135 Autoimmune Ocular disease: selfies gone wrong
Sunday, May 07, 2017 1:30 PM–3:15 PM
Exhibit/Poster Hall Poster Session
Program #/Board # Range: 528–564/B0083–B0119
Organizing Section: Immunology/Microbiology

Program Number: 528 Poster Board Number: B0083
Presentation Time: 1:30 PM–3:15 PM

Immune mechanisms underlying relapsing-remitting or monophasic experimental autoimmune uveitis (EAU) with neovascularization
Gerhild Wildner1, Maria Diedrichs-Möhring1, Ulrike Kaufmann1, Christine von Toerne2, Stephan R. Thurau1, 3, 4
1Ophthalmology, Clinic of the University of Munich LMU, Munich, Germany; 2Research Unit Protein Science, Helmholtz-Zentrum München, Neuherberg, Germany; 3NYU Langone Medical Center, New York, NY.

Purpose: We investigated spontaneously relapsing-remitting EAU, induced with the interphotoreceptor retinoid-binding protein peptide R14 or monophasic/chronic disease with choriretinal neovascularization induced with retinal S-Antigen peptide PDSAg to elucidate immune mechanisms underlying the different disease courses.

Methods: Lewis rats were immunized with peptide PDSAg or R14 or both to induce EAU and to generate T cell lines. T cell lines were analyzed for gene and protein expression/secretion. Rats with EAU induced by both antigens were treated with chemokine mutants, analyzed for intracellular T cell populations or injected intraocularly with antibodies to IFN-g, IL-17 and IL-10 to investigate the effect on EAU.

Results: Gene expression analysis revealed 26 genes upregulated in R14-specific T cells upstream or downstream of IFN-g and belonging to various intracellular signaling pathways. PDSAg-specific T cells produced more IL-17, IL-6, IL-10, CCL2 and VEGF (resulting in CNV) than R14-specific T cells, which had higher secretion of IFN-g, IL-18, CCL3 and CCL5. R14-induced relapsing EAU was significantly ameliorated by intravascular injection of anti-IFN-g and anti-IL-10 and deteriorated by anti-IL-17. PDSAg-induced, monophasic EAU was only reduced with anti-IL-17. Cells co-expressing IFN-g, IL-17 and IL-10 increased during monophasic and decreased during relapsing disease, Foxp3-expression increased late in monophasic EAU. A CCL5-mutant only suppressed PDSAg-, but not R14-mediated EAU, and co-immunization of rats with both antigens revealed that the monophasic EAU dominated with respect of the disease course and intracellular T cell populations.

Conclusions: The two EAU models in Lewis rats will help us to understand the interplay of various immune mechanisms in relapsing autoimmunity and to develop and test new therapies for uveitis in ongoing disease.

Commercial Relationships: Gerhild Wildner, AbbVie (R), Panoptes Pharma GmbH (F), Pharm-Allergan (R); Maria Diedrichs-Möhring, Panoptes Pharma (F); Ulrike Kaufmann, None; Christine von Toerne, None; Stephan R. Thurau, AbbVie (R), Santen Inc (R), Pharm-Allergan (R), Panoptes Pharma GmbH (C)

Program Number: 529 Poster Board Number: B0084
Presentation Time: 1:30 PM–3:15 PM

Preparation of tolerogenic GM-CSF murine bone marrow cells for potential use in autoimmune experimental uveoretinitis model
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Purpose: Autoimmune uveitis, a potentially sight-threatening disease is induced by immunodendritic cells (DCs) via Th1/Th17 cells. Studies have shown that tolerogenic (tol) DCs prevent development of autoimmunity including autoimmune experimental uveoretinitis (EAU). However, studies have shown that conventionally prepared GM-CSF derived bone marrow (BM) DC population yielded a low percentage of CD11c+CD11b+ MHCII+CD135+ DCs, with CD115+ macrophage/monocyte myeloid cells as the predominant population. Therefore, we aimed to generate a tolDC population from bone marrow progenitor cell population by depleting lineage-defined cells, particularly cells expressing MHCII prior to culture.

Methods: Bone marrow cells (BMCs) were extracted from C57Bl/6 mice and depleted for MHCII+, CD4+, CD8+ and B220+ cells. These Lin+ cells were cultured for 6 days in GM-CSF and then depleted for granulocytes (Gr-1). Further, BMCs were cultured for 24 hours (h) in the presence of tolerogenic agent (Vitamin D3+Dexamethasone) and an immunogenic stimulant (Mycobacterium tuberculosis (Mtb) extract (15µg/ml) (24h) or lipopolysaccharide (LPS) (1µg/ml) (1h)). Using multicolour flow cytometry, these cells were tested for maturation status (MHCII, CD40), and characterised using progenitor (CX3CR1, c-kit) and differentiation (CD11b, CD11c, Ly6C, Zbtb46) markers. Additionally, BMCs were investigated for their functional capacity in T cell proliferation induction using OTII specific assay.

Results: Our data suggests that after 7 days of culture more than 60% of untreated BMCs are committed cDC progenitors expressing Zbtb46, yet have low levels of activation markers (MHCII, CD40). These results are similar to the Vitamin D3+Dexamethasone treated BMCs. DC-enriched BMCs induced T regulatory cell (Tregs) (FoxP3+) as effectively as the Vitamin D3+Dexamethasone treated BMCs. In contrast LPS stimulated cells failed to promote Tregs but induced Th1 (Tbet+) cells. Our protocol reduced the CD115+ macrophage contamination from 80% to 30% and yielded a maximum of 98.5% of committed progenitor cell population in the DC fraction.

Conclusions: Lin+ BMCs generated in GM-CSF were highly enriched for progenitor/tolDC population that expressed low levels of MHCII and CD40. The tolerogenic state of these cells was confirmed by Treg induction in vitro. These cells are currently investigated in the in vivo model of EAU.

Commercial Relationships: Maria Christofi, None; Lucia Kuffova, None; John V. Forrester, None

Program Number: 530 Poster Board Number: B0085
Presentation Time: 1:30 PM–3:15 PM

Comparison of Inflammatory Aqueous Cell Populations from Experimental Autoimmune Uveitis (EAU) and Primed Mycobacterial Uveitis (PMU) in Lewis Rats
Kathryn L. Pepple, Russell N. Van Gelder. Ophthalmology, University of Washington, Seattle, WA.

Purpose: Experimental Autoimmune Uveitis (EAU) and Primed Mycobacterial Uveitis (PMU) are two models of uveitis that have demonstrated mechanistic differences by histology, cytokine, and
proteomic analysis. We sought here to compare the intraocular effector cells present on the day of peak inflammation using flow cytometry.

**Methods:** EAU and PMU were initiated in female Lewis rats (total=16, EAU=5, PMU=11). Aqueous humor was collected on day 14 for EAU and on day 2 for PMU (days of peak inflammation). Eyes were analyzed individually. Cell surface labelling was performed for CD3, CD4, CD8, CD45R, and CD11b. Viability was established by dye exclusion. Flow cytometry data was collected on a CANTO II and analyzed with FlowJo. Populations were compared using Student’s t-test with Bonferroni correction (p<0.008 for significance).

**Results:** Between 10 and 15 microliters of aqueous humor was obtained from each eye. The average cell concentration was 1.47x10^6 cells/ml for EAU eyes and 1.28 x 10^6 cells/ml for PMU eyes. There were two distinct populations defined by side scatter (SSC-A). 89% of the PMU cell population demonstrated high SSC-A compared to only 46% of the EAU population. CD3, CD45, CD11b, and CD8 cell surface markers also identified significantly different population by percentage in each model. CD3+ cells made up 5% of the PMU vs 24% of the EAU population (p=0.0001). CD45+ cells made up 4% of the PMU vs 19% of the EAU populations (p<0.0001). CD11b+ cells made up 53% of the PMU population and 30% of the EAU population (p=0.002). There was no significant difference in the percentage of CD3+CD4+ cells (78% in PMU and 71% in EAU, p=0.02). However, there was a significantly larger percentage of CD3+CD8+ cells in EAU (21%) vs PMU (7%) (p=0.0001).

**Conclusions:** There are significant differences in the effector cell infiltrate found in the eyes of rats with PMU and EAU. PMU has more granulocytes and CD11b+ cells consistent with a predominantly innate immune effector cell response. EAU has a predominant small lymphocytic infiltrate with a significant T and B cell presence consistent with an adaptive immune driven response. The differences identified here continue to highlight the mechanistic differences between PMU and EAU, and support PMU as an alternative model to EAU.

**Commercial Relationships:** Kathryn L. Pepple, Russell N. Van Gelder, None

Support: NIH NEI K08EY023998, and an unrestricted departmental grant from RPB

**Program Number:** B0086

**Presentation Time:** 1:30 PM–3:15 PM

**Treatment with repository corticotropin injection reduces the progression of experimental autoimmune uveitis in rats**

_Dale Wright, Ben Zweifel, Rick Fitch_. Biological Sciences, Mallinckrodt Pharmaceuticals, Hazelwood, MO.

**Purpose:** Previous studies have suggested that melanocortin receptor (MCR) agonists play a role in regulating the progression and resolution of experimental autoimmune uveitis (EAU). Repository corticotropin injection (RCI: H.P. Acthar® gel) is a complex formulation containing a porcine ACTH analogue. ACTH is a melanocortin peptide that binds to the 5 known MCRs. Because RCI is an FDA-approved treatment for certain inflammatory ocular disorders, the aim of this study was to investigate the effects of RCI on a preclinical model of EAU.

**Methods:** Lewis rats were immunized with interphotoreceptor retinoid binding protein (IRBP) peptide in complete Freund’s adjuvant. Inflammation was observed under a dissection microscope on days 14 for EAU and on day 2 for PMU (days of peak inflammation). Rats were dosed with RCI (10, 40, or 400 U/kg), Placebo gel (5mL/kg) or Prednisolone (0.1, 1, or 5 mg/kg) every other day starting on the first day of the study. Sections were stained with hematoxylin & eosin and scored.

**Results:** Clinical assessment within the anterior chamber of the eye demonstrated that RCI administered at 40 or 400 U/kg significantly reduced the ocular clinical disease score on day 14 compared to placebo (0.93 ± 0.18 and 0.85 ± 0.17 versus 1.98 ± 0.22, respectively, (p≤0.01). In contrast, prednisolone marginally reduced the clinical disease score, at the doses tested, with only the 1 mg/kg dose having significance (1.05 ± 0.18; p≤0.05). In addition, the clinical findings for RCI were supported by the histological data, showing protection to the retinal architecture with a reduction in inflammation at all 3 doses evaluated.

**Conclusions:** The treatment of EAU with RCI resulted in the suppression of the ocular clinical score and inflammation reducing retinal damage. These responses by RCI are thought to be through the different melanocortin receptors. While multiple melanocortin receptors have been shown to control inflammation, these data are the first to explore the effects of RCI in a preclinical model of experimental autoimmune uveitis.

**Commercial Relationships:** Dale Wright, Mallinckrodt Pharmaceuticals (E); Ben Zweifel, Mallinckrodt Pharmaceuticals (E); Rick Fitch, Mallinckrodt Pharmaceuticals (E)

**Program Number:** B0087

**Presentation Time:** 1:30 PM–3:15 PM

**Preventing relapses and chorioretinal neovascularization in EAU with a novel small molecule suppressing rat and human T cells, but not retinal pigment epithelial cells**

_Stephan R. Thurau¹, Maria Diedrichs-Möhring¹, Claudia Priglinger¹, Franz Obermayr¹, Gerhild Wildner¹. Clinic of the University of Munich, Munich, Germany; ²Panoptes Pharma, Wien, Austria.

**Purpose:** Experimental autoimmune uveitis (EAU) in rats is a suitable model for the respective intraocular inflammatory disease in humans. We investigated the in vivo effect of the small molecule dihydroorotate dehydrogenase inhibitor PP-001 in spontaneously relapsing-r致命ing and monophasic/chronic EAU with chorioretinal neovascularization and in vitro on rat and human lymphocytes and human RPE cells.

**Methods:** Lewis rats were immunized with S-Antigen peptide PDSAg or interphotoreceptor retinoid-binding protein peptide R14 to induce monophasic or relapsing EAU, respectively. PP-001 was fed daily or injected intraocularly once. Uveitis was graded clinically and histologically, neovascularization was determined by histology. Rat lymph node cells and human peripheral lymphocytes of a healthy donor were stimulated with PHA and cocultured with PP-001. The human RPE cell line ARPE 19 and primary human RPE cells were cocultured with PP-001 or anti-VEGF, cytokines in culture supernatants were measured by bioplex bead assay.

**Results:** Daily oral treatment with PP-001 after onset or peak of EAU induced with retinal S-Antigen peptide PDSAg (monophasic uveitis) significantly reduced neovascularization. A significant reduction of the number and intensity of relapses in R14-induced EAU was observed when daily oral PP-001 treatment was initiated or a single intraocular injection performed after resolution of the first attack of uveitis. Proliferation of autoantigen-specific rat T-cell lines and secretion of IFN-γ, IL-17, IL-10, IP-10 and VEGF were efficiently suppressed by PP-001. PP-001 also suppressed proliferation and cytokine secretion of PHA-stimulated human PBL without affecting the viability of the cells. Treating the human retinal pigment epithelial cell line ARPE-19 with PP-001 no suppressive effect on proliferation, viability and VEGF production was observed, in contrast to treatment with anti-VEGF bevacizumab. PP-001 showed no toxic effect on rat eye tissues after intraocular injection.
Conclusions: Here we present a novel drug for systemic and local treatment of autoimmune uveitis without adverse effects on resident ocular cells. Furthermore, our data show that neovascularization as a sequel in uveitis can be caused by VEGF-producing autoreactive T cells only and not by RPE.

Commercial Relationships: Stephan R. Thurau, AbbVie (R), Panoptes Pharma (C), Santen (R), Pharm Allergan (R); Maria Diedrichs-Möhring, Panoptes Pharma (F); Claudia Priglinger, None; Franz Obernay, Panoptes Pharma (E); Gerhild Wildner, AbbVie (R), Panoptes Pharma (F), Pharm Allergan (R)

Program Number: 533 Poster Board Number: B0088
Presentation Time: 1:30 PM–3:15 PM
The Lipoxin A_4 Circuit is Essential to Prevent Development of Experimental Autoimmune Uveitis
Jessica Wei, Victoria Ly, Allison Chan, Julia Yoo, Karsten Gronert.
Vision Science, UC Berkeley, Berkeley, CA.

Purpose: The prevalence of autoimmune diseases has steadily increased in developed countries in the past decade, with annual health care costs at $100 billion. Current treatment for autoimmune uveitis is systemic immune suppression, which often causes opportunistic infections and secondary glaucoma. We previously showed that lipoxin A_4 (LXA_4) exerts protective effects during uveitis pathogenesis by limiting inflammation and disease progression of experimental autoimmune uveitis (EAU). We investigated whether LXA_4 is an effective treatment and endogenous protective circuit of EAU in C57B6/J and B10RIII mouse strains and set out to define the mechanism of action and cellular targets for the LXA_4 circuit.

Methods: Autoimmune uveitis was induced in both C57B6/J and B10RIII mice to assess the role of LXA_4 during uveitis pathogenesis. Expression of 5-lipoxygenase (5-LOX), 12/15-lipoxygenase (12/15-LOX) and LXA_4 receptor gene expression was assessed in the eye and lymph nodes to determine the role of LXA_4 circuit during healthy homeostasis and EAU. Endogenous LXA_4 formation in tissues and isolated effector cells was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Immune cell infiltration, identification and EAU pathogenesis was assessed by flow cytometry, immunohistochemistry and clinical scoring of the fundus and retinal morphology.

Results: 5-LOX and 12/15-LOX are dramatically upregulated in the choroid and retina respectively, and LXA_4 receptor expression was cell-type specific indicating novel site- and cell-specific actions of the LXA_4 circuit in the eye and lymph nodes. Endogenous formation of LXA_4 was dramatically abrogated in the draining lymph nodes by a factor of 4 (n=6) during peak inflammation, which correlated with autoimmune disease progression and development. More importantly, treatment with LXA_4 prevented EAU development in both C57B6/J and B10RIII mice.

Conclusions: Findings suggest that the LXA_4 circuit in the eye and lymph nodes is essential in maintaining healthy homeostasis and balanced T-cell activation, and is a key factor for the limiting the progression and development of EAU. This endogenous protective and immune regulatory circuit can be amplified therapeutically and thus is a target of interest for treating ocular autoimmune diseases.

Commercial Relationships: Jessica Wei; Victoria Ly; None; Allison Chan, None; Julia Yoo, None; Karsten Gronert, None
Support: EY026082

Program Number: 534 Poster Board Number: B0089
Presentation Time: 1:30 PM–3:15 PM
PD-1 Receptor Blockade Decreases IRBP-induced Uveitis in Mice
Negin Ashki1, Ann M. Chan1, Ralph D. Levinson1, Yu-Ling Chang2, Lynn K. Gordon3.
1 Ophthalmology, University of California Los Angeles, LOS ANGELES, CA; 2 Pathology, University of California Los Angeles, LOS ANGELES, CA.

Purpose: Uveitis is a potentially blinding, immune mediated, intraocular inflammatory disease. Although programmed death-1 (PD-1) binding to its two ligands is believed to down-regulate autoimmunity, we previously observed that blockade of PD-1Ligand 1 prevents uveitis in a mouse model of experimental autoimmune uveitis (EAU). To further investigate these findings, we tested the hypothesis that PD-1 blockade using an antibody against PD-1 receptor would decrease EAU.

Methods: Uveitis was induced in C57Bl/6 mice using an IRBP peptide according to published protocols. All experiments were carried out in strict accordance with ARVO guidelines and the Guide for the Care and Use of Laboratory Animals. Mice were treated with 5mg/kg of anti-PD-1 antibody (BioXCell) or a control IgG (EAU+IgG) once a week for three weeks. Analysis of uveitis severity was done through a masked examination of the fundus and the animals were subject to euthanasia and histologic evaluation at 3 weeks after peptide immunization; both on a four-point scale.

Results: Clinical scores were significantly decreased (P<0.0001) in animals that received early treatment with anti-PD-1 antibody compared to either EAU or EAU+IgG groups. In concordance with the clinical examination, histological evaluation of the mice at the peak of inflammation (day 21) revealed that 100% of control EAU and EAU+IgG mice developed uveitis with an average histological score of 1.19 and 0.83, respectively. Only 25% of EAU+PD-1 antibody treated animals developed uveitis with an average histological score of 0.094; (p<0.0001). No spontaneous clinical or histological evidence for uveitis was seen in any of the control wild type mice.

Conclusions: The decrease in uveitis susceptibility following treatment with anti-PD-1 antibody confirmed our previous findings; suggesting that it is the blockade of PD-1, PD-ligand interaction, and not an alternative PD-ligand pathway that is protective for uveitis development in EAU. This observation may lead to a new understanding of the role of the PD-1 system in inflammation pathogenesis. The availability of blocking antibodies for PD-1 and PD-ligands, recently approved for use in cancer immunotherapy, presents the potential for clinical use of these agents in uveitis prevention. Additional studies are required to determine if blockade of this system would improve inflammation in ongoing uveitis.

Commercial Relationships: Negin Ashki; Ann M. Chan, None; Ralph D. Levinson, None; Yu-Ling Chang, None; Lynn K. Gordon, None

Program Number: 535 Poster Board Number: B0090
Presentation Time: 1:30 PM–3:15 PM
Anti-Uveitic Treg Cells Function Through PD-1 and a Subset of Uveitis Patients Express PD-1 with A2Ar Stimulation
Darren J. Lee, Dawei Wang, Fauzyya Muhammad. Ophthalmology/Dean McGee Eye Institute, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Purpose: Resolution of experimental autoimmune uveoretinitis (EAU) is marked by antigen specific regulatory immunity in the spleen. This post-EAU regulatory immunity requires adenosine 2A (A2Ar) expression on post-EAU Treg cells that are PD-1⁺ PD-L1⁺Nrp-1⁺CD25⁺CD4⁺ (PD-1 Tregs). These Tregs suppress T eff cells through PD-1, but it has not been demonstrated if the suppression is mediated by signaling through the T cell or APC. It is also not known...
Experimental autoimmune uveitis (EAU) was induced by subcutaneous injection of human interphotoreceptor retinoid-binding protein peptide (IRBP, New England Peptide) in male C57BL/6 mice (8-10 weeks old) using immunization with mycobacterium tuberculosis (Difco) in Complete Freund’s adjuvant, and pertussis toxin (Sigma). Spatial visual thresholds were measured in the mice with a virtual optokinetic system (OptoMotry, CerebralMechanics Inc.), and a fundus evaluation was obtained weekly. In Study-1, visual function was measured at baseline and during the course of EAU. Spatial frequency and contrast thresholds in both eyes were measured, and the baseline results were consistent with published values for normal C57BL/6 mice (Prusky et al., 2004; 2006). In Study-2, Animals in EAU groups, were intraperitoneally injected with either an active anti-IL-6R antagonist (anti-mFc-6R, 100 mg/kg) or control protein (mFc, 33mg/kg) every 3 days from days 5 post EAU. Animals were terminated on day 28 to collect ocular tissues for histology analysis.

**Results:** We found that visual decline commenced in the uveitis group within 2 weeks of EAU induction, and reached ~40% after 4 weeks. No visual decline was found in non-immunized controls. Visual decline was mitigated in Anti-IL-6R antibody-treated groups: decline was delayed until 3 weeks after induction. In EAU animals treated with with mFc the decline was unchanged. Improved visual function was accompanied by improved fundus scores.

**Conclusions:** Ocular inflammation due to EAU was mitigated by inhibiting C5 activity, either by genetic deletion or pharmacologic inhibition with a specific monoclonal antibody. These results indicate that C5 is a potential therapeutic target for autoimmune uveitis.

**Commercial Relationships:** Aixu Sun, Ming Yuan, Regeneron Pharmaceutical Inc. (E); Hua Yang, Regeneron Pharmaceutical Inc. (E); Thomas C. MacPherson, Regeneron Pharmaceutical Inc. (E); Adrianna Latuszek, Regeneron Pharmaceutical Inc. (E); Henry Chen, Regeneron Pharmaceutical Inc. (E); Ying Hu, Regeneron Pharmaceutical Inc. (E); Jingtai Cao, Regeneron Pharmaceutical Inc. (E); Carl Romano, Regeneron Pharmaceutical Inc. (E)

**Program Number:** 536 Poster Board Number: B0091
**Presentation Time:** 1:30 PM–3:15 PM
**Systemic administration of an anti-IL-6R antibody mitigated visual decline in a murine model of Experimental Autoimmune Uveitis (EAU)**

**Purpose:** Uveitis is a major source of human visual disability. Previous studies demonstrated that a murine IL-6R antibody delivered systemically inhibits the retinal and choroidal inflammation and reduces infiltrates in the vitreous in C57BL/6 mice (Cao et al, IOVS 2013; E-Abstract 5193). The present study aimed to determine if visual function (i.e. spatial frequency and contrast thresholds) declined with EAU and if so, to determine if anti-IL6R treatment would mitigate the decline.

**Methods:** Experimental autoimmune uveitis (EAU) was induced in male C57BL/6J mice (8-10 weeks old) using immunization with human interphotoreceptor retinal-binding protein (IRBP) peptide (New England Peptide), mycobacterium tuberculosis (Difco) in Complete Freund’s adjuvant, and pertussis toxin (Sigma). Spatial visual thresholds were measured in the mice with a virtual optokinetic system (OptoMotry, CerebralMechanics Inc.), and a fundus evaluation was obtained weekly. In Study-1, visual function was measured at baseline and during the course of EAU. Spatial frequency and contrast thresholds in both eyes were measured, and the baseline results were consistent with published values for normal C57BL/6 mice (Prusky et al., 2004; 2006). In Study-2, Animals in EAU groups, were intraperitoneally injected with either an active anti-IL-6R antagonist (anti-mFc-6R, 100 mg/kg) or control protein (mFc, 33mg/kg) every 3 days from days 5 post EAU. Animals were terminated on day 28 to collect ocular tissues for histology analysis.

**Results:** We found that visual decline commenced in the uveitis group within 2 weeks of EAU induction, and reached ~40% after 4 weeks. No visual decline was found in non-immunized controls. Visual decline was mitigated in Anti-IL-6R antibody-treated groups: decline was delayed until 3 weeks after induction. In EAU animals treated with with mFc the decline was unchanged. Improved visual function was accompanied by improved fundus scores.

**Conclusions:** Ocular inflammation due to EAU was mitigated by inhibiting C5 activity, either by genetic deletion or pharmacologic inhibition with a specific monoclonal antibody. These results indicate that C5 is a potential therapeutic target for autoimmune uveitis.

**Commercial Relationships:** Aixu Sun, Ming Yuan, Regeneron Pharmaceutical Inc. (E); Hua Yang, Regeneron Pharmaceutical Inc. (E); Thomas C. MacPherson, Regeneron Pharmaceutical Inc. (E); Adrianna Latuszek, Regeneron Pharmaceutical Inc. (E); Henry Chen, Regeneron Pharmaceutical Inc. (E); Ying Hu, Regeneron Pharmaceutical Inc. (E); Jingtai Cao, Regeneron Pharmaceutical Inc. (E); Carl Romano, Regeneron Pharmaceutical Inc. (E)
Conclusions: These data indicate that systemic administration of an anti-IL-6R antibody may hold promise to treat autoimmune uveitis.

Commercial Relationships: Nazia M. Alam, CerebralMechanics, Inc (E); Glen T. Prusky, CerebralMechanics Inc (P);
Thomas C. MacPherson, Regeneron Pharmaceuticals, Inc (E); Jingtai Cao, Regeneron Pharmaceuticals, Inc (E);
Stanley J. Wiegand, Regeneron Pharmaceuticals, Inc (E);
George Yancopoulos, Regeneron Pharmaceuticals, Inc (E);
Carl Romano, Regeneron Pharmaceuticals, Inc (E)

Program Number: 539 Poster Board Number: B0094
Presentation Time: 1:30 PM–3:15 PM

Deficiency in Nod2 is associated with dysregulation of Th17-related responses in the eye
Ellen J. Lee1,2, Paige Snow1, Joao M. Furtado1, Ruth Napier1,2, Emily E. Vance3,4, Phyllis Silver2, Justine Smith2, Rachel R. Caspi1, Holly L. Rosenzweig2,4.1 Email code: R&D 14, VA Portland Health Care System, Portland, OR; 2Molecular Microbiology & Immunology, Oregon Health & Science University, Portland, OR; 3Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto, Brazil; 4Laboratory of Immunology, National Eye Institute, Bethesda, MD; 5School of Medicine, Flinders University, Adelaide, SA, Australia.

Purpose: Mutation in NOD2 results in Blau syndrome, which is characterized by granulomatous uveitis in association with dermatitis and arthritis. NOD2 plays a role in innate immune receptors that function in host defense, but we recently identified a novel suppressive role for Nod2 in experimental autoimmune uveitis (EAU), a T cell-dependent model of uveitis. Nod2 deficiency resulted in greater expansion, but similar proportion, of CD4+ T cells in eyes. Here we sought to study the cellular mechanisms behind modulation of EAU by Nod2.

Methods: Mice deficient in Nod2 or congenic wild type (WT) C57BL/6J controls were immunized for EAU with a mixture of interphotoreceptor retinoid-binding protein (IRBP) and IRBP20 peptide. Flow cytometry analysis of CD4+ T cells isolated from whole inflamed eyes harvested d21 post-immunization was used to evaluate Th1 (IFNγ+) and Th17 (IL-17+) effector subsets. Single cell suspensions isolated from inflamed eyes were re-stimulated in vitro with IRBP20 peptide to assess antigen-recall cytokine responses by flow cytometry after intracellular cytokine staining for IL-17A, TNF, IFNγ, GM-CSF, and IL-22. Relative contributions of IL-17 and IFNγ to disease in vivo were evaluated by fundus imaging and histopathology following antibody neutralization (100 μg/dose), on days 1, 2, 5, 9, and 13 relative to immunization.

Results: Nod2 deficiency resulted in increased percentages of ocular CD4+ T cells that produced IL-17 (6.1% vs. 1.52% in WT) or IFNγ (9.38% vs. 1.57% in WT). Antigen-recall studies of purified ocular cells revealed a dramatic increase in the proportion of IL-17+CD4+ cells in Nod2 KO compared to WT mice (12.3% vs. 3.5%). Further evaluation of IRBP-stimulated cytokine production of IL-17+CD4+ cells revealed enhanced production of all cytokines tested, including IFNγ (i.e. IL-17+IFNγ + cells). Blockade of IL-17 in vivo significantly diminished EAU severity for both genotypes, but to a greater extent in Nod2 KO mice. In contrast, blockade of IFNγ significantly worsened EAU severity in WT mice, but not alter disease severity in Nod2 KO mice.

Conclusions: These data suggest that the exacerbated uveitis accompanying Nod2 deficiency is mediated primarily through dysregulation of the Th17-related rather than Th1-related response.

Commercial Relationships: Ellen J. Lee, None; Paige Snow, None; Joao M. Furtado, None; Ruth Napier, None; Emily E. Vance, None; Phyllis Silver, None; Justine Smith, None; Rachel R. Caspi, None; Holly L. Rosenzweig, None
Support: NIH/NEI (grant EY025250), the Department of Veterans Affairs Biomedical Laboratory (101 BX002180; IK2 BX001295); NEI intramural support (Project # EY00184), and ARC (FT130101648)

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**Purpose:** Autoimmune retinopathy (AIR) is a condition that manifests in acute and progressive vision loss in humans. Many AIR patients have responded with stabilization and improvement of vision following treatment with general immune suppressive medications. Laboratory diagnostic tests for AIR have been limited to detection of specific anti-retinal antibodies (ARA) that are thought to drive pathogenesis. Antibodies against the retinal protein recoverin are frequent ARAs detected in AIR patients, which prompted us to study the cellular immune responses against recoverin.

**Methods:** We have established a murine model to provide a mechanistic approach toward a better understanding of disease processes in AIR. Mice were immunized subcutaneously with recombinant mouse recoverin (100-200 mg) emulsified in complete Freund’s adjuvant. Also, mice were injected intraperitoneally with pertussis toxin (400 ng) on the same day. After three weeks, the immunized mice were scanned with optical coherence tomography (OCT) for detection of pathological changes in the eyes. Spleen and draining lymph nodes were harvested and cells were restimulated with recoverin (5 mg/ml) for 4 days. Supernatants were collected for ELISA cytokine assay. Eyes were harvested and infiltrating lymphocytes were isolated after digestion with collagenase at 37°C for flow cytometry.

**Results:** Immunization of C57BL/6 and BALB/c mice with mouse recoverin resulted in recoverin-specific T cell responses. Production of IFN-γ and IL-17 were observed in cells harvested from draining lymph nodes and spleen of the immunized mice upon restimulation in vitro with recoverin. Immunization also led to production of high titers of recoverin-specific antibodies (1/400) in the immunized mice. The recoverin-specific Th1 and Th17 cell response was augmented in IL-10 KO mice, whereas humoral response was unaffected. More importantly, the number of lymphocytes infiltrated into the eyes dramatically increased in IL-10 KO mice upon immunization with recoverin. Immunization of Fas ligand deficient GLD mice with recoverin antigen led to a predominant Th17 cell response toward recoverin.

**Conclusions:** These new insights regarding the anti-recoverin immune response in wild-type and mutant mice have led us to hypothesize that skewing of recoverin-specific T cell response toward inflammatory Th1 and/or Th17 responses can drive the pathogenesis of AIR.
patients were re-randomized to one of the two active treatment arms. The primary endpoint (PE) was a mean composite score that reflected corneal fluorescein staining (CFS) as assessed by the modified Oxford scale, need for rescue medication, and occurrence of corneal ulceration during 4 months.

**Results:** The PE of superiority of active treatment over vehicle was met; the difference in the least-squares (LS) mean vs vehicle was statistically significant for both the high-dose group (0.76, p = 0.007) and the low-dose group (0.67, p = 0.010). Treatment effect was mainly driven by CFS score. A majority of the patients did not need rescue medication, and while the mean number of rescue medication courses was significantly different between the high-dose and vehicle groups (p = 0.010), it was only marginally different between the low-dose and vehicle groups (p = 0.055). The symptoms and quality of life also improved; outcomes remained stable over the 8-month follow-up. Overall, the proportions of patients with ≥1 adverse event were 58.3% and 50.0% in the high- and low-dose groups, respectively. The most frequently reported AEs in the high-dose group were instillation site pain, instillation site pruritus, cough, influenza, and ocular hyperemia.

**Conclusions:** Patients who received active doses (high and low doses) of CsA CE 1 mg/mL showed significant improvement in signs of VKC when compared with patients receiving the vehicle alone. Follow-up at 8 months suggested a good tolerability and efficacy profile, with maintenance of beneficial treatment effects.

**Commercial Relationships:** Andrea Leonardi, Santen (C), Serge Doan, Horus (C), Thea (C), Bausch&Lomb (C), Santen (C), Allergan (C), Alcon (C); Mourad Amrane, Santen (E); Dahlia Ismail, Santen (E); Jesus Montero, None; VVI Narasimha Rao, None; Janos Nemeth, None; Dominique Bremond-Gignac, None

**Clinical Trial:** 2012-005060-10

**Program Number:** 542 Poster Board Number: B0097
**Presentation Time:** 1:30 PM–3:15 PM

**Phagocytosis is active in retinal microglia during Experimental Autoimmune Uveitis (EAU)**

**Tat Fong Ng, Andrew W. Taylor.** Ophthalmology, Boston University School of Medicine, Boston, MA.

**Purpose:** Although activated microglia are detected in the retina during uveitis, their functions remain unknown. Recently we have demonstrated that healthy retinal pigment epithelial cells suppress the process of phagocytosis. Since, activated microglia may play a role in presenting antigen to effector T cells during EAU, we hypothesized retinal microglia exhibit any phagocytic activity.

**Methods:** Mice (C57BL/6) were immunized with complete Freund’s adjuvant and IRBP followed by Pertussis Toxin injections to induced EAU. The EAU was scored once a week by fundus examination. When the EAU reached a score of 3, the eyes were collected. The eyes were dissected and the neural retinas were separated into two groups. The neural retinas in Group 1 were incubated with opsonized-AlexaFluor488-E. coli bioparticles in serum free medium for 24 hours. They were fixed in 4% paraformaldehyde and immunostained for Iba-1. The density of Iba-1 positive cells containing bioparticles was counted. In Group 2, the retinal microglia were isolated from the neural retina by centrifugation through a discontinuous 35% and 70% Percoll gradient, the microglial cells were collected at the 35% and 70% interface. The microglial cells were incubated with opsonized pHrodo-S. aureus bioparticles for 24 hr. Peritoneal macrophages were used as a positive control for phagocytosis. The fluorescence intensity determined as a measure of phagocytic activity.

**Results:** In healthy whole mount neural retina (n=3) the density of Iba-1 positive microglia cells in the nerve fiber layer (NFL) was 60.4±0.5 cells/mm², and none in the outer nuclear layer (ONL). In the EAU whole mount neural retina (n=3), the density of Iba-1 positive microglia cells in the NFL was 179.3±54.4 cells/mm², and in the ONL was 121.8±41 cells/mm². In the EAU neural retina the density of Iba-1 positive cells containing AlexaFluor488-E. coli bioparticles was 11.1±4.8 cells/mm² in NFL, and 42.8±24.5 cells/mm² in ONL. In contrast, there was no detectable phagocytized bioparticles in healthy neural retina. The isolated retinal microglia showed similar differences in that microglial cells from retina with EAU demonstrated phagocytic activity, whereas, the microglial cells from naive mice showed none.

**Conclusions:** The results demonstrate little phagocytic activity by microglial cells in healthy retina, which is activated in EAU.

**Commercial Relationships:** Tat Fong Ng, None; Andrew W. Taylor

**Support:** NEI Grant EY025961

**Program Number:** 543 Poster Board Number: B0098
**Presentation Time:** 1:30 PM–3:15 PM

**IL-12p35 induces expansion of IL-10- and IL-35-expressing regulatory B cells (Bregs) and ameliorates autoimmune disease**

**Jin Kyeong Choi, Venkat Mohanram, Chengrong Yu, Anita Uche, Daniel Gebreselassie, Hyunsu Lee, Charles Egwuagu.** Laboratory of Immunology, NEI, National Institutes of Health, Bethesda, MD.

**Purpose:** Interleukin 35, a heterodimeric cytokine composed of IL-12p35 and Ebi3 subunits, suppresses autoimmune diseases, prevents sterilizing immunity to pathogens and limits anti-tumor immunity by inducing IL-35-producing regulatory B (i35-Breg) and T cells (iTR35). Thus far, biologically active, native or recombinant heterodimeric IL-35 is not commercially available due to technical challenges. Despite sharing IL-12p35 subunit, IL-12 (IL-12p35/IL-12p40) promotes inflammatory responses that mediate autoimmune diseases while IL-35 (IL-12p35/Ebi3) induces regulatory responses that maintain self-tolerance and suppresses chronic inflammatory diseases, suggesting that IL-12p35 may possess unknown intrinsic immune-regulatory functions regulated, in part, by its heterodimeric partner.

**Methods:** Using experimental autoimmune uveitis (EAU), an animal model of Uveitis, we have produced mouse recombinant IL-12p35 (rIL-12p35) and rEbi3 and examined whether either protein can recapitulate the inhibitory activities of IL-35 in uveitis.

**Results:** We demonstrate that the IL-12p35 subunit exhibits immunoregulatory functions hitherto attributed to IL-35: It suppresses lymphocyte proliferation, induces expansion of IL-10- and IL-35-expressing regulatory B-cells (Bregs) and suppresses CNS autoimmune disease by antagonizing pathogenic Th17 responses. The demonstration that IL-12p35 can recapitulate essential immunosuppressive activities of IL-35 offers tremendous promise for therapeutic use of IL-12p35, particularly for in vivo expansion of Breg cells and autologous Breg immunotherapy.

**Conclusions:** Our data showing that IL-12p35 might be an effective drug for the treatment of autoimmune uveitis further suggest that intrinsic immune-regulatory activities of other single-chain IL-12 family proteins can also be exploited therapeutically in other autoimmune or infectious diseases.

**Commercial Relationships:** Jin Kyeong Choi, None; Venkat Mohanram, None; Chengrong Yu, None; Anita Uche, None; Daniel Gebreselassie, None; Hyunsu Lee, None; Charles Egwuagu, None
ARVO 2017 Annual Meeting Abstracts

Program Number: 544 Poster Board Number: B0099
Presentation Time: 1:30 PM–3:15 PM

MHC class II Expression In The Retina During Experimental Autoimmune Uveitis
Deborah A. Lipski1,2, Remi Dewispelaere3,4, Vincent Fouchart1,4, Laure E. Caspers1, Matthieu Defrance1, Catherine A. Bruyns1, François Willermain1,2,4, Ophthalmology, CHU Saint-Pierre, Brussels, Belgium; 2Ophthalmology, Hôpital Erasme, Brussels, Belgium; 3Ophthalmology, CHU Saint-Pierre, Brussels, Belgium; 4Ophthalmology, CHU Brugmann, Brussels, Belgium; 2Laboratory of Cancer Epigenetics, ULB, Brussels, Belgium.

Purpose: Controversy exists regarding which cell types are responsible for auto-antigen presentation in the retina during experimental autoimmune uveitis (EAU) development. In this study, we use a combination of techniques to characterize retinal resident and infiltrating cells susceptible to express MHC Class II (MHCII).

Methods: EAU is induced by adoptive transfer of IRBP1-20-specific activated T cells in female C57BL/6 mice aged 6-8 weeks. At day 21, cryosections are prepared and MHCII, GFAP, endoglin or CD31 and IBA-1 expression analyzed by immunofluorescence (IF). For flow cytometry analysis (FC), retinas are processed into single cell suspensions by enzymatic digestion and tested for MHCII, CD11b, CD45, Ly6C, CD31, CD40, CD80 and CD86 membrane expression using specific antibodies coupled to different fluorochromes. RNA-Seq is performed on different retinal cell populations sorted by FC.

Results: IF images demonstrate a strong induction of MHCII expression in EAU, especially in the inner retina at the level of vasculitis, extending to the outer nuclear layer in severely inflamed eyes, at the level of the retinal pigment epithelium and on cells infiltrating the vitreous. Most MHCII+ cells express the hematopoietic marker IBA-1. FC analysis demonstrates that retinal cell MHCII expression is significantly correlated with disease severity (p<0.001, N=20) and associated with upregulation of co-stimulatory molecules CD40, CD80 and CD86. Although FC studies pick up a CD45+CD11b+ cell population with low MHCII expression, they confirm that most cells expressing high levels of MHCII are of hematopoietic origin. Both Ly6C+ and Ly6C− cells contribute to the increase in MHCII+CD45−CD11b− cells that occurs during EAU, possibly corresponding to macrophage infiltration and resident microglial cell proliferation. RNA-Seq analysis of both FC-sorted cell populations leads to a clear sample clustering with some differential inflammatory gene expression. However, no clear difference in cell lineage is evidenced.

Conclusions: MHCII induction during EAU is correlated to disease severity and accompanied by upregulation of co-stimulatory molecule expression. Hematopoietic cells express higher levels of MHCII than non-hematopoietic cells and various levels of Ly6C. In these experimental conditions, Ly6C expression seems to be associated with different expression of some inflammatory genes but not to a single specific cell type.

Commercial Relationships: Deborah A. Lipski; Remi Dewispelaere, None; Vincent Fouchart, None; Laure E. Caspers, None; Matthieu Defrance, None; Catherine A. Bruyns, None; François Willermain, None
Support: Fonds Erasme, FRO

Program Number: 546 Poster Board Number: B0101
Presentation Time: 1:30 PM–3:15 PM

Alteration of gut microbiota by antibiotics impacts spontaneous ocular autoimmunity
Ryan S. Salvador; Reiko Horai, Carlos Zárate-Bladés, Yingyos Jittayasothorn, Rachel R. Caspi. Laboratory of Immunology, NEI, National Institutes of Health, Bethesda, MD.

Purpose: The R161H spontaneous uveitis model permits to study natural triggers of uveitis. We have previously shown that elimination of commensals from R161H mice by oral antibiotic treatment or germ-free conditions attenuated uveitis and reduced Th17 cells in the gut. Because oral antibiotics do not completely deplete commensals, we set out to associate modulation of gut microbiota by alternative antibiotic treatments with spontaneous disease and immune responses.

Methods: R161H mice and WT littermates were treated with antibiotics (ampicillin, metronidazole, neomycin and vancomycin, either individually or as a mix (AMNV), or with gentamicin added (AMNV/G)) in drinking water from before birth. Antibiotic-treated mice were compared to age-genotype-matched specific-pathogen-free (SPF) mice that received normal water. Uveitis was evaluated by fundoscopy and histology. Proteinaceous extracts ofecal contents were used to stimulate R161H lymphocytes in vitro; activation was assessed by induction of CD69 positivity (flow cytometry) and IL-2 secretion into the supernatant (ELISA). Fecal pellets were collected

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Commercial Relationships: Jun Chen; Weiwei Wang, None; Zilin Chen, None; Hongyan Zhou, None; Eric F. Wawrousek, None; Igal Gery, None; Rachel R. Caspi, None
Support: Project 985 Grant 83000-31121300

Program Number: 548 Poster Board Number: B0103
Presentation Time: 1:30 PM–3:15 PM
IL-17A inhibits expression of IL-17 lineage cytokines through a negative feedback loop involving IL-24 and controls autoimmune uveitis

Kumarkrishna Raychaudhuri, Wai Po Chong, Reiko Horai, Phyllis Silver, Yingyos Jittayasothorn, Chi-Chao Chan, Jun Chen, Rachel R. Caspi.

Purpose: The Th17 response has been associated with autoimmune diseases in patients and in animal models. IL-17A is recognized as the Th17 signature cytokine and IL-17-producing T cells are pathogenic effectors in models of autoimmunity, including experimental autoimmune uveitis (EAU). Paradoxically, injection of IL-17 was shown by others to ameliorate the disease (PMID: 19234216).

Methods: Using a model of spontaneous uveitis in IRBP T cell receptor transgenic R161H mice, we investigated the susceptibility to disease of mice on an IL-17A−/− background. Additionally, T cells from IL-17A-deficient and deficient R161H mice were polarized under Th17 conditions and were adoptively transferred to WT recipients to examine their cytokine profile and their ability to induce EAU.

Results: Surprisingly, IL-17A−/− R161H mice developed essentially undiminished uveitis and IL-17−/− R161H T cells, polarized to Th17 and infused into wild-type recipients, induced similar disease to IL-17A−/− R161H T cells. Interestingly, IL-17A−/− R161H T cells polarized under Th17 conditions produced elevated amounts of other Th17-related cytokines, i.e. IL-17F, GM-CSF and IL-22. Supplementing these cultures with recombinant IL-17A normalized the elevated production of those cytokines. RNAseq analysis revealed that IL-17A−/− T cells displayed lower IL-24 expression compared to their IL-17 sufficient counterparts. Mechanistic studies indicated a negative feedback loop where IL-17A induces Th17 cells to produce IL-24, which suppresses production of Th17 lineage cytokines. Finally, injection of recombinant IL-24 ameliorated adoptive Th17-induced EAU, and conversely, depletion of IL-24 in Th17 cells increased their pathogenicity and elevated disease severity.

Conclusions: These data suggest that: (a) IL-17A exerts a negative feedback on uveitogenic Th17 cells via IL-24 production, and (b) IL-24 limits the expression of other Th17-related cytokines and controls their pathogenicity.

Commercial Relationships: Kumarkrishna Raychaudhuri, None; Wai Po Chong, None; Reiko Horai, None; Phyllis Silver, None; Yingyos Jittayasothorn, None; Chi-Chao Chan, None; Jun Chen, None; Rachel R. Caspi, None

Program Number: 549 Poster Board Number: B0104
Presentation Time: 1:30 PM–3:15 PM
Antenatal inflammation induced by interleukin-1β causes retinal and sub-retinal vasculopathy in progeny

Alexandra Beaudry-Richard, Mathieu Nadeau-Vallée, Jose C. Rivera, Anusha Madaan, Emilie HECKEL, Amariyous Boudreaux, Xin Hour, Christiane Quiniou, David Olson, Jean- Sebastian Joyal, Sylvain Chemtob.

University of Montreal, Montreal, QC, Canada; 2CHU Sainte-Justine, Montreal, QC, Canada; University of Alberta, Edmonton, AB, Canada; 3Maisonneuve-Rosemont Hospital, Montreal, QC, Canada.

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Purpose: Preterm birth (PTB; < 37 weeks gestation) involving inflammation has numerous harmful effects on foetal development, but so far the underlying mechanism is unclear. With our inflammation-induced murine model of PTB, we intend to unveil the role of interleukin (IL)-1β in the developing retina and choroid.

Methods: CD-1 pregnant mice were injected in utero with IL-1β at gestation day (G)16 to cause PTB, and injections with the receptor’s antagonists Kinera (competitive inhibitor, already commercialised) and 101.10 (non-competitive inhibitor, recently developed by our lab) were performed sub-cutaneously at every 12th until birth. Eyes were collected from pups before (G16.5, 17, 17.5 and 18; obtained by C-section) and after birth (post-term days (P1, 4 and 15) to measure the induction of pro-inflammatory genes with qPCR and of proteins with ELISA methods (N=5/gr). Then, retinal flat mounts and choroidal cryosections were stained for lectin to quantify vascular surface/density and thickness (N=5/gr). Finally, electroretinogram was performed on fully developed pups (P30) to assess retinal function (N=8/gr). Only stillborn animals have been excluded from the analyses. Ordinary one-way ANOVA with Tukey’s multiple comparison test were used to determine significance (p<0.05).

Results: Results show that an inflammatory response is generated before birth in the eye of the pup, which starts with an induction of Caspase-1 gene, closely followed with IL-1β and CXCL-16 genes, and a final increase in IL-12 gene preceding birth. Activated microglia is also observed in the retina and choroid of the pups before birth. During development, there is a delay in retinal vessels growth (78% decrease in IL-1 group vs. sham at P1; 49% at P4; 27% at P15; SD=3%; p<0.001) and a long lasting thinning of the choroid (50% at P15; SD=5%; p<0.001). These phenotypes lead to functional alteration of the retina, as shown by decreased a- and b-wave amplitudes. Antenatal injections of 101.10 was efficient in preventing all deleterious effects induced by IL-1β observed, whereas Kinera had smaller effect.

Conclusions: To summarize, uterine inflammation engenders an inflammatory response in the foetal eye, which leads to deficient vessel growth in the retina, thinning of the choroid and retinal dysfunction. In addition, antenatal administration of 101.10 prevents these phenotypes and protects the eyes from damaging inflammation.

Commercial Relationships: Alexandre Beaudry-Richard, Mathieu Nadeau-Vallée, Jose C. Rivera, Ankush Madaan, Emile HECKEL, Amarilys Boudreault, Xin Hou, Christiane Quiniou, David Olson, Jean-Sebastien Joyal, Sylvain Chemtob, None; Boram Lee, Dae-Young Park, Chan Min Yang, Kyung In Woo, Yoon Duck Kim, Jisang Han, Tae-Young Chung, Dong-Hui Lin, None; Tae-Young Chung, None; Ka-Jin Lim, None; Kyung In Woo, None; Yoon Duck Kim, None; Tae-Young Chung, None; Dong-Hui Lin, None; Yoon Duck Kim, None; Tae-Young Chung, None; Dong-Hui Lin, None

Program Number: 550 Poster Board Number: B0105

Presentation Time: 1:30 PM–3:15 PM

Orbital adipogenesis in a mouse model of autoimmune arthritis, Zymosan-induced SKG mice

Boram Lee, Dae-Young Park, Chan Min Yang, Kyung In Woo, Yoon Duck Kim, Jisang Han, Tae-Young Chung, Dong-Hui Lim. Ophthalmology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea (the Republic of); ‘Ophthalmology, Myongji Hospital, Seonam University School of Medicine, Goyang, Korea (the Republic of).

Purpose: Inflammation and remodeling of orbital tissue associated with enhanced adipogenesis commonly occurs in the thyroid ophthalmopathy and idiopathic orbital inflammation. However, the underlying mechanisms that link immune cells and adipocytes in orbital inflammation are little known due to the absence of appropriate animal model. The purpose of this study was to elucidate how a genetically determined shift in the T-cell repertoire toward self-reactive T cells could drive orbital adipogenesis in zymosan-induced SKG mice, an experimental model of autoimmune arthritis.

Methods: SKG mice (BALB/c mice that harbor the point mutation in ZAP-70) were housed under pathogen–free conditions. To induce arthritis, zymosan-A (or vehicle in a control) was intraperitoneally injected once at 8 weeks. The exenterated orbits, lid, and thyroid gland were evaluated 8 weeks after injection by histology.

Results: The eyes of SKG mice in response to zymosan displayed proptosis and inflammatory responses as indicated by significant increase in the thickness of lid and meibomian gland, and subepithelial cellular infiltration. The detailed analysis of orbital adipose tissue in zymosan-induced SKG mice showed enhanced orbital adipogenesis with fat burning but no distinct cellular infiltration or fibrosis compared to control. The extraocular muscles in zymosan-induced SKG mice exhibited angulated fibers without swelling indicating atrophic change in muscle. No significant difference was observed in the follicles of thyroid gland between control and zymosan-induced SKG mice.

Conclusions: The orbital adipogenesis was demonstrated in zymosan-induced SKG mice with self-T cell activation. This study allows us to understand the complexity surrounding the pathogenesis of orbital adipogenesis associated with T-cell dysfunction and may provide the therapeutic potential target alternative to steroid and surgical treatment in orbital inflammation disease.

Commercial Relationships: Boram Lee, None; Dae-Young Park, None; Chan Min Yang, None; Kyung In Woo, None; Yoon Duck Kim, None; Jisang Han, None; Tae-Young Chung, None; Dong-Hui Lin, None

Program Number: 550 Poster Board Number: B0106

Presentation Time: 1:30 PM–3:15 PM

Interleukin-7 and -15 Maintain Memory T Helper 17 Cells in Dry Eye Disease

Yihe Chen, Sunil Chaushman, Xuhua Tan, Reza Dana. Scheepens Eye Research Ins /MEEI, Boston, MA.

Purpose: Memory T helper 17 (mTh17) cells have been found crucial in mediating the chronicity of various refractory autoimmune disorders, including dry eye disease (DED); however, the underlying mechanisms maintaining mTh17 cells have remained elusive. This study was designed to investigate whether interleukin (IL)-7 and IL-15 maintain mTh17 cells in chronic DED.

Methods: Chronic DED was induced by exposing C57BL/6 mice to desiccating stress using a controlled environment chamber for 14 days and then housing mice in a standard environment for additional 14 days. Expression of IL-7 receptor (IL-7R) and IL-15 receptor (IL-15R) by mTh17 cells (CD4+CD44hiIL-17A+) derived from chronic DED mice was analyzed using flow cytometry. IL-7 and IL-15 mRNA and protein expressions in DED mice were quantified by real-time PCR and ELISA. In addition, draining lymph nodes isolated from chronic DED mice were cultured in the presence of IL-7, IL-15, IL-7 and IL-15, anti-IL-7 antibody (Ab), anti-IL-15 Ab, anti-IL-7 and IL-15 Abs, or control IgG for 72 hours, and then examined for the frequencies of mTh17 cells using flow cytometry. Finally, therapeutic effects of topical neutralization of either IL-7, IL-15 or IL-17 were evaluated in chronic DED.

Results: mTh17 cells from chronic DED mice expressed IL-7R and IL-15R. IL-7 and IL-15 expression was significantly higher in chronic DED mice compared to normal mice at both mRNA and protein levels (2-fold increase, p < 0.05) and protein (37.3±6.2 and 99.2±14.3, vs. 2.0±0.3 and 43.1±4.8, p < 0.05) levels in the conjunctiva. They were constitutively expressed at comparable levels in the DLNs of normal and DED mice. Furthermore, DLNs from chronic DED mice cultured in the presence of IL-7, IL-15, or IL-7 and IL-15 together, displayed...
higher frequencies of mTh17 cells (0.20±0.03%, 0.18±0.02%, 0.16±0.02%, respectively) compared with those cultured with anti-IL-7 Ab (0.057±0.002%), anti-IL-15 Ab (0.055±0.017%), anti-IL-7 and anti-IL-15 Abs together (0.081±0.006%), or control IgG (0.087±0.006%). Topical neutralization of ocular IL-7 or IL-15 effectively reduced DED severity and significantly suppressed frequencies of mTh17 cells in both conjunctiva and DLN, while topical neutralization of IL-17 did not affect mTh17 frequencies in the DLN.

Conclusions: These findings demonstrate that both IL-7 and IL-15 maintain mTh17 cells and could serve as novel therapeutic targets for chronic DED.

Commercial Relationships: Yihe Chen; Sunil Chauhan, Schepens Eye Research Institute (P); xuhua tan, None; Reza Dana, Schepens Eye Research Institute (P)

Support: NIH R01 EY 20889

Program Number: 552 Poster Board Number: B0107
Presentation Time: 1:30 PM–3:15 PM

Clinical statistics for secondary glaucoma in patients with scleritis
Tomoyuki Kunishighe, Kohei Miyata, Satoko Yui, Kenji Nakamoto, Junko Hori. Nippon Medical School, Bunkyo-ku, Japan.

Purpose: We have previously reported clinical statistics for secondary glaucoma in ocular inflammatory diseases including sarcoidosis and Behcet’s disease. The purpose of the present study is to provide a clinical statistical study of secondary glaucoma in patients with scleritis at the Ocular Inflammation Service, Nippon Medical School Hospital.

Methods: This retrospective study was based on a review of medical records. Among patients with a defined diagnosis of scleritis (n=203) who were evaluated as outpatients in the Ocular Inflammation Service, Department of Ophthalmology at Nippon Medical School Hospital between April 2004 and May 2016, and who also developed secondary glaucoma (n=59). This study comprised 59 patients, 83 eyes (36 men, 52 eyes; 23 women, 31 eyes) in whom tonometry and Humphrey visual field testing with measurement of mean deviation (MD) had been performed at least twice.

Results: 29% (59 out of 203) scleritis patients developed secondary glaucoma. Among them, steroid-induced glaucoma was diagnosed in 81%. Causative scleritis associated with secondary glaucoma included episcleritis in 16%, diffuse anterior scleritis in 60%, nodular anterior scleritis in 18%, necrotizing anterior scleritis in 4%, and posterior scleritis in 1% of cases. At initial evaluation in scleritis patients with secondary glaucoma, mean intraocular pressure was 19.1±6.1 mmHg and MD was -2.0±4.0 dB. At final evaluation, mean intraocular pressure was 15.5±4.2 mmHg and MD was -2.6±4.6 dB (Mean follow-up period 29.6±32.6 months). In compare with our previous studies for sarcoidosis (-7.26±7.80dB at initial evaluation, -8.86±9.68dB at final evaluation) and Behcet’s disease (-7.64±5.99dB at initial evaluation, -10.5±7.98dB at final evaluation), scleritis was less progressive visual field defect. Glaucoma surgery was performed in 5 eyes of scleritis patients, and the procedure involved trabeculectomy in 2 eyes and trabeculotomy in 3 eyes.

Conclusions: Scleritis was frequent in eyes that developed steroid-induced glaucoma. Careful selection of treatment and management of intraocular pressure is important in scleritis.

Commercial Relationships: Tomoyuki Kunishighe, None; Kohei Miyata, None; Satoko Yui, None; Kenji Nakamoto, None; Junko Hori, None

Program Number: 553 Poster Board Number: B0108
Presentation Time: 1:30 PM–3:15 PM

Immunohistochemical examination of the B lymphocyte infiltrate in human uveitis
Simon Epps1, Natalie Coplin2, Philip J. Luthert3, Andrew D. Dick4, Sarah E. Coupland1, Lindsay B. Nicholson5. 1Ophthalmology, University of Bristol, Bristol, United Kingdom; 2Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom; 3Clinical and Cancer Medicine, University of Liverpool, Liverpool, United Kingdom; 4Institute of Ophthalmology, UCL, London, United Kingdom.

Purpose: Ectopic lymphoid-like structures (ELS) are focal aggregations of B lymphocytes with features of secondary lymphoid follicles that develop in non-lymphoid tissue. ELS are found in chronic inflammatory disorders, and with respect to uveitis, have been demonstrated in murine experimental autoimmune uveoretinitis and equine recurrent uveitis but have yet to be shown in man. This histopathological observational study examined a series of 14 human uveitic eyes by immunohistochemistry (IHC) to assess the nature of B cell infiltration and determine whether features of ELS were present.

Methods: 14 formalin-fixed paraffin-embedded enucleated human eyes from 14 patients with uveitis were obtained from the Liverpool Ocular Oncology Biobank (Liverpool, UK) and Moorfields Biobank (London, UK) with ethical approval. Samples were identified by reviewing histopathology reports from each institution and selected if reported to contain visible lymphocytes within the uveoretina on haematoxylin- and eosin-stained sections. Further sections were examined by IHC for CD3+ T cells and CD20+ B cells. If CD20+ cells were present additional IHC was performed for lymphoid follicle markers CD21, CD23, BCL6 and AID. ELS were defined as focal B and T cell aggregates with T/B cell segregation and a CD21+ network.

Results: B cells were present in the majority of cases (10 out of 14, 71%). The B cell infiltrate was either diffuse (3 out of 14 cases, 21%), focal (5 out of 14 cases, 36%), or focal with features of ELS (2 out of 14 cases, 14%). B cells were most frequently seen within the uvea and not the retina (uvea only: 9 out of the 10 cases where B cells were evident, 90%; uvea and retina 1 out of 10 cases, 10%).

Conclusions: Within the uvea of patients with uveitis a B cell infiltrate is often present as either diffuse, focal or organised aggregations with T cells and lymphoid follicle markers consistent with ELS. These ELS-like structures within the uvea may represent sites of antigen-specific B cell proliferation, mutation and maturation, and could be of clinical relevance with respect to B-cell immunomodulatory therapies in uveitis.

Figure: IHC image of choroid of patient 2 showing focal CD20+ B and CD3+ T cell infiltrate with CD21+ follicular dendritic cell network, CD23+ follicular B cells and BCL6 expression consistent with an ELS (x400 magnification)
Purpose: Autoimmune retinopathy (AIR) is a rare disorder of the retina characterized by vision loss, visual field deficits, and photoreceptor dysfunction in the presence of anti-retinal antibodies. Cystoid macular edema (CME) may be seen in patients with this disease. This study aimed to elucidate the relationship between CME and disease progression relationship by using ellipsoid zone (EZ) length as a surrogate for disease severity, and correlating this to the presence of CME.

Methods: A retrospective chart review of AIR patients presenting between 2008 and 2016 was conducted. A single reader determined the presence or absence of CME at each visit using the volume scan and measured the preserved ellipsoid zone (EZ) length on the centermost 9 mm SD-OCT 7-line volume B scan. A second reader confirmed the EZ borders on equivocal images. All scans were of good quality and shadowing did not affect the ability to calculate EZ length.

Results: 14 patients (28 eyes) were included in this study, 8 males and 6 females with average age at presentation of 59 years and mean follow-up of 38 months. All patients had positive anti-retinal antibodies, evidence of ERG dysfunction, and a negative malignancy evaluation. In 8 eyes that did not have CME at initial presentation and did not develop CME, the mean EZ length was 6083 microns at initial visit and 6014 microns at final follow-up. In 7 eyes that did have CME at initial presentation, the mean EZ length was 3020 microns at initial visit and 6014 microns at final follow-up. In 3 eyes that did not have CME at initial presentation but developed CME during the course of their disease, the mean EZ length was 5570 microns at initial presentation and 2351 microns at final follow-up. Additionally, in these 5 eyes, an average decrease in EZ length of 1400 microns was seen between the visit prior to the development of CME and after the development of CME.

Conclusions: Eyes without CME have longer EZs than those that do. Furthermore, in eyes that previously did not have CME there was a disease progression inflection point as manifested by a marked decrease in EZ length after CME developed. While this study is limited by its small sample size, the development of CME may suggest more advanced disease in patients with AIR.

Table summarising IHC data; + indicates present, - indicates absent

<table>
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<tr>
<th>Protein</th>
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and high ACE were more in younger group than in elder group with statistical significance.

Conclusions: It was indicated that the characteristic findings are fewer in ocular sarcoidosis patients older than 65-year-old compared with the younger patients.

Commercial Relationships: Kouzo Harimoto, None; Takahiro Kuraishi, None; Manzo Taguchi, None; Takayuki Kanda, None; Masaru Takeuchi, None

Program Number: 556 Poster Board Number: B0111
Presentation Time: 1:30 PM–3:15 PM
Importance of Checking for Active Autoimmunity in Genetically-Confirmed Retinal Dystrophy Patients

Steven K. Lundy1,2, Athanasios J. Karoukis3, Ray Ohara1, Enayat Nikoopour2,3, Mohammad I. Othman3, Maria Fernanda Abalem3, Thiran Jayasundera4, Kari E. Branham1, John R. Heckenlively5

1Internal Medicine-Rheumatology, University of Michigan Medical School, Ann Arbor, MI; 2Graduate Program in Immunology, University of Michigan Medical School, Ann Arbor, MI; 3Ophthalmology and Visual Sciences, University of Michigan Medical School, Ann Arbor, MI.

Purpose: Anti-retinal antibodies (ARA) have been found in patients with retinal dystrophies, and used as a lab diagnostic tool to detect retinal autoimmunity. The importance of this to patient care is that immune suppressive treatment can often slow progression of retinal degeneration that is greatly accelerated by autoimmunity. However, the methods usually used to detect ARA do not provide information about the pathogenesis or current activity of the disease. We have set out to define more robust criteria for active retinal autoimmunity and to discover clues to the mechanisms involved in immune-mediated retinal degeneration.

Methods: We randomly selected a set of 49 consented patients with genetically confirmed retinal dystrophies. Blood samples underwent an extensive immune profile consisting of standard measurement of ARA by Western blot, as well as detection of anti-recoverin IgG and IgM antibodies by ELISA. Recoverin-induced cytokine (IFNγ, TNFα and IL-10) production by patient peripheral blood mononuclear cells was analyzed by ELISA. Immune cell lineages and activation status were compared using four 10-color flow cytometry panels. Whole blood mRNA was isolated to measure gene expression using NanoString™ assays. Comparisons were made to define autoimmune and non-autoimmune dystrophy patients.

Results: We used several criteria to classify patients as autoreactive consisting of: 1) three or more ARA; 2) a high titer of recoverin-specific IgG or IgM; and/or 3) very high production of IFNγ or TNFα in response to recoverin. The data summarized in the table show the number of patients meeting each of the criteria. We found that 28/49 (57%) of dystrophy patients had elevated anti-recoverin antibody titers or inflammatory cytokine responses. These parameters closely correlated with disease activity and were sensitive to treatment with immune suppression. High levels of natural killer cells (>30%) and B lymphocytes (>20%) were frequently found in the blood of autoimmune patients but not the non-autoimmune group. Immune cell activation and gene expression in the patients were also affected by treatments, but the patterns of ARA were not altered in most of the treated patients.

Conclusions: Retinal dystrophies sometimes have comorbidity associated with autoimmunity. Cellular and humoral immune responses against recoverin mark disease activity and can be favorably altered by immune suppressive treatment.

Commercial Relationships: Steven K. Lundy, Chugai Pharmaceuticals (F), Merck Corporation (F); Athanasios J. Karoukis, None; Ray Ohara, None; Enayat Nikoopour, None; Mohammad I. Othman, None; Maria Fernanda Abalem, None; Thiran Jayasundera, None; Kari E. Branham, None; John R. Heckenlively, None

Support: NIH Grant EY028136

Program Number: 557 Poster Board Number: B0112
Presentation Time: 1:30 PM–3:15 PM
Characterization of corneal endothelial pseudoguttata in the setting of anterior uveitis

Doran Spencer1, Stephen D. Anesi2, C. Stephen Foster1, 2
1Massachusetts Eye Research and Surgery Institution, Waltham, MA; 2Harvard Medical School, Boston, MA.

Purpose: Corneal endothelial pseudoguttata are a poorly recognized physical finding that have been described previously primarily as a sign of corneal pathophysiology; their association with intraocular inflammation is currently undetermined and unknown in ophthalmology. We describe the novel characteristics of pseudoguttata in patients with anterior uveitis (AU) of various etiologies seen at a Uveitis referral center. Per our experience, pseudoguttata represent a reliable and highly clinically relevant sign that may allow for more specific treatment in these patients.

Methods: We systematically reviewed a set of 14 AU patients who were noted, including their age, gender, diagnosis, Standardization of Uveitis Nomenclature (SUN) anterior chamber cell severity, presence of pseudoguttata, flaremeter reading, specular and slit lamp microscope findings; unaffected, contralateral eye characteristics were reported when appropriate. The evolution of these characteristics in response to treatment is also described.

Results: 14 patients with active AU were seen. All patients with non-operative AU were noted to have pseudoguttata. Etiologies of AU included HLA-B27-associated (4), idiopathic (3), Juvenile Idiopathic Arthritis (JIA)-associated (1), Behçet’s (1) and post-operative (5). AU SUN cell severity of study patients ranged from trace to 3+, with absence of keratic precipitates; pseudoguttata prominence was noted to correlate directly with SUN severity in non-operative patients, ranging from trace to near confluent. Flaremeter readings correlated with SUN cell and pseudoguttata severity in non-operative patients. Pseudoguttata were readily visible via specular microscopy when noted at the slit lamp. Pseudoguttata were noted to disappear in response to treatment in direct correlation with SUN AU cell severity and flaremeter readings. Post-operative cataract patients (POD#1) were observed not to have prominent pseudoguttata on slit lamp or specular microscopic examination nor elevated flaremeter readings, despite typical SUN anterior chamber cell scores of 1+.

Conclusions: We have described the novel finding of corneal endothelial pseudoguttata in 14 AU patients. In our experience, pseudoguttata represent a reliable, clinically relevant and readily discernible finding that associates with active, non-surgical AU. We believe that this represents a significant advancement in terms of uveitis assessment and evaluation of treatment efficacy.

Commercial Relationships: Doran Spencer; Stephen D. Anesi, None; C. Stephen Foster, None
Multiple analysis of flow cytometry data

Hi

CD38

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1:30 PM–3:15 PM

We utilized, developed and applied a data analysis strategy

L'EFS en Rhône-Alpes-Auvergne Recherche et Développement

The expansion of human i35-Bregs by CpG and

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Purpose:

To develop, test and apply a multidimensional flow
cytometry-based protein expression analysis approach allowing
classification and grouping of states of health and disease in human
cellular autoimmune disorders.

Methods: We utilized, developed and applied a data analysis strategy taking into account all mathematically possible combinations of protein markers in a given flow cytometry panel for the analysis of selected mined flow cytometry data generated using peripheral blood samples derived from human healthy, sarcoidosis or Behcet’s uveitis patients. Original FACS data files were mined from Dryad Digital Repository http://dx.doi.org/10.5061/dryad.v6ste with reference to http://dx.doi.org/10.1371/journal.pcbi.1003215, gated utilizing FlowJo software according to population partitioning in bivariate plots. We used combinatorial mathematics to generate a matrix quantifying the representation of all possible cell populations using a given set of staining antibodies (markers) within the respective starting population and coded the algorithm in Java to create a platform enabling the computation of input values derived from the measurements of a theoretically unlimited number of markers. The resulting data sets were visualized in a heat map approach using R to classify patient samples according to states of health and disease.

Results: Our approach clustered healthy vs diseased subjects with minimal error using only 4 common markers (CD3, CD8, CD197 and CD45), and allowed differential clustering of uveitis patients with sarcoidosis and Behcet’s disease.

Conclusions: Multi-dimensional analysis of flow cytometry data allows meaningful large-scale screening of biologically relevant markers at the protein level enabling classification and characterization of states of health and autoimmune disease, using measurement of only a few common markers. The approach is unbiased as all mathematically possible marker combinations enter analysis, thus enabling the discovery of cell populations with relevance as potential biomarkers or biological research targets.

Commercial Relationships: Johannes Nowatzky, None;

Julia Manasson, None; Ezra Resnick, None; Cristy Stagnar, None; Olivier Manches, None

Support: NIH K08 EY025324-02

Program Number: 559 Poster Board Number: B0113

Presentation Time: 1:30 PM–3:15 PM

High Output Flow Cytometry Array Protein Expression Profiling Facilitates Discriminant Phenotyping of Behcet’s and Sarcoidosis Patient-derived Peripheral Whole Blood Cells Revealing Distinct Immunophenotypes of Autoimmune Uveitides in the Context of Systemic Autoimmunity

Johannes Nowatzky1, Julia Manasson1, Ezra Resnick2, Cristy Stagnar1, Olivier Manches1. 1Medicine-Rheumatology, NYU School of Medicine, New York, NY; 2Google Inc., New York, NY; 1L'EFS en Rhône-Alpes-Auvergne Recherche et Développement “Immunobiology and Immunotherapy in Chronic Diseases”, French National Institute of Health and Medical Research, Beynost, France.

Purpose:

To develop, test and apply a multidimensional flow
cytometry-based protein expression analysis approach allowing
classification and grouping of states of health and disease in human
cellular autoimmune disorders.

Methods: We utilized, developed and applied a data analysis strategy taking into account all mathematically possible combinations of protein markers in a given flow cytometry panel for the analysis of selected mined flow cytometry data generated using peripheral blood samples derived from human healthy, sarcoidosis or Behcet’s uveitis patients. Original FACS data files were mined from Dryad Digital Repository http://dx.doi.org/10.5061/dryad.v6ste with reference to http://dx.doi.org/10.1371/journal.pcbi.1003215, gated utilizing FlowJo software according to population partitioning in bivariate plots. We used combinatorial mathematics to generate a matrix quantifying the representation of all possible cell populations using a given set of staining antibodies (markers) within the respective starting population and coded the algorithm in Java to create a platform enabling the computation of input values derived from the measurements of a theoretically unlimited number of markers. The resulting data sets were visualized in a heat map approach using R to classify patient samples according to states of health and disease.

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Conclusions: Multi-dimensional analysis of flow cytometry data allows meaningful large-scale screening of biologically relevant markers at the protein level enabling classification and characterization of states of health and autoimmune disease, using measurement of only a few common markers. The approach is unbiased as all mathematically possible marker combinations enter analysis, thus enabling the discovery of cell populations with relevance as potential biomarkers or biological research targets.

Commercial Relationships: Johannes Nowatzky, None;

Julia Manasson, None; Ezra Resnick, None; Cristy Stagnar, None; Olivier Manches, None

Support: NIH K08 EY025324-02

Program Number: 559 Poster Board Number: B0114

Presentation Time: 1:30 PM–3:15 PM

Human IL35-producing B cells (i35-Bregs) are induced by CpG DNA

Chengrong Yu, Charles Egwuagu. Laboratory Immunology, National Eye Inst/NIH, Bethesda, MD.

Purpose: In the mouse, in vitro activation of B cells by LPS induces the expansion of regulatory B cells that produce IL-35 (i35-Bregs) and i35-Bregs play critical roles in suppressing ocular inflammation and amelioration of experimental autoimmune uveitis (EAU). In this study we have investigated whether TLR agonists also induce the expansion of human i35-Breg cells.

Methods: CD4+ T or CD19+ B cells were isolated from human PBMC of healthy subjects by using CD4+ or CD19-magnetic bead sorting. The cells were stimulated with LPS, CD40L, CpG, PGN, anti-CD40 or anti-IgM in presence or absence of hIL-35 for 2 days. The immunophenotype was characterized by FACS using labeled mAbs specific to CD4, CD19, CD20, CD24, CD25, CD27, CD38, CD45RA, CD45RO, CD138, IgM, IgD, IL-10, IL-12p35, EB13, IL-12beta2. ELISA was used for IL-10 detection. PCR and Western blotting assays were used for cytokine detections. [H]-thymidine incorporation was performed with five replicate cultures.

Results: Among the TLR ligands examined (LPS, CpG, PGN), CpG was the most effective inducer of IL-10- and IL-35-producing Bregs. Similar to mouse IL-35, hIL-35 induced autocrine production of IL-35 by promoting the expansion of i35-Bregs and mediated its effects through activation of STAT1 and STAT3. We also show that human i35-Breg cells are CD27+CD38+CD138+ CD19- plasmablast.

Conclusions: The expansion of human i35-Bregs by CpG and discovery that hIL-35 can induce the conversion of B-cells into IL-10- producing B cells (Bregs) or i35-Bregs allows ex-vivo production of large amounts of Bregs and i35-Bregs. These findings may also facilitate the development of adoptive Breg cell immunotherapy for autoimmune diseases.

Commercial Relationships: Chengrong Yu, None;

Charles Egwuagu, None

Program Number: 560 Poster Board Number: B0115

Presentation Time: 1:30 PM–3:15 PM

MUCOUS MEMBRANE GRAFTS IN OCULAR CICATRICIAL PEMPHIGOID: SCHIRMER’S TEST AND LONG TERM FORNIX DEPTH OUTCOMES

Arturo E. Grau1, Valerie P. Saw2, Garijeet Jutley2, David Verity3, John K. Dart1, Richard Collin1, 1Ophthalmology, Pontificia Universidad Católica de Chile, Santiago, Chile; 2Corneal & External disease, Moorfields Eye Hospital, London, United Kingdom; 3Adnexal, Moorfields Eye Hospital, London, United Kingdom.

Purpose: Conjunctival fornix reconstruction using a labial, buccal or hard palate mucous membrane graft to restore mucosal surface area, is sometimes necessary for correction of advanced fornix contracture in ocular cicatricial pemphigoid (OCP). The aim of this study was to evaluate long term outcomes of mucous membrane grafts in OCP including fornix depth measurements, and identify optimum criteria for successful fornix reconstruction.

Methods: Retrospective review of 25 OCP eyes receiving mucous membrane graft fornix reconstruction at Moorfields Eye Hospital between 1997 – 2010. Average follow up duration was 54 ± 30 months.

Results: Indications included ankyloblepharon (32%), exposure with lagophthalmos (32%) and entropion with lid shortening (36%). For the 12 wet eyes with Schirmer’s test ≥5mm, mean fornix depth appeared to be maintained long term and measured 6 ± 1 mm in the lower fornix, and 10 ± 3 mm in the upper fornix. For the 13 dry eyes with Schirmer’s test <5mm, mean lower fornix depth was smaller 3 ± 2 mm (p=0.005), and upper fornix depth 9 ± 3 mm. Final visual acuity was 0.34 ± 0.3 in the wet eyes, and 0.18 ± 0.3 in dry eyes (p=0.24). All eyes were immunosuppressed for at least 4 months prior to the mucous membrane graft, and systemic immunosuppression was increased post-operatively in 4 eyes. Complications including bacterial keratitis (20%), persistent epithelial defect (16%), surface failure (8%) and recurrent entropion (28%) occurred most frequently in the dry eyes.
Conclusions: Eyes with Schirmer’s test 5mm or more do best with mucous membrane grafting in OCP, with long term maintenance of inferior conjunctival fornix and good visual acuity. Control of inflammation with systemic immunosuppression prior to mucous membrane graft surgery, and increased postoperative immunosuppression where necessary, is a requirement for success of the procedure.

Commercial Relationships: Arturo E. Grau, None; Valerie P. Saw, None; Gurjeet Butley, None; David Verity, None; John K. Dart, None; Richard Collin, None

Program Number: 561 Poster Board Number: B0116
Presentation Time: 1:30 PM–3:15 PM

Ocular Involvement in Mucous Membrane Pemphigoid
Gloria H. Hong, Irfan Khan, Amde Shifera, Chinwenwa Okeagu, Jennifer E. Thorne. Johns Hopkins University School of Medicine, Baltimore, MD.

Purpose: Mucous membrane pemphigoid (MMP) is a potentially life threatening, autoimmune disease affecting various mucosal surfaces including the conjunctiva. Ocular MMP can lead to blindness if untreated. The purpose of this study is to describe the demographic and clinical features of MMP patients with and without ocular disease at presentation; to calculate the risk of developing ocular MMP or new extra-ocular site of MMP during follow up; and to identify risk factors for new-onset disease.

Methods: We performed a retrospective chart review of 162 biopsy-proven MMP patients. Wilcoxon rank-sum test and chi-square test were used to compare characteristics of those with and without ocular MMP. Kaplan-Meier curves were used to describe incidence of ocular MMP and development of a secondary extra-ocular location of activity. Cox regression models were used to identify risk factors for new-onset ocular MMP and new extra-ocular site of MMP.

Results: At presentation, 109 of 162 MMP patients (67.3%) had ocular involvement. The median [interquartile range, IQR] age was higher for ocular MMP patients than those without ocular disease (68 [59, 78] years vs. 61 [49, 69] years, p=0.004). Patients with ocular involvement were more likely to be male (49.5% vs. 30.2%, p=0.02). A history of trichiasis was more common among patients with ocular MMP (60.6% vs. 5.7%, p=0.001). At presentation, 78 (71.6%) of patients with ocular MMP also had extra-ocular involvement, with the oropharynx (89.7%) most commonly involved site. Over a median IQR follow-up time of 7.1 [2.6, 12.5] years (range=3 months to 22 years), the risk of developing ocular MMP was 0.014 per eye-year (EY) (95% confidence interval [CI]: 0.005/EY, 0.034/EY). The risk of developing a new location of extra-ocular MMP was 0.020/EY (95% CI: 0.007/EY, 0.043/EY). Smoking was a risk factor for developing an additional extra-ocular MMP location (hazard ratio=4.09, p=0.05).

Conclusions: MMP patients with ocular involvement were more likely to be older, male, and have a history of trichiasis than those without ocular involvement. Patients presenting with only extra-ocular MMP are at risk for developing ocular MMP, and all MMP patients are at risk for developing secondary extra-ocular MMP locations, although these risks are low. Long-term follow-up is necessary to detect new-onset disease.

Commercial Relationships: Gloria H. Hong, Irfan Khan, None; Amde Shifera, None; Chinwenwa Okeagu, None; Jennifer E. Thorne, None

Program Number: 562 Poster Board Number: B0117
Presentation Time: 1:30 PM–3:15 PM

Measurements of aqueous flare objectively using an ocular fluorometer
PAVANI MURTHY PENGUNODLA1, RR Sudhir2, Sirisha Tadepalli2, Deepti Talele1, ARUSHI GOYAL1, Sanjay Mahadik2, Prema Padmanabhan3, Karthikeyan Rangaswamy2, Sangly P. Srinivas1, Sankara Nethralaya, Chennai, India; 2Electronics, Amrita School of Engineering, Bengaluru, India; 3Optometry, Indiana University, Bloomington, IN.

Purpose: The quantification of aqueous flare in post-cataract or uveitis patients is performed subjectively or using a laser flare meter. In this project, we have employed a newly constructed confocal spot fluorometer equipped with a lock-in amplifier for flare measurements in a cohort of post-cataract patients. Since the inclusion of lock-in amplifiers can overcome the influence of external light, we expect to make flare measurements with higher sensitivity and dynamic range than the conventional laser flare meter.

Methods: We used a confocal ocular fluorometer to measure the light scatter from the anterior chamber (a/c) as an index of aqueous flare. The fluorometer has a depth resolution of < 300 μm, and hence, can yield depth-resolved measurements from the a/c. Moreover, as a non-depth scanning device, one can place the focus of the excitation beam at any spot in the a/c. The instrument is also equipped with a white LED as a light source and a lock-in amplifier for emission/scatter detection. For scatter measurements, we removed the excitation filter, and placed the focal diamond in the a/c using either 0.5/0.25 mm slits in front of the LED. The excitation and emission arms were held 45° apart for all measurements.

Results: The aqueous flare intensities (measured as scatter intensity in mV) in normal subjects were 0.177±0.006mV (n = 12 eyes) and 0.069±0.005mV (n = 13 eyes) at 0.5mm and 0.25mm slit widths, respectively. In the post cataract subjects (1 day), the mean flare intensities were 0.4±0.023 and 0.231±0.018mV (n = 16 eyes) at 0.5mm and 0.25mm, respectively, which were significantly different from normal eyes (p < 0.05). The corresponding eyes were scored at 0 to 2+ as per the subjective classification of Standardization of Uveitis Nomenclature (SUN) flare scoring system. Further comparison showed that the flare intensity measured with 0.25 mm is significantly different from contralateral eyes in cataract patients (p < 0.05).

Conclusions: Our data indicates that the ocular spot fluorometer can be employed to grade the aqueous flare quantitatively on a finer scale. A slit width of 0.25 mm, which enables higher depth resolution, is more reliable for measurements of aqueous flare. Overall, we have shown a simple technique for assessing aqueous flare that could be used for monitoring the efficacy of pharmacological strategies quantitatively, and possibly, aid in early detection of relapses in uveitis patients.

Commercial Relationships: PAVANI MURTHY PENGUNODLA, None; RR Sudhir, None; Sirisha Tadepalli, None; Deepti Talele, None; ARUSHI GOYAL, None; Sanjay Mahadik, None; Prema Padmanabhan, None; Karthikeyan Rangaswamy, None; Sangly P. Srinivas, None
Support: Obama-Singh Initiative award (PI-SP)
Inhibition of recurrent experimental autoimmune uveitis by blockade of the receptor for advanced glycation end products (RAGE)

Hui Shao1, Juan Yun1, Tong Xiao1, Yuan Zhao1, Deming Sun2, Henry J. Kaplan1. 1Ophthalmology, University of Louisville, Louisville, KY; 2Doheny Eye Institute, Los Angeles, CA; 3Sullivan University College of Pharmacy, Louisville, KY.

Purpose: The mechanism of recurrent autoimmune uveitis is unknown. The receptor for advanced glycation end products (RAGE) has been linked to chronic inflammation. It is able to bind ligands of AGE and some damage associated molecules such as HMGB1 and S100A8/A9. In the current study, using anti-RAGE antibody (Ab), we examined the role of RAGE in recurrent experimental autoimmune uveitis (r-EAU) induced by uveitogenic T cells.

Methods: r-EAU was induced in Lewis rats by adoptive transfer of interphotoreceptor retinoid-binding protein (IRBP) peptide R16-specific T cells. These rats were intraocularly treated with anti-RAGE Ab. Intraocular inflammation was examined by fundoscopy and histology. Proliferation, cytokine production and disease inducing ability of responder T cells from treated or non-treated rats were determined and compared.

Results: Administration of anti-RAGE Ab in r-EAU significantly reduced the severity of intraocular inflammation and the frequency of recurrence. In addition, rats treated with anti-RAGE Ab generated decreased numbers of IFN-γ and IL-17 uveitogenic T cells, but increased numbers of Foxp3 regulatory T cells. Mechanistic studies showed that the effect of the injected anti-RAGE Ab was on antigen presenting cells (APCs). Anti-RAGE Ab–treated APCs caused R16-specific T cells to lose their uveitogenic activity and acquire immunosuppressive activity, which suppressed the induction of EAU by additional pathogenic R16-specific effector T cells.

Conclusions: Ligands-RAGE axis plays an important role in r-EAU by driving uveitogenic T cell pathogenicity through APCs. Anti-RAGE Ab can protect from the development of r-EAU by inducing regulatory APCs that convert pathogenic T cells into regulatory T cells.

Commercial Relationships: Hui Shao, None; Juan Yun, None; Tong Xiao, None; Yuan Zhao, None; Deming Sun, None; Henry J. Kaplan, None

Support: This work was supported in part by NIH grant EY024051 and an unrestricted grant from Research to Prevent Blindness (RPB) Inc.

Characterization of progressive cicatrical conjunctival disease with negative immunofluorescence

Jae Young You, Esen Akpek. Cornea, Wilmer Eye Institute, Baltimore, MD.

Purpose: Progressive cicatrical conjunctivitis with no detectable immunoreactant deposition in the basement membrane zone (BMZ) by direct immunofluorescence microscopy (DIF) is a subset of mucosal autoimmune disease that is poorly understood. This study aims to characterize the clinical features and outcomes of those patients who have undergone light microscopy (LM) and DIF examination of biopsied conjunctivas for suspected mucus membrane pemphigoid (MMP).

Methods: We created a list of all patients who have undergone diagnostic conjunctiva biopsy between January 2007 and October 2015 using the Current Procedural Terminology code 68100. 118 patients were identified. Of them, 30 patients who had a conjunctiva biopsy for cicatrising conjunctivitis and had both LM and DIF examinations were included for a retrospective chart review. Information regarding demographics, clinical presentation, medical history and clinical course was collected. The primary outcome was the rate of the clinical diagnosis of MMP which was defined as progression of the conjunctival scarring in cases which initial conjunctival biopsy was negative for immunofluorescence.

Results: Conjunctiva biopsy was consistent with MMP in three patients (3/30, 10.0%) with 3 months of mean presentation-to-treatment time (PTT) (range, 1 to 4 months). 26 patients (26/30, 86.7%) had negative DIF with mean follow-up of 40 months (range, 1-138 months). Eight of the patients (8/26, 30.8%) showed progression of cicatrical scarring. Two of the eight patients had positive repeat biopsy, 3 and 21 months after the initial biopsy; six patients required immunosuppression with mean PTT of 4 months (range, 1 to 7 months). Three other patients (3/26, 11.5%) were diagnosed with psoriasis (n=1), Sjogren’s syndrome (n=1) and sarcoidosis (n=1) based on microscopic examination of biopsied conjunctiva and required immunosuppression for progressive scarring in six months of PTT (range, 4 to 8 months). Remaining 15 patients with negative biopsy showed no signs of worsening disease over the mean follow-up of 25 months (range, 1-103 months). Six of the 15 patients had a follow-up that was less than one year.

Conclusions: Progressive conjunctival cicatization is not uncommon in patients with negative DIF for auto-antibodies to BMZ. This study emphasizes the importance of vigilant clinical follow-up in all patients with cicatrical conjunctivitis to prevent vision loss.

Commercial Relationships: Jae Young You, None; Esen Akpek, None