.5±3.2 mmHg, respectively. IOPs were taken prior to MRI imaging. The lack of enhancement in the vitreous suggests that the structural integrity of the aqueous-vitreous and the blood-retinal barriers are relatively preserved in the transplanted eye 3 weeks after WET.

Fig. 3 Time profiles of Gd-enhanced aqueous humor dynamics



The anterior chambers of both naïve and transplanted eyes showed gradual Gd-enhancement after systemic Gd administration. No apparent vitreous enhancement was noted for either eye. Preliminary data suggests the normalization of aqueous humor dynamics at 10 weeks.

Fig. 4 Quantitative comparisons of initial rate of Gd increase, peak % Gd signal enhancement, and time to peak in the anterior chamber (AC) and vitreous (V) of uninjured left (l) eye and transplanted right eye at 3 weeks after whole eye transplantation (Two-tailed paired t-tests: **p<0.01)



8:01 AM - 8:06 AM

The Effect of Different Cellular Therapies on Donor-Specific Chimerism and Composite Tissue Allograft Survival in Face Transplantation Model

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[1]Gulhane Military Medical Academy, College of Medicine, [2]Gulhane Military Medical Academy, Haydarpasa Training Hospital, [3]University of Illinois at Chicago

Introduction: Composite tissue allografts such face transplant require life-long immunosuppression causing significant side effects of these highly toxic immunosuppressive New less toxic therapies of tolerance induction are developing to solve this problem, The aim of

this study is to determine the effect of different bone marrow based cellular therapies on donor-specific chimerism in face transplantation model.

Material and Method: Bone marrow cells (BMC) were harvested and prepared from ACI ($RT1^a$) donors. Bone marrow stromal cells (BMSC) were obtained from ACI ($RT1^a$) donors by culturing whole bone marrow cells in alpha-MEM medium for 5-8 passages. Chimeric animals were created by intraosseous injection of donor BMC to Lewis ($RT1^l$) recipients. These chimeric animals were treated with 7-day αβ-TCR/CsA and after 21 days donor/recipient chimeric cells (DRCC) were isolated from chimeric animals by MACS technique.

Twenty hemiface allograft transplantations were performed between ACI ($RT1^a$) donors and Lewis ($RT1^l$) recipients.

Group I was allograft rejection group. Intraosseous cellular therapy injections were delivered: In group II,: BMC ($100x10^6$ donor derived BMC) group III :BMSC ($10x10^6$ donor derived BMSC) and in Group IV: Chimeric Cell injection ($10x10^6$ donor/recipient chimeric cells). None of the groups was supported with immunosuppresion protocol.

Gene expression for proinflammatory (IL-2, TNFα, IL-6, IFNγ) and tolerogenic (IL-10, TGFβ, IL-4) cytokines were evaluated in donor and recipient face skin biopsies using Taqman® real-time PCR.

Results: In group I, composite grafts rejected on day 8-9, posttransplant. In groups II and III, composite grafts rejected on posttransplant 10-11 and 10-12 days, respectively. Extended face allograft survival was observed (13-16 days) in recipients under chimeric cellular therapy (Group IV). This correlated with lower level of chimerism (below 1 %) in groups I, II and III, and higher chimerism (2.5 %) under chimeric cell therapy.

Increased gene expression (4.7 fold) of tolerogenic IL-4 cytokine was seen chimeric therapy recipients.

Conclusion: Cellular therapy with donor/recipient chimeric cell resulted in 62 % extension of face allograft survival, without immunosuppression. This was supported by higher chimerism level and tolerogenic cytokine expression when compared with BMC and BMSC therapy groups confirming tolerogenic properties of chimeric cells.

8:06 AM - 8:11 AM

Migration, Engraftment and Safety of Human Cord Blood Derived Ex-vivo Created Di-Chimeric Cells Therapy tested in NOD SCID Mouse Model: A Preliminary Report

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Background: Cellular therapies are considered the most promising approach for prolonging survival and tolerance induction in vascularized composite allograft (VCA) transplantation. This

study aimed to evaluate the phenotype, migration, engraftment and safety of ex-vivo fused human cord blood derived di-chimeric cell therapy (DCC) in the NOD SCID mouse model.

Methods: A total of 30 fusions of mononuclear cells isolated from human umbilical cord blood (UCB) were performed. UCB from two unrelated donors were separately stained with PKH26 and PKH67 dyes. Fused with polyethylene glycol, double (PKH26/ PKH67) stained DCC were sorted and subjected to the following in vitro evaluations (15 fusions): lymphocytotoxicity (LCT) test, PCR-rSSOP, STR-PCR, viability, apoptosis (Annexin-V, TUNEL assay), colony forming unit (CFU) assay, phenotype, and COMET assay. DCC (3-5x10^6 cells) from 15 fusions (n=5/Group) were delivered: Group 1: intraosseous, Group 2: intramuscular, and Group 3: subcutaneous to NOD SCID recipient mice. Control mice in Groups 4, 5, and 6 (n=5) received 3x10^6 UCB utilizing the same three delivery methods. Mice were observed daily for 180 days for changes in weight, activity, posture, and hair loss. Moreover, mice were evaluated three times per week by palpation and at 90 and 180 days post-op by magnetic resonance imaging (MRI) for tumor growth. DCC presence in the peripheral blood was determined by complete blood count (CBC) and flow cytometry (FC). The DCC migratory pathways, peripheral blood, bone marrow, lymph nodes, spleen, lung, and liver were assessed at 90 and 180 days using immunofluorescent staining.

Results: The presence of HLA class I and II from both UCB donors was confirmed by LCT, PCR-rSSOP, and STR. CFU determined proliferative properties of DCC comparable to the UCB. COMET assay showed no damage to the DNA of DCC following fusion procedure. Human derived cells (CD45+, CD19+, HLA class I and CD4+) were detected at a level up to 2% in the peripheral blood as tested by CBC and flow cytometry at 90 days following delivery. The presence of human derived cells was confirmed by PCR. No DCC derived tumor-like growth was observed by palpation or MRI, thus confirming safety of the therapy.

Conclusions: We have characterized the phenotype, viability, and migratory properties of DCC and confirmed their safety. The unique concept of DCC supportive therapy introduces cells representing phenotype characteristics of both the transplant donor and the recipient. Thus, DCC represent a novel, promising approach for tolerance induction in solid organ and VCA transplants.

8:11 AM - 8:15 AM
Discussion