**Program Number:** 4730  
**Presentation Time:** 11:00 AM–12:45 PM  
**Wednesday, May 04, 2016**

### Corneal Immunology and Contact Lenses

#### Abstract Title: Infiltration and cytokine induction in dry eye mouse

**Eunyoung Choi1, Hyungoo Kang1, Wungrak Choi1, Moon In hee1, Moon In hee1, Areum Yeo1, Hyemi Noh1, Eung Kweon Kim1-2, Hyeon Chang Kim1, Hyung Keun Lee1, 2**  

**Purpose:** Langerhans cells (LCs) located within the epithelial layer of normal cornea are suspected to function as one of major antigen-presenting cells (APCs) in pathologic status of ocular surface. In previous study we reported that increased infiltration and morphologic changes of epithelial DCs in dry eye (DE) were visualized by confocal microscope. It was noticeable that in DE patients with less severe symptoms, the average length of LC processes and LC areas were observed to be increased when compared to symptomatically severe DE. We performed experimental study using in vivo CD207-depleted murine DE model to investigate the functional role of LCs during ocular surface inflammation in DE disease.

**Methods:** C57BL/6 wild-type (WT) and CD207-depleted Langerin-diphtheria toxin receptor (CD207-DTR) mice were used in the study. To keep the depleting status of CD207+ cells in ocular surface, DT (0.1 microgram/ml) was injected every other days to the CD207-DTR mice. Before and during DE induction in mice, corneal erosion was scored with fluorescent staining. After seven days of DE induction in mice, corneas with adjacent conjunctival tissues were acquired. Inflammatory cytokines and CD4+, CD11b+, and Gr1+ cell numbers were analyzed with fluorescence activated cell sorting (FACS) and compared between WT and CD207-deleted mice after DE induction.

**Results:** Corneal erosion score was significantly elevated from the immediate after the DE induction in CD207+ cell depleted mice. Ocular surface IL-6 and IL-17 levels in CD207+ deleted DE-induced mice were 3.9-fold and 5.2-fold higher, respectively, than the WT DE model. TNF-a, and IL-1b levels were showed no differences. In addition, inflammatory cell (e.g., CD4+, CD11b+, and Gr1+ cells) numbers were significantly higher in langerin knockout than WT mice after DE induction.

**Conclusions:** We demonstrated that LCs have a role of negatively regulating ocular surface inflammation during DE disease and might be important to maintaining disease tolerability. Considering with the findings of previous clinical study, the activation parameters, rather than the cell density of corneal LCs, are important for reducing DE severity and predicting DE-induced immunopathologic change.

### Commercial Relationships: Eunyoung Choi, Hyungoo Kang, None; Wungrak Choi, None; Moon In hee, None; Areum Yeo, None; Hyemi Noh, None; Eung Kweon Kim, None; Hyeon Chang Kim, None; Hyung Keun Lee, None

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**Program Number:** 4731  
**Presentation Time:** 11:15 AM–12:30 AM  
**Allogeneic sensitization and tolerance induction post corneal endothelial cell injection into the anterior chamber**

**Jun Yamada1-2, Morio Ueno1, Munetoyo Toda1, Katsuhiko Shinomiya2, Chie Sotozono2, Shigeru Kinoshita1, Junji Hamuro1**  

**Purpose:** Cultivated allogeneic corneal endothelial cell (CEC) injection therapy for CEC dysfunction is now available. To evaluate allogeneic response post CEC injection into the anterior chamber (AC), we developed new experimental models and examined allosensitization and acquisition of transplantation tolerance.

**Methods:** After detachment of the epithelial layer from the corneal eyecups of C57BL/6 mice by EDTA, mice-derived primary CECs (mpCECs) were collected by using trypsin. mpCECs (1 x 10⁶) were then injected into the AC, subcutaneously (SC) into the neck, or intravenously (IV) into BALB/c mice. In the murine CEC injection models, a 2mm central area of the cornea was pretreated by cryoinjury to eliminate CECs. Allogeneic cell survival, allo-specific delayed-type hypersensitivity (DTH) response, and AC-associated immune deviation (ACAID) induction were evaluated at 1-week post CEC injection. Long-term transplantation tolerance was evaluated by observing the secondarily performed penetrating keratoplasty (PKP) from C57BL/6 donor mice at 8-weeks postoperative.

**Results:** SC injection of mpCECs induced a DTH response, while AC and IV injection of mpCECs did not. ACAID was induced by injection of mpCECs into the AC of normal eyes. In contrast, after the eyes were inflamed by cryo-injury, the AC injection of mpCECs also did not induce DTH response in the absence of ACAID induction (N=5 each, p<0.001). Labeled mpCECs in the cryo-injured eyes survived for at least 1 week (N=5). C57BL/6 PKP allografts at 8-weeks post mpCEC injection never displayed episodes of allogeneic rejection (N=10, 100%, p<0.001), yet PKP allografts were rejected in 60% of the mice with IV injection of mpCECs and 70% of the mice with AC injection of mpCECs but without cryoinjury.

**Conclusions:** Allogeneic mpCECs injected into the AC, but not SC, display low allogenicity and lack the capacity to induce DTH, and mice with surviving allogeneic mpCECs in the AC for 8 weeks acquired transplantation tolerance of the full-thickness corneal allograft. Thus, CECs injection into the AC is apart from the allogeneic rejection. Cultivated allogeneic human CEC injection therapy may be immunologically safe to maintain long-term survival post attachment at the side of Descemet’s membrane in the proposed therapy. Future studies using cultivated mCECs are necessary to further confirm the safety.

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**Program Number: 4732**
**Presentation Time: 11:30 AM–11:45 AM**

An in vitro model to study antigen-induced macrophage activation and cytokine production in corneal allograft rejection

**Paola Kammrath Bet ancor, Antonia Hildebrand, Florian Emmerich, Thomas Reinhard, Daniel Boehringer, Gunther R. Schlu nck, Thabo Lapp.** 1 Eye Center, Medical Center - University of Freiburg, Freiburg, Germany; 2 Institute for Cell and Gene Therapy, University Medical Center Freiburg, Freiburg, Germany.

**Purpose:** To establish an in vitro system for assessment of corneal antigen detection and processing, cell activation, and cytokine production of human monocyte-derived macrophages.

**Methods:** Human monocytes were isolated from peripheral blood mononuclear cells (PBMC) and differentiated into monocyte-derived macrophages (MDM). A protocol was established to standardize the fragmentation of human corneal tissue. MDMs were stimulated with processed human corneal material. Lipopolysaccharide (LPS) or interferon-gamma (IFNγ) served as controls for MDM activation.

Chemokines were detected in MDM supernatants using a Luminex bead-based multiplex assay for 37 cytokines and compared to data from clinical aqueous humour samples. Transwell migration experiments, cell viability assays, and fluorescence-activated cell sorting were used to assess cell recruitment, immunogenicity of corneal endothelium (CEC), and monocyte survival.

**Results:** Various inflammatory and chemotactic cytokines were released by MDMs after stimulation with human corneal antigen (paired t-test after 48h stimulation: MIP-1β [p<0.001], MCP-1 [p<0.001], MDC [p<0.001], IP-10 [p<0.001]). These in vitro samples shared distinct similarities with human samples of clinical rejections. The presence of CEC material in the stimulation fragments attenuated the upregulation of distinct pro-inflammatory chemokines, (3-way ANOVA; FGF-2 [p=0.01], VEGF [p<0.001]). Medium conditioned by stimulated MDMs induced a selective recruitment of monocytes in a transwell migration model (monocytes versus lymphocytes; 3-way ANOVA with neat supernatant [p=0.001] and with a 1:10 dilution of the MDM supernatant [p<0.001]). Antigen detection and cell activation are not changing MDM cell morphology, but the supernatant modulates immune cell viability and attracts further immune cells.

**Conclusions:** Our data indicate that distinct aspects of corneal transplant rejection can be successfully modeled in vitro. The observations further support the hypothesis that macrophages play a significant role in the initiation of transplant rejection. Macrophages process allogenic corneal tissue fragments and generate an inflammatory milieu to recruit further immunocompetent cells and modulate cell survival and differentiation.

**Commercial Relationships:** Paola Kammrath Betancor, Antonia Hildebrand, Florian Emmerich, Thomas Reinhard, Daniel Boehringer, Gunther R. Schlunck, Thabo Lapp

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**Program Number: 4733**
**Presentation Time: 11:45 AM–12:00 PM**

Pathological Conversion of Regulatory T Cells Cause Disruption of Immune Privilege

**Jing Hua1, Takenori Inomata1, William Stevenson2, Tina Shiang2, Yihe Chen3, Jeffrey Bluestone4, Reza Dana5.** 1 Ophthalmology, Harvard Medical School, Cambridge, MA; 2 Diabetes Center, University of California San Francisco, San Francisco, CA.

**Purpose:** Disruption of immune privilege is associated with premature transplant rejection and autoimmunity, often leading to devastating inflammation and irreversible tissue damage. We examine

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the central and mid-peripheral temporal cornea. LC morphology was graded using a grading scale.

**Results:** LC density in the central cornea (19±25 cells/mm²) and in the mid-periphery (14±12 cells/mm²) was not significantly different. There were no regional differences in LC morphology. Central and mid-peripheral LC density was strongly correlated (r=0.73, P<0.001), but there was no such association for LC morphology. Age or contact lens wear did not affect these relationships. In both corneal regions, higher LC density was associated with more mature LC morphology (C 0.44, P<0.004; MP 0.36, P=0.03). Age was not associated with LC density or morphology and this relationship was not altered by contact lens wear. There were no differences in LC density or morphology between contact lens wearers and non-wearers, nor between soft contact lens and orthokeratology wearers. A greater proportion of more mature LC phenotypes was observed in the non-wearers.

**Conclusions:** Corneal Langerhans cells do not appear to be influenced by age or contact lens wear in this healthy young population. Exploration in a larger cohort and during inflammatory events is required to understand the role of Langerhans cells in contact lens related inflammation in this at-risk population.

**Commercial Relationships:** Blanka Goebelowski; Kim Bui, None; Winnie Lam, None; Cecilia Chao, None; Kathryn Richdale, None; Fiona Stapleton, None

**Program Number:** 4735  
**Presentation Time:** 12:15 PM–12:30 PM  
**Bulbar conjunctival microcirculation and microvascular network in habitual contact lens wearers**

Liang Hu1, Jin Zhou1, WAN CHEN1, Ye Yang1, Min Li1, Hong Jiang1, Jianhua Wang1. Bascom Palmer Eye Institute, Miami, FL; 2School of Ophthalmology and Optometry, Wenzhou Medical University, Wenzhou, China; 3Guangzhou Women and Children’s Medical Center, Guangzhou, China; 4Zhongshan Ophthalmic Centre, Sun Yat-sen University, Guangzhou, China; 5Department of Ophthalmology, Shanghai First People’s Hospital, Shanghai Jiaotong University, Shanghai, China.

**Purpose:** This study was to investigate the morphometry and hemodynamics of the bulbar conjunctival microvasculature between habitual contact lens wearers and non-contact lens wearers.

**Methods:** Custom built Functional Slit-lamp Biomicroscope (FSLB) was used to image the temporal bulbar conjunctiva of contact lens wearers. The averaged blood flow velocity of habitual contact lens wearers was 9.1±0.24 mm/s, which was significantly faster than non-contact lens wearers (10.4±0.23 mm/s, P<0.01). There was no significant difference in vessel diameter between contact lens wearers (16.5±2.3 µm) and non-contact lens wearers (17.3±1.7 µm, P>0.05).

**Conclusions:** Although there was no difference in microvascular network between two groups, increased blood flow velocity in habitual contact lens wearers indicates the vascular responses to contact lens, which may have a protective mechanism for successful lens wear. Future longitudinal studies with large sample sizes may validate the view point.
TEN. Age at consultation, time from consultation to PROSE fitting, etiology of SJS/TEN, change in visual acuity (VA) with treatment, and change in visual functioning (VF) at 6 months using the National Eye Institute 25-item Visual Functioning Questionnaire (NEI-VFQ25) was recorded.

Results: 236 patients with SJS/TEN underwent PROSE treatment. 40 patients were under 18 years old (YO) at the time of presentation to BFS, with a range of 4-17 YO (median 9 YO, 23 females, 17 males). Of these pediatric patients, time from presentation to dispense of prosthetic device varied from .04-5.41 years. There was a trend toward longer customization and training time for those ≤7 YO compared to those 8-18 YO (1.28 years vs. 0.73 years, p=0.452). Of the 40 pediatric patients, 18 patients (32 eyes) had complete VA and NEI-VFQ data (9 females, 9 males). Age at PROSE treatment ranged from 4-16 YO (median 8.5 YO). Suspected etiologies included antibiotics (n=6, amoxicillin=5/6), ibuprofen (2), quinine (1), phenytoin (1), lamotrigine (1), phenobarbital (1), viral infection (2), unknown (4). Median VA at presentation was 20/100 (range, LP-20/25). Median VA after PROSE treatment was 20/30 (range, HM-20/20, p<0.0001). Follow-up ranged from 6-42 months (mean 13.9 months). Mean NEI-VFQ25 score prior to PROSE treatment was 65.57. Mean score 6 months after was 78, p=0.016.

Conclusions: PROSE treatment can improve VA and VF in pediatric patients with chronic OSD from SJS/TEN, with significant positive impact in these patients who might otherwise be poor contact lens candidates. Time from consultation to dispense of prosthetic device may be longer in the youngest of pediatric patients but our data show that children with a history of SJS/TEN, including as young as 4 YO, are good candidates for PROSE treatment.

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