Purpose: Vitamin A (VitA) and its derivative retinoic acid are essential for immunological responses. Acquisition of effector responses is impeded in VitA deficient (VAD) mice. However, little is known about maintenance and expression of previously acquired effector function in the VAD environment or its impact on progression of autoimmune diseases. We examined this using experimental autoimmune uveitis (EAU) induced by immunization and spontaneous uveitis in IRBP TCR transgenic (R161H) mice.

Methods: VAD was induced by dietary lack of VitA from before birth or by daily injections of a pan-retinoic acid receptor inhibitor BMS493 in adult mice. EAU was induced by immunization with IRBP161-180 peptide. R161H mice develop uveitis spontaneously by 2–3 months of age. R161H T cells were activated in vitro in the presence of IRBP peptide for 3 days and adoptively transferred into control or VAD recipients. As an additional autoimmune model we used experimental autoimmune encephalomyelitis (EAE) induced by immunization with myelin basic protein and pertussis toxin.

Results: VAD mice were essentially resistant to EAU or EAE and displayed impaired effector T cell responses. Defective priming/acquisition of effector function by VAD T cells was also evident in vitro. Interestingly, effector T cells primed in a VitA-sufficient environment were able to function in VAD recipients, as evidenced by maintenance of high lineage-specific effector cytokine production and induction of EAU. Furthermore, R161H mice fed with VAD diet, in which the priming of pathogenic T cells had occurred before onset of VAD, developed exacerbated spontaneous uveitis compared to VitA-sufficient R161H mice.

Conclusions: We conclude that although priming of naïve T cells in the VAD environment is defective, effector function acquired under VAD sufficient conditions is maintained and can be expressed under VAD conditions. Our findings may shed light on immunity and autoimmunity in geographical regions where dietary VitA is limiting.

Commercial Relationships: Reiko Horai, None; Ru Zhou, None; So Jin Bing, None; Kaska Wloda, None; Jun Chen, None; Phyllis Silver, None; Yingyos Jittayasothorn, None; Rachel R. Caspi, None

Purpose: Disruption of immune homeostasis at the ocular surface is associated with discomfort, inflammation and potential loss of vision. Immune cells are present within the conjunctiva and can be affected by environmental factors, potentially including microorganisms as seen in other classical mucosal sites like the intestine. However, proof that a resident ocular microbiome exists and influences local immunity has been elusive. We used a mouse model of ocular surface disease to study whether commensal microbes are present in ocular mucosa and modulate immunity.

Methods: Mice were either treated with PBS, topical antibiotics, or were ocularly inoculated with a Corynebacterium sp. that we show influences the immune signature within the conjunctiva. Tears were assessed for anti-microbial components and functionality. Conjunctivae were isolated and assessed for neutrophilic infiltration and IL-17 production. We used an ocular model of Candida albicans to assess the functional implications of commensal bacteria colonization at the ocular surface.

Results: We found that IL-17 is constitutively produced within the conjunctiva-associated lymphoid tissue (CALT) and is necessary to recruit neutrophils to the ocular surface in the steady state and after a bacterial challenge. IL-17 sources in CALT include γδ T cells, γδ T cells and innate lymphoid cells (ILCs), in that order. Notably, a strain of Corynebacterium isolated from ocular tissue of mice, and known to also colonize the ocular mucosa of humans, induced the conjunctival γδ T cells to secrete IL-17, which modified the local inflammatory signature. We found that when this bacterium colonized the ocular mucosa it cannot be passed horizontally; however, it can be passed vertically from one generation to the next. This interaction appears necessary to regulate local immunity at the ocular surface, since elimination of these bacteria by antibiotic treatment, or their introduction into non-colonized mice, correlated inversely with severity of an experimental Candida albicans or Pseudomonas aeruginosa infection.

Conclusions: By satisfying all four of Koch’s postulates, we have shown, for the first time, this Corynebacterium sp. directly induces a γδ/IL-17 driven protective immunity at the ocular surface. Thus showing that microbes can exist in ocular mucosa, are immunologically relevant, and can play a role in ocular disease.

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Program Number: 843
Presentation Time: 3:45 PM–4:00 PM

Vitamin D supplementation modulates Th2 immune response by inducing T regulatory cells in allergic conjunctivitis

Purpose: The prevalence of allergic diseases is rapidly increasing worldwide, and allergic conjunctivitis (AC) is one of the most common diseases in eye clinics. Recently, Vitamin D deficiency has been shown to be associated with allergic disorders. However, the therapeutic potential of vitamin D for AC and the underlying mechanisms of its actions remain still unknown. This present study was designed to evaluate the efficacy of vitamin D to suppress the development of ovalbumin (OVA)-sensitized AC in a murine model.

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Methods: Seven- to eight-week-old BALB/c mice were sensitized with OVA and aluminum hydroxide via intraperitoneal injection. Two weeks later, mice were challenged by OVA eyedrops for 12 days with intraperitoneal injection of vehicle or 1, 25-dihydroxyvitamin D3 (1,25(OH)2D3). We evaluated clinical signs, the infiltration of inflammatory cells into conjunctiva, regulatory T cells (Treg) in drainage LN, serum levels of OVA-specific IgE production, and Th2 cytokines secretion in vitro T cells assay through flow cytometry and ELISA. In addition, to evaluate inhibitory function of Treg cells, anti–CD25 blocking antibody or isotype control antibody was injected intravenously 2 day prior to, on the day of, and 5 day after topical OVA challenge.

Results: AC development and conjunctival infiltration of eosinophils (CD45+ Siglec-F+; p = 0.018 vs. vehicle) and mast cells (CD45+ c-kit+; p = 0.022 vs. vehicle) were significantly impaired with 1,25(OH)2D3 treatment. In addition, 1,25(OH)2D3 suppressed production of OVA-specific IgE in serum (p < 0.001 vs. vehicle) and Th2 cytokines in vitro T cell assays, such as IL-4 (p = 0.032 vs. vehicle) and IL-13 (p = 0.016 vs. vehicle), compared to vehicle group. Interestingly, 1,25(OH)2D3 led to increase Treg cells population in draining LNs and suppressive levels of clinical signs and inflammatory cells infiltration into conjunctiva were reversed by depleting Treg cells (p = 0.028 vs. isotype control).

Conclusions: Our results suggest that vitamin D supplement alleviate allergic conjunctivitis, indicated by suppressing Th2 response in draining LNs and inflammatory cells infiltration into conjunctiva. Vitamin D also increased population of Treg cells in draining LNs, which inhibited Th2 response. Therefore our results demonstrated new insight into the therapeutic potential of vitamin D for allergic diseases including asthma or atopic dermatitis.

Commercial Relationships: Hyun Soo Lee, None; Ji Young Kwon, None; Chang Rae Rho, None; Jeewon Mok, None; Choun-Ki Joo, None.

Program Number: 844
Presentation Time: 4:00 PM–4:15 PM
The effect of dietary omega-3 fatty acids on allergic conjunctivitis in mice
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Purpose: Allergic conjunctivitis is one of the most common diseases of ocular surface. Previous studies showed that omega-3 fatty acids have anti-allergic and anti-inflammatory properties. It is unknown, however, whether omega-3 fatty acids have any therapeutic effect on allergic conjunctivitis. Thus, we evaluated the effectiveness of feeding of omega-3 fatty acids using a mouse model of allergic conjunctivitis.

Methods: BALB/c mice were fed either an omega-6 rich control diet or an omega-3 rich diet for one month. The mice were sensitized twice with Ragweed pollen (RW) in alum adjuvant and challenged with RW in eye drops. Clinical score was evaluated and eosinophil infiltration into the conjunctiva was counted. Levels of serum immunoglobulin E and cytokines in the conjunctival tissues were quantified. Eicosanoid profiling in the conjunctiva was performed using HPLC-ESI/MS/MS.

Results: Omega-3 fed mice showed a lower clinical score and a decrease in the number of infiltrated eosinophils in the conjunctiva. The expression level of Il4, Il13, Ccl5 and Ccl11 was lower in omega-3 fed mice compared to omega-6 fed mice. The production of inflammatory eicosanoids such as prostaglandin D2 and leukotriene B4 was decreased in the conjunctiva of omega-3 fed mice.

Conclusions: Dietary omega-3 fatty acids might exert therapeutic effects on allergic conjunctivitis by regulating multiple processes including eicosanoid production, eosinophil infiltration and the expression of Th2 cytokines and chemokines.

Commercial Relationships: Toshiaki Hirakata, None; Kentetsu Lee, None; Mai Ohba, None; Akira Matsuda, None; Akira Murakami, None; Takehiko Yokomizo, None.

Program Number: 845
Presentation Time: 4:15 PM–4:30 PM
Low vitamin D is associated with different types of ocular inflammation
Stephanie M. Llop, Samaneh Davoudi, Lindsay Grotting, Lisa Tom, George Papaliodis, Lucia Sobrin. Uveitis/Ocular Immunology, Massachusetts Eye and Ear Infirmary, Boston, MA.

Purpose: Vitamin D plays an immunoregulatory role. Low vitamin D status has been associated with some autoimmune diseases including multiple sclerosis, Vogt-Koyanagi-Harada and anterior uveitis. Our aim is to determine whether there is any association between vitamin D levels and different types of autoimmune ocular inflammation including scleritis, posterior, intermediate, anterior and panuveitis.

Methods: 61 patients (31% male, mean age 48 years) and 90 controls (26.6% male, mean age 51 years) were enrolled. Fellowship-trained specialists diagnosed patients with scleritis, posterior, intermediate, anterior and panuveitis after exclusion of infectious and neoplastic causes. Controls were patients without any history of eye inflammation. All subjects had a recorded total 25-hydroxy-vitamin D measured by mass spectroscopy or immunoassay. Clinical and demographic information was recorded from patients’ medical records including age, gender, race, smoking status, history of vitamin D supplements and vitamin D draw date.

Logistic regression models were created to examine the relationship between hypovitaminosis D and the presence of ocular inflammation using Stata (College Station, TX). In a subanalysis, we also examined vitamin D as a continuous variable. Age, gender and race were included in all multivariate models.

Results: Smoking status, history of vitamin D supplements and vitamin D draw date were not statistically different between two groups. Vitamin D level means and standard deviations were 33 ± 12.6 and 24.4 ± 11.7 nanograms per milliliter in controls and cases, respectively. The odds of having ocular inflammation was 2.4 higher in patients with low vitamin D status compared with normal vitamin D level in univariate analysis [(odds ratio (OR) = 2.4, 95% Confidence Interval (CI) = 1.24-4.73, P = 0.009)]. The association persisted in multivariate regression, after adjusting for age, gender and race (OR = 2.7, 95% CI = 1.30-5.63, P = 0.008). The odds of developing ocular inflammation was 7% lower for every unit increase in vitamin D level (OR = 0.93, CI = 0.89-0.98, P = 0.005) in multivariate regression.

Conclusions: Hypovitaminosis D was associated with increased risk of ocular inflammation in this retrospective study. This data adds to the increasing body of knowledge showing that vitamin D may play a role in autoimmune diseases.

Commercial Relationships: Stephanie M. Llop, None; Samaneh Davoudi, None; Lindsay Grotting, None; Lisa Tom, None; George Papaliodis, None; Lucia Sobrin, None.
Program Number: 846
Presentation Time: 4:30 PM–4:45 PM

**Gut Microbiome in Uveitis**

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**Purpose:** Our understanding of the etiology of uveitis and its driving mechanisms remain limited, although anecdotal evidence has linked flares of uveitis to microbial infections in some cases. Alteration in gut microbiota (dysbiosis) has been associated with other autoimmune diseases both in humans and animal models, however such studies in uveitis have been limited to animal models. The purpose of this study is to assess whether there are specific alterations in gut microbiota among uveitis patients.

**Methods:** Twenty uveitis patients were enrolled in a clinical study at the NEI (NCT01859299) to evaluate the rectal microbiome. Rectal fluids collected according to a standardized protocol obtained through anoscopy following saline enema administration were analyzed using 16S RNA sequencing with the F515/R806 primers on a HiSeq 2500 to a depth of greater than 100,000 sequences per sample. Results were compared to rectal fluids of healthy controls collected at UCLA for a HIV microbiome study.

**Results:** Rectal fluid samples of thirteen of the twenty patients have been analyzed. Average age was 54 years (48 yrs among controls), majority were posterior segment uveitis and were on systemic treatment. The degree of microbiome diversity among uveitis patients was not significantly different than in controls, though there was a trend towards increased diversity in uveitis patients. However, prominent differences in microbial composition were noted between uveitis and control samples. Among these, we highlight the genus Prevotella, which was undetectable among uveitis patients (0 of 13 samples) whereas it was present in 13 of 20 control samples (p=3x10^-4). Median abundance of *Prevotella* among controls was 29.63% and among uveitis it was 0.54% (p<0.001). Additionally, unclassified Enterobacteriaceae (56 fold; q=1x10^-10) and *Fusobacterium* (31 fold; q=5x10^-10) were enriched among uveitis samples.

**Conclusions:** While these results are preliminary, there seem to be significant differences between uveitis patient samples and healthy controls, however we cannot rule out potential effects of treatment. The unprecedented association of *Prevotella* as a health-associated taxon merits further investigation. Studies to further validate the changes in gut microbiome in untreated new onset uveitis patients are underway.

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Presentation Time: 4:45 PM–5:00 PM

Short-term high fat diet feeding reduces corneal wound healing in mice

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**Purpose:** Obesity is a serious public health problem. The influence of the pre-diabetic metabolic syndrome (MS) on corneal structure and function is poorly understood. We utilized a murine model to study the effects of a short term high milk fat diet (HFD) on the cornea, and on the cornea’s ability to heal surface wounds.

**Methods:** Six-week old C57BL/6J mice were maintained under a 12-h light/12-h dark (LD) cycle and fed *ad lib* the HFD (42% milk fat) for 10 days (a condition that did not alter fasting glucose levels). Control mice were fed a normal chow diet (CD). Some mice were analyzed for neutrophil influx in the corneal limbus and corneal expression of the circadian clock gene Rev-erβ (data collected at 3 h intervals over a 24 h cycle). Corneal sensitivity to touch was measured using the Cochet-Bonnet aesthesiometer. Other mice were given 2mm diameter corneal epithelial abrasions and analyzed for re-epithelialization, mitosis, leukocyte and platelet influx, recovery of subbasal nerve plexus density and corneal sensitivity to touch.

**Results:** Corneas from mice on the CD exhibited significant flux in tissue neutrophils in the limbus (peaking at zeitgeber time (ZT) 18 and nadir at ZT2), and expression of Rev-erβ (peak, ZT11; nadir, ZT20). Mice on the HFD failed to exhibit these fluxes, and as previously reported, (Hargrave et al., IOVS 2016; 57(12)) corneal sensitivity was significantly reduced (p<0.05), though nerve density was not reduced by the HFD. Corneal wound closure in the CD group was complete within 24 hours, but remained open at 30 hours in the HFD group (p<0.01) and epithelial cell division was significantly reduced (p<0.01). Neutrophil and γδT cell migration to the wound area, and platelet accumulation in the limbus at 18 hours after wounding were reduced by 80%, 25% and 40% respectively (p<0.01) in the HFD group. Recovery of the subbasal nerve plexus density and nerve sensitivity were also reduced (56% and 50%, respectively, p<0.01).

**Conclusions:** These data indicate that a short-term HFD, prior to inducing changes in fasting blood glucose levels, alters circadian rhythms in the cornea as has been reported in other tissues, alters nerve sensitivity in the cornea, and significantly reduces key parameters of corneal wound healing.

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