IOSS - 2009

International Ocular
Surface Society

12th Ocular Surface & Tear Workshop

The Aging Ocular Surface

Saturday, May 2, 2009
8:30 am to 5:00 pm

Lakeview Room, Lago Mar Resort and Club
1700 S. Ocean Lane
Fort Lauderdale, FL 33316, USA

Reception 5:00 pm until 7:00 pm

Time: **8:35-8:47AM**

Title: **Sirolimus Versus Conventional Immunosupressor Regimen in a High Risk Keratoplasty Patient**

Presentor: **Gabriela Blanco, MD**

Co-Authors: **Nelson Hernandez, MD, David Arana, MD**

Institution: **Hospital Militar Dr. Carlos Arvelo, Caracas, Venezuela**

Abstract:

Background: If syrolimus has been used in diabetic patients with severe kidney damage, where steroids and calcineurin inhibitors produce further descompensation of systemic pathology, we have used sirolimus as an only modulator.

Purpose: To assess the efficacy of new surgical techniques combined with new immunosuppressor regimen in a patient with high-risk corneal and limbal grafts due to previous rejected graft.

Methods: After removal of conjunctivalilzed tissue over the cornea and intraoperative Mit C in subconjunctival surrounding tissue, we performed transplantation of criopreserved amniotic membrane using fibrin glue as a basal membrane. During the same procedure, we performed first keratolimbal allograft from cadaver, then, criopreserved amniotic membrane as a patch with nylon 10.0 surget and temporal tarsorrhaphy for three weeks. Beside the surgery he received conventional combination of immunosuppressors with tacrolimus, mycophenolate mofetil and systemic corticosteroids only for the immune acute state or three first months, then, he received monotherapy with systemic syrolimus since the patient had three months POP.

Results: The follow up period was 10 months. The vision improved, cornea is more clear and there is any sign of irreversible graft rejection while receiving monotherapy with syrolimus.

Discussion: The immunosuppressive efficacy of Syrolimus beside strategical surgical reconstruction is effective in prevention of rejection in this patient with high risk corneal and limbal grafts despite previous rejected graft.

Syrolimus therapy is effective after immune acute phase despite the dose of corticosteroids and others immusuppressors are discontinued and without toxicity effects Further studies are required to establish the longterm efficacy and safety of sirolimus alone or in combination.

Time: **8:47-8:59AM**

Title: **Bilateral Descemetoceles in an Older Stevens-Johnson Patient - a Case Report and Treatment Review**

Presentor: **Darren Gregory, M.D.**

Institution: **Rocky Mountain Lions Eye Institute**

Abstract:

A 61 year old woman initially treated with bilateral Prokeras during an acute episode of Stevens-Johnson syndrome was referred for further care 1 month into the episode due to worsening of ocular surface inflammation and pain. Amniotic membrane transplantation (AMT) was performed to both eyes and all 4 eyelids. Patient continued to have bilateral persistent corneal epithelial defects which progressed to descemetoceles over the next 2 months despite treatment with autologous serum drops, cyanoacrylate glue application, and multiple amniotic membrane applications (including multilayered).

Right eye continues to have the original cyanoacrylate patch in place 5 months after placement. Left eye underwent manual lamellar keratoplasty 3 months into episode followed by fitting for a Boston scleral lens. Case is presented to discuss the various treatments used and the extreme difficulty in achieving a stable ocular surface despite the many therapies employed. Case also highlights the importance of early intervention with AMT to the entire ocular surface and lids during the first 2 weeks of the disease.

Time: **8:59-9:11AM**

Title: **S100 proteins in Ocular Surface Disease**

Presentor: **Roger Beuerman, M.D.**

Co-Authors: **Zhou Lei[1,2], Louis Tong[1,3], Li Jing[1,2], Chan Choi Mun[3]**

Institution: **Singapore Eye Research Institute[1], Ophthalmology, Yong Loo Lin School of Medicine, National University of Singapore[2], Singapore National Eye Center[3]**

Abstract:

Introduction: The calcium binding S100 proteins are found only in vertebrates and have intracellular and extracellular roles in the regulation of many diverse processes such as protein phosphorylation, cell growth and motility, cell-cycle regulation, transcription, differentiation and cell survival. Several of 25 members of this protein family are strongly associated with inflammatory diseases such as rheumatoid arthritis.

Methods: We have analyzed the tears of patients with pterygium, and dry eye disease. Tears from patients with pterygium (n=12, contralateral normal eyes as controls) were collected by glass capillary just prior to surgery and analyzed by SELDI and proteins identified by nano-LC and tandem mass spectrometry. For pterygium tissue immunohistochemisry was carried out and compared with non-involved conjunctival tissue from normal eyes. Tear samples from patients with dry eye (n=28) and controls (n=20) were collected by Schirmer's strip. We have used a quantitative proteomic approach, iTRAQ coupled with 2D nanoLC-nanoESI-MS/MS to compare the relative changes of tear protein profiles from controls and patients with dry eye.

Results: The tears from the pterygium patients showed significant levels of S100 A8 and A9 compared to the contralateral eyes. Immunoprocedures showed that S100 A6, A8 and A9 were upregulated in pterygium tissue compared to non-involved conjnctiva. The results from the dry eye study showed that S100 A4, A8, A9 and A11 were significantly upregulated in tears from patients with dry eye compared to normals.

Conclusions: These results began to suggest that S100 proteins have an important role in ocular surface inflammatory disease. Pharmacological control of these proinflammatory proteins would likely have significant benefits to modulate inflammation.

Time: **9:11-9:22AM**

Title: **Corneal Epithelial Opacity in Dysfunctional Tear Syndrome**

Presentor: **Stephen C. Pflugfelder, M.D.**

Co-Authors: **Joseph J. Chen, Kavita Rao**

Institution: **Baylor College of Medicine**

Abstract:

Purpose. To compare the appearance of the superficial corneal epithelium in patients with dysfunctional tear syndrome (DTS) patients and an asymptomatic control group using laser scanning confocal microscopy and determine the correlations between confocal microscopic findings and clinical severity parameters.

Methods. 31patients with newly diagnosed DTS and 21 asymptomatic control subjects were evaluated. DTS subjects were classified into four levels of clinical severity (DTS 1-4) based on the Delphi dry eye panel report criteria. The Heidelberg Retina Tomograph 2 Rostock Cornea Module (HRT2-RCM) laser scanning confocal microscope was used to image the superficial corneal epithelium. Areas of single or multiple opaque superficial epithelial cells were measured as a percentage of the 400 x 400sq μm field area in 4 randomly selected images from each eye. Spearman correlations between the confocal findings and clinical parameters were calculated.

Results. The mean area of opaque superficial corneal epithelial cells was significantly greater in DTS patients than normal subjects ($p<0.0001$). Significant differences were observed between the DTS severity groups and the control group ($p<0.001$), except for the DTS 1 group. The area of opaque cells significantly increased with level of clinical severity. The confocal findings showed significant correlation with clinical severity parameters, including blurred vision symptoms ($r = 0.86$, $p = 0.0001$), best corrected visual acuity (BCVA) (Spearman $r = 0.4$, $p= 0.03$), conjunctival lissamine green staining scores (Spearman $r= 0.4$, $p =0.026$), corneal fluorescein staining scores (Spearman $r= 0.5$, $p =0.002$) and videokeratoscopic surface regularity index (Spearman $r = 0.5$, $p =0.02$).

Conclusion. Morphological changes in the superficial corneal epithelium of DTS patients detected by laser scanning confocal microscopy correlates with blurred vision symptoms and objective severity parameters.

Time: **9:22-9:34AM**

Title: **Use of the Boston Ocular Surface Prosthesis in Patients 65 and older**

Presentor: **Deborah S. Jacobs, MD (1, 2)**

Co-Authors: **Perry Rosenthal, MD (1)**

Institution: **1. Boston Foundation for Sight, 464 Hillside Avenue, Suite 205, Needham, MA**
2. Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, MA

Abstract:

Purpose: To report clinical experience using the Boston Ocular Surface Prosthesis (BOS-P) in patients 65 and older.

Methods: A clinical database of patients fitted with the BOS-P, a fluid-ventilated gas-permeable scleral lens, from January 2002 – December 2008 was sorted by age. Of the 1319 patients fitted with the BOS-P during this time period, 116 patients were identified who were 65 years or older at the time of fitting (age: 73.1+/- 6.5; M:F= 40:76). The patients were sorted by age into groups of increments of five years. The number of eyes and their underlying diagnoses are discussed.

Results: In the 65-69 year old age group (age: 67.2; M:F= 15:27), 75 eyes of 42 patients were fitted with the BOS-P. In the 70-74 year old age group (age: 71.8; M:F= 10:25), 59 eyes of 35 patients were fitted with the BOS-P. In the 75-79 year old age group (age: 76.9; M:F= 10:11), 30 eyes of 21 patients were fitted with the BOS-P. In the 80-84 year old age group (age: 82.1; M:F= 5:7), 19 eyes of 12 patients were fitted with the BOS-P. In the 85-89 year old age group (age: 87.0; M:F= 0:2), 2 eyes of 2 patients were fitted with the BOS-P. In the 90-94 year old age group (age: 91; M:F= 0:3), 5 eyes of 3 patients were fitted with the BOS-P. In the 95-100 year old age group (age: 95; M:F= 0:1), 1 eye of 1 patient was fitted with the BOS-P. The top five diagnoses for patients 65 and older were Keratoconjunctivitis Sicca (n=100), post-penetrating kertoplasty (n=25), keratoconus (n=15), Steven-Johnson Syndrome (n=13) and anatomical exposure (n=8). Patients with Keratoconjunctivitis Sicca were subdivided into those with Sjogren's Syndrome (n=30), graft versus host disease (n=20), rheumatoid arthritis (n=6), post-Lasik (n=4), post-radiation (n=1) and others (n=39).

Conclusions: The BOS-P is a useful treatment device for patients 65 and older across a wide variety of ocular disorders.

Time: 9:34-9:46AM

Title: Modulation of wound healing by caveolin-1 status in human corneal epithelium with aging

Presentor: Jae Hoon Jeong[2]

Co-Authors: Ji-Heon Rhim[1], Jae Hoon Kim[2], Jae Chan Kim[2] and Sang Chul Park[1]

Institution: 1. Department of Biochemistry and Molecular Biology, Aging and Apoptosis Research Center, Seoul National University College of Medicine, Seoul, Korea
2. Department of Ophthalmology, Chung-Ang University College of Medicine, Seoul, Korea.

Abstract:

Purpose: To identify the factors for delay of wound healing in the elderly, we carried out the comparison study between young and old patients on protein profiles of the corneal epithelia and the wound healing pattern after excimer laser photoablation surgery, especially focusing on expression of age related proteins such as caveolin-1.

Methods: Corneal epithelial cells were maintained from fifty-three people who had under-gone excimer laser photoablation. After the surgery, the patients were followed-up every 24 hour until the corneal epithelium completely closed and the healing time of corneal wounds was recorded. We observed that expression pattern of caveolin-1 in the corneal epithelia by Western blotting and immunofluorescence studies. Correlation between wound healing time and caveolin-1 status of elderly patients compared to those of young was analyzed.

Results: Higher level of caveolin-1 was observed in the corneal epithelia of elderly patients than those of the young concomitantly with the delayed wound healing time. The level of caveolin-1 was significantly ($p<0.05$) higher in the corneal epithelia from the elderly (60 ± 5 yr, $n=10$) than those from the young (23 ± 3 yr, $n=10$). In average, wound healing time increased significantly with aging from 55 hours at young patients group to 92 hours at elderly patients group.

Discussion: It might be suggested that caveloin-1 status would be responsible for wound healing capacity and that it might be utilized as a new biomarker for wound healing efficiency and a novel target for adjustment in vivo.

Time: **9:46-9:58AM**

Title: **Oxidative Stress and Age Related Dysfunction in the Lacrimal Gland of the Cu,Zn-Superoxide Dismutase-1 (*Sod-1*) Knockout Mice.**

Presentor: **Tais Hitomi Wakamatsu**

Co-Authors: **M. Dogru[1,3], Y. Ogawa[2], Y. Imamura[2], A. Igarashi[3], Y. Sasaki[2], O. Ibrahim[1,2], T. Inaba[2], S. Ward[1], T. Shimizu[4], S. Noda[5], T. Shirasawa[4], J. Shimazaki[3], K. Tsubota[2].**

Institution:
1. **Johnson & Johnson Ocular Surface Visual Optics Department, Keio University, Tokyo Japan.**
2. **Ophthalmology Department, Keio University, Tokyo, Japan**
3. **Ophthalmology Department, Tokyo Dental College, Chiba, Japan**
4. **Gerontology, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan**
5. **Tokai University School of Health Sciences, Kanagawa, Japan**

Abstract:

Purpose: The purpose of our study was to determine the age related alterations in the lacrimal gland of Copper/zinc superoxide dismutase (*Sod*1)–knock-out (–/–) mice .

Methods: Tear function test (cotton thread) and corneal fluorescein staining tests were performed on *Sod*1 –/– mice (n=18) aged 10 and 50 weeks and wild type (+/+) mice (n=14) aged 10 and 50 weeks. The mice were sacrificed and the lacrimal glands were collected for histopathology analyses. Haematoxylin/Eosin (HE), Periodic Acid Schiff, Mallory staining, immunohistochemistry for CD45, lipid and DNA oxidative stress (4HNE and 8OhdG respectively), apoptosis (TUNEL and Caspase-3 staining), necrosis (Ig staining) and mitochondrial marker (anti-mitochondria imunogen) were performed. Micro-structural alterations were demonstrated by transmission electron microscopy from 10 and 50 weeks mice. We performed ELISA for IL-6 and TNF-α in serum and tear samples of 10 and 50 weeks mice. Histolopathogical alterations were also investigated in human lacrimal gland cadaver tissues by HE and the same oxidative

stress markers. The study was conducted in compliance with the ARVO statement for the use of animals in Ophthalmic and Visual Research and the Tenets of Declaration of Helsinki.

Results: Tear quantity values in *Sod*1 -/- mice were significantly lower compared to the *Sod*1 +/+ mice detected by the cotton thread test throughout the study. Fluorescein staining scores were also consistently and significantly higher in the *Sod*1 -/- mice compared to the wild type mice. Histopathological analysis showed lacrimal gland inflammation with presence of intense lymphocytic infiltration, periductal fibrosis and periacinar CD45 positivity in the lacrimal glands of 50 weeks *Sod*1 -/- mice. Immunohistochemistry using 4-Hydroxy-2-nonenal (4-HNE) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) monoclonal antibodies showed prominent positive staining in the lacrimal glands of aged *Sod*1 -/- mice. Transmission electron microscopy (EM) reveled structural alterations in the mitochondria of the lacrimal glands including swelling and disorganization of the mitochondrial inner membranes (cristae) in the 50 weeks knockout mice. EM also showed us the increase of the secretory vesicles, signs of apoptosis, inflammatory cells and increase of fibrotic tissue in the 50 weeks *Sod*1-/- mice. Evidence of apoptosis and necrosis by positive TUNEL, Caspase-3 and Ig stainings suggested these as the pathological mechanisms of cell death and dysfunction of the lacrimal gland. The same alterations observed in the 50 weeks *Sod*1-/- mice lacrimal gland for inflammatory and oxidative stress markers were also confirmed in the aged human lacrimal gland.

Conclusion: Although the S*od*1 -/- mouse is known as a model mouse of aging, abnormal quantitative and qualitative findings observed in relation to the tear functions, ocular surface and lacrimal gland histopathology suggest that it can also serve as a mouse model of age related dry eyes.

Time: **10:30-10:42AM**

Title: **Aging Worsens Sjogren's Syndrome Like Keratoconjunctivitis in IL-2r alpha -/- mice**

Presentor: **Cintia S. De Paiva[1]**

Co-Authors: **Cindy S. Hwang[1], John D. Pitcher III[1], Solherny B. Pangelinan[1], Ehsan Rahimy[1], W. Chen[1,2], K.-C. Yoon[3], William J. Farley[1], Jerry Y. Niederkorn[4], Michael E. Stern[5], De-Quan Li[1], Stephen C. Pflugfelder[1]**

Institution: **[1]Ocular Surface Center, Department of Ophthalmology, Cullen Eye Institute, Baylor College of Medicine, Houston, Texas; [2]School of Ophthalmology and Optometry, Eye Hospital, Wenzhou Medical College,Wenzhou, Zhejiang, China [3] Department of Ophthalmology, Chonnam National University Medical School, Dong-Gu, Gwangju, South Korea [4]Department of Ophthalmology, University of Texas Southwestern Medical Center, Dallas, Texas. [5] Department of Biological Sciences, Allergan Inc., Irvine, California**

Abstract:

Purpose: IL-2ra (CD25)-/- mice develop autoimmunity and lymphoproliferative disorders, including Sjögren's Syndrome-like disease. The objective of this study was to evaluate the severity of corneal epithelial disease and T cell cytokine profile in the ocular surface of the CD25KO mice.

Methods: CD25KO mice were evaluated at 8, 12 and 16 weeks of age. The smoothness and epithelial permeability to Oregon green dextran (OGD) was measured. Lymphocytic infiltration was evaluated by immunohistochemistry. T helper (Th)-1, -2 and -17 associated cytokines were measured by real time PCR in cornea and conjunctiva and by Luminex bead assay in tears.

Results: Compared to 8-week old wild-type mice, CD25KO mice of the same age had significantly greater corneal irregularity and a significant increase in number of CD4+ and CD8+T cells infiltrating the cornea and conjunctiva. CD25KO mice had significant higher levels of IL-6, TGF-β1, IL-23R, IL-17A, IL-17F, IL-21, CCL20, IL-10, GATA-3 and IFN-γ mRNA transcripts in their cornea and conjunctiva than C57BL/6 control mice

at 8 weeks. IL-17A/F mRNA transcripts further increased to maximal levels at 12 weeks, while IFN-γ peaked at 16 weeks in CD25KO mice. Increased expression of IL-17A/F at 12 weeks was accompanied by a worsening of corneal surface regularity and OGD permeability in CD25KO mice.

Conclusions: Disruption of IL-2 signaling in CD25KO mice results in age dependent Sjögren's syndrome like auto-immune lacrimal-keratoconjunctivitis. A mix of Th-1 and Th-17 cytokines were detected, with an early predominance of IL-17 and a late dominance of IFN-γ. The peak severity of corneal epithelial disease corresponded to the peak of IL-17 expression.

Support: NIH Grants EY11915 (SCP), Fight for Sight Grants-in-aid (CSDP), Hartford Foundation (CSDP), RPB, Oshman Foundation, William Stamps Farish Fund, Hamill Foundation and Allergan Inc.

Time: **10:42-10:54AM**

Title: **Corneal sensitivity evaluation with the Belmonte's gas esthesiometer: influence of age, gender, and several ocular parameters**

Presentor: **Margarita Calonge,[1,2]**

Co-Authors: **Marisa Teson,[1,2] M. Carmen Acosta,[3] A. Morejon,[1] S. Sancho,[1] D. Velasco,[1] I. Fernandez,[2,1] María J. Gonzalez-Garcia[1,2]**

Institution: **[1]IOBA, University of Valladolid, Valladolid; [2]CIBER-BBN, Valladolid; [3]Neuroscience Institute, Alicante, Spain.**

Abstract:

Purpose: The purpose of this study was to evaluate corneal sensitivity in healthy subjects by gas esthesiometry with 3 aims: 1) to establish the mechanical (MT), chemical (ChT), thermal hot (THT), and thermal cold (TCT) sensitivity thresholds; 2) to assess its reproducibility and safety; and 3) to analyze the putative influence of some ocular variables.

Methods: 1) MT, ChT, THT, and TCT were determined with the Belmonte's gas esthesiometer in the central cornea of 80 eyes from 40 normal subjects (20 males, 20 females) equally distributed in 5 age ranges. 2) To evaluate reproducibility, two evaluations were performed at two different days (interval, 1-6days). Safety of the technique was assessed by corneal fluorescein staining and bulbar hyperemia evaluation before and after esthesiometry. The evoked sensations were evaluated with a verbal scale; 3) The influence of best corrected visual acuity, (BCVA), contrast sensitivity, ocular sensorial dominance, and refractive error in corneal sensitivity thresholds was analyzed.

Results: 1) No significant differences were found in any corneal sensitivity threshold related with gender or age; there were no differences between both eyes from the same individual. 2) Reproducibility indexes showed similar values regardless of gender and age. MT and THT reproducibility was higher than that of ChT and TCT. The majority of correct identifications were for ChT. Intensity perceived by females was significantly lower than that by men for ChT. No changes were observed in corneal staining or hyperemia before and after the evaluation. 3) MT was significantly lower in those eyes

with better BCVA and in myopic eyes. No differences were found with contrast sensitivity or ocular dominance.

Conclusions: Thresholds for 4 different types of sensitivity in central cornea have been established in healthy males and females of different age groups. These thresholds were comparable between both eyes regardless of gender and age. The use of the gas eshesiometer was safe and reproducible. The fact that factors such as visual acuity and refractive error (myopia) seemed to influence mechanical corneal sensitivity deserves further investigation.

Time: **10:54-11:06AM**

Title: **Anti-Aging Approach for the Treatment of Dry Eye**

Presentor: **Kazuo Tsubota, M.D.**

Institution: **Keio University School of Medicine**

Abstract:

Eyestrain, associated visual symptoms, and dry eye have shown a marked increase due to visual display terminal (VDT) use, and have become significant health problems affecting the quality of life in industrialized countries. People regularly using a VDT have demonstrated a higher incidence of eyestrain, ocular pain and dry eyes. Excess evaporation of tear fluid due to reduced blinking while focusing has been considered to be a major causative factor in VDT-associated dry eye.

A rat model, mimicking the situation of office workers, was used to evaluate lacrimal function. We invented a unique model based on the concept that gazing is not necessarily only observed in the concentrated tasks such as VDT use, but also can be observed in the spatial orientation that is required for the maintenance of posture, similar to that seen in tightrope walkers. The rat office worker model, placing rats onto a swing in combination with exposure to an evaporative environment, showed chronic reduction of tear secretion. This reduction was recovered when rats were moved to general conditions for 10 days without the swing. VDT syndrome was believed to be related to evaporative dry eye. However, according to our findings, it was a combination of evaporative dry eye and tear deficiency type dry eye. We found that the accumulation of the secretory vesicles was a new mechanism of dry eye, resulting in a new concept for dry eye syndrome.

Since dry eye also increases with aging, we hypothesized that free radicals, which are considered to be the major cause of aging, may be related to dry eye. Superoxide dismutase knockout mouse, in which free radicals were shown to increase in the body, showed dry eye with the accumulation of secretory vesicles in the lacrimal gland, as well as age-related retinal changes. We have also performed the preliminary experiment of calorie restriction (CR), which is believed to have an anti-aging effect for the decreased tearing with aging in rats. We found that the tear volume is maintained in the CR rat. These findings may reveal the mechanism of decreased tear production and secretion in aging and can be the treatment target. At last, I would like to share my personal experience of the anti-aging approach for the treatment of dry eye as a dry eye patient myself.

Time: **11:06-12:00**

Title: **Genes Associated with Longevity in Mice**

Presentor: **Gretchen Darlington, PhD**

Institution: **Baylor College of Medicine**

Abstract:

Mutations in the growth hormone/IGF-1/insulin pathway have a major impact on lifespan and extend longevity in multiple species. This lecture will address the metabolic alterations observed in long-lived mice and speculate on the possible benefits conferred by growth hormone deficiency.

Time: **1:30-1:42PM**

Title: **The Presence of ΔNp63 (+)/Pax 6(-) Human Limbal Basal Epithelial Progenitor Cells**

Presentor: **Shuangling Chen, M.D.**

Co-Authors: **Ying-Ting Chen and Scheffer C. G. Tseng**

Institution: **Tissue Tech, Inc., Ocular Surface Center, and Ocular Surface Research & Education Foundation, Miami, FL.**

Abstract:

Purpose: Because of the lack of consensus biomarker(s) to identify limbal stem cells (SCs), it remains a great challenge to pinpoint their exact location in the limbal basal layer known to express p63. Pax 6, a well known master homeobox gene that directs eye morphogenesis, is expressed in the nucleus of all postnatal ocular surface epithelium. Because downregulation of Pax 6 is an early step during transdifferentiation of adult rabbit corneal transient amplifying cells to epidermal cells when engrafted in mouse embryonic epidermis as well as a hallmark during physiological and pathological squmamous metaplasia of the ocular surface epithelium, we speculate that there exist ΔNp63(+)/Pax 6(-) progenitor cells in the limbal basal epithelium.

Methods: Double immunostaining to ΔNp63 and Pax 6 were performed with counter nuclear staining by Hoechst 33342 in cross-sections and flat mount preparation of human limbal epithlelia, cytospin of dispase-isolated and trypsin/EDTA-treated single cells, and clones generated by single limbal epithelial progenitor cells enriched by rapid adhesion to collagen I-coated dishes cocultured with feeder layers made of murine 3T3 fibroblasts or human amniotic epithelial cells.

Results: Double immunostaining to Pax 6 and ΔNp63 or p63(+) revealed clusters of limbal basal epithelial cells that were positive to nuclear staining of p63 but negative to nuclear staining of Pax 6 in both cross-sections and flat mount preparations. The same double immunostaining also confirmed that these ΔNp63(+)/Pax 6(-) cells were derived from the limbal epithelium, but not from the limbal stroma, by isolating the intact human limbal epithelial sheets by dispase, dissociating them into single cells, and submitting them to the cytospin preparation. When these single limbal epithelial cells were enriched by seeding on collagen I-coated dishes for 15 min and cocultured with 3T3 fibroblasts, all 134 resultant clones were ΔNp63(+)/Pax 6(+). In contrast, 24 clones generated on the feeder layer made of human amniotic epithelial cells consisted of ΔNp63 (+)/Pax 6(-) cells (n=9), ΔNp63(+)/Pax 6(-/+) cells (n=6), and ΔNp63(+)/Pax

6(+) cells (n=9). Furthermore, immunostaining to ABCG2 was uniformly strong for the latter clones, but heterogeneous for the former clones.

Conclusions: Collectively, these results indicate that there exist ΔNp63(+)/Pax 6(-) cells in the limbal basal epithelium. These progenitor cells can be maintained by human amniotic epithelial feeder layers but not by murine 3T3 fibroblast feeder layers, raising a question about the efficacy of using 3T3 fibroblasts to preserve/expand limbal epithelial progenitors. The SC status and plasticity of this p63(+)/Pax 6(-) progenitors awaits further characterization by subcloning experiments.

Support: NIH, NEI, R01EY06819 and R43EY15735 (to SCGT)

Time: **1:42-1:56PM**

Title: **Biochemical Characterization and Anti-inflammatory and Anti-scarring Efficacies for the Covalent Complex of Hyaluronan (HA) and the Heavy Chain (HC) of Inter-α-trypsin Inhibitor Purified from AM Extract**

Presentor: **Hua He, M.D.**

Co-Authors: **[1]Wei Li, [2]David Y. Tseng, [3]Anthony J. Day and [1]Scheffer C. G. Tseng**

Institution: **[1]TissueTech, Inc. and Ocular Surface Center, and [2]Ocular Surface Research Education Foundation, Miami, FL, USA; [3] Wellcome Trust Centre for Cell-Matrix Research, Faculty of Life Sciences, University of Manchester, Manchester, UK.**

Abstract:

Purpose: Abundant hyaluronan (HA) is present in AM stroma, but it remains unknown whether HA is covalently linked with any proteins and contributes to AM's activity against scarring and inflammation.

Methods: Cryopreserved human AM was successively extracted with buffers containing increasing salt concentrations: 150 mM NaCl (A), 1 M NaCl (B), and 4 M guanidine-HCl (C). Additionally, HC-HA complex was purified by water-soluble AM extract (P) via two runs of CsCl/Guanidine HCl ultracentrifugation and reconstituted *in vitro* by HA, IαI and TSG-6. The amount and sizes of HA in these AM extracts were determined by HA ELISA and agarose gel electrophoresis analyses, respectively. The binding of inter-α-trypsin inhibitor (IαI) or tumor necrosis factor-stimulated gene-6 (TSG-6) with HA in AM extracts was examined with hyaluronidase (HAase) digestion or NaOH treatment followed by Western blotting. Anti-inflammatory and anti-scarring assays were examined by macrophage apoptosis and TGF-β1 promoter assays, respectively.

Results: HA was present in all four AM extracts, but was mostly (70.1 ± 6.0 %) in water-soluble Extracts A and P, had an average molecular weight (MW) about $6x10^6$ Daltons (Da), and was covalently linked with heavy chains (HCs) of IαI via a NaOH-

sensitive bond. This complex of HC-HA was also more abundant in Extracts A and P and could be purified by ultracentrifugation and *in vitro* reconstitution. Bikunin (or the light chain of IαI) and TSG-6 were mainly present in Extracts A and C, and not covalently linked with HA. A TSG-6 immunoreactive 50-kDa band was mostly present in Extract A. HC-HA complex purified from water-souluble Extracts A and P or reconstituted *in vitro* was effective in promoting macrophage apoptosis and suppressing TGF-β1 promoter activation. The latter suppression was not mimicked by HMW HA alone and was abolished by digestion with HAase or heat treatment.

Conclusions: The covalent complex of HMW HA and HC is predominantly extracted by a low salt buffer, can be purified from AM, and contributes to the anti-inflammatory and anti-scarring actions of AM and AM extract.

Support: NIH, NEI, R01EY06819 and R43EY15735 (to SCGT)

Time: **1:56-2:08**

Title: **β-catenin gain-of-function mutant leads to hyperplasia but loss-of-function mutant prevents FGF-7-induced neoplasia in the mouse cornea**

Presentor: **Chia-Yang Liu, PhD**

Co-Authors: **Mindy Call[1], Yujing Zhang[1], Tyler Kohl[1], Makoto M. Taketo[2], Winston W.-Y. Kao[1] and Chia-Yang Liu[1]**

Institution: **[1]Crawley Vision Research Center/Department of Ophthalmology, College of Medicine, University of Cincinnati,_[2]Department of Pharmacology, Graduate School of Medicine, Kyoto University**

Abstract:

β-catenin signaling has been implicated in two major biological processes of embryonic development and tumorigenesis. We have previously reported that β-catenin activation was associated with excess FGF-7 induced ocular surface squamous neoplasia (OSSN) in *Krt12$^{rtTA/rtTA}$;tetO-FGF-7* transgenic mice fed doxycycline (Dox) (Chikama *et al*. *Am J Pathol*. 2008, 172: 638-649). To further probe the role of β-catenin in corneal epithelial morphogenesis, homeostasis, and tumorigenesis, conditional loss (ΔE2-6) and gain (ΔE3) -of-function mutations of the β-catenin gene (*Ctnnb1*) were expressed in differentiated corneal epithelium, respectively. Loss of β-catenin from the corneal epithelium did not impact corneal homeostasis, but effectively prevented FGF-7-induced OSSN in Dox-treated *Krt12$^{rtTA/rtTA}$;tetO-FGF-7;tetO-Cre;Ctnnb1$^{lox(E2-6)/lox(E2-6)}$* mice. On the other hand, expression of dominant stable ΔE3 β-catenin in Dox-treated *Krt12$^{rtTA/wt}$;tetO-Cre;Ctnnb1$^{floxedE3/Wt}$* mice resulted in hyperplastic epithelial nodules which invaded into corneal stroma concomitant with profound neovascularization. Moreover, using TOPGAL β-catenin reporter line, X-gal stained cells were highly associated with the epithelial hyperplasia and stromal invasion in the cornea of *Krt12$^{rtTA/Wt}$;tetO-Cre;Ctnnb1$^{floxedE3/Wt}$;TOPGAL* quadruple transgenic mice upon being induction with Dox. Immunofluorescent staining demonstrated that these hyperplastic lesions increased BrdU uptake and the expression of PCNA and p63, but decreased Pax-6 expression in the hyperproliferative nodules. Expression of ΔE3 β-catenin resulted in the loss K12 but gain ectopically K15 expression in the corneal epithelium. Interestingly, MMP-7, known to be a direct β-catenin target gene, was robustly

upregulated but basement membrane components i.e., laminins were drastically down-regulated from E17.5 to P2 and completely lost at P22. Finally, the expression of Shh, Ptc, and Gli3, members in sonic hedgehog signaling pathway, were nearly diminished. Taken together, Loss of β-catenin did not impact corneal morphogenesis and homeostasis, but effectively prevented FGF-7-induced tumor. Aberrant activation of β-catenin signaling leads to hyperplastic transformation and disrupts corneal homeostasis. These results suggest that critical regulation of β-catenin signaling warrants a normal corneal epithelial homeostasis and inhibitors against activated β-catenin maybe a potential therapeutic strategy for treating OSSN.

Time: **2:08-2:20**

Title: **Significance of p120/Kaiso Signaling in Unlocking the Mitotic Block Mediated by Contact-inhibited Human Corneal Endothelial Cell Monolayers**

Presentor: **Scheffer Tseng, M.D., PhD**

Co-Authors: **Ying-Ting Zhu, Szu-Y Che**

Institution: **TissueTech, Inc., Ocular Surface Center, and Ocular Surface Research Education Foundation, Miami, FL, USA.**

Abstract:

Purpose: The mitotic block mediated by contact inhibition is universal and explains why human corneal endothelial cells (HCEC) have a limited proliferative capacity *in vitro* and *in vivo,* a phenomenon also strongly correlated with aging. When cadherins are disrupted in the adherent junction, two signaling pathways can potentially be elicited by liberating β-catenin and p120 catenin (abbreviated as p120), respectively, to reach the nucleus. The former acts as a transcriptional coactivator through binding with TCF/LEF transcription factor, while the latter releases the repressor activity of Kaiso. We speculate that these two signaling pathways play a different role in unlocking the mitotic block caused by contact-inhibited HCEC. **Methods:** In contact-inhibited HCEC monolayers, we have discovered that the aforementioned two pathways could be activated by 2 mg/ml EDTA for 1 h followed by 20 ng/ml bFGF and by transfection with siRNA to p120, respectively. Twenty four hours before termination, monolayers were labeled with 10 μM BrdU for 24 h. Immunostaining to p120, Kaiso, β-catenin, TCF/LEF, ZO-1, N-cadherin, Na^+, K^+, ATPase, S100A4, and BrdU were compared. **Results:** Phase contrast microscopy showed that the hexagonal pattern of HCEC was disrupted by EDTA/bFGF leading to more spindle cells, but maintained by transfection with p120 siRNA. Immunostaining confirmed that β-catenin, but not p120, was disrupted from the adherent junction and translocated to the nucleus with an increase of TCF/LEF nuclear staining in EDTA/bFGF-treated cultures. In contrast, p120, but not β-catenin, from the adherent junction, was disrupted by p120 siRNA transfection, leading to nuclear translocation and release of Kaiso from the nucleus. Although both treatments resulted in significantly higher BrdU labeling than their respective controls, the normal HCEC phenotype was restored upon withdrawal of p120 siRNA leading to effective *ex*

vivo expansion. In contrast, the EDTA/bFGF treatment resulted in the loss of intercellular distribution of N-cadherin, and nuclear localization of S100A4. Upon addition of TGF-β. there was evident phenotypic change consistent with endothelial-mesenchymal transformation into fibrous metaplasia. **Conclusions:** These results collectively indicate that activation of the p120/Kaiso pathway unlocks the mitotic block mediated by contact inhibition via the formation of adherent junction, but retaining the normal phenotype. Although proliferation is also promoted by activation of the β-catenin/Wnt pathway, the resultant cellular change risks in the development of endothelial mesenchymal transformation into a pathologic state of retrocorneal membrane. They shed new insight on not only how HCEC can be best engineered *ex vivo*, but also how new therapeutics may be developed to switch on and off cellular proliferation without disrupting the intercellular junction *in vivo* in tissues where mitosis is known to be inhibited because of contact inhibition in the aging process

Author Disclosure: **S.C.G. Tseng**, Tissue Tech Inc, E, I, and P; **S. Chen** and **Y.T. Zhu**, Tissue Tech, Inc., E.

Support: NIH, NEI, R01EY06819 and R43EY15735 (to SCGT)

Time: **2:20-2:32PM**

Title: **The Boston Ocular Surface Prosthesis in the management of severe exposure keratopathy in hospitalized burn patients**

Presentor: **Sheri DeMartelaere, M.D.**
Perry Rosenthal, M.D.

Co-Authors: **Anthony J. Johnson, MD (1), Evan M. Renz, MD (2), Perry Rosenthal, MD (3)**

Institution: **1 San Antonio Uniformed Services Health Education Consortium, San Antonio, TX**
2 United States Army Institute of Surgical Research, Fort Sam Houston, TX
3 Boston Foundation for Sight, 624 Hillside Ave, Suite 205, Needham, MA

Abstract:

Introductory Sentence: We herein present a year-long review of the Boston Ocular Surface Prosthesis (BOSP) in the treatment of refractory exposure keratopathy in hospitalized burn patients.

Methods: A retrospective chart review of burn patients at the United States Army Institute of Surgical Research (USAISR) from January 2008 to June 2009.

Results: Placement of the BOSP was performed on 7 patients, 3 unilaterally and 4 bilaterally, for the treatment of severe exposure keratopathy. All were male, age 21 to 37 years, TSA 34 to 92%; all with deep 2nd to 4th degree burns to the eyelids and severe cicatricial lagophthalmos. 5 patients had prior release of eyelid cicatrix with skin or allografting; all had prior tarsorrhaphies. 4 patients had associated corneal infiltrates. All epithelial defects and corneal infiltrates resolved with application of the BOSP.

Conclusions: The Boston Ocular Surface Prosthesis provides an effective treatment modality for patients with severe exposure keratopathy who failed to respond adequately to other treatment modalities. This treatment also allows for the placement of fortified antibiotics in the reservoir of the lens to aid in the prevention and management of infectious keratitis. Challenges remain for managing ocular exposure of eyes with cicatricial lagophthalmos during periods of non-wear.

Bibliography:

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The views of these authors are their own and do not purport to reflect the position of the Army Medical Department, the Department of the Army, or the Department of Defense.

Time: **2:32-2:44PM**

Title: **Extreme Exposure**

Presentor: **Deborah Jacobs, M.D.**

Co-Authors: **Trisha Hussoin, BS/BA (1), Perry Rosenthal, MD (1)**

Institution: **1. Boston Foundation for Sight, 464 Hillside Avenue, Suite 205, Needham, MA**
2. Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, MA

Abstract:

Purpose: To report our experience with the Boston Ocular Surface Prosthesis (BOS-P) in the rehabilitation of patients with "Extreme Exposure."

Methods: Retrospective medical record review of 5 cases referred for lagophthalmos in which there was keratopathy unresponsive to conventional therapy. In each case exposure keratitis was compounded by other factors, or tarsorrhaphy was insufficient or problematic for vision.

Results:

Case #1: 44 y.o. M s/p resection, radiation, and reconstruction of R sided facial sarcoma in setting of underlying chronic Graft-versus-Host Disease. BCVA 20/25 and 20/20 in BOSP OU.

Case #2: 36 y.o. M with burns to face and upper extremities. BCVA improved 20/25 to 20/15 in BOSP OD, 20/80 with tarsorrhaphy OS.

Case #3: 69 y.o. M with Graves' orbitopathy and progressive external ophthalmolplegia. BCVA 20/15 and 20/20 in BOSP OU after substantial tarsorrhaphies reversed OU.

Case #4: 57 y.o.M with paralytic exposure and neurotrophic keratitis s/p PK X 2, s/p resection of acoustic neuroma. BCVA improved 20/400 to 20/50 in BOSP OD, 20/20 OS.

Case #5: 54 y.o. F s/p enucleation after hatchet assault, with cicatricial and paralytic exposure of remaining eye. BCVA 20/20 in BOSP in only eye, after failure of multiple tarsorrhaphies.

Conclusions: The BOS-P is a useful adjunct in the rehabilitation of "Extreme Exposure."

Time: **2:44-2:56PM**

Title: **Bevacizumab delivered via the Boston Ocular Surface Prosthesis in the treatment of corneal neovascularization**

Presentor: **Deborah Jacobs, M.D.**

Co-Authors: **Mira Lim, MD (2), Perry Rosenthal, MD (1), Karen G. Carrasquillo, PhD, OD (1)**

Institution: **1. Boston Foundation for Sight, 464 Hillside Avenue, Suite 205, Needham, MA**
2. Massachusetts Eye and Ear Infirmary, 243 Charles Street, Boston, MA

Abstract:

Purpose: To report clinical experience with topical bevacizumab delivered by the Boston Ocular Surface Prosthesis (BOS-P) in the treatment of corneal neovascularization.

Methods: This is a retrospective review of medical records on 5 patients with ocular surface disease, already wearing the BOS-P on a daily basis, who were treated with non-preserved bevacizumab 1%, 1 drop delivered BID via the reservoir of the BOS-P, for sight threatening superficial corneal neovascularization. Data collected included best-corrected visual acuity, slit lamp exam, and slit-lamp photography at each visit. Patients were monitored at 1 week, 1 month, then monthly for several months. Treatment was continued for 3 months and then tapered over 1-2 months.

Results: Experience with male and female patients, ages 26-65, with a range of underlying diagnoses including neurotrophic keratitis, secondary Sjogren's syndrome, and Stevens-Johnson syndrome is reported. There was improvement in vision or comfort in all patients. No adverse local or systemic effects were noted. Regression of active vessels, when it occurred, was durable, with no recurrence after cessation of the drug with follow-up, in some cases, of more than 1 year.

Conclusions: Controlled studies and long term follow up are required to determine the optimal dose and duration of topical bevacizumab therapy for corneal neovascularization. The role of the BOS-P in enhancing delivery and/or protecting the corneal surface warrants further investigation. The clinical impact in these first five patients shows great promise.

Time: **3:30-3:42PM**

Title: **Adaptive Immune Profile of Squamous Metaplasia Development in Autoimmune Regulator-deficient Dry Eye**

Presentor: **Ying Ting Chen, M.D.**

Co-Authors: **Shimin Li, Karina Nikulina, Travis Porco, Marianne Gallup, Nancy McNamara**

Institution: **Francis I. Proctor Foundation, University of California, San Francisco**

Abstract:

Purpose: Sjögren's syndrome (SS) is one of the most prevalent autoimmune disorders. Nine out of tens patients are women and the average age of onset is late 40's. Squamous metaplasia is reported to be the most frequently observed cytological change of the ocular surface in SS patients and increasing evidence suggests that it may be a primary manifestation of cellular immune dysregulation. While the detailed immunopathological mechanism underlying keratinization of the ocular muco-epithelium in SS remains unclear, mice deficient in the autoimmune regulator gene (*Aire*) demonstrate SS-like pathological changes in the exocrine organs and ocular surface, including squamous metaplasia. Using small proline rich protein 1B (SPRR1B) as a biomarker for squamous metaplasia, we sought to determine the specific immune events that predict progression of squamous metaplasia in the setting of Aire-deficiency.

Methods: Lissamine green staining, goblet cell density (GCD) and corneal SPRR1B were compared in Aire-sufficient and -deficient mice at ages 4, 8 and 16 weeks. Corneal, limbal and conjunctival infiltration of $CD4^+$ and $CD8^+$ T cells, as well as $CD11c^+$ and MHC class II ($I\text{-}A^{d+}$) dendritic cells (DCs), were examined at the same time points. Ordinary least squares regression was used to model SPRR1B's relationship with lissamine green staining, GCD and immune cell infiltration. Adoptive transfer of $CD4^+$ and/or $CD8^+$ lymphocytes from Aire-deficient mice to SCID recipients was performed to verify the role of T cells in the development of squamous metaplasia.

Results: Lissamine green staining was present in Aire-deficient mice by 4 weeks of age and increased over time. Compared to Aire-sufficient controls, conjunctival GCD decreased and corneal SPRR1B increased in Aire-deficient mice, with significant differences noted at both 8 and 16 weeks. Immune-mediated $CD4^+$ T cell infiltration of

the conjunctiva and limbus peaked at 8 weeks and then decreased. In contrast, corneal T cell infiltration continued to increase over time, reaching a maximum cell number at 16 weeks. $CD11c^+$ myeloid-derived DCs were found in the conjunctiva and limbus at all time points. As the mice aged, there was a notable increase in corneal $CD11c^+$ cell counts, while the dynamic of activated MHC class II^+ DCs was nearly identical to that of $CD4^+$ T cells; peaking first in the limbus at 8 weeks with maximum infiltration of the cornea by 16 weeks. As expected, corneal SPRR1B levels were strongly related to lissamine green staining of the ocular surface. The presence of activated DCs in the cornea was most prognostic of corneal SPRR1B. SPRR1B expression was also largely dependent on the presence of precursor DCs, activated DCs and $CD4^+$ T cells in the limbus. Elevated levels of corneal SPRR1B in SCID mice following adoptive transfer of Aire-deficient $CD4^+$ T cells, confirmed a direct link between effector $CD4^+$ T cells and squamous metaplasia.

Conclusions: Aire-deficient mice represent a useful model to study Sjögren-like autoimmune-mediated ocular surface disease. Results of the current study suggest that squamous cell precursor protein, SPRR1B, provides an important read-out to evaluate ocular surface damage and specific events related to immune-mediated inflammation. Results define an appropriate time frame for interventional studies and identify $CD4^+$ T cells as the target for potential immunotherapy in the treatment of inflammation-mediated keratinizing ocular surface disease.

Time: **3:42 – 3:54 PM**

Title: **Evaluation of Th1 and Th17 Responses in a Mouse Model of Experimental Lacrimal Keratoconjunctivitis.**

Presentor: **Michael E. Stern, PhD**

Co-Authors: **Stephen C. Pflugfelder[2], Karyn F. Siemasko[1], Christopher Schaumburg[1], Jianping Gao[1], Cintia De Paiva[2], Virginia L. Calder[3], Margarita Calonge[4], Jerry Y. Niederkorn[5]**

Institution: **[1]Allergan, Irvine, CA; [2]Baylor College of Medicine, Houston, TX; [3]University College London, United Kingdom; [4] IOBA, University of Valladolid, Spain; [5]Ophthalmology, University of Texas Southwestern Medical Center, Dallas, TX.**

Abstract:

Purpose: Emerging evidence suggests that Th17 cells are involved in the autoimmune-mediated immunopathogenesis of dry eye. We have reported that mouse corneas exposed to desiccating stress are immunogenic and induce proliferation of CD4+ T cells from mice with experimental lacrimal keratoconjunctivitis (LKC). The purpose of this study was to determine if proliferating CD4+ T cells from LKC mice are Th17 cells.

Methods: CD4+ T cells "responders" from control or 7 days desiccating stress (DS; low humidity <40%, scopolamine treatment, and direct air flow from a fan) C57BL/6 wild type mice were isolated from superficial cervical lymph nodes and spleens, labeled with carboxyfluorescein succinimidyl ester (CFSE) to track proliferating cells (low CFSE=proliferating; high CFSE=non proliferating), and co-cultured with control or 7 day DS corneal and conjunctival wedges "stimulators." At the end of 4 days in co-culture, cells were stained with anti-T-bet (Th1), anti-RORγt (Th17) and anti-GATA3 (Th2) antibodies and analyzed by flow cytometry. Cells positive for the T cell-subset-specific transcription factors were gated and evaluated for CFSE expression *i.e.* proliferation.

Results: Co-culture of control CD4+ T cells with control wedges yielded 3.04% GATA-3-specific CD4+ T cells that were proliferating (low-level CFSE expression), suggesting that the normal homeostatic environment of the ocular surface favors Th2 polarization.

In contrast, 7 day DS CD4+ T cells co-cultured with 7 day DS conjunctival and corneal wedges yielded marked Th1 (T-bet, 5.18%) and Th17 (RORγt, 5.62%) specific CD4+ T cell proliferation. No GATA-3 positive cells were detected under the DS conditions. Furthermore, there was minimal Th1 (T-bet, 0.76%), Th2 (GATA-3+, 0.72%) and Th17 (RORγt, 0.96%) specific T cell proliferation when control CD4+ T cells were co-cultured with 7 Day DS wedges; similar results were found when 7 day DS CD4+ T cells were co-cultured with control wedges (T-bet, 0.30%; GATA-3, 0.84%; RORγt, 0.36%).

Conclusions: Proliferating CD4+ T cells isolated from DS mice and co-cultured with DS conjunctival and corneal wedges expressed the Th17-specific transcription factor RORγt and the Th1 transcription factor T-bet. These results provide additional evidence that Th17 cells play a role in the immunopathogenesis of experimental dry eye and are a valid target for therapeutic intervention.

Time: **3:54-4:06PM**

Title: **Transforming growth factor beta (TGFβ) and cytokine gene expression in dry eye patients**

Presentor: **Srihari Narayanan, PhD, FAAO**

Co-Authors: **Corrales RM,[2,3] Herreras JM,[3, 2] Enriquez-de-Salamanca A,[3, 2] Saez V,[3, 2] Cordero Y,[3] Calonge M [3, 2]**

Institution:
1. **Salus University, Pennsylvania College of Optometry, Elkins Park, PA (USA)**
2. **CIBER-BBN, Valladolid, (Spain)**
3. **Ocular Surface Group-IOBA, Universidad de Valladolid, Valladolid, (Spain)**

Abstract:

Purpose: Previous studies have described expression of some inflammatory cytokines in human and experimental dry eye (DE). One of these cytokines is TGF-β, which plays a relevant role in cell growth, differentiation and death and is an important ligand in modulation of cell behavior in ocular tissues. We investigated the expression of TGF-β (isoforms and receptors) and several cytokine genes, in the conjunctival epithelium of DE patients compared to that of healthy subjects.

Methods: Conjunctival impression cytology (CIC) samples were obtained from 48 DE patients (16 males, 29 females; mean age 62.7±8.3 years) and 76 healthy subjects (36 males, 40 females; mean age 62.6±8.3 years). The DE patients had significantly higher scores on the McMonnies DE questionnaire, were tear-deficient (Schirmer-1 < 5mm) and had a tear break-up time < 10 seconds. Following RNA extraction and cDNA synthesis, real-time PCR was performed using specific primers to evaluate expression of genes reported previously in DE: MMP-1, MMP-3, MMP-9, TRAIL, TGF-β1, TGF-β2, hBD-1, and hBD-2, as well as genes not studied before in DE: IFN-γ, TRAIL-R1, TRAIL-R2, TGF-β3, TGFβ-RI, TGFβ-RII, and TGFβ-RIII. Two different detection systems were used: Taqman Probes and SYBR® Green I. A housekeeping gene (GAPDH) was amplified to normalize the amount of expression levels. Non-template control and RNA were used to test contamination. All samples were tested in duplicate. A dissociation curve was performed to verify the identity of each gene amplification product when SYBR® Green I dye detection was used. Results were analyzed by

applying the comparative Ct method using the data obtained from healthy subjects as calibrator. The student t-test for independent samples was used for statistical analysis.
Results: All genes were expressed in all samples. There was no difference in expression in conjunctival TRAIL, TRAIL-R1, TRAIL-R2, MMP-1, IFN-γ and hBD-1 between the two groups. Conjunctival MMP-3 ($p<0.0001$), MMP-9, and hBD-2 ($p<0.05$) were significantly upregulated in the DE group. There were no differences in TGF-β1, TGFβ-RI and TGFβ-RII expression between DE patients and healthy subjects. TGF-β2 and TGF-β3 were significantly ($p<0.01$) upregulated, almost 3 and 2-fold, respectively, in the DE samples compared to healthy subjects. TGFβ-RIII was significantly downregulated ($p<0.01$), almost 2-fold, in the DE group.
Conclusion: Our results support previous experimental as well as human data with respect to an inflammatory basis for the pathogenesis of DE disease. We postulate that hBD-2 might play a protective role at the ocular surface of DE patients who may be at risk for microbial keratitis. MMP-3 upregulation in conjunctival epithelia of DE patients indicates an important role for this metalloproteinase in the pathogenesis of ocular surface inflammation in DE. Additionally, this study reinforces the relevance of TGFβ in DE and the importance of studying its three known isoforms and receptors.

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Time: **4:06-4:18PM**

Title: **Amniotic Membrane Application in the Treatment of Acute Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis: Improved Outcome in a Series of Seven Biopsy-proven Cases**

Presentor: **Kimberly Sippel, M.D.**

Co-Authors: **Edward Lai, M.D., Jennifer Yang, M.D., Jayati Sarkar, M.D.**

Institution: **Weill Cornell Medical School**

Abstract:

Purpose: To describe the results of a novel treatment approach to the acute ophthalmic management of Stevens-Johnson syndrome and toxic epidermal necrolysis.

Methods: Patients with biopsy-proven Stevens-Johnson syndrome or toxic epidermal necrolysis admitted to either the Pediatric Intensive Care Unit or the Burn Unit at the New York-Presbyterian Hospital were treated with the application of amniotic membrane to the ocular surface within twelve days of the onset of the disease. The method of application varied from full coverage of the entire ocular surface, including the palpebral conjunctiva and lid margins, to application of a Prokera™ device alone, affording coverage of only the cornea and perilimbal conjunctiva.

Results: Fourteen eyes of seven patients were treated. Two patients expired during the hospitalization. Mean follow-up time of the surviving patients was nine months. All surviving patients in whom the entire ocular surface was treated retained best corrected visual acuities of 20/25 or better, an intact ocular surface with the exception of mild lid margin keratinization, and good ocular comfort. One patient in whom the ocular surface was treated with the exception of the lid margins displayed significant lid margin keratinization, but has otherwise retained an intact ocular surface and visual acuities of 20/20 in each eye. The patient treated with the Prokera™ device alone developed a corneal perforation in one eye.

Conclusions: The use of amniotic membrane coverage of the ocular surface during the acute phase of Stevens-Johnson syndrome and toxic epidermal necrolysis is associated with the preservation of good visual acuity, an intact ocular surface, and good ocular comfort. It is, however, imperative that the entire ocular surface, including the palpebral conjunctiva, as well as lid margins, be covered with amniotic membrane.

Time: **4:18-4:30PM**

Title: **Human Corneal Epithelium-Derived Thymic Stromal Lymphopoietin Links the Innate and Adaptive Immune Responses via Toll-Like Receptors and Th2 Cytokines**

Presentor: **De-Quan Li, M.D., PhD**

Co-Authors: **Ping Ma, MD; Fang Bian, MD, PhD; Xiaofen Zheng, MD; Stephen C Pflugfelder, MD.**

Institution: **Ocular Surface Center, Cullen Eye Institute, Department of Ophthalmology, Baylor College of Medicine, Houston, Texas, USA**

Abstract:

This study was to explore the crucial role of human corneal epithelium-derived pro-allergic cytokine thymic stromal lymphopoietin (TSLP) in initiation and regulation of immune responses. TSLP was found to be expressed by human corneal epithelial cells. When challenged by 11 microbial ligands respective to 9 TLRs, TSLP mRNA and protein in corneal epithelial cells were largely induced in a concentration-dependent fashion by polyI:C, Flagellin and FSL-1, ligands for TLRs, 3, 5 and 6, respectively. Compared with control, TSLP mRNA, evaluated by real-time PCR, increased up to 60-, 12- and 8-fold, and TSLP protein, measured by ELISA, increased by 67-, 19- and 7-fold, by these 3 ligands, respectively. Proinflammatory cytokines (TNF-α and IL-1β) and Th2 cytokines (IL-13 and IL-4) moderately induced TSLP production. IL-4 or IL-13 strongly synergized with polyI:C, Flagellin and TNF-α to promote TSLP production in ex vivo tissues and in vitro cultures of corneal epithelium. Western blot and immunostaining showed that polyI:C, Flagellin or TNF-α induced NF-kB p65 nuclear translocation. NF-kB inhibitor quinazoline blocked both NF-kB activation and TSLP production induced by these stimuli. These findings provide the first evidence of TSLP induction in human eye, and demonstrate a novel phenomenon that corneal epithelium-derived TSLP links the innate and adaptive immune responses. It suggests that TSLP could be a novel therapeutic target for TSLP-mediated allergic and inflammatory conditions.

Time: **4:42-4:54PM**

Title: **Azithromycin suppresses pro-inflammatory mediators stimulated by a TLR2 ligand zymosan in human corneal epithelial cells.**

Presentor: **Nan Zhou, M.D.**

Co-Authors: **Ping Ma, M.D., De-Quan Li, M.D., Ph.D., Stephen C. Pflugfelder, M.D.**

Institution: **Ocular Surface Center, Cullen Eye Institute, Department of Ophthalmology, Baylor College of Medicine, Houston, Texas**

Abstract:

This study was to explore the suppressive effects of azithromycin on pro-inflammatory mediators stimulated by a fungus component zymosan, a TLR2 ligand, in human corneal epithelial cells (HCECs). Primary HCECs were treated with zymosan (1-50 μg/ml) in the absence or presence of azithromycin (1-50 μg/ml), TLR2 antibody or NFkB inhibitor (NFkB-I) for 4-48 hours. The cells were subjected to total RNA extraction, RT-real-time PCR, Immunofluorescent staining and Western blot analysis. The supernatants of culture media for 48 hours were collected for Luminex immunobead assays. The results showed that zymosan concentration-dependantly stimulated the mRNA expression of pro-inflammatory mediators including TNF-α, IL-1β, IL-6, IL-8, RANTES, MMP-1, MMP-3 and MMP-9, in HCECs. Luminex immunobead assays confirmed the stimulation at protein levels. Azithromycin, pre-added in the cultures for one hour, suppressed the zymosan-induced mRNA expression and protein production of these pro-inflammatory cytokines and chemokines. Immunofluorescent staining and Western blot analysis showed TLR2 antibody and NF-κB-inhibitor blocked the NF-κB p65 nuclear translocation and further reduced the up-regulation of TNF-α, RANTES and MMP-3, induced by zymosan. These findings indicate that azithromycin has a potential efficacy to suppress inflammatory responses stimulated by zymosan through TLR2 and NF-κB pathways in human corneal epithelial cells.

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