**540 Immune mechanisms in Eye disease: perception and reality**
Thursday, May 11, 2017 11:30 AM–1:15 PM
Exhibit/Poster Hall Poster Session
**Program #/Board # Range:** 5728–5759/B0280–B0311
**Organizing Section:** Immunology/Microbiology

**Program Number:** 5728 **Poster Board Number:** B0280
**Presentation Time:** 11:30 AM–1:15 PM
***In vitro* effects of IL-6 and IL-6R blockade on the blood-retinal barrier**
*Marina Mesquida[1, 2], David A. Copland[3], Philippa J. Lait[3], Victor Llorens[2], Maite Sainz De La Maza[2], Alfredo Adan Civera[2], Andrew D. Dick[3], Richard W. Lee[3], Blanca Molins[1]*. [1]Ophthalmology, Clinic Foundation for Biomedical Research, Barcelona, Spain; [2]Ophthalmology, Hospital Clinic Barcelona, Barcelona, Spain; [3]School of Clinical Sciences, University of Bristol, Bristol, United Kingdom.
**Purpose:** Macular edema (ME) is a leading cause of visual loss in uveitis patients. The pathogenesis of uveitic ME includes disruption of the blood-retinal barrier (BRB). IL-6 is a pro-inflammatory cytokine that has been implicated in ME pathogenesis, and clinical cohort studies showed that IL-6R blockade may be beneficial in the treatment of uveitic ME. The aim of this study was to interrogate the effect of IL-6 and its blockade with the IL-6R inhibitor tocilizumab (TCZ) on the barrier properties of an *in vitro* model of the inner and outer BRB.
**Methods:** The paracellular permeability of human retinal pigment epithelial cell (ARPE-19) and human retinal microvascular endothelial cell (HRMEC) monolayers was assessed by measuring the passive permeation of 40 kDa FITC-dextran across confluent cells grown on transwell filters. At day 19 cells were treated with IL-6 (200, 400 ng/mL) for 48h. In some cases TCZ (200 ng/mL) was added at day 20. At day 21, FITC-dextran was added to the apical compartment of the chamber and samples from the basal medium were collected 120 min later. Transepithelial/endothelial electrical resistance (TEER) was also measured in both cell monolayers grown on filters.
The distribution of the tight junction (TJ) protein ZO-1 in ARPE-19 and HRMEC monolayers was then examined by immunofluorescence. Cells grown on coverslips were incubated with or without IL-6 (200 ng/ml or 400 ng/ml) for 48 hours. When indicated, TCZ was added 24h after IL-6 stimulation. Then cells were fixed and immunolabeled with anti-ZO-1 and imaged by confocal microscopy.
**Results:** Treatment with IL-6 for 48h significantly increased the diffusion rate of FITC-dextran and decreased TEER in both ARPE-19 cells and HRMEC compared to untreated cells. TCZ restored the diffusion rate of FITC-dextran and TEER in IL-6-treated cells to the level observed in untreated cells. Immunofluorescence showed a normal distribution of ZO-1 in untreated ARPE-19 and HRMEC monolayers, although ZO-1 expression was markedly disrupted following exposure to IL-6 for 2 days. TCZ restored ZO-1 distribution in IL-6-treated cells.
**Conclusions:** These *in vitro* data support the hypothesis that IL-6 disrupts the BRB and contributes to the pathogenesis of ME. IL-6-induced disrupted ZO-1 expression was accompanied by an increase in paracellular permeability, and decreased TEER of ARPE-19 and HRMEC monolayers, whereas TCZ restored IL-6-induced BRB breakdown.
**Commercial Relationships: Marina Mesquida**, None; **David A. Copland**, None; **Philippa J. Lait**, None; **Victor Llorens**, None; **Maite Sainz De La Maza**, None; **Alfredo Adan Civera**, None; **Andrew D. Dick**, None; **Richard W. Lee**, None; **Blanca Molins**, None

**Program Number:** 5729 **Poster Board Number:** B0281
**Presentation Time:** 11:30 AM–1:15 PM
**Low concentration of thrombospondin-derived CD47 agonist inhibits IL-17 mediated leukocyte adhesion to vascular endothelial cells**
*Sharmila Masli[1], Laura Soriano-Romani[2], Bruce Turpie[1]*. [1]Ophthalmology, Boston University School of Medicine, Boston, MA; [2]IOBA, University of Valladolid, Valladolid, Spain.
**Purpose:** In chronic inflammatory diseases putative pathogenic Th17 cells can promote tissue infiltration of leukocytes by upregulating VCAM-1 expression on vascular endothelium. Previously thrombospondin-derived CD47 agonist peptide was reported to reduce severity of chronic ocular surface inflammation associated with Sjögren's syndrome in a mouse model. However, it was not clear whether this is achieved by direct influence on vascular endothelium. We hypothesize that CD47 agonist peptide inhibits IL-17 mediated VCAM-1 expression and associated leukocyte adhesion.
**Methods:** Expression of VCAM-1 on primary cultures of mouse (C57BL/6) lung vascular endothelial cells (ECs) untreated or treated with IL-17 (10 ng/ml) and TSP-derived CD47 agonist peptide (10 nM) for 24 hr was determined by immunostaining and real-time PCR. Adhesion of leukocytes was assessed by overlaying treated endothelial cells with fluorescence-labeled leukocytes for 1 hr followed by the removal of non-adherent cells and measurement of fluorescence of adherent cells. In vivo leukocyte adhesion was visualized in retina flatmounts after infusion of fluorescence-conjugated Con.A in C57BL/6 mice induced to develop chronic uveitic inflammation via IRBP/CFA immunization and treated topically with CD47 agonist or control peptide 10 mg/mouse for 1 week post-immunization.
**Results:** While VCAM-1 expression in IL-17-treated ECs was significantly increased, treatment with 10 nM CD47 agonist peptide significantly reduced this expression as compared to the control peptide (AUF 3.2+0.7 vs. 1.4+0.5 resp., $p<0.05$). These changes were consistent with changes in VCAM-1 message detected by real-time PCR. Similarly in vitro leukocyte adhesion was significantly reduced in CD47 agonist treated ECs compared to control peptide treated cells (13,122+1861 vs. 18,831+493, $p<0.05$). Furthermore, leukocyte adhesion in uveitic retinal vessels was significantly reduced in mice treated with CD47 agonist peptide as compared to those treated with control peptide (69.95+12.34 vs. 11.98+1.18, $p<0.05$).
**Conclusions:** Our results support the hypothesis that TSP-derived CD47 agonist peptide inhibits IL-17 induced leukocyte adhesion to vascular endothelium. Therefore this peptide may offer a viable alternative to VLA-4 blockade approach that is although beneficial in the treatment of chronic inflammation is associated with a severe adverse event.
**Commercial Relationships: Sharmila Masli**, None; **Laura Soriano-Romani**, None; **Bruce Turpie**, None
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**Program Number:** 5730 **Poster Board Number:** B0282
**Presentation Time:** 11:30 AM–1:15 PM
**Corneal alkali injury induces retinopathy via inflammation that is preventable with anti-TNF-α treatment**
*Fengyang Lei[1, 2], Chengxin Zhou[1, 2], Vassiliki Kapoulea[1, 2], James Chodosh[1], Claes H. Dohlman[1], Eleftherios I. Paschalis[1, 2].*
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**Purpose:** Ocular alkali burns can lead to blindness, even if promptly treated, due to cornea and retina damage. Our recent work ruled out the possibility of direct physical damage, as the cause of retinal injury. Instead, we found that upregulation of tumor necrosis factor alpha (TNF-α) causes blood-retinal barrier disruption and activation of retinal immune cells and retinal damage. We now explore the role of myeloid cells and retinal glial cells in retinal degeneration in alkali burns.
**Methods:** C57BL/6J, CX3CR1$^{+/eGFP}$ and TNFRSF1A1B$^{-/-}$ mice were used. Bone marrow chimerism was achieved by transferring CX3CR1$^{+/eGFP}$ donor cells into busulfan-myelodepleted C57BL/6J mice. Corneal alkali burns were performed using a filter paper soaked with 1N NaOH, placed on cornea for 20 seconds, and then irrigated with saline for 15 minutes. Anti-TNF-α treatment (6.25mg/kg of infliximab intraperitoneally) was given promptly after irrigation. Mice were euthanized at 24 hours after the burn and the eyes were studied by quantitative PCR, flow cytometry, immunofluorescence and confocal microscopy.
**Results:** Cornea alkali burn induced significant corneal and retinal cell death at 24 hours and significant accumulation of CD45$^+$CD11b$^+$Ly6C$^+$ immune cells in the iris (268-fold increase) of which 40% expressed MHC-II. This led to a marked upregulation of inflammatory cytokine genes, such as TNF-α (iris: 90-fold; retina: 50-fold) and IL-1β (iris: 190-fold; retina: 200-fold). Burns in the bone marrow chimera model showed that CX3CR1$^+$ macrophages (3.64%±2.0% of CD45$^+$ cells versus 0.58%±0.58% in controls) have crossed the blood-retinal barrier and entered into the retina. Subsequently this was associated with astrocyte and Müller glial cell activation, as indicated by GFAP upregulation ($p<0.05$) and further release of inflammatory mediators. Prompt anti-TNF-α treatment suppressed immune cell activation and CX3CR1$^+$ cell infiltration, and led to retinal protection. The protective role of prompt anti-TNF-α therapy was further validated by using TNFRSF1A1B$^{-/-}$ mice.
**Conclusions:** Corneal alkali burns cause significant retinal damage via cytokine upregulation and activation/infiltration of immune cells. Prompt inhibition of TNF-α by using monoclonal antibody inhibits monocyte infiltration and glial cell activation and protects the retina. Therefore, anti-TNF-α therapy may be a useful adjunct to standard emergency therapy after burns.
**Commercial Relationships: Fengyang Lei**, None; **Chengxin Zhou**, None; **Vassiliki Kapoulea**, None; **James Chodosh**, None; **Claes H. Dohlman**, None; **Eleftherios I. Paschalis**, None
**Support:** Boston KPro Fund

**Program Number:** 5731 **Poster Board Number:** B0283
**Presentation Time:** 11:30 AM–1:15 PM
**Corneal nerve ablation in one eye induces Langerhans cell infiltration into both eyes and abolishes immune privilege in both eyes**
*Jerry Y. Niederkorn, Juan Mo, Sudha neelam, jessamee Mellon, amber Wilkerson*. Ophthalmology, Univ Texas Southwestern Med Ctr, Dallas, TX.
**Purpose:** Circular corneal incisions made prior to penetrating keratoplasty abrogate immune privilege for future corneal transplants, even in the opposite eye. We examined the effect of corneal incisions on the distribution and activation of corneal antigen presenting Langerhans cells (LC) and on the immune privilege of corneal allografts and immune privilege in the anterior chamber (AC).
**Methods:** Corneal incisions (2.0 mm) were made in the central corneal epithelium of BALB/c mice prior to receiving C57BL/6 corneal allografts or injection of antigens into AC. Immune privilege in the AC was evaluated by assessing anterior chamber-associated immune deviation (ACAID). MHC class II+, CD207+, CD11c+ LC were identified by immunofluorescence. LC were depleted by subconjunctival injection of clodronate-containing liposomes.
**Results:** Corneal incisions in one eye induced activation and centripetal migration of LC into both eyes, which persisted for over 140 days. The presence of infiltrating LC abolished immune privilege in the AC as ACAID could not be induced in eyes of mice subjected to corneal incisions. ACAID was restored by purging infiltrating LC by subconjunctival injection of clodronate liposomes or by i.p. injection of the SP receptor antagonist, Spantide II. Although purging LC restored ACAID, it did not restore immune privilege of corneal grafts in mice treated with corneal incisions prior to keratoplasty. Although the neuropeptide substance P (SP) is associated with the abrogation of ACAID and the loss of immune privilege of corneal allografts, blocking SP with Spantide II did not affect LC migration in response to circular incisions.
**Conclusions:** Although placing circular incisions into the cornea in one eye abolishes both ACAID and immune privilege of corneal allografts in both eyes, the underlying mechanisms are different. SP contributes to the loss of immune privilege in the AC and for corneal allografts. However, activation and immigration of LC into the central corneal epithelium is independent of SP.
**Commercial Relationships: Jerry Y. Niederkorn**, None; **Juan Mo**, None; **Sudha neelam**, None; **jessamee Mellon**, None; **amber Wilkerson**, None
**Support:** NIH grant EY007641 and an unrestricted grant from Research to Prevent Blindness

**Program Number:** 5732 **Poster Board Number:** B0284
**Presentation Time:** 11:30 AM–1:15 PM
**Novel characterization and intravital imaging of lymphangiogenesis and valvulogenesis after lamellar keratoplasty**
*Lu Chen[1, 2], Liwei Zhang[1, 2], David G. Hwang[3], Guangyu Li[1, 2].*
[1]Graduate Group in Vision Science, University of California at Berkeley, Berkeley, CA; [2]Center for Eye Disease and Development, Program in Vision Science, and School of Optometry, University of California at Berkeley, Berkeley, CA; [3]Ophthalmology, University of California at San Francisco, Berkeley, CA.
**Purpose:** Despite the recent emergence of lamellar keratoplasty (LK) as the dominant surgical technique in clinical practice, supplanting full-thickness keratoplasty, we remain in our infancy in our understanding of the mechanisms to lamellar corneal graft rejection. This study was to establish a murine model of LK in Prox-1-GFP mice and to investigate whether graft rejection in this setting is associated with lymphangiogenesis (LG) and valvulogenesis (VG).
**Methods:** Anterior lamellar keratoplasty was performed between fully mismatched BALB/c (donor) and Prox-1-GFP mice in C57BL/6 background (recipient). Corneal LG and VG were assessed in vivo by a custom built live imaging system. Corneal grafts were evaluated by ophthalmic slit-lamp microscopy and anterior segment o*ptical coherence tomography (OCT)*.
**Results:** Over 70% of the lamellar grafts were rejected at 6 weeks post-transplantation. Progressive LG and VG were observed, which were both strongly associated with graft rejection. The thickness of

corneal grafts in the rejection group was significantly higher than in the survival group.
**Conclusions:** This study reveals novel and fundamental mechanisms leading to lamellar graft rejection. Therapeutic strategies targeting LG and VG should offer new approaches to promote graft survival in this setting.
**Commercial Relationships: Lu Chen**, None; **Liwei Zhang**, None; **David G. Hwang**, None; **Guangyu Li**, None
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**Presentation Time:** 11:30 AM–1:15 PM
**Combined blockade of VEGFR-3 and integrin alpha9beta1 inhibits corneal lymphangiogenesis and valvulogenesis in vivo and promotes high-risk graft survival**
*Guangyu Li[1, 2], Gyeong Jin Kang[1, 2], Narae Lee[1, 2], Anna A. Gong[1, 2], Yasuyuki Yokosaki[3], Lu Chen[1, 2]*. [1]Graduate Group in Vision Science, University of California at Berkeley, Berkeley, CA; [2]Center for Eye Disease and Development, Program in Vision Science, and School of Optometry, University of California at Berkeley, Berkeley, CA; [3]Cell-Matrix Frontier Laboratory, Biomedical Research Unit, Hiroshima University, Hiroshima, Japan.
**Purpose:** Corneal transplantation is the last hope for vision restoration for patients who are blind from corneal diseases. The rejection rate of high-risk grafts can be 50-90%, irrespective of current treatment modalities. VEGFR-3 (vascular endothelial growth factor receptor-3) is known for its critical role in lymphangiogenesis (LG), and we recently reported that integrin alpha9beta1 mediates luminal valve formation (valvulogenesis, VG) inside the lymphatic vessels during corneal LG. In this study, the effects of combined blockade of VEGFR-3 and integrin alpha9beta1 were evaluated after high-risk corneal transplantation.
**Methods:** High-risk corneal transplantation was performed between fully mismatched BALB/c (donor) and Prox-1-GFP (green fluorescent protein) mice in C57BL/6 background (recipient). The recipient mice were randomized to receive neutralizing antibodies of VEGFR-3 (kindly provided by Eli Lilly and Company) and integrin alpha9beta1. Processes of corneal LG and VG were assessed in vivo by our live imaging system, and corneal grafts were evaluated by ophthalmic slit-lamp microscopy as well.
**Results:** Combined blockade of VEGFR-3 and integrin alpha9beta1 significantly suppressed both LG and VG after corneal transplantation, and this treatment led to a markedly promoted survival rate in the high-risk setting.
**Conclusions:** This study offers new insights into high-risk transplant rejection. It may also provide a novel pharmaceutical therapy to treat other lymphatic- and immune-related diseases in the body.
**Commercial Relationships: Guangyu Li**, None; **Gyeong Jin Kang**, None; **Narae Lee**, None; **Anna A. Gong**, None; **Yasuyuki Yokosaki**, None; **Lu Chen**, None
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**Program Number:** 5734 **Poster Board Number:** B0286
**Presentation Time:** 11:30 AM–1:15 PM
**Role of B7-H3/TLT-2 pathway in immune privilege of corneal allografts**
*Hiroko Taniguchi[1], Hidenori Hase[2], Hisaya Akiba[3], Hideo Yagita[3], Miyuki Azuma[2], Junko Hori[1]*. [1]Ophthalmology, Nippon Medical School, Bunkyo-ku, Japan; [2]Molecular Immunology, Tokyo Medical and Dental University, Tokyo, Japan; [3]Immunology, Juntendo University School of Medicine, Tokyo, Japan.
**Purpose:** B7-H3 belongs to the B7 superfamily, a group of molecules that co-stimulate or down-modulate T-cell responses. Recently, triggering receptor expressed on myeloid cells-like transcript-2 (TLT-2) has been identified as a B7-H3 receptor. We have previously reported that B7-H3 is constitutively expressed in ocular tissue and B7-H3/TLT-2 pathway is necessary for corneal allograft survival. To further investigate the mechanisms B7-H3-associated immune suppression, we examined anterior chamber associated immune deviation (ACAID) *in vivo* and destruction of corneal endothelial cells (CECs) by allo-reactive $CD4^+$ T cells *in vitro*.
**Methods:** Allo-antigen-specific ACAID model was used. BALB/c mice received anterior chamber (AC) injection of C57BL/6 (B6) splenocytes 2 weeks prior to subcutaneous (SC) immunization. Induction of allo-specific ACAID was assessed by ear challenge with B6 splenocytes at 1 week after immunization. Recipients were administrated intraperitoneally with 0.2 mg of anti-B7-H3 mAb, anti-TLT-2 mAb, or control rat IgG, three times a week for 3 weeks after AC injection. As a different set of experiments, B6 cornea pre-treated with anti-B7-H3 mAb or control rat IgG were incubated with $CD4^+$ T cells for 6 h. $CD4^+$ T cells were purified from the spleen of BALB/c that were presensitized by SC immunization with B6 splenocytes or with third-party (C3H/He) splenocytes, or from the spleen of naive BALB/c, B6 or C3H/He mice. Dead CECs stained with propidium iodide were counted and compared.
**Results:** ACAID was abolished in the recipients treated with either anti-TLT-2 or anti-B7-H3 mAb. The number of dead CECs was significantly larger in anti-B7-H3 mAb-treated corneas than in control IgG-treated corneas after incubation with alloreactive $CD4^+$T cells. The number of dead CECs was also significantly larger in anti-B7-H3 mAb-treated corneas than in control corneas after incubation with $CD4^+$T cells activated against third-party allo-antigens.
**Conclusions:** B7-H3/TLT-2 pathway is involved in the induction of ACAID. B7-H3 expressed on CECs plays a role in protecting CECs from destruction by activated $CD4^+$ T cells. Thus, B7-H3/TLT-2 pathway maintains acceptance of corneal allografts by inducing ACAID as a systemic effect and suppressing allo-reactive $CD4^+$ T cells within the eye as a local effect.
**Commercial Relationships: Hiroko Taniguchi**, None; **Hidenori Hase**, None; **Hisaya Akiba**, None; **Hideo Yagita**, None; **Miyuki Azuma**, None; **Junko Hori**, None
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**Program Number:** 5735 **Poster Board Number:** B0287
**Presentation Time:** 11:30 AM–1:15 PM
**Retinal leukostasis in diabetic mice immunized with Keyhole Limpet Hemocyanin**
*Manzo Taguchi[1], Makoto Inada[1], Toshihiko Murata[1], Kozo Harimoto[1], Yoko Karasawa[1], Masataka Ito[2], Masaru Takeuchi[1]*. [1]Ophthalmology, National Defense Medical College, Tokorozawa, Japan; [2]Developmental Anatomy, National Defense Medical College, Tokorozawa, Japan.
**Purpose:** Akita mouse, which has a point mutation in exon3 of insulin 2 gene, is a non-obese type 1 diabetes (DM) model animal. Mice heterozygous of this mutant show development of hyperglycemia and hypo-insulinemia that are detectable after 4 weeks of age. However, no significant diabetic retinopathy (DMR) occurs. In both human and experimental animals, DM causes adhesion of leukocytes to vascular endothelial cells. We have recently indicated that T helper type 2 (Th2)- and Th17-related cytokines are elevated in the vitreous of proliferative diabetic retinopathy (PDR) patients. Keyhole Limpet Hemocyanin (KLH) originally isolated from the mollusc *Megathura crenulata* is a copper-containing protein found

in the hemolymph of many arthropods and mollusca. It has been reported that, in mammals immunized by KLH, more Th cells differentiate into Th2 and Th17 cells. In this study, we evaluated retinal leukostasis, as an early sign of DMR, in Akita mouse immunized by KLH.
**Methods:** Six-week-old male Akita mice were injected subcutaneously with 50 μg of KLH (immunized group). Control group of Akita mice were injected with the same amounts of vehicle subcutaneously. Two weeks later, fundus observations and Spectral-Domain (SD) -OCT images were evaluated, followed by analysis of leukostasis in the retinal blood vessels using Con A-conjugated FITC perfusion.
**Results:** Ophthalmoscopic observations showed no retinal lesion characteristic of DMR, and no abnormal retinal structure was observed in SD-OCT. However, the leukostasis was increased in immunized group compared with control group with statistical significance ($12.2 \pm 1.4$ / retina in the immunized group vs. $9.4 \pm 1$ / retina in the control group, $P = 0.0101$).
**Conclusions:** In the retinas of Akita mice immunized by KLH, mild inflammatory changes were observed, suggesting that Th17-related cytokines are involved in the etiology of DMR.
**Commercial Relationships: Manzo Taguchi**; **Makoto Inada**, None; **Toshihiko Murata**, None; **Kozo Harimoto**, None; **Yoko Karasawa**, None; **Masataka Ito**, None; **Masaru Takeuchi**, None

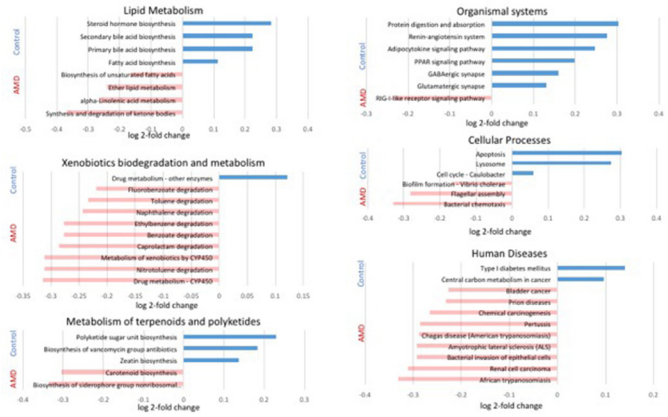
**Program Number:** 5736 **Poster Board Number:** B0288
**Presentation Time:** 11:30 AM–1:15 PM
**Conditional, genetically-encoded, small molecule-regulated inhibition of NF-κB signaling in RPE cells**
*John Hulleman, Khiem Vu*. Ophthalmology and Pharmacology, Univ of Texas Southwestern Med Center, Dallas, TX.
**Purpose:** Nuclear factor-κB (NF-κB) is a proinflammatory transcription factor that controls the expression of hundreds of genes involved in metastasis, angiogenesis and inflammation. Classical activation of NF-κB ultimately causes upregulation of pro-forms of inflammatory cytokines such as interleukin-1β (IL-1β) and interleukin-6 (IL-6). A number of studies have implicated IL-1β/IL-6 as being detrimentally involved in age-related macular degeneration (AMD) and retinal dystrophies, thus making inhibition of NF-κB signaling a potential therapeutic target. While a plethora of pharmacologic NF-κB inhibitors exist, the complex intraocular delivery of these drugs, combined with their potential for off-target effects, limits their utility in the eye.
**Methods:** We developed a genetic-based, trimethoprim (TMP)-regulated approach that allows for conditional inhibition of NF-κB by fusing a destabilized domain (DD) of E. *coli.* dihydrofolate reductase (DHFR) to IkBα (DHFR-IkBα). In the absence of TMP, DHFR fusion proteins are degraded and not present in the cell at appreciable levels. However, after TMP addition, DHFR is stabilized, and the fusion protein can function (in this case as an inhibitor of NF-κB). We used lentivirus to generate stable ARPE-19 cells expressing dox-inducible DHFR-YFP (as a control) or DHFR-IkBα. We then used western blotting, qPCR, EMSA, ELISA and reporter assays to assess whether DHFR-IkBα could be utilized to prevent NF-κB signaling and inflammasome priming.
**Results:** In the absence of dox/TMP, DHFR-YFP and DHFR-IkBα protein levels were tightly regulated in ARPE-19 cells. Addition of dox/TMP led to induction and stabilization of the fusion proteins in a concentration-dependent manner, and this strategy could be turned off by removal of dox/TMP for 48-72 h. We were able to cycle this strategy 'on' and 'off' at least three times. Without dox/TMP, DHFR-IkBα cells demonstrated identical NF-κB-related responses after stimulation with IL-1α (i.e., NF-κB nuclear translocation, IL-1β/IL-6 upregulation and secretion, etc.). Addition of dox/TMP to DHFR-IkBα cells significantly prevented NF-κB-mediated signaling, reducing these same responses by as much as 90% ($p<0.05$).
**Conclusions:** This DD strategy is effective at conditionally preventing NF-κB-mediated signaling and inflammasome priming. We believe that it has the potential to be a unique strategy to prevent diseases such as AMD.
**Commercial Relationships: John Hulleman**; **Khiem Vu**, None
**Support:** Research to Prevent Blindness Career Development Award, Karl Kirchgessner Foundation Vision Research Grant

**Program Number:** 5737 **Poster Board Number:** B0289
**Presentation Time:** 11:30 AM–1:15 PM
**Investigation of the interactions between macrophages and retinal pigment epithelium (RPE) cells in AMD**
*Takahiro Yamawaki, Eiko Ito, Jun Yamada, Shigeru Kinoshita, Chie Sotozono, Junji Hamuro*. Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto City, Japan.
**Purpose:** Age-relate macular degeneration (AMD) is partly caused by chronic inflammation. The purpose of this study is to clarify the interactions between macrophages(MPs) and RPE cells in coculture systems.
**Methods:** Adherent peritoneal cells or murine MPs cell line Raw 264.7 was cocultured with primary RPE cells taken from C57BL/6 mice. MCP-1, IL-6, VEGF, and TNF-α in the culture supernatants(CSs) were quantified by ELISA. The expression profiles, in cocultures, of complement-associated genes, TNF-α, and angiogenesis-associated genes were analyzed by quantitative real-time PCR. To investigate the effect of MPs polarization, MPs cultured with LPS and IFNγ or IL4 were also used.
**Results:** The production of MCP-1, IL-6, and VEGF were synergistically elevated when primary MPs or RAW264.7 and RPE cells were co-cultured compared with those derived from sole cultures of MPs and RPE cells. TNFα production by MPs was suppressed by RPE cells. Coculture of RPE cells with RAW264.7 cells increased the gene expression of C3, CFB, and VEGF genes, whereas it reduced those of complement regulatory factors CFH, CD59, clusterin, TNFα, and PEDF. The synergistic effect was more increased when MPs were polarized into M1 compared with when polarized into M2.
**Conclusions:** Our findings indicate the presence of ingenious interactions between MPs and RPE cells that forces the inflammation and complement activation in the vicinity of RPE cells, and the interactions were more prominent when MPs were polarized into M1.
**Commercial Relationships: Takahiro Yamawaki**, None; **Eiko Ito**, None; **Jun Yamada**, None; **Shigeru Kinoshita**, None; **Chie Sotozono**, None; **Junji Hamuro**, None

**Program Number:** 5738 **Poster Board Number:** B0290
**Presentation Time:** 11:30 AM–1:15 PM
**The role of interleukin-33 in retinal tissue fibrosis after laser injury**
*Akira Matsuda[1], Toshiaki Hirakata[1, 2], Takehiko Yokomizo[2], Susumu Nakae[3]*. [1]Department of Ophthalmology, Juntendo Univ School of Med, Tokyo, Japan; [2]Biochemistry, Juntedo University School of Medicine, Tokyo, Japan; [3]Frontier Research Initiative, Institute of Medical Science, University of Tokyo, Tokyo, Japan.
**Purpose:** Interleukin-33 (IL-33) is an IL-1 family cytokine, known to have pro-fibrotic function as other Th2 cytokines. We previously reported that IL-33 expression was observed in the nucleus of Muller cells and recombinant IL-33 injection to the vitreous induced the expression of TGF-beta1, TIMP-1 and COL1A1 in the retinal tissue

(ARVO 2014). In this study we examined the roles of IL-33 in tissue fibrosis after laser injury in the mouse retinal tissue using IL-33 knockout (KO) mice and congenic wild-type mice.
**Methods:** Laser photocoagulations (50 micrometer, 100 mW, 100 ms) were performed in unilateral eye of C57BL/6-IL-33 KO and congenic wild-type mice. *Il33, Il4, Il5, Il13, Il6, and Ccl2* expression after laser injury of the retina was evaluated chronologically (24, 72, 168 hours after injury) with realtime PCR. Expression of fibrosis-related genes (*Col1a1* and *Acta2*) was also quantified.
**Results:** Statistically significant *Il33* upregulation was observed from 24 to 72 hour after laser injury in the retinal tissue in wild-type mice. Old mice (16week-old) showed higher *Il33* expression (4.21 fold) than young mice (8week-old). Upregulation of Th2 cytokine (*Il4, Il5, Il13*) expression was observed in wild-type mice after laser injury and significant attenuation of Th2 cytokine expression (*Il4*; 0.2fold, *Il5*; 0.36fold, *Il13*; 0.47fold) was noted in IL-33 KO mice 24 hour after laser injury. The expression of macrophage activation-related genes (*Ccl2* and *Il6)* was peaked at 24 hour after laser injury, and significant reduction of the expression was observed in the IL-33 KO mice (*Ccl2*; 0.17fold, *Il6*; 0.23fold) compared to the wild type mice. The expression of fibrosis related genes (*Col1a1* and *Acta2)* was peaked at 168 hours after laser injury and significant attenuation (*Col1a1*; 0.4fold, *Acta2*; 0.33fold) of expression was noted in IL-33 KO mice.
**Conclusions:** Laser injury increased IL-33 expression in mouse retinal tissue and may promote retinal fibrotic changes through activation of Th2 type inflammation-related and macrophage activation-related signaling pathways.
**Commercial Relationships: Akira Matsuda**, None; **Toshiaki Hirakata**, None; **Takehiko Yokomizo**, None; **Susumu Nakae**, None
**Support:** JSPS 25670738

**Program Number:** 5739 **Poster Board Number:** B0291
**Presentation Time:** 11:30 AM–1:15 PM
**The gut microbiome in advanced age-related macular degeneration**
*Lee Kiang[1], Scott McClintic[1], Mohamed Saleh[2], Christina Metea[2], Kevin Mitio[2], Mark Asquith[2], Tammy M. Martin[2], Michael L. Klein[1], Lisa Karstens[2], Phoebe Lin[1]*. [1]Ophthalmology, Casey Eye Institute, Oregon Health & Sciences University, Portland, OR; [2]Oregon Health and Science University, Portland, OR.
**Purpose:** Genetic risk factors for age-related macular degeneration (AMD) and the benefit of oral vitamin supplementation in slowing AMD progression are well-established. The link between the gut and AMD is not well understood but innate immune system defense against bacterial pathogens has been implicated in AMD pathogenesis. We performed a case-control observational study to test the hypothesis that alterations in the gut microbiota in AMD affect pathways involved in the pathogenesis of AMD.
**Methods:** Case control study. Subjects with AMD and controls answered a questionnaire regarding health history with focus on inflammatory/autoimmune conditions, diet, medications, family history and allergies and provided stool and/or serum samples. Gut microbial composition was determined by DNA extraction from stool samples, PCR amplification of 16s DNA and gene sequencing using Illumina MiSeq. DNA sequences were grouped into operational taxonomic units (OTUs) at 97% similarity. Functional potential was inferred using Piphillin and Kyoto Encyclopedia of Genes and Genomes (KEGG) to identify metabolic pathways inferred by the constituent bacteria (97% identity cutoff). Subjects underwent genotyping for 25 genes/loci implicated in AMD.
**Results:** There were 85 subjects with AMD (mean age 82.5 years, 36% male, number obese 26.1, 60% with >=1 comorbidity) and 49 controls (mean age 76.4 years [p <0.0001], 47% male [p = 0.23], 27.6 obese [p=0.57], 43% >=1 comorbidity [p=0.06]). Of cases, the identified CFH rs1061170 CC risk allele was present in 36.8% (14.7% of controls [p=0.02]), and the ARMS2 rs10490924 TT risk allele was present in 23.7% (2.9% of controls [p=0.008]). 72 metabolic pathways were represented by gut microbiota with significant differences identified using DeSeq2 (p<0.05, adjusted for multiple comparisons metabolic pathways). These were enriched for lipid metabolism, metabolism of terpenoids and polyketides, and cellular processes in both AMD and control; for xenobiotics degradation and human diseases in AMD; and for organismal systems in controls (Figure).
**Conclusions:** Gut bacteria enriched in patients with AMD versus controls implicate differential functions in pathways for lipid and drug metabolism, carotenoid biosynthesis, bacterial chemotaxis and cell death suggesting that the gut microbiota may affect the pathogenesis of AMD.




Metabolic pathways represented by gut microbial constituents, enriched in controls and in AMD
**Commercial Relationships: Lee Kiang**, None; **Scott McClintic**, None; **Mohamed Saleh**, None; **Christina Metea**, None; **Kevin Mitio**, None; **Mark Asquith**, None; **Tammy M. Martin**, None; **Michael L. Klein**, None; **Lisa Karstens**, None; **Phoebe Lin**, None
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**Program Number:** 5740 **Poster Board Number:** B0292
**Presentation Time:** 11:30 AM–1:15 PM
**Association between macular phenotype and serum auto-antibodies (AAbs) against macular antigens in age-related macular degeneration (AMD): Preliminary studies**
*Alessandro Iannaccone[1], TJ Hollingsworth[3], Natalia Lenchik[2], Sarka Beranova-Giorgianni[4], Ivan Gerling[2], Marko Z. Radic[5], Francesco Giorgianni[4]*. [1]Duke Eye Center, Duke University Medical Center, Durham, NC; [2]Internal Medicine/Endocrinology, University of Tennessee Health Science Center, Memphis, TN; [3]Neuroscience, University of Tennessee Health Science Center, Memphis, TN; [4]Pharmaceutical Sciences, University of Tennessee Health Science Center, Memphis, TN; [5]Microbiology, Immunology and Biochemistry, University of Tennessee Health Science Center, Memphis, TN.
**Purpose:** We have documented that AMD sera exhibit circulating AAbs recognizing antigens expressed in the human macula [*PLOS One* 2015; 10: e0145323]. While these antigens [heat shock proteins HSPA8, HSPA9, and HSPB4, best known as alpha-crystallin A

chain (CRYAA); Annexin A5 (ANXA5); Protein S100-A9; and the CD5-like (CD5L) protein/apoptosis inhibitor of macrophages (AIM)] are not "macula-specific", they share a biologically plausible mechanistic connection to AMD via having key roles in autophagy, immunomodulation, and protection from oxidative stress and apoptosis. CD5L/AIM also has a role in oxidized (ox)LDL uptake, whereby it could also play a role in drusen biogenesis. Here we begin exploring possible correlates between macular phenotype and circulating AAbs recognizing these antigens in a discovery sample of AMD sera known to be positive for at least one of these autoreactivities to gauge their possible relevance to specific disease features.

**Methods:** Autoreactivities were measured by standard ELISA against corresponding purified or recombinant proteins as previously described [*PLOS One* 2015; 10: e0145323]. Macular AMD phenotype was investigated based on masked photo grading performed at baseline on all participants (n=18) by U. Wisconsin Reading Ctr. AREDS criteria. Characterized features included: maximum drusen size; drusen area; neovascular (nv)AMD; and presence and size of geographic atrophy (GA).

**Results:** All AAb levels but anti-ANXA5 tended to be higher in bilateral advanced AMD (AREDS-4) than early/mid-stage AMD (AREDS-3) – especially anti-HSPA8, HSPA9 and S100A9. Anti-HSPA8 and anti-HSPA9 AAb levels were specifically higher in subjects with nvAMD but not with GA. Compared to the lowest quartile, the upper quartile of anti-HSPA8 and anti-CD5L/AIM AAb levels was associated with a 5- and 2-step higher bilateral drusen area, respectively.

**Conclusions:** These preliminary exploratory comparisons suggest that certain AMD features and disease stages may correlate with different autoreactivities, whereas others like anti-ANXA5 are elevated in AMD regardless of either feature. The association with greater drusen area for anti-CD5L/AIM AAbs is consistent with its emerging biological role in oxLDL uptake. The strong association with anti-HSPA8 with drusen area and nvAMD was not entirely expected and deserves further investigation.

**Commercial Relationships: Alessandro Iannaccone**, None; **TJ Hollingsworth**, None; **Natalia Lenchik**, None; **Sarka Beranova-Giorgianni**, None; **Ivan Gerling**, None; **Marko Z. Radic**, None; **Francesco Giorgianni**, None

**Support:** NEI/NIH grant R01 EY022706 (FG/AI); BrightFocus Foundation (FG/AI)

**Program Number:** 5741 **Poster Board Number:** B0293
**Presentation Time:** 11:30 AM–1:15 PM

**Regulation of antigen processing in macrophages by RPE**

*Andrew W. Taylor, Robert Shannon, Issac Benque, Tat Fong Ng*. Ophthalmology, Boston Univ School of Medicine, Boston, MA.

**Purpose:** Our previous work demonstrated that healthy retinal pigment epithelial cell (RPE) monolayers promote suppressor cell functions in macrophages, and regulate the process of phagocytosis. The neuropeptides alpha-melanocyte stimulating hormone (α-MSH) and Neuropeptide Y (NYP) produced by healthy (RPE) mediate the suppression of phagolysosome activation in macrophages that have phagocytized opsonized-material. This suggested that the RPE neuropeptides are potentially regulating antigen processing in resident macrophages and microglial cells by altering phagosome maturation. Therefore, we assayed the content of vesicles with phagocytized materials for markers of phagosome/endocytic maturation.

**Methods:** The macrophage cell line RAW 264.7 were treated with the neuropeptides (1 ng/ml each), and fed opsonized-Ovalbumin (OVA)-coated magnetic Dynabeads under serum-free conditions. After 24 hours the cells were lysed in 250 mM Sucrose, 3mM Imidazole lysing buffer to preserve the intracellular vesicles. The magnetic bead containing vesicles were isolated using a magnet. The isolated vesicles were assayed by immunoblotting for OVA protein, Rab5 (early phagosome marker), Rab7 (late phagosome marker), Lamp1 (marker of phagolysosme formation), and MHC class II-beta chain (an indicator of fusion with the MHC class II compartment for peptide loading onto the MHC II molecule). Band intensity was measured, and the relative intensities of Rab7, Lamp1, and MHC class IIβ to Rab5 were calculated. For OVA analysis the relative levels of whole verses fragmented OVA was measured.

**Results:** The intracellular vesicles containing phagocytized magnetic beads from α-MSH/NPY treated macrophages had significantly more intact OVA than the vesicles from untreated phagocytizing macrophages. The immunoblotting showed that there was a high expression of Rab5 from the treated macrophages relative to the expression of Rab7, Lamp1, and MHC class IIβ. In contrast, there was significantly higher relative expression of Rab7, Lamp1 and MHC class IIβ in the vesicles from the untreated macrophages.

**Conclusions:** The results demonstrate that RPE neuropeptides α-MSH and NPY suppress the maturation of phagosomes in macrophages. This suggests that the mechanisms of retinal immune privilege include a mechanism to alter the handling of phagocytized materials in macrophages and microglial cells to help prevent presentation of autoantigen peptides and induction of autoimmune disease.

**Commercial Relationships: Andrew W. Taylor**, None; **Robert Shannon**, None; **Issac Benque**, None; **Tat Fong Ng**, None

**Support:** Massachusetts Lions Eye Research Foundation and NIH Grant EY025961

**Program Number:** 5742 **Poster Board Number:** B0294
**Presentation Time:** 11:30 AM–1:15 PM

**Activation of NLRP3 by intracellular aggregates in RPE cells**

*Anu Kauppinen[1], Niina Piippo[1], Eveliina Korhonen[1], Maria Hytti[1], Kati Kinnunen[3], Kai Kaarniranta[2, 3]*. [1]School of Pharmacy, University of Eastern Finland, Kuopio, Finland; [2]Department of Ophthalmology, University of Eastern Finland, Kuopio, Finland; [3]Department of Ophthalmology, Kuopio University Hospital, Kuopio, Finland.

**Purpose:** NLRP3 inflammasome promotes inflammation by the caspase-1-mediated release of IL-1β and IL-18, and its activation has been associated with the pathogenesis of age-related macular degeneration (AMD). We have previously shown that insufficient degradation of intracellular waste material serves as an activation signal for NLRP3 in human retinal pigment epithelial (RPE) cells. NLRP3 is a pattern recognition receptor (PRR) with several activation mechanisms, and in this study, we have explored the mechanism(s) contributing to the inflammasome activation induced by dysfunctional intracellular cleaning.

**Methods:** NLRP3 was activated in IL-1α-primed human ARPE-19 cells by the proteasome inhibitor MG-132 and the autophagy inhibitor Bafilomycin A. We measured extracellular ATP using a commercial kit, and inhibited ATP-receptors by a $P_2X_7$ inhibitor in cell cultures. For preventing potassium efflux, cell cultures were exposed to high extracellular potassium concentration or glyburide. Cathepsin B activity was measured using a commercial assay. In order to study the role of oxidative stress, mitoTEMPO, APDC (ammonium pyrrolidine dithiocarbamate), or NAC (N-acetylcysteine) were added to the cell cultures. Cellular reactive oxygen species (ROS) were detected using the DCFDA (2',7'-dichlorofluorescin diacetate) assay.

**Results:** Inhibition of $P_2X_7$ receptors did not prevent the secretion of IL-1β although increased levels of extracellular ATP could have been

able to initiate the response. Blocking the potassium efflux showed a weak response but cathepsin B seemed not to play any role in the inflammasome activation in our model. The treatment increased intracellular oxidative ($P < 0.001$), and ROS inhibitors mitoTEMPO ($P < 0.05$) and APDC ($P < 0.001$) but not NAC alleviated the release of IL-1β.
**Conclusions:** According to our results, oxidative stress plays a major role in the activation of NLRP3 inflammasome in human RPE cells with declined functionality of intracellular clearance systems. Especially inhibitors of NADPH oxidase and mitochondria-derived ROS appeared efficient.
**Commercial Relationships: Anu Kauppinen**, None; **Niina Piippo**, None; **Eveliina Korhonen**, None; **Maria Hytti**, None; **Kati Kinnunen**, None; **Kai Kaarniranta**, None
**Support:** The Academy of Finland (grants 297267 and 307341), the Emil Aaltonen Foundation

**Program Number:** 5743 **Poster Board Number:** B0295
**Presentation Time:** 11:30 AM–1:15 PM
**Chronic exposure to TNFα impairs RPE barrier and immunosuppressive functions**
*Sara Touhami[1, 2], Fanny Beguier[2], Sébastien Augustin[2], Sacha Reichman[2], Olivier Goureau[2], Emeline F. Nandrot[2], Xavier Guillonneau[2], Bahram Bodaghi[1], Florian Sennlaub[2].*
[1]Ophthalmology, Pitie Salpetriere Hospital, Paris, France; [2]Institut de la Vision, Paris, France.
**Purpose:** The retinal pigment epithelium (RPE) is a monolayer of pigmented cells with important functions in the outer blood-retinal barrier and subretinal immune suppression. Failure of RPE functions and chronic inflammation have been both hypothesized to play a role in the pathophysiology of age related macular degeneration (AMD). We have previously shown that acute stimulation of RPE cells by TNFα down-regulates gene expression of OTX2 (Orthodenticle homeobox 2) and that of major visual cycle-related genes. We here investigated the long-term effects of TNFα on RPE morphology and function in vitro.
**Methods:** Primary porcine RPE cells were cultivated until confluence, then recombinant TNFα was added daily in the culture medium (at 0.8, 4, 20 or 100ng/ml=C1,C2,C3 and C4) for 10 days. RPE cell morphology and gene expression, barrier, phagocytosis and immunosuppressive functions were assessed.
**Results:** *Cell morphology and gene expression:* 10 day stimulation by TNFα (i) decreased RPE cell numbers (3653.6, 3428, 3227, 2791 and 2020 cells/mm2 respectively for control, C1,C2,C3 and C4, all $p<0.01$); (ii) increased cell size (+5.3, +12.6, +13.9 and +9.5% for C1,C2,C3 and C4 as compared to control, all $p<0.05$); (iii) increased the number of multinucleated cells (5.7, 7.7, 9.4, 9.9, 15.9% of multinucleated cells for control, C1,C2,C3 and C4, all $p<0.05$); (iv) and decreased OTX2 expression (-11.1, -19.7, -52 and -82.9% for C1,C2,C3 and C4 as compared to control, all $p<0.05$). The number of apoptotic TUNEL+ cells was increased upon TNFα adjunction (112,3, 158.7 and 189.9% of control for C2,C3 and C4, all $p<0.05$). *Barrier function:* 10 day stimulation by TNFα (i) disturbed Zonula Occludens 1 cellular distribution and cytoskeletal architecture (actin F distribution) and (ii) significantly decreased RPE transepithelial resistance in a dose-dependent manner (-70, -88.5 and -90.8% of control for C2,C3 and C4, $p<0.05$). *Immunossupressive function*: 10 day pre-stimulation with -TNFα significantly decreased RPE capacity to induce monocyte death after 24h of co-culture ($p<0.05$).
**Conclusions:** Chronic exposure to TNFα deteriorates major RPE functions that are essential to visual function and might play a key role in the pathophysiology of AMD.
**Commercial Relationships: Sara Touhami**, None; **Fanny Beguier**, None; **Sébastien Augustin**, None; **Sacha Reichman**, None; **Olivier Goureau**, None; **Emeline F. Nandrot**, None; **Xavier Guillonneau**, None; **Bahram Bodaghi**, None; **Florian Sennlaub**, None

**Program Number:** 5744 **Poster Board Number:** B0296
**Presentation Time:** 11:30 AM–1:15 PM
**Optic Nerve as a Source of Retinal Mononuclear Cells Post-Injury**
*Dale S. Gregerson, Neal D. Heuss, Mark Pierson, Scott W. McPherson*. Ophthalmology & Visual Neurosciences, University of Minnesota, Minneapolis, MN.
**Purpose:** We previously found mononuclear cells in murine CNS tissues, including retina, that expressed GFP from a transgenic CD11c promoter. Their origin is of interest. Their frequency was elevated in retina following several types of stress or injury or inflammation, and they possessed the ability to process and present antigen to naïve antigen-specific T cells in vivo.
**Methods:** Retinal ganglion cells in adult mice were injured in one of three procedures; an ON crush, a partial ON transection, and a full ON transection; all procedures spared the ophthalmic artery to preserve retinal circulation. ON injuries were done in CD11c-GFP mice, in beta-actin GFP parabiotic mice, in mice with tamoxifen-induced depletion of CX3CR1+ cells, and in radiation bone marrow chimeras, to further examine the origins of the responding GFPhi cells. ON and retina were collected for analysis by immunofluorescence and flow cytometry.
**Results:** The origin of GFPhi cells in CD11c-GFP mice depended on the injury. Of particular interest was the observation that full ON transection (artery was spared) gave a limited GFPhi cell response relative to an ONC ($p<0.05$) or partial transection ($p<0.05$), despite the injury of a much larger number of ganglion cell axons following a full transection. All ON injuries led to appearance of a significant number of cells with Ki67+ nuclei, while few cells in retina post-ONC were Ki67+. The significance of the small number of Ki67+ cells in ONC retina was further examined by comparison with retinas inflamed by experimental autoimmune uveoretinitis. Inflamed retinas had many Ki67+ cells including T lymphocytes, CD11b+ cells and GFPhi cells. ON injury in parabiotic mice gave no evidence of recruitment from the circulation ($p<0.01$). ON injury in tamoxifen-depleted mice induced a small mononuclear cell response that was largely GFPhi ($p<0.05$). The large mononuclear cell response in the ON post-injury may lead to migration of cells from the ON into retina, following the severed axons.
**Conclusions:** While Ki67+ cells were found in injured ON, Tam-depletion substantially delayed the retinal response to the ON injury. Precursors of GFPhi cells may be more prevalent or active in the ON, and contribute to the GFPhi cells found associated with RGC and nerve fibers post-ON injury. The reduced yield of retinal GFPhi cells after a full ON transection may reflect macrophage migration along injured axons, lost following a full transection.
**Commercial Relationships: Dale S. Gregerson**, None; **Neal D. Heuss**, None; **Mark Pierson**, None; **Scott W. McPherson**, None
**Support:** 1 R01 EY025209-01A1; RPB, Inc.; Minnesota Lions Clubs; Wallin Neuroscience Discovery Fund

**Program Number:** 5745 **Poster Board Number:** B0297
**Presentation Time:** 11:30 AM–1:15 PM
**Evidence of melanoma immunoreactivity in patients with Birdshot retinochoroidopathy**
*Lynn Hassman[1], Michelle Warren[2], Krystel R. Huxlin[1], Mina M. Chung[1], lei xu[2]*. [1]Ophthalmology, University of Rochester, Flaum Eye Institute, Rochester, NY; [2]Biomedical Genetics, University of Rochester, Rochester, NY.
**Purpose:** Birdshot retinochoroidopathy (BSRC) is an inflammatory disease characterized by hypopigmented spots in the retina. These spots resemble cutaneous vitiligo, which results from immune-mediated destruction of melanocytes. Vitiligo can arise spontaneously, or in the context of a successful immune response to melanoma. Notably, there are multiple reports of BSRC occurring in patients with a history of melanoma. Given the similarities between BSRC and vitiligo, we tested the hypothesis that BSRC might also be associated with an immune response to melanoma by searching for anti-melanoma antibodies in serum from patients with BSRC.
**Methods:** Serum was obtained from patients with BSRC, as well as from melanoma patients and normal patients without ocular disease or systemic autoimmunity. Importantly, our BSRC patients had no known personal or family history of skin cancer. Western blots were made from melanoma cell lysates and probed with patient or control sera. Lysate from human fibroblasts was also used as a control for non-melanoma autoimmunity.
**Results:** Thus far, sera from 6 patients with BSRC, 4 patients with melanoma, and 5 normal patients have been analyzed. Prominent bands were seen at 100 kDa, 70 kDa, 55 kDa and 45-50 kDa on blots probed with BSCR patient serum, indicating immunoreactivity to specific proteins present in melanoma cell lysates. The bands were absent or extremely faint on blots probed with normal patient serum, but variably present on blots probed with melanoma patient serum, suggesting a similar immune response in BSRC and melanoma patients.
**Conclusions:** These results demonstrate the presence of anti-melanoma antibodies in serum from patients with BSRC. While our BSRC patients have no clinical history of melanoma, the presence of these antibodies suggests that they may have had subclinical tumors that were eliminated by a successful immune response. This work suggests that the immune trigger for the HLA-A29-restricted inflammation seen in BSRC may be melanoma, and that BSRC may represent an immune cross-reactivity to melanin-associated antigens in the choroid and RPE. Alternatively, BSRC may trigger an immune response to melanin-associated chorioretinal antigens that cross-react with melanoma cell antigens.
**Commercial Relationships: Lynn Hassman**, None; **Michelle Warren**, None; **Krystel R. Huxlin**, None; **Mina M. Chung**, None; **lei xu**, None
**Support:** Unrestricted grant from Research to Prevent Blindness

**Program Number:** 5746 **Poster Board Number:** B0298
**Presentation Time:** 11:30 AM–1:15 PM
**Functional characterisation of CD11c-eYFP$^+$ cells during systemic inflammation reveals that the mouse retina is devoid of antigen presenting cells**
*Samantha Dando[1], Renee Kazanis[1], Holly R. Chinnery[2], Claude C. Bernard[3], Paul McMenamin[1]*. [1]Monash Biomedicine Discovery Institute and Department of Anatomy and Developmental Biology, Monash University, Clayton, VIC, Australia; [2]Optometry and Vision Sciences, University of Melbourne, Parkville, VIC, Australia; [3]Australian Regenerative Medicine Institute, Monash University, Clayton, VIC, Australia.
**Purpose:** The question of whether the CNS contains professional antigen presenting cells (APCs) has been debated for many years. We previously reported that putative dendritic cells in the neural retina of CD11c-eYFP *Crb1*$^{wt/wt}$ mice are phenotypically indistinguishable from microglia in the normal eye; however, their functional capacity has not been investigated. We hypothesised that retinal CD11c-eYFP$^+$ cells are a subset of microglia and thus would have a limited capacity to present antigen to naïve T cells.
**Methods:** We first tested the ability of retinal CD11c-eYFP$^+$ cells to upregulate APC markers following systemic inflammation. CD11c-eYFP *Crb1*$^{wt/wt}$ mice received an i.p injection of LPS (9mg/kg, n=23) or saline (n=18) and retinas were collected 24h later for confocal microscopy and flow cytometry. Myeloid cells from the uveal tract (known to contain APCs) of Cx3cr1-GFP mice were also included for comparison. For functional studies, retinal CD11c-eYFP$^+$ cells and microglia (CD45$^{lo}$ YFP$^-$) were isolated from CD11c-eYFP *Crb1*$^{wt/wt}$ mice (n=20) 24h post-LPS injection by FACS. CD11c-eYFP$^+$ cells from spleen and meninges were included as controls. Isolated cells were co-cultured with naïve OT-II CD4$^+$ T cells and ovalbumin for 72h. T cell proliferation was measured by flow cytometry.
**Results:** Retinal CD11c-eYFP$^+$ cells retracted their processes and displayed a stout morphology 24h post-LPS injection. Despite this, retinal CD11c-eYFP$^+$ cells did not upregulate I-A/I-E, or the co-stimulatory molecules CD80 and CD86, and were phenotypically identical to CD45$^{lo}$ CD11b$^+$ F4/80$^{lo}$ I-A.I-E$^-$ microglia. In contrast, Cx3cr1-GFP$^+$ cells in the iris/ciliary body and choroid expressed I-A/I-E, CD86 and significantly upregulated CD80 following LPS challenge ($p<0.05$). Both retinal CD11c-eYFP$^+$ cells and microglia from LPS treated mice failed to induce T cell proliferation *in vitro* (0.9±0.4% and 0.5±0.07% proliferation respectively), whereas splenic and dural CD11c-eYFP$^+$ cells were potent APCs that induced T cell proliferation and upregulation of CD44 ($p<0.05$).
**Conclusions:** Retinal CD11c-eYFP$^+$ cells do not upregulate APC markers following systemic inflammation and are incapable of presenting antigen to naïve CD4$^+$ T cells *in vitro*. Moreover, retinal CD11c-eYFP$^+$ cells are phenotypically and functionally identical to microglia. These data provide new evidence that the retina is devoid of APCs.
**Commercial Relationships: Samantha Dando**, None; **Renee Kazanis**, None; **Holly R. Chinnery**, None; **Claude C. Bernard**, None; **Paul McMenamin**, None
**Support:** Rebecca L. Cooper Medical Research Foundation grant 10470

**Program Number:** 5747 **Poster Board Number:** B0299
**Presentation Time:** 11:30 AM–1:15 PM
**Anti-Transgene Cellular Immune Reponses can be Induced by Subretinal Gene Transfer with rAAV in a Dose-Dependent Manner**
*Sylvain Fisson[1, 2], Julie Vendomele[1, 2], Quentin Khebizi[1, 2], Safa Dehmani[1, 2], Mirella Mormin[1], Sabrina Donnou-Triffault[1, 2], Anne Galy[1, 2]*. [1]INSERM UMRS951, Genethon, Evry, France; [2]University of Evry Val d'Essonne, Evry, France.
**Purpose:** From animal experiments to the first human clinical trials in 2007, recombinant adeno-associated virus (rAAV)-mediated ocular gene therapy has shown successful results which have been attributed in part to the immune-privileged situation of the eye. Recently, some ocular gene therapy clinical trials have reported that visual acuity returned to baseline 6 to 12 months after therapy. The involvement of anti-transgene immune responses may lead to loss of therapeutic efficacy. This prompted us to evaluate in a murine model if rAAV gene transfer leads to an anti-transgene immunization. In this study,

we characterized the CD4 and CD8 T-cell responses specifically directed toward the transgene product in a murine model following rAAV2/8-mediated subretinal gene transfer.
**Methods:** A rAAV2/8 encoding for the GFP-HY fusion protein under the ubiquitous PGK promoter was used. The transgene expresses the HY male antigen which contains MHC class I and MHC class II-restricted T cell epitopes (UTY and DBY, respectively), that are immuno-dominant in female mice. Two µL of endotoxin-free, PBS-formulated rAAV2/8 PGK GFP-HY were injected subretinally in C57Bl/6 female mice. At day 7, mice were challenged subcutaneously with the UTY and DBY peptides adjuvanted in CFA, and the immune response was analyzed at day 14 by IFNg ELISpot, cytokine titration and proliferation assays.
**Results:** Our results revealed that: *(i)* The subretinal injection of 10E8 to 2.10E9 vg/mouse of rAAV2/8 PGK GFP-HY did not induce a significant HY-specific peripheral immune modulation in contrast to the ACAID obtained after subretinal injection of HY peptides (50µg each); *(ii)* Higher doses of rAAV2/8 PGK GFP-HY (5.10E10 vg/mouse) triggered increased Th1 and Tc1 cellular immune responses against the transgene product in peripheral lymphoid organs.
**Conclusions:** We show that rAAV2/8 vector-mediated subretinal gene transfer is not necessarily ignored at the immunological level. High doses of vector can effectively trigger anti-transgene T-cell responses with the potential for elimination of transgene-expressing cells. Clearly, anti-transgene-specific immune monitoring should be refined at least in preclinical models, to improve the biosafety and the long-term efficacy of rAAV-mediated ocular gene transfer. Moreover, immunosuppressive strategies concomitant to the AAV injection are currently being tested.
**Commercial Relationships: Sylvain Fisson**, None; **Julie Vendomele**, None; **Quentin Khebizi**, None; **Safa Dehmani**, None; **Mirella Mormin**, None; **Sabrina Donnou-Triffault**, None; **Anne Galy**, None
**Support:** French Academic ATIGE grant

**Program Number:** 5748 **Poster Board Number:** B0300
**Presentation Time:** 11:30 AM–1:15 PM
**Analysis of Factors Necessary for Recruitment and Retention of Mononuclear Cells in the Retina**
*Scott W. McPherson, Neal D. Heuss, Mark Pierson, Dale S. Gregerson*. Ophthalmology and Visual Neurosciences, University of Minnesota, Minneapolis, MN.
**Purpose:** Previously we reported that the retinal environment imparts significant control over the phenotype and function of mononuclear cells. However, the origin and function of any particular phenotype of mononuclear cell in either retinal homeostasis or injury response are matters of active investigations. To determine the control exerted by resident mononuclear cells or other retinal cells in recruiting and retaining mononuclear cells, we analyzed the retinal expression of chemokines and cytokines associated with mononuclear cell function in mice that had been depleted of mononuclear cells and allowed to recover with or without injury to the retina.
**Methods:** CD11c-DTR/GFP mice (GFP and diphtheria toxin receptor under control of a transgenic CD11c promoter), CX3CR1-YFP-CreER mice, (CX3CR1+ cells express yellow fluorescent protein and are depletable by tamoxifen, TAM), and F1 crosses were used. Fluorescence activated cell sorting (FACS) was used to analyze mononuclear cell populations in the retina. Gene expression analysis was done by quantitative RT-PCR on mRNA prepared from whole retinas or pools of phenotypically defined mononuclear cells sorted from the retina by FACS. Retinal injury was induced by optic nerve crush (ONC).
**Results:** Expression analysis from whole retinas of various F1 mice groups (untreated, TAM depleted only, ONC only, and TAM depleted plus ONC) was performed. Some genes (IL-34, CXCL12, and CXCL13) were unchanged between any groups suggesting they were not made by any retinal mononuclear cell or not involved in recruitment or injury responses. CSF-1 was upregulated in ONC only mice suggesting it is made by non-mononuclear retinal cell(s) in response to injury. Other genes (CCL2, CCL3, CCL5, CXCL10) appeared to be made by mononuclear cells that were recruited to, or only became activated within the retina, in response to injury. CCL4, CCL21, and CXCL1 expression was lower in TAM only mice compared to controls suggesting that they are constitutively expressed by resident mononuclear cells, but only CCL4 was increased upon ONC stimulation.
**Conclusions:** These results have allowed us to categorize the retinal cellular origin of certain chemokines and cytokines associated with mononuclear cells, and to determine if those factors are important in retaining and/or recruiting those cells to the retina.
**Commercial Relationships: Scott W. McPherson**, None; **Neal D. Heuss**, None; **Mark Pierson**, None; **Dale S. Gregerson**, None
**Support:** NIH grant RO1 EY025209-01A1; Research to Prevent Blindness; Minnesota Lions Club; Wallin Neuroscience Discovery Fund

**Program Number:** 5749 **Poster Board Number:** B0301
**Presentation Time:** 11:30 AM–1:15 PM
**Association of Th17-cell related cytokines between Aqueous Humor and Vitreous Fluid in Proliferative Diabetic Retinopathy Patients**
*Masaru Takeuchi, Tomohito Sato, Manzo Taguchi, Yoko Karasawa, Yutaka Sakurai, Kozo Harimoto, Masataka Ito*. Ophthalmology, National Defense Medical College, Tokorozawa, Japan.
**Purpose:** Inflammation involved in progression of diabetic retinopathy has been known. We have recently indicated that vitreous levels of IL-4, IL-17A, IL-22, IL-31, and TNFα in proliferative diabetic retinopathy (PDR) patients were higher than those in the serum, and vitreous levels of these cytokines were also higher in PDR than in other non-inflammatory vitreoretinal diseases or in uveitis associated with sarcoidosis. The aqueous humor can be collected more easily and repeatedly compared with vitreous fluid. In the present study, we investigated aqueous humor levels of inflammatory cytokines elevated in the vitreous fluid of eyes with PDR including Th17-cell related cytokines, and analyzed the association of respective cytokines between the aqueous humor and vitreous fluid.
**Methods:** The study group consisted of consecutive 31 type 2 diabetic patients with PDR who underwent cataract surgery and vitrectomy for vitreous hemorrhage and/or tractional retinal detachment between January 1, 2016 and July 31, 2016 in National Defense Medical College. Approximately 0.2 to 0.5 mL of undiluted aqueous humor was collected during cataract surgery when the anterior chamber was replaced by viscoelastic substance. After cataract surgery, vitreous fluid was obtained using a 25G vitreous cutter inserted into the mid-vitreous cavity at the beginning of vitrectomy before active infusion. IL-1β, IL-4, IL-6, IL-10, IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, IFN-γ, soluble CD40 ligand (sCD40L), and TNFα in the aqueous humor and vitreous fluid were measured by beads-array system.
**Results:** Although percent detectable of IL-10, IL-17A, IL-22, and TNFα were significantly lower in the aqueous humor than in the vitreous fluid, the correlation of IL-17A with IL-10, IL-22, and TNFα were observed in the aqueous humor as well as in the vitreous fluid.

In addition, IL-17A in the aqueous humor was significantly correlated with IL-10, IL-17A, and TNFα in the vitreous fluid.
**Conclusions:** The present study demonstrated that in patients with PDR, IL-17A is detected in the aqueous humor which is lower than that in the vitreous fluid, and is significantly correlated with IL-17A in the vitreous fluid and with IL-10, IL-22, and TNFα both in the aqueous humor and in the vitreous fluid.
**Commercial Relationships: Masaru Takeuchi**; **Tomohito Sato**, None; **Manzo Taguchi**, None; **Yoko Karasawa**, None; **Yutaka Sakurai**, None; **Kozo Harimoto**, None; **Masataka Ito**, None
**Support:** Grant-in-Aid 16K11337 for Scientific Research from the Japan Society for the Promotion of Science

**Program Number:** 5750 **Poster Board Number:** B0302
**Presentation Time:** 11:30 AM–1:15 PM
**Autophagy marker expression in Non-Sjögren and Sjögren syndrome dry eyes**
*Soojung Shin[1, 2], Yong-Soo Byun[1, 2], Hyun Jung Lee[1, 2], So-Hyang Chung[1, 2].* [1]Department of Ophthalmology and Visual Science, Seoul St. Mary's Hospital, Catholic University of Korea, College of Medicine, Seoul, Korea (the Republic of); [2]Catholic Institute for Visual Science, Catholic University of Korea, College of Medicine, Seoul, Korea (the Republic of).
**Purpose:** Autophagy is a lysosome-mediated catabolic process that maintains cellular homeostasis through the degradation and recycling of cytoplasmic components and organelles. Recent data showed that enhanced autophagy was involved in the autoantibody redistribution in secretory epithelial cells in Sjögren syndrome (SS). We investigated autophagy markers (ATG5, LC3B) in tears and conjunctiva from non- SS and SS dry eyes (DE), and also analyzed their correlation with clinical features to determine whether autophagy dysregulation might be a key finding in SS DE.
**Methods:** Forty SS DE patients (78 eyes), 24 non-SS DE patients (48 eyes), and 16 normal healthy subjects (25 eyes) were included in the present study. Schirmer I test, tear film breakup time (TBUT), ocular surface staining (OSS) scores, and ocular surface disease index (OSDI) were assessed. ATG5, LC3BII/I conversion ratio in tears were analyzed by Western blot. Immunostaining and qPCR of ATG5 and LC3BII were also performed using conjunctival impression cytology. Additionally, two markers were observed in immunostained lacrimal glands of NOD/LtJ mice (SS animal model).
**Results:** SS DE showed lower values in Schirmer and TBUT and higher values in OSS staining scores compared to non-SS DE. ATG5 protein and LC3BII/I conversion ratio were significantly higher in SS DE than non-SS DE tears of patients and control. The levels of encoding ATG5 and LC3BII in the conjunctiva were also upregulated in SS DE than non-SS DE and control. The immunofluorescent staining of conjunctival impression cytology specimen showed a punctate staining pattern of ATG5 and LC3BII in SS DE, whereas the characteristic staining pattern was not observed in those of non-SS DE and control. Among clinical parameters, conjunctival staining scores were strongly correlated with ATG5 protein abundance in tears and ATG5 mRNA level in conjunctiva. After corticosteroid treatment in SS DE (32 eyes), ATG5 and LC3BII expression in tears and conjunctiva were significantly reduced.
**Conclusions:** Autophagy markers (ATG5 and LC3B) were upregulated in tears and conjunctiva. ATG5 expression was correlated with conjunctival staining scores in SS DE. Anti-inflammatory treatment induced downregulation of autophagy markers accompanied by improvement of clinical parameters in SS DE.

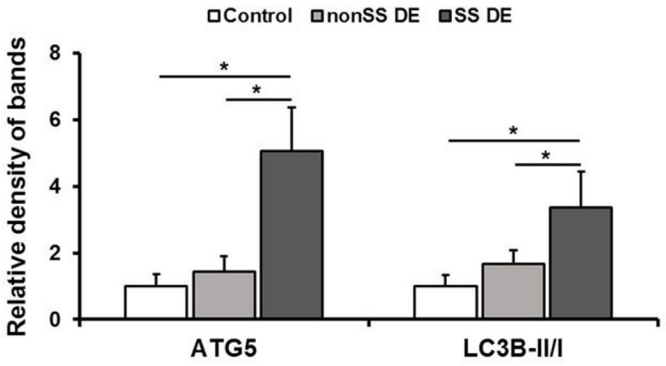

Fig 1. Protein abundance in tears

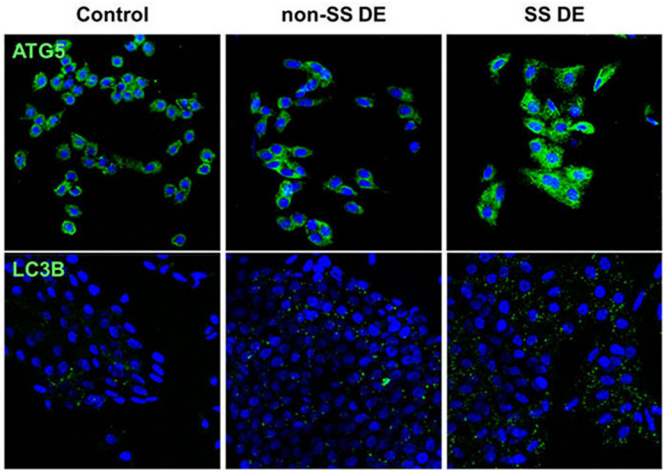

Fig 2. Immunostaining of conjunctival epithelium
**Commercial Relationships: Soojung Shin**, None; **Yong-Soo Byun**, None; **Hyun Jung Lee**, None; **So-Hyang Chung**, None
**Support:** This work was supported by a grant of the Korea Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (HI13C0016).

**Program Number:** 5751 **Poster Board Number:** B0303
**Presentation Time:** 11:30 AM–1:15 PM
**Retrospective Analysis of Increment Instillation of Allergen During the Conjunctival Allergen Provocation Test (CAPT)**
*Holly I. Lorentz, Victoria Nelson, Nerizsa Tenorio, Anne Marie Salapatek.* Inflamax Research Inc, Mississauga, ON, Canada.
**Purpose:** To assess the differences in ocular response during the allergen concentration dose finding phase of the conjunctival allergen provocation test (CAPT) in birch, ragweed and grass allergic conjunctivitis subjects.
**Methods:** After obtaining informed consent, 169 SPT and allergy positive subjects were screened for their ocular allergic response by performing the allergen concentration dose finding phase (titrating phase) of the CAPT model. Titrating CAPT (tCAPT) involved instilling increasing concentrations of standardized allergen extracts into both eyes until a Positive Ocular Allergic Response (POAR) occurred or until the maximum dose was reached. Subjects reported symptoms of ocular itching and tearing using an electronic Patient Diary Acquisition Tablet (ePDAT™), while staff assessed signs of conjunctival redness. A POAR is elicited when a score ≥ 2 for ocular itching and a score > 2 for ocular redness (in at least one region for both eyes) is established at any time point.
**Results:** 151 subjects responded with a POAR during tCAPT, at varying, subject-dependent allergen concentrations. Overall, as

the concentration of allergen increased, subjective and objective scores also increased. Subjects who reached the POAR with lower concentrations of allergen were found to have greater mean change in their symptom and sign scores between concentrations for ocular itching, tearing and redness compared to those who reach their POAR at higher allergen concentrations, at matched timepoints ($p<0.05$). For example, the 10 min timepoint, those who reached a POAR at the second lowest allergen concentration (125 PNU/mL) had a max mean change between the lowest two CAPT concentrations for itching, tearing, and redness of 1.28±0.16, 0.79±0.12, and 1.67±0.25 respectively, whereas those who reached a POAR at the highest allergen concentration (4000 PNU/mL) had a max mean change of 0.36±0.35, 0.06±0.10, 0.36±0.21 respectively, $p<0.05$.
**Conclusions:** In this study, the CAPT model was able to show differences in ocular signs and symptoms between POAR at low and high allergen concentrations, possibly indicating that those who respond to lower concentrations may be more symptomatic or have a more severe allergic responses. This approach could be used to screen for allergy severity in trials.
**Commercial Relationships: Holly I. Lorentz**, Inflamax Research Inc. (E); **Victoria Nelson**, Inflamax Research Inc (E); **Nerizsa Tenorio**, Inflamax Research Inc (E); **Anne Marie Salapatek**, Inflamax Research Inc (E)

**Program Number:** 5752 **Poster Board Number:** B0304
**Presentation Time:** 11:30 AM–1:15 PM
**Use of Conjunctival Allergen Challenge as a Tool for Predicting Response to Oral Food Challenge in Food Allergic Patients**
*Paul J. Gomes[1], Emily Schoemmell[1], Sarah Ellen Godek[1], Endri Angjeli[2], Keith J. Lane[2]*. [1]Allergy, ORA, Andover, MA; [2]R&D, Ora, Inc, Andover, MA.
**Purpose:** Current diagnostic tests for assessing food allergies include Skin Prick Tests (SPT) and Oral Food Challenges (OFC). Due to the overwhelming false positives associated with a SPT, many patients are required to undergo an OFC, which poses a significant risk to the patient due to the potential for a severe systemic allergic response. We proposed that the conjunctival allergen challenge (CAC) may be able to predict which patients are likely to experience an adverse event during an OFC. A moderate to severe ocular response following CAC (level 2 on a 0-4 Scale) has been shown to correlate well with a positive food response during an escalating OFC, and might be a better predictor of food allergies than a skin test and serum specific IgE combined.
**Methods:** Seventeen subjects ≥ 5 years of age who exhibited a positive SPT to a food allergen and received an OFC within the prior year were included in the study. The subjects received a CAC in titrated incremental allergen doses in a maximum of 8 steps, starting at a 1:320 dilution. Conjunctival redness, itchy palate, and ocular itch were assessed 10 minutes after each dose. A positive result was defined as ≥ 2 for ocular itch and ≥ 2 for conjunctival redness in both eyes. The food allergens were peanut, milk, egg, shellfish, soy and almond. The primary endpoint was mean allergen dose step at which a positive reaction was observed.
**Results:** Of the 17 subjects, 5 had a positive OFC and 12 had a negative OFC. Of the 5 subjects who had a positive OFC, all had a positive CAC response at a low allergen dose (mean dose step of 3.3±3.1). Of the 12 subjects who had a negative OFC, 5 had a negative CAC response, and 4 subjects had a positive CAC at a high allergen dose (mean step of 7.0±1.4). The remaining 3 subjects (mean dose step of 4.3±2.1) had a high score in only ocular itching or redness, and therefore challenges were discontinued.
**Conclusions:** A low-threshold CAC is an effective predictor for a positive response to an OFC. A positive high-threshold CAC and negative OFC might indicate a food sensitivity rather than allergy. The CAC can be used as an effective screening tool for identifying patients who exhibit food allergy without the risks of anaphylaxis associated with an OFC.
**Commercial Relationships: Paul J. Gomes**, Ora, Inc (E); **Emily Schoemmell**, Ora, Inc (E); **Sarah Ellen Godek**, Ora, Inc (E); **Endri Angjeli**, Ora, Inc (E); **Keith J. Lane**, Ora, Inc (E)

**Program Number:** 5753 **Poster Board Number:** B0305
**Presentation Time:** 11:30 AM–1:15 PM
**The role of Oncostatin M in the pathogenesis of severe allergic conjunctivitis**
*Keitaro Mashimo, Ayumi Usui, Nobuyuki Ebihara*. Juntendo university Urayasu hospital, Urayasu city, Japan.
**Purpose:** Vernal Keratoconjunctivitis (VKC) is severe allergic conjunctivitis. In the conjunctival giant papillae of VKC, many inflammatory cells infiltrate and produce various cytokine and chemokine. However, to date, there are no reports that which cytokine has a crucial role in the giant papillae formation of VKC. Oncostatin M (OSM) is IL-6 family. OSM signals through the receptor, stimulates the JAK/STAT signal pathways. OSM has multiple functions such as inflammation, proliferation, remodeling, fibrosis, apoptosis and more. The purpose of this study is to investigate the role of OSM in the pathogenesis of VKC.
**Methods:** The primary human conjunctival epithelial cells (HConEpiCs) and human conjunctival fibroblasts (HConFs) were cultured. Expression of OSM receptor (OSMR) in HConEpiCs and HConFs was assessed by flow cytometry and immunocytochemistry. We estimated the intensity of expression in OSMR of HConEpiCs treated with IL-4, IL-13, TGFβ and TNFα, using flow cytometry. We investigated OSM induced STAT1 and STAT3 phosphorylation in HConEpiCs by western blotting. We also analyzed the comprehensive gene expression following OSM treatment in HConEpiCs and HConFs by microarray and real-time PCR. And we measured the concentration of OSM in the tears of seven VKC patients using ELISA. The expression of OSM in giant papillae of VKC patients was determined by immunohistochemistry and real-time PCR.
**Results:** OSMR was expressed in HConEpiCs and HConFs. However, OSMR was not upregulated with IL-4, IL-13, TGFβ and TNFα. Phosoho-STAT1 and 3 were increased in HConEpiCs treated with OSM. The mRNA of Matrix metallopeptidase (MMP)1, MMP3, Suppressor of cytokine signaling 3 (SOCS3), Serpin peptidase inhibitor, clade B (SERPINB3), IL-20, IL-24 and Tenascin C were upregulated in HConEpiCs treated with OSM.($p<0.05$) Meanwhile, in HConFs, mRNA of SOCS3, SERPINB3, IL-33 and Vascular endothelial growth factor (VEGF) were upregulated by OSM. ($p<0.05$) Concentrations of OSM in the tears of VKC patients were increased. Many infiltrating cells in giant papillae produced OSM.
**Conclusions:** OSM may contribute to inflammation and tissue remodeling in giant papillae of VKC patients.
**Commercial Relationships: Keitaro Mashimo**, None; **Ayumi Usui**, None; **Nobuyuki Ebihara**, None

**Program Number:** 5754 **Poster Board Number:** B0306
**Presentation Time:** 11:30 AM–1:15 PM
**Immediate Hypersensitivity vs Late Phase Responses in Allergic Conjunctivitis Can Be Traced to Differences in Tear Cytokine Profiles Prior to Allergen Challenge**
*Rachel Smith[1], Farid Gizatullin[2], Andy Whitlock[2], Paul J. Gomes[1]*. [1]Allergy, Ora, Inc., Andover, MA; [2]Pre-Clinical Department, Ora, Inc., Andover, MA.
**Purpose:** Allergic conjunctivitis responses range from immediate hypersensitivity reactions involving mast cell degranulation to late

phase responses involving lymphocyte and eosinophil infiltration. Due to the vast range of allergic responses, we sought to determine if there are any indicative differences in tear cytokine profiles among atopic individuals prior to allergen challenge.
**Methods:** Tears were collected from three atopic and three non-atopic human subjects outside of the allergy season during an internal pilot study. Tears were collected from both eyes at the tear meniscus, taking care not to touch the lid or corneal surface, and not to induce reflex tearing. Samples were dispensed into a microcentrifuge tube, immediately capped, and placed on dry ice. Tears were collected at baseline from all subjects, and additionally from atopic subjects post- allergen challenge. An allergic reaction was induced using conjunctival allergen challenge (Ora CAC®) system. Tears were analyzed for IFN-γ, IL-1β, IL-2, IL-4, IL-6. IL-8, IL-10, IL-12p70, IL-13, and TNF-α using the MSD V-PLEX Platform and Meso QuickPlex SQ 120 plate reader.
**Results:** Even prior to allergen challenge, IL-6 and IL-8 were increased in atopic baseline tears compared to non-atopic baseline tears. In one late phase responder, the cytokine profile at baseline displayed elevated IL-6 compared to the baseline of the remaining atopic subjects. Differences in changes in IL-6 and IL-8 were also seen among the atopic subjects post-allergen challenge. The late phase allergy responder showed a much larger change in IL-6 post-CAC compared to the other atopic subjects at the same post-CAC time-points.
**Conclusions:** Allergic subjects who manifest a late phase response might have a different tear cytokine profile than atopic subjects who do not. Differences can be seen even prior to allergen challenge, when compared to other atopic subjects and to non-atopic subjects. This data may be valuable in establishing predictive profiles of atopic individuals for study sorting, and may provide clues as to why certain atopic individuals respond poorly to traditional anti-histamine treatments.
**Commercial Relationships: Rachel Smith**, Ora, Inc. (E); **Farid Gizatullin**, Ora, Inc. (E); **Andy Whitlock**, Ora, Inc. (E); **Paul J. Gomes**, Ora, Inc. (E)

**Program Number:** 5755 **Poster Board Number:** B0307
**Presentation Time:** 11:30 AM–1:15 PM
**Effect of resolvin on allergic conjunctivitis in mouse**
*Satoshi Iwamoto[2], Mikiko Okano[2], Takehiko Yokomizo[1], Akira Matsuda[2]*. [1]Biochemistry, Juntendo Univ School of Med, Chiyodaku, Japan; [2]Ophthalmology, Juntendo Univ School of Med, Tokyo, Japan.
**Purpose:** Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are the ω3 poly unsaturated fatty acids that are found in fish oil. Resolvins are the metabolites of EPA and DHA. Previous reports showed that resolvins plays anti-inflammatory roles in vasculitis and asthma models in mice. In this study, we investigated the roles of resolvin D2 in allergic conjunctivitis using a mouse experimental allergic conjunctivitis model.
**Methods:** 10-12 weeks-old female BALB/c mice were used. Mice were sensitized and challenged for 4 days by ragweed pollen. Resolvin $D_2$ (100 ng) was injected intraperitoneally before sensitization and before each challenge. 24 hours after the last challenge, conjunctival tissue was analyzed by giemsa staining. Furthermore, serum total IgE and Th2 cytokines in the conjunctiva were quantified by Q-PCR.
**Results:** The number of infiltrating eosinophil was reduced by resolvin $D_2$ treatment. On the other hand, concentration of total IgE and expression of Th2 (*Il4, Il5* and *Il13*) cytokines in the conjunctiva were not affected by resolvin $D_2$ treatment.
**Conclusions:** Resolvin $D_2$ attenuated eosinophil infiltration in a mouse allergic conjunctivitis model. The effect of resolvin is not attributed to total IgE and Th2 cytokine regulation. Resolvin might be a new therapeutic modality of allergic conjunctivitis.
**Commercial Relationships: Satoshi Iwamoto**, None; **Mikiko Okano**, None; **Takehiko Yokomizo**, None; **Akira Matsuda**, None

**Program Number:** 5756 **Poster Board Number:** B0308
**Presentation Time:** 11:30 AM–1:15 PM
**Suppressed Signaling of Interleukine 27, a Novel Immunosuppressive Cytokine, in Experimental Allergic Conjunctivitis Induced by Short Ragweed Pollen**
*XIN CHEN[1, 2], Wei Chi[1, 3], Changjun Wang[1], Zhao Liu[1], Xia Hua[1], Ruzhi Deng[1, 2], Fang Bian[1], Stephen C. Pflugfelder[1], De-Quan Li[1]*. [1]OPHTHALMOLOGY, BAYLOR COLLEGE OF MEDICINE, Houston, TX; [2]School of Ophthalmology and Optometry, Wenzhou Medical University, Wenzhou, China; [3]Zhongshan Ophthalmic Center, Sun Yan-Sen University, Guangzhou, China.
**Purpose:** While most studies focused on pro-allergic cytokines, the protective role of immunosuppressive cytokines in allergic inflammation is largely unknown. This study was to explore a novel anti-inflammatory role and molecular mechanism of IL-27 in allergic inflammation induced by short ragweed pollen.
**Methods:** A murine model of experimental allergic conjunctivitis (EAC) was induced in BALB/c or IL-27Rα deficient (*WSX-1*$^{-/-}$) mice by short ragweed (SRW) pollen, which was first immunized with 50μg in 5 mg Imject Alum by footpad injection on day 1, followed by topical challenge of 1.5mg SRW in 10μl PBS into each eye once a day from days 11 to 13. Mice without treatment (UT) or treated with PBS along will be used as controls. The serum, eyeballs, conjunctiva and cervical lymph nodes (CLN) were collected on days 14 for evaluation. The mRNA expression was determined by RT-qPCR. The protein production was evaluated by immunostaining, ELISA, Western blotting and/or flow cytometry.
**Results:** Typical allergic signs, stimulated TSLP signaling and Th2 cytokine overproduction were observed in ocular surface of BALB/c mice with EAC induced by SRW. The decrease of IL-27 at mRNA (IL-27A/EBI3 two chains) and protein levels, accompanied by suppressed levels of IL-10 and signaling molecules STAT1, C-Maf, ICOS and PD-L1 with increased STAT6 and GATA3, was observed in conjunctiva and draining CLN tissues of SRW-EAC mice when compared with UT or PBS control mice, as evaluated by RT-qPCR, immunofluorescent staining, ELISA and/or Western blotting. IL-27 was identified to be produced mainly by dendritic cells (DCs) and macrophages, but not epithelial or T cells by flow cytometry and immunostaining with specific cell surface markers CD11c, F4/80 and CD4. Furthermore, EAC model induced by SRW in *WSX-1*$^{-/-}$ mice showed more severe allergic signs with significantly higher expression of TSLP by corneal and conjunctival epithelial cells, OX40L by DCs, and Th2 cytokines IL-4, IL-5 and IL-13 by $CD4^+$ T cells than EAC-BALB/c mice.
**Conclusions:** Our findings for the first time uncovered a novel mechanism by which SRW pollen triggers Th2-dominant allergic inflammation by suppressing IL-27 anti-allergic signaling pathway. These findings shed light on the understanding of pollen-induced allergic inflammation, and may create new therapeutic targets to treat allergic diseases.
**Commercial Relationships: XIN CHEN**, None; **Wei Chi**, None; **Changjun Wang**, None; **Zhao Liu**, None; **Xia Hua**, None; **Ruzhi Deng**, None; **Fang Bian**, None; **Stephen C. Pflugfelder**, None; **De-Quan Li**, None

**Support:** NIH NEI Grants EY023598 (DQL) and EY011915 (SCP), Core Grant for Vision Research EY002520, Research to Prevent Blindness, Oshman Foundation, William Stamps Farish Fund.

**Program Number:** 5757 **Poster Board Number:** B0309
**Presentation Time:** 11:30 AM–1:15 PM
**Ocular Allergy and Quality of Life: Patient Survey**
*Emily Schoemmell[1], Kara Sicard[1], Rachel Smith[1], Yesha Raval[1], Paul J. Gomes[1], David A. Hollander[2]*. [1]Allergy, Ora, Inc, Adover, MA; [2]Ora, Inc, Andover, MA.
**Purpose:** We conducted a survey to assess the impact of ocular allergy on quality of life from a patient's perspective. Our goal was to compare our local population to national and global averages, and to identify potential emerging trends.
**Methods:** Subjects from an ocular allergy clinical trial database and agreed to participate in a clinical trial were asked to participate in this IRB-approved questionnaire.Sixty subjects completed questionnaires and were included in the survey analysis. Subjects provided information on their disease characteristics, their treatment strategies, and their satisfaction with their current therapeutic regimes.
**Results:** The mean age of respondents was 45.7 years (52% male). The overwhelming majority (95%) reported experiencing nasal as well as ocular allergy symptoms, while smaller percentages (18-46%) stated that they also suffered from food allergies, skin allergies, or asthma. Approximately 1 in 5 reported some type of allergy to medication. As a group, the respondents reflect recent national trends: 46% experience allergic symptoms year-round, while 62% experience seasonal allergic disease. The second-most reported complaint (after ocular itching) among all patients was excessive tearing or watery eyes, not ocular redness. A high percentage of patients (61%) reported not seeking treatments for their allergies, less than half of those who do seek treatments use prescription eyedrops as a treatment. While a majority had reported that they tried over the counter medications two-thirds stated that these products were effective "some or none of the time". Subjects reported preferring a treatment that could relieve both itch and redness, and half of all respondents reported also experiencing some degree of dry eye.
**Conclusions:** This survey confirms that our study population accurately reflects national and global trends regarding incidence of perennial and seasonal allergic disease[1], and highlights the need for improved treatments for those with year-round allergy. We find that an overwhelming majority of patients experience both ocular and nasal symptoms, and many suffer with additional allergic symptoms. The survey results highlight the importance of encouraging patients to take advantage of existing therapeutic options that are likely to improve their quality of life.
1. Rosario N. Epidemiology of allergic conjunctivitis. *Curr.Opin. Allergy Clin. Immun*. 2011; 11:471–476.
**Commercial Relationships: Emily Schoemmell**, Ora, Inc (E); **Kara Sicard**, Ora, Inc (E); **Rachel Smith**, Ora, Inc (E); **Yesha Raval**, Ora,Inc (E); **Paul J. Gomes**, Ora, Inc (E); **David A. Hollander**, Ora, Inc (E)

**Program Number:** 5758 **Poster Board Number:** B0310
**Presentation Time:** 11:30 AM–1:15 PM
**Crosslink between lipids and uveitis: a lipidomic analysis**
*Haiyan Wang*. ophthalmology, shanghai first people's hospital affiliated to Jiaotong University, Shanghai, China.
**Purpose:** We try to explore the roles of a variety of phospholipids and sphingolipids in the inflammatory process of uveitis.
**Methods:** Experiments were conducted in Endotoxin-induced uveitis (EIU) rat model. Aqueous humor (AH) and retina were obtained from control and EIU rats. Lipids were extracted and then subjected to mass spectrometric identification and ratiometric quantification. Relative intensity analysis (EIU/control) was performed to evaluate the amount change of common lipids between EIU and control groups.
**Results:** EIU rat model was successfully established according to clinical, biochemical, and histological evaluation. Increase in total sphingolipids and phospholipids was observed in EIU AH and retina. Unique lipid species were found in control as well as in EIU AH and retina. Several phospholipid and sphingolipid species were found common to both groups but with remarkable differences in amount in individual group. Commensurate with significant increased level of lysophospholipids in EIU AH and retina, the ratio of lysophospholipids to total phospholipids was found significantly increased too. Further study revealed that PIs, regarded as immune homeostasis maintainer, remarkably increased in EIU AH by 2.3-fold and EIU retina by 4.2-fold. Pro-inflammatory factor PGs showed noteworthy increase in EIU AH by 1.5-fold and EIU retina by 8.0-fold. We also detected a significant increase of 18:0 lysophosphatidylcholine, which can activate G protein-coupled receptor G2A-mediated leukocyte response, in EIU group (fold change=6.4 in AH and 3.8 in retina). Cer240, Cer241, and SM240 remarkably increased in EIU AH. Concurrently the amount of total sphingolipids also significantly increase in EIU retina by 2.8- fold change, especially with increased C12 C-1-P, C16 C-1-P, C24 C-1-P, upregulated Cer160, Cer240, SM120, and SM240. Signaling molecule C-1-P is believed to transfer inflammation to homeostasis restoration by inhibiting NF-κB activation. However we still found elevated NF-κB in EIU retina, indicating that this level of enhancement in endogenous C-1-P cannot reverse the inflammation process induced by LPS.
**Conclusions:** Taken together, specific lipids might have exert function in EIU inflammation. Exogenous topical application of these protective lipids or inhibition of these pro-inflammatory lipids may be useful therapeutic strategies for the resolution of EIU. And the underlying mechanism deserves further study
**Commercial Relationships: Haiyan Wang**, None
**Support:** National Natural Science Foundation of China (No. 81300776).

**Program Number:** 5759 **Poster Board Number:** B0311
**Presentation Time:** 11:30 AM–1:15 PM
**The superoxide dismutase 1 knock out (SOD1 KO) mouse develops age-dependent auto reactivity to ocular antigens**
*Diwa Koirala[1], Alessandro Iannaccone[2], TJ Hollingsworth[3], Sarka Beranova-Giorgianni[1], Ivan Gerling[4], Marko Z. Radic[5], Francesco Giorgianni[1]*. [1]Pharmaceutical Sciences, University of Tennessee Health Science Center, Memphis, TN; [2]Duke Eye Center, Duke University Medical Center, Memphis, TN; [3]Neuroscience, University of Tennessee Health Science Center, Memphis, TN; [4]Internal Medicine/Endocrinology, University of Tennessee Health Science Center, Memphis, TN; [5]Microbiology, Immunology, and Biochemistry, University of Tennessee Health Science Center, Memphis, TN.
**Purpose:** We have evidence that sera from AMD patients exhibit increased levels of auto-antibodies (AAbs) against macular antigens (PLOS One 2015; 10:e0145323). Here we have tested the hypothesis that the SOD1 KO mouse model, analogously to patients with AMD, develops an age-dependent increase in auto-reactivity against ocular antigens, and that this phenotype develops earlier and more severely in the SOD1/APOE double knock out (DKO) on high fat diet (HFD).
**Methods:** Wild type (WT), SOD1 KO and DKO mice were aged up to 14 months. For the WT, and the DKO genotypes a subgroup of animals were maintained on HFD for 3 months before collection

of sera and ocular tissues. At 9, 12, and 14 months sera and ocular tissues were collected for all animal groups. Sera were used to test for auto reactivity against a pool of ocular proteins from WT mice. Autoreactive bands were detected by immunoblotting and fluorescence imaging.

**Results:** Our preliminary data suggest that the SOD1 KO and the DKO exhibit similar, increased autoreactivities against ocular antigens starting at 9 months of age as compared to WT. We found no significant differences in the number of detected autoreactive bands between animals kept on regular diet as compared to animals on HFD. The strongest autoreactivity was observed against ocular antigens in the 17 to 35 kDa molecular weight range.

**Conclusions:** We have obtained preliminary evidence that animals with impaired antioxidative defense mechanisms display an age-dependent increase in autoreactivity against ocular antigens. The observed phenotype was not worsened in the DKO on HFD, suggesting that oxidative stress might play a more prominent role in AMD than impaired lipid metabolism and/or diet. Further investigation of our animal models will focus on the characterization of other retinal phenotypic differences and on identification of the ocular proteins targeted by the AAbs.

**Commercial Relationships: Diwa Koirala**, None; **Alessandro Iannaccone**, None; **TJ Hollingsworth**, None; **Sarka Beranova-Giorgianni**, None; **Ivan Gerling**, None; **Marko Z. Radic**, None; **Francesco Giorgianni**, None
**Support:** NEI/NIH grant R01 EY022706 (FG/AI)