468 Conjunctiva, Lacrimal and Meibomian glands and contact lenses
Wednesday, May 10, 2017 3:45 PM–5:30 PM
Ballroom 2 Paper Session
Program #/Board # Range: 4722–4728
Organizing Section: Cornea

Program Number: 4722
Presentation Time: 3:45 PM–4:00 PM
Resolvin D2 increases conjunctival goblet cell intracellular [Ca^{2+}] by increasing cellular cAMP to activate protein kinase A
Darlene A. Dartt[1, 4], Nora Botten[1, 2], Robin R. Hodges[1, 4], Dayu Li[1, 4], Jeffrey Bair[1, 4], Marie Shatos[1, 4], Tor Utheim[1, 3], Charles Serhan[5, 6]. [1]Schepens Eye Research Institute/MEEI, Boston, MA; [2]Medical Biochemistry, University of Oslo, Oslo, Norway; [3]Medical Biochemistry, University of Oslo, Oslo, Norway; [4]Ophthalmology, Harvard Medical School, Boston, MA; [5]Anesthesiology, Perioperative and Pain Medicine, Brigham and Womens Hospital, Boston, MA; [6]CET&RI, Harvard Institutes of Medicine, Harvard Medical School, Boston, MA.
Purpose: Specialized substances of resolution (SPMs) such as the resolvins, lipoxins, and protectins function to actively resolve pathological inflammatory processes. Another critical function is to maintain tissue homeostasis in health. The purpose of this study was to determine if an SPM of the resolvin (Rv) family, RvD2, regulates conjunctival goblet cell (GC) function and uses the same cellular signaling pathways as observed with RvD1, RvE1, and lipoxin A_4.
Methods: Rat conjunctival GCs were grown from tissue explants and first passage cells were used. Presence of the RvD2 receptor GPR18 was determined by RT-PCR. To measure intracellular [Ca^{2+}] ([Ca^{2+}]$_i$) cells were incubated with the calcium indicator dye fura2 and [Ca^{2+}]$_i$ measured. RvD2 (10^{-10}-10^{-8}M) was added alone or inhibitors were added 30 min prior to RvD2 and [Ca^{2+}]$_i$ quantified. RvD1, VIP, or the cholinergic agonist carbachol were used as positive controls.
Results: Message for the RvD2 receptor GPR18 was expressed in GCs at 163 base pairs, the correct base pair number. RvD2 significantly increased [Ca^{2+}]$_i$ to the same magnitude as RvD1 with 10^{-8} M giving the maximal response for both Rvs. The protein kinase A inhibitor H-89 blocked the RvD2, but not the RvD1, stimulated increase in [Ca^{2+}]$_i$. Blockage of the response to VIP was the positive control. Forskolin that increases cellular cAMP levels independent of receptor stimulation and IBMX that prevents breakdown of cAMP each significantly increased [Ca^{2+}]$_i$. Removal of extracellular Ca^{2+} and inhibition of the IP3 receptor with 2-APB blocked the RvD2 stimulated increase in [Ca^{2+}]$_i$. This finding suggests that RvD2 uses IP3-dependent intracellular Ca^{2+} stores similarly to the positive control carbachol.
Conclusions: Unlike RvD1, RvE1, and lipoxin A_4, RvD2 uses the cAMP/protein kinase A signaling pathway to increase intracellular Ca^{2+}levels and could play a role in maintaining conjunctival GC homeostasis in health.
Commercial Relationships: Darlene A. Dartt, None; **Nora Botten**, None; **Robin R. Hodges**, None; **Dayu Li**, None; **Jeffrey Bair**, None; **Marie Shatos**, None; **Tor Utheim**, None; **Charles Serhan**, Resolvyx (P)
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Program Number: 4723
Presentation Time: 4:00 PM–4:15 PM
Pathological changes of meibomian gland and ocular surface in Apolipoprotein E knockout mice
JingHua Bu[1, 2], Yang Wu[1, 2], Xin He[1, 2], chengyou zuo[1, 2], Shangkun Ou[1, 2], ZUGUO LIU[1, 2], Wei Li[1, 2]. [1]Eye Institute of Xiamen University, Xiamen, Fujian, China; [2]Fujian Provincial Key Laboratory of Ophthalmology and Visual Science, Xiamen, Fujian, China.
Purpose: To investigate the pathological changes of meibomian gland (MG) and ocular surface tissues in Apolipoprotein E knockout ($ApoE^{-/-}$) mice, and to illustrate the effect of ApoE on meibomian gland function.
Methods: The ocular surface of $ApoE^{-/-}$ male mice (n=30) aged from 3 months to 7 months, and age and sex matched wild type mice (n=30) was observed under slit lamp microscope. MG tissue sections underwent H&E staining, Oil Red O staining, TUNEL assay, and immuno-fluorescence staining for K10, Fabp5, Ki67, K6a, p63, PPAR-gamma, NF-kB p65, p-NF-kB p65, caspase 3, caspase 8, and immune-histochemical staining for CD45. Real-time RT-PCR and Western blot was performed to detect above mentioned gene expression in MG tissues. Lipid metabolism related genes expression in MG were also detected by real-time RT-PCR.
Results: Eyelid thickening, keratinization and corneal neovascularization occurred in $ApoE^{-/-}$ mice at the age of 5 months, and the changes were more obvious at 7 months. H&E staining showed hypertrophy of the meibomian gland acinus, and Oil red O staining showed accumulation of lipid in MG acinus of $ApoE^{-/-}$ mice. Both K10 and Fabp5 expression were increased, while Ki67, K6a, p63 were decreased in $ApoE^{-/-}$ mice compared to the wild type mice. Cytoplasmic and nuclear expression of PPAR-gamma was decreased, NF-kB p65, p-NF-kB p65, caspase 3, and caspase 8 expression were higher in the $ApoE^{-/-}$ mice compared to the wild type mice. TUNEL assay showed more positive cells in the meibomian gland acinus of $ApoE^{-/-}$ mice. CD45 positive cell infiltration increased from 5 months to 7 months in $ApoE^{-/-}$ mice. Lipid metabolism related genes such as FFAR1, PAR3, ACSL3, and LIPC were decreased in $ApoE^{-/-}$ mice compared to the wild type mice.
Conclusions: ApoE knockout mice represented abnormal meibomian gland acinar cell proliferation, differentiation, and lipid metabolism, they also showed eyelid and corneal pathological changes. These mice may be used as model to study the pathophysiology of meibomian gland dysfunction.
Commercial Relationships: JingHua Bu; **Yang Wu**, None; **Xin He**, None; **chengyou zuo**, None; **Shangkun Ou**, None; **ZUGUO LIU**, None; **Wei Li**, None

Program Number: 4724
Presentation Time: 4:15 PM–4:30 PM
THE ROLE OF PANX1 GLYCOPROTEIN IN LACRIMAL GLAND INFLAMMATION
Helen P. Makarenkova[1], Anastasia Gromova[1], Xin Tang[1], Driss Zoukhri[2], Valery I. Shestopalov[3, 4]. [1]Cell and Molecular Biology, The Scripps Research Institute, La Jolla, CA; [2]Department of Diagnosis and Health Promotion, Tufts University School of Dental Medicine, Boston, MA; [3]Department of Ophthalmology, Bascom Palmer Eye Institute, University of Miami school of Medicine, Miami, FL; [4]Department of Cell Biology, Bascom Palmer Eye Institute, University of Miami school of Medicine, Miami, FL.
Purpose: Sjögren's Syndrome is a systemic chronic autoimmune inflammatory disease that targets primarily the salivary and lacrimal glands (LG). Currently there is no cure, therefore cell-based regenerative therapy may represent a viable option.

We have shown that progenitor cell engraftment is more efficient during LG regeneration phase vs acute inflammation phase. The induction of inflammation in the LG is facilitated by extracellular ATP and mediated by the pannexin-1 (Panx1) membrane channel glycoprotein. We hypothesized that suppression of inflammation through manipulation of Panx1 pathway activity can stimulate epithelial cell progenitors (EPCPs) engraftment.
Methods: The expression of pannexins in mouse LG was assayed by qRT-PCR, immunohistochemistry and RNA-sequencing. Acute LG inflammation was induced by intraglandular injection of 1 μg interleukin-1 alpha (IL1α). Thrombospondin-1-null (TSP-1-null) mouse was used as a model of chronic LG inflammation. EPCPs transplantation was performed to study cell engraftment. Panx1-specific blocking peptide ^{10}panx (100 μm, sequence WRQAAFVDSY) prior to EPCP cell transplantation. Cell engraftment and area of inflammation were analyzed by microscopy. The unpaired two-tailed Student's t-test was used to determine statistical significance.
Results: Two pannexin isoforms, Panx1 and Panx2 were detected in the LG epithelial cells at both mRNAs and protein level. In the acute model of regeneration, Panx1 and pro-inflammatory cytokines and caspases 1 and 4 were strongly upregulated during the inflammatory phase, 1-3 days after IL-1 injection. In contrast, Panx2 was increased only during LG regeneration. The analysis of EPCP engraftment showed a significant and reproducible positive correlation between the ^{10}panx peptide treatment and the number of engrafted cells per cross section, with an average increase of engrafted cells of approximately 63% relative to untreated controls. The same strategy showed efficacy in the TSP-1-null mouse chronic model: blocking inflammation by LG treatment with either ^{10}panx peptide or by silencing caspase 4 using dsRNAi showed a significant decrease of total area (cumulative foci) affected by inflammation.
Conclusions: Our results suggest that the manipulation of Panx1 and/or Caspase4(11) is a new beneficial strategy to enhance donor cell engraftment and LG regeneration through the reduction of inflammation.
Commercial Relationships: Helen P. Makarenkova, None; **Anastasia Gromova**, None; **Xin Tang**, None; **Driss Zoukhri**, None; **Valery I. Shestopalov**, None
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Presentation Time: 4:30 PM–4:45 PM
Dual roles of IL-33 in ragweed-induced mouse conjunctivitis models under lacrimal gland excision
Yosuke Asada[1, 2], Susumu Nakae[2], Nobuyuki Ebihara[3], Akira Matsuda[1]. [1]Ophthalmology, Juntendo University School of Medicine, Tokyo, Japan; [2]Laboratory of Systems Biology, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan; [3]Ophthalmology, Juntendo Urayasu Hospital, Chiba, Japan.
Purpose: We previously reported the essential roles of IL-33 in ragweed(RW)-induced experimental allergic conjunctivitis (EAC), identified existence of group 2 innate lymphoid cells (ILC2s) in lacrimal glands (LG) and effects of IL-33 with LG excision (ARVO2014, 2016). In this study, we further examined the roles of IL-33 in RW-EAC models with LG excision.
Methods: LG excision was performed unilaterally on 8-week-old female BALB/c-IL-33 deficient and BALB/c wild-type mice. The mice were sensitized with RW in alum, and then challenged 4 times with RW eye drops. Twenty minutes after the last eye-drop challenge, the EAC was evaluated using the scores for chemosis, redness, lid edema, tearing, discharge, and the number of scratching behaviors was counted for 20 minutes.
24 hours after the last challenge, the eyeballs were collected for histological analyses.
Results: Significant reduction of the numbers of scratching behaviors was observed in the RW-EAC models using IL-33 deficient mice compared to wild type mice. Histological examination revealed augmented epithelial cell defects and neutrophil predominant inflammatory cell infiltration in the ocular surface of the LG-excised RW-EAC models using IL-33 deficient mice compared to those of wild-type mice.
Conclusions: The existence of IL-33 in mouse ocular surface caused ocular irritation and induced scratching behaviors in RW-EAC models. On the other hand, IL-33 may contribute to protect eye surface from neutrophil predominant ocular surface inflammation in RW-EAC models with LG excision.
Commercial Relationships: Yosuke Asada, None; **Susumu Nakae**, None; **Nobuyuki Ebihara**, None; **Akira Matsuda**, None

Program Number: 4726
Presentation Time: 4:45 PM–5:00 PM
Attenuation of Murine Lacrimal Gland Chronic Graft-Versus-Host Disease by Oral Injection of Tranilast
Eisuke Shimizu, Shin Mukai, Yoko Ogawa, Kazuo Tsubota. Ophthalmology, Keio University, Shinjuku-ku, Japan.
Purpose: Graft-versus-host disease (GVHD)-related dry eye disease is one of the most frequent complications after allogeneic hematopoietic stem cell transplantation. Tranilast was synthesized for the first time in Japan and then approved for medical use. Over the last decades, this molecule has been utilized to treat asthma, atopic dermatitis and keloid. We have shown a significant role of fibroblasts in cGVHD induced pathogenic fibrosis including the epithelial mesenchymal transition (EMT) in lacrimal gland affected by cGVHD. Using cell-based screening methods, tranilast was found to have a potential to suppress EMT in vitro. The purpose of this study is to investigate whether tranilast is capable of mitigating inflammation, fibrosis and oxidative stress in lacrimal gland disordered by cGVHD.
Methods: Whole bone marrow transplantation (BMT) was conducted in order to obtain a mouse model of cGVHD. In the case where the donors are B10.D2 mice and the recipients are BALB/c mice, it is allogeneic transplantation and furnishes a mouse model of cGVHD (Zhang, Y. et al J Immunol. 2002). The allogeneic BMT recipients were treated with tranilast or the solvent-vehicle by oral or intraperitoneal injection. We performed (1) histological investigation, (2) immunohistochemical examination, (3) quantitative PCR (qPCR), (4) immunoblot assays and (5) electron microscopic analysis to explore whether oral or intraperitoneal injection of tranilast could mitigate cGVHD in lacrimal glands.
Results: Histopathological assessments and immunofluorescence staining suggest that oral or intraperitoneal injection of tranilast suppressed cGVHD-induced, inflammatory cell infiltration, fibrosis and oxidative stress in lacrimal glands. In addition, the results of immunoblot analysis and qPCR indicate that the protein or mRNA expression of NF-kB, CXCL9, TGF-b, and thioredoxin interaction protein (TXNIP) was vastly lower in the lacrimal glands treated with TL compared with those treated with the solvent-vehicle. As indicated by our data, oral injection of tranilast can repress lacrimal gland cGVHD in a more effective manner than intraperitoneal injection.
Conclusions: This study suggests that oral administration of tranilast alleviates inflammation and fibrosis caused by ocular cGVHD and

therefore can be a promising therapeutic intervention to attenuate cGVHD related dry eye disease.
Commercial Relationships: Eisuke Shimizu, None; **Shin Mukai**, None; **Yoko Ogawa**, None; **Kazuo Tsubota**, None
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Program Number: 4727
Presentation Time: 5:00 PM–5:15 PM
Development of a dynamic co-culture ocular cell *in vitro* model for ocular biocompatibility testing
Maud Gorbet[1, 2], Dana Toameh[1], Joyce Zhang[1], Chau-Minh Phan[2], Hendrik Walther[2], Lyndon W. Jones[2]. [1]Systems Design and Biomedical Engineering, University of Waterloo, Waterloo, ON, Canada; [2]School of Optometry and Vision Science, CCLR, University of Waterloo, Waterloo, ON, Canada.
Purpose: As contact lenses (CL) are evolving towards becoming diagnostic and therapeutic devices, there is a growing need for *in vitro* models that can efficiently assess ocular surface toxicity and biocompatibility of the technologies embedded in CL. While current *in vitro* ocular cell models allow for the characterization of cellular mechanisms, they can be too complex and costly for rapid testing. The aim of this project was to develop a co-culture cell model using the OcuFlow, an *in vitro* system which mimics tear flow between an eyelid and eyeball piece.
Methods: Eyelid and eyeball pieces were synthesized with Sylgard 184; the two pieces fit together and permit a CL to be set on the corneal surface of the eyeball piece. After sterilization, HPV-immortalized and primary human corneal epithelial cells (HCEC) were grown to confluence on the outer curved eyeball surface and primary human conjunctival epithelial cells (HConjEC) on the inner concave surface of the eyelid piece. Cells were cultured in serum-free keratinocyte medium (KM). The pieces with confluent cells on their surfaces were then fitted together, placed on the OcuFlow and transferred to a cell incubator. Experiments were performed at 0 (immersion), 2 and 10μL of KM/min for up to 6 hours. Viability was then assessed using XTT, or cells were harvested and expression of ICAM-1, integrin $\alpha_3\beta_1$ was characterized by flow cytometry.
Results: Experiments with confluent monolayers of immortalized-HCEC on both the eyeball and eyelid pieces indicated that greater cell survival was obtained with a 10μL/min tear (KM) flow rate. The presence of a PBS-soaked CL did not reduce cell viability, while a BAK-soaked CL led to significant cell death. Comparing HCEC on the OcuFlow at 0 and 10 μL/min, no significant upregulation of ICAM-1 occurred while changes were observed in the expression of α_3 and β_1; exposure to flow-induced shear stress is likely responsible for the latter. Successful experiments were performed with HConjEC (eyelid piece) and HCEC (eyeball piece) mounted on the OcuFlow with 10μL/min flow-rates.
Conclusions: Our results present a novel *in vitro* model where corneal and conjunctival epithelial cells can be exposed simultaneously to a CL and tear flow using the OcuFlow system. The OcuCell model mimics interactions at the ocular surface and has significant potential for the toxicological evaluation of therapeutic, diagnostic and conventional CL.
Commercial Relationships: Maud Gorbet, None; **Dana Toameh**, None; **Joyce Zhang**, None; **Chau-Minh Phan**, None; **Hendrik Walther**, None; **Lyndon W. Jones**, None
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Presentation Time: 5:15 PM–5:30 PM
Activity of antimicrobial peptides against *Stenotrophomonas, Delftia, Elizabethkingia,* and *Burkholderia* isolated from contact lens related adverse events
Debarun Dutta[1], Larke M. Holmlund[2], Mark D. Willcox[1]. [1]Optometry, School of Optometry and Vision Science, Sydney, NSW, Australia; [2]Department of Biology, University of Copenhagen, Copenhagen, Denmark.
Purpose: *Stenotrophomonas maltophilia, Delftia acidovorans, Elizabethkingia meningoseptica,* and *Burkholderia cepacia* have been associated with corneal infiltrative events during contact lens wear. These bacteria are naturally resistant against many first line antibiotics. The purpose of this study was to determine the activity of the antimicrobial peptide Mel4 in solution and when attached on contact lens surface against these Gram-negative bacteria.
Methods: *In vitro* antimicrobial activity of Mel4 was determined against the four Gram-negative bacteria by investigating growth curves for 24 hours by spectrophotometry followed by determination of minimum inhibitory concentration (MIC). The Gram negative bacteria were isolated from cornea or contact lens cases of adverse events during contcact lens wear. Mel4 was attached onto contact lenses following 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) coupling and characterized by amino acid analysis. Antimicrobial activity of the coated contact lenses against these bacteria was determined by viable plate count.
Results: Mel4 was active against all the bacteria tested. Antimicrobial activity was highest against *S. maltophilia* (MIC = 31.2 μg/ml), and weaker activity was found against *D. acidovorans, E. meningoseptica* and *B. cepacia* (MIC = 500 to 1000 μg/ml). Amino acid analysis revealed that Mel4-coated contact lenses were associated with 93 ± 13 μg of peptide. Following surface attachment the inhibition of adhesion against the four bacteria was 90%, 91%, 77% and 53% respectively ($P<0.05$).
Conclusions: Mel4 has varying antimicrobial activity against the Gram negative bacteria that are naturally resistant to antibiotics. Surface attachment this peptide to contact lenses was able to significantly reduce the attachment of these bacteria
Commercial Relationships: Debarun Dutta; **Larke M. Holmlund**, None; **Mark D. Willcox**, US7282214 B2 (P)