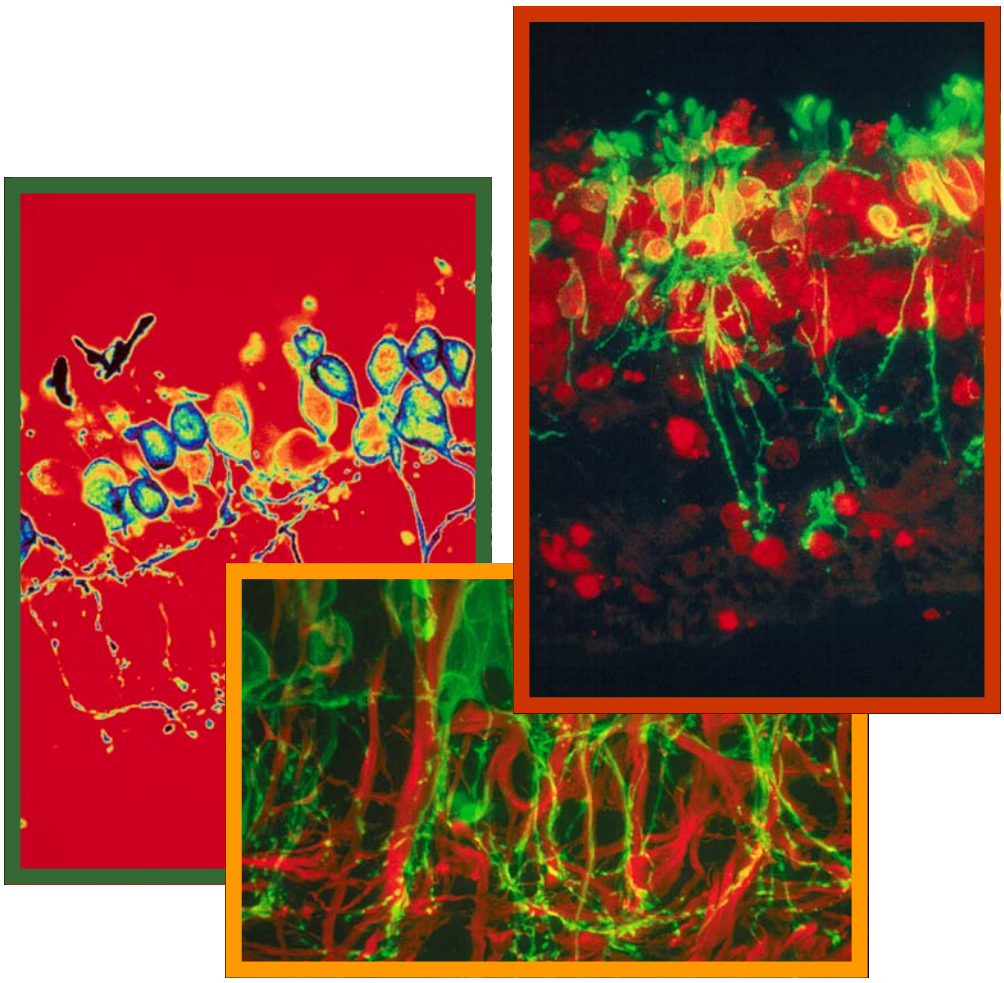
NEI Research Day



Rod neurite outgrowth in the retinas of patients with retinitis pigmentosa.
Photos by Robert Fariss, LMOD

October 31, 2003

The Cloisters, NIH

National Eye Institute
National Institutes of Health

NEI Intramural Research Day

October 31, 2003

Agenda

8:00 a.m.	**Registration and Continental Breakfast**
9:00 a.m.	**Paul Sieving, M.D., Ph.D.** Welcome Remarks *(in Cloister's Lecture Hall)*
9:15 a.m.	**Sheldon Miller, Ph.D.** Introductory Remarks
9:30 a.m.	**Graeme Wistow, Ph.D.** "Gene Discovery and Functional Genomics in the Eye"
10:00 a.m.	**Pat Becerra, Ph.D.** "Regulation of the Neurotrophic and Anti-angiogenic Activities of PEDF in the Retina"
10:30 a.m.	**Karl Csaky, M.D.** "Lost in Translation: How Do Clinical Medicine and Basic Science Communicate?"
11:00 a.m.	**Emily Chew, M.D.** "The Age-Related Eye Disease Study: Its Beginning and Its Future"
11:30 a.m.	**Lunch *(Cafeterias are available in Buildings 10, 31, 1, 45)***
1:00–3:30 p.m.	**Poster Session *(in Cloister's Lecture Hall/Chapel)*** *(Light refreshments and beverages will be served)*
3:30 p.m.	**Adjourn**

Poster Session

1:00–3:30 p.m.

The Poster Session is to take place in the Cloister's Lecture Hall and Chapel.

Posters are numbered in alphabetical order according to the first author's last name.

During the Poster Session there will also be information tables from Core Resources that will have information and handouts available. Please visit these tables and learn more about some of the resources available to NEI scientists. The Core Resource Information Tables are:

Core Facility	Chief Contact Person
Central Transgenic Facility	Eric Wawrousek, Ph.D.
NEI Biological Imaging Core	W. Gerald Robison, Jr. Ph.D.
Histopathology Core Facility	Chi-Chao Chan, M.D.
EyeBank	Graeme Wistow, Ph.D.
Colony Management & Research Support	Jorge Sztein, Ph.D.
Division of Bioengineering and Physical, Office of Research Services	Henry Eden, Ph.D.

POSTER 1

Peripheral expression of a Sequestered Autoantigen by DNA Vaccination Protects from Autoimmune Disease

Rajeev K. Agarwal, Phyllis B. Silver, Shao Bo Su, Chi-Chao Chan, Rachel Caspi.
Lab of Immunology, NEI, NIH, 9000 Rockville Pike, Bethesda, MD 20892.

Purpose: Experimental autoimmune uveitis (EAU) induced in mice with retinal antigens such as IRBP serves as an experimental equivalent to human autoimmune uveitis. The eye is an immunologically privileged organ that becomes separated from the immune system early in ontogeny. We hypothesized that sequestration may subvert peripheral tolerance to these antigens and enhance susceptibility to EAU. ***Methods:*** We used hydrodynamic injections of IRBP-DNA to express IRBP in the periphery and thus revoke its immune privileged status. ***Results:*** IRBP expression in the tissue could be demonstrated by Western blotting. Vaccinated mice were highly protected from EAU in a prevention protocol, but protection was only partial in a reversal protocol when disease was induced by a uveitogenic T cell line. This suggested that vaccination may preferentially target naïve cells, and is less effective in tolerizing cells that have already been primed. Mechanistic studies revealed hyporesponsiveness to IRBP without evidence for immune deviation. Tolerance was not transferable, arguing against induction of regulatory cells, and appeared not to require apoptosis either by the Fas or by the mitochondrial pathway. ***Conclusion:*** The data are compatible with the interpretation that peripheral presentation of the normally sequestered retinal antigen IRBP acts to desensitize the high-affinity self-reactive T cells relevant to disease.

POSTER 2

Subconjuntival Delivery of Pigment Epithelium-derived Factor (PEDF) Protein to the Choroid and Retina

J.Amaral[1], R. N. Fariss[2], M. Campos[2], W. G. Robison, Jr. [2], D. Himmelberg[3], H. Kim[3]R. Lutz[3], S.P. Becerra[1]
[1]LRCMB and [2]LMOD; NEI; [3]DBEPS, ORS/OD

Purpose: PEDF is a potent neurotrophic and antiangiogenic factor for the retina. The purpose of this work was to evaluate protein diffusion through the sclera-RPE to establish novel routes for PEDF delivery to the retina. ***Methods:*** Fluorescein–conjugated PEDF and ovalbumin were prepared. Monkey retinal pigment epithelial cells were established in culture (RPE). Fluorescein was detected by spectrophotometry, immunoblotting, fluorometry and confocal microscopy. Transepithelial resistance and voltage were measured by an electrical resistance system. Sclera was dissected from porcine eyes and cultured in vitro. Protein implants were prepared with PVA. In vivo delivery was performed in rat eyes. ***Results:*** Transepithelial resistance and impermeability to trypan blue and horseradish-peroxidase confirmed confluency, polarization, monolayer and tight junction formation of the RPE. FI-PEDF passed through the monolayer from either the apical or basal side. FI-ovalbumin diffused through the scleral tissue at a constant rate. Subconjunctival FI-PEDF injections or FI-ovalbumin implants revealed diffusion into the retina as early as 1 hour, with maximum diffusion by 24 hours. ***Conclusion:*** These results indicated that PEDF protein can traverse through the sclera- RPE to the retina, suggesting that subconjunctival PEDF delivery can be feasible and less invasive than other known routes of protein delivery to the retina.

POSTER 3

CD28-Mediated Stabilization of IL-2 mRNA in Activated T Cell is Independent of HuR Binding to the 3'UTR

Hooman Azmi, Yuko Seko and Jack A. Ragheb
Laboratory of Immunology, National Eye Institute, National Institutes of Health

Purpose: To determine whether the HuR protein binds the IL-2 mRNA in vivo and whether such binding is related to CD28-mediated stabilization of the mRNA. ***Methods:*** Experiments were performed with a normal mouse T-cell clone. Confocal microscopy was used to show translocation of HuR from the nucleus to the cytoplasm in vivo. Immunoprecipitation RT-PCR (IP RT-PCR) with an anti-HuR monoclonal antibody and IL-2 mRNA specific primers was used to demonstrate that HuR binds the Il-2 mRNA in vivo. ***Results:*** We found that HuR is present in the nucleus but not the cytoplasm of resting cells. Upon TCR signaling, translocation of HuR from the nucleus to the cytoplasm occurs. However, engagement of both the TCR and CD28 receptors does not result in any further increase in the amount of cytoplasmic HuR. Specific binding of HuR to the IL-2 mRNA is demonstrated in vitro and in vivo but such binding appears unrelated to CD28-mediated stabilization of the mRNA. **Conclusion:** The data indicates that HuR binding to the IL-2 mRNA is constitutive in activated T cells and suggests that binding is not associated with IL-2 mRNA stabilization.

POSTER 4

Role of Histidines in Mouse b-Carotene 15,15'-Monooxygenase Activity

Eugenia Batyreva[1], Susan Gentleman[1], Francis X. Cunningham[2], F. Ella Greene[3], Nancy J. Miller-Ihli[3] and Michael Redmond[1]
[1]National Eye Institute, NIH, 6/337, Bethesda, MD 20892-2740, [2]Department of Plant Biology, University of Maryland, College Park, Maryland 20742
[3]FoodComposition Laboratory, USDA, Beltsville, MD 20705

Purpose: The mouse b-carotene 15,15'-monooxygenase (b-CM) belongs to a family, including RPE65, that has four histidine, one aspartate and four glutamate residues perfectly conserved. Our objective was to determine the role of the conserved residues in the catalytic mechanism of b-CM. ***Methods:*** We replaced conserved residues and four non-conserved histidines with alanine by site-directed mutagenesis. Enzymatic activity was measured *in vitro* using affinity purified proteins and *in vivo* by expressing constructs in a strain of *E.coli* that produces b-carotene. B-carotene and retinal were quantified by reverse phase HPLC. Iron content analyses were performed by Inductively Coupled Plasma Emission spectrometer. ***Results:*** Our *in vitro* and *in vivo* data showed that mutation of four conserved histidines and glutamate 405 led to a total loss of activity. However, no significant differences were observed in activity of wild-type b-CM and mutants with replaced non-conserved histidines. The iron bound to protein was found to be a 4.4 mol/mol for wild type b-CM, but for inactive mutants bound iron was not significantly different from blanks. ***Conclusion:*** Therefore, conserved histidines are absolutely required for activity of b-CM, whereas mutant proteins are unable to bind iron, thus indicating that these histidines are probably involved in coordinating the iron.

POSTER 5

A Study to Investigate the Safety and Efficacy of Daclizumab to Treat the Ocular Complications Related to Behçet's Disease

Ronald R. Buggage[1], Darby J. Thompson[3], H. Nida Sen[1], Roxana. Ursea[1], Allison T. Bamji[1], Thomas Waldmann[2], Robert B. Nussenblatt[1]
[1]Laboratory of Immunology, National Eye Institute and [2]Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD,
[3]The EMMES Corporation, Rockville, MD

Purpose: Ocular Behçet's disease is characterized by recurrent, explosive episodes of intraocular inflammation. Treatment with systemic immunosuppressive agents is often complicated by the side effects of the therapy. Consequently, a safer and effective therapy is an important research goal. We investigated daclizumab for the treatment of ocular Behçet's disease. ***Methods:*** Randomized placebo-controlled double masked study. ***Results:*** Seventeen patients were followed 1-34 months without experiencing a safety endpoint. Visual acuity remained stable in all patients. More adverse events perceived as greater in severity occurred in patients receiving placebo than daclizumab. 6/9 daclizumab and 4/8 placebo patients experienced ocular attacks requiring therapy with a median yearly attack rate of 1.27 and 0.17, respectively. Patients in the placebo arm experienced a greater reduction in the level of prescribed immunosuppressive medications compared to patients receiving placebo.(-4.0 vs -1.0). ***Conclusions:*** The observed results in the placebo group demonstrate that careful follow-up and treatment with standard combination immunosuppressive therapy can be effective for management of ocular Behçet's. In our small study, there was no suggestion of benefit of daclizumab in comparison with placebo. However, the low observed attack rate in the placebo group limited our ability to make a definitive treatment group comparison.

POSTER 6

Ciliary Neurotrophic Factor (CNTF) Delivered By An Encapsulated Cell-Based Intraocular System: Effect On The Electroretinogram (ERG) And Retinal Histology of Normal Rabbit.

[1,3]Ronald A. Bush, [1,5]Bo Lei, [2]Weng Tao, [4]Chi–Chao Chan, [4]Terry A. Cox, [1,3,4]Paul A. Sieving

[1]Department of Ophthalmology and Visual Science, Kellogg Eye Center, University of Michigan, Ann Arbor, MI; [2]Neurotech USA, Lincoln, RI; [3]National Institute of Deafness and Communication Disorders and [4]National Eye Institute, NIH, Bethesda, MD; [5]Department of Veterinary Medicine and Surgery, College of Veterinary Medicine & Department of Ophthalmology, School of Medicine, University of Missouri-Columbia, MO

Purpose: To investigate the effect of ciliary neurotrophic factor (CNTF) delivered intravitreally at therapeutic doses and higher by encapsulated engineered human RPE cells (ECT) on retinal function and morphology of the normal rabbit retina. ***Methods:*** Fifteen adult New Zealand albino rabbits had ECT devices secreting CNTF at high (22 ng/day), low (5 ng/day), or zero doses implanted in the superior temporal quadrant of the left eye. Right eyes served as untreated controls. Full-field dark- and light-adapted ERGs, and outer nuclear layer (ONL) histology was evaluated at 25 days after implantation. ***Results:*** The rod a- and b-waves were unaffected by device implantation. The cone b-wave in implanted eyes of high dose animals was significantly reduced and the ONL was qualitatively more dense and/or thicker than control eyes. Measurements revealed that these eyes had a significantly greater ONL area, particularly in the dorsal retina. ***Conclusions:*** Controlled intraocular delivery of CNTF by ECT device at doses shown to protect photoreceptors in animal models of retinal degeneration does not reduce the rod or cone ERG in normal rabbit. Higher doses may affect cone pathway function and produce changes in outer nuclear layer morphology.

POSTER 7

Microarray Analysis of AlphaA and AlphaB-Crystallin Knockout Mouse Lenses.

D.A. Carper[1], M. John-Aryankalayil[1], C.J. Jaworski[1], K. Brown[2], D.A. Stephan[2], E.F. Wawrousek[3].

SMT, LMOD, NEI[1], Children's National Medical Center, Washington, D.C[2], TAGMS, LMDB, NEI[3].

Purpose: Examine differential gene expression associated with cataract in aA-crystallin knockout mice. ***Methods:*** Oligonucleotide microarray analysis of 12,000 genes was performed on lens RNA from two litters of 11-day-old aA-crystallin knockout ($aA^{-/-}$), aB-crystallin knockout ($aB^{-/-}$), and wild type 129Sv mice. At this age, $aA^{-/-}$ mice have apparent nuclear opacities, which progress to nearly complete lens opacity by 10 weeks. Cataracts are not observed in $aB^{-/-}$ mice. Hybridization data were analyzed using Affymetrix microarray suite v5.0. RT-PCR was used for confirmation of the differential gene expression. ***Results:*** Eighteen genes were consistently differentially expressed in $aA^{-/-}$ mice when compared to wild type mice. Seven genes were up regulated and 11 genes were down regulated. Up-regulated genes from $\alpha A^{-/-}$ lenses that have been confirmed by RT-PCR include ApoA1 binding protein, protein disulfide isomerase-related protein, and preproglucagon. Five genes were differentially expressed in $aB^{-/-}$ mice compared to wild type. ***Conclusions:*** Microarray analysis indicates that both $aA^{-/-}$ and $aB^{-/-}$ mice have differences in gene expression patterns when compared to wild type. The subset of genes altered in $aA^{-/-}$, but not in $aB^{-/-}$, may be involved in cataract formation and provide novel suggestions for processes that may prove to be relevant to cataractogenesis.

POSTER 8

Molecular signatures of *Helicobacter pylori (H. pylori)* in conjunctival mucosa-associated lymphoid tissue (MALT) lymphoma

Chi-Chao Chan, Janine A. Smith, DeFen Shen, Roxana Ursea, Phuc LeHoang, Hans E. Grossniklaus

Purpose: Conjunctival MALT lymphoma is an extranodal marginal zone B-cell lymphoma characterized by a clonal expansion of B cells, which implicates a pathological response to dominant antigen(s) including bacteria. *Helicobacter pylori (H. pylori)* infection is recognized as one of the causative agents of gastric MALT lymphoma; however, it is not reported in extragastric MALT lymphoma. We examined the association of conjunctival MALT lymphoma and *H. pylori*. ***Methods:*** Conjunctival biopsies were obtained from 5 patients with conjunctival MALT lymphoma. Lymphoma and normal conjunctival cells were microdissected using laser capture microscopy or manual techniques. DNA was extracted and subjected to PCR amplification using H. pylori gene-specific primers from the urease B and vac/m2 gene. The amplified products were verified by Southern blot hybridization. Cells from chronic conjunctivitis (normal lymphocytes), conjunctival human T-cell lymphotropic virus type-1/adult T-cell leukemia/lymphoma (HTLV-1/ATL), and orbital B-cell lymphoma were served as the controls. ***Results:*** *H. pylori* DNA was detected in the conjunctival MALT lymphoma cells of 4/5 cases. The negative case was the one with abnormal bone marrow. In contrast, *H. pylori* gene was not detected in the control samples. Conclusion: These data suggest that *H. pylori* may play a role in conjunctival MALT lymphoma.

POSTER 9

Profound Fluctuations in Expression of the Chemokine Receptor CXCR3 by Inflammation-Inducing Th1 Cells

J. Chen,[1] B.P. Vistica,[1] D.I. Ham,[1] R.N. Fariss,[2] W.G. Robison, Jr,[2] C.C. Chan,[1] E.F. Wawrousek,[3] and I. Gery[1].
Laboratories of [1]Immunology, [2]Mechanisms of Ocular Diseases, [3]Molecular and Developmental Biology, National Eye Institute

Purpose: CXCR3 plays a major role in immune-mediated inflammation and its expression characterizes Th1 lymphocytes. Here, we studied the expression of CXCR3 on Th1 cells at different stages prior to and during the induction of ocular inflammation. ***Methods:*** The experimental system of Th1-induced ocular inflammation in mice is detailed in IOVS, 2002, 43:758. Expression of CXCR3 on Th1 cells was determined by flow cytometry, RT-PCR, and fluorescent immunohistochemistry. ***Results:*** CXCR3 expression was up-regulated following primary activation of Th1 cells, but unexpectedly, it dramatically declined upon antigenic reactivation, which also down-regulated the level of CXCR3 mRNA transcript. However, reactivated Th1 cells exhibited the highest level of pathogenicity when adoptively transferred into recipients. These cells re-expressed CXCR3, while residing in the recipient mouse, reaching the peak just before their massive migration into the target eyes. Importantly, infiltrating Th1 cells underwent profound phenotypic changes in the eye that closely resembled those seen during reactivation of Th1 cells in vitro and included down-regulation of CXCR3 expression. ***Conclusion:*** CXCR3 expression on inflammation-inducing Th1 cells is restricted to their migration to the target organ and the phase that precedes it. The receptor expression is down-regulated, however, by re-exposure of the Th1 cells to their specific antigen.

POSTER 10

RNA Single Stranded Index: A New Parameter for the Optimal Design of Short Interfering RNA (siRNA) for Gene Silencing

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Laboratory of[1] Mechanisms of Ocular Diseases and Division of Epidemiology and Clinical Research[3], National Eye Institute, National Institutes of Health, Bethesda, Maryland; Cable & Wireless USA, Reston, Virginia[2]

Purpose: It is not clear how to accurately determine an effective gene target region for siRNA. It is also controversial if the predicted target mRNA secondary structure should have significant impact on the gene silence by the siRNA. Understanding the relationship between the secondary structure and gene silencing ratio (GSR) by siRNA could help to design siRNA optimally in high throughput function-based genetic screens and potentially for development as therapeutic tools. Our hypothesis is that the selection of a single stranded region as the target region should increase the efficiency of siRNA silencing. ***Methods:*** A formula and software were developed to select the single stranded region for siRNA silencing. We also statistically analyzed quantitative relationships between GSR and 10 parameters from 309 published siRNA sequences. ***Results:*** We identified four significant nonlinear parameters for designing siRNA, such as RNA Single-Stranded Index (SSi), a new parameter that identifies the target position and its related secondary structures, GC content, delta G of the siRNA, and the 5'GC content of the first four bases of the antisense RNA. ***Conclusion:*** The target RNA secondary structure is a statistical significant determinant in gene silencing and should now be used to optimize RNAi technology.

POSTER 11

Dietary Carotenoids and Risk for Age-related Macular Degeneration

E.Y. Chew, J.P. SanGiovanni, R.D. Speduto, N.Kurinij, F.L. Ferris, and The AREDS Research Group

Purpose: To evaluate the relationship between dietary carotenoid intake and prevalence of age-related macular degeneration (AMD) in the Age-Related Eye Disease Study (AREDS). ***Background:*** Lutein and zeaxathin are isomeric compounds that compose macular pigment. They may protect the retina by: (1) absorbing/attenuating spectral wavelengths associated with photochemical damage; and (2) quenching reactive oxygen species. ***Methods:*** This is a case-control analysis of the 4,513 AREDS participants. AMD severity at enrollment was assessed centrally from stereo color fundus photographs. Subjects completed a semi-quantitative food frequency questionnaire at enrollment. Nutrient intake estimates were energy-adjusted with the nutrient density model. We used multiple logistic regression methods to evaluate the relationship of dietary carotenoids with AMD status. **Results:** Compared with subjects without AMD, the likelihood of advanced AMD (either neovascular or geographic atrophy) was significantly decreased for the highest vs lowest quintiles of lutein/zeaxanthin intake (ORs = 0.7; 95% CI, 0.5 - 0.9 and 0.5; 95% CI, 0.2 – 0.9 respectively), after statistical adjustment for all nutrient- and nonnutrient-based covariates. ***Conclusion:*** Higher intake of lutein/zeaxanthin was associated with a decreased likelihood of having advanced AMD after adjusting for nutrient- and nonnutrient-based predictors and correlates of AMD.

POSTER 12

Autoantibodies in a patient with cone-rod degeneration triggers death of retinal ganglion (RGC-5) cells.

Chin MS[1A], Nagineni CN[1A], Caruso R[1B], Agarwal N[2], Detrick B[3] and Hooks JJ[1A]

[A]Laboratory of Immunology, [B]Ophthalmic Genetics and Visual Function Branch, [1]NIH/NEI; [2]Department of Pathology and Anatomy, University of North Texas Health Science Center, Fort Worth, TX; Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD.

Purpose: We have identified anti-retinal antibodies in a patient with progressive retinal degeneration. In this study we evaluated mechanisms by which these anti-retinal antibodies induce retinal cell death. ***Methods:*** Serum from a patient with cone-rod degeneration was evaluated for the presence of anti-retinal antibodies by immunohistochemistry on ocular tissues. Celltiter 96 cell proliferation assay (Promega, Madison, WI) was used to evaluate viability of a rat retinal ganglion cell (RGC-5) line after incubation with the patient sera. ***Results:*** The patient serum showed reactivity to retinal ganglion cells and the inner nuclear layer. Immunofluorescent staining of RGC-5 with patient sera produced a punctate pattern in nuclei. Incubating RGC-5 with patient sera produced 50% and 75% cell death after 2 hours and 6 hours, respectively. Control sera and heat-inactivated patient sera had no effect on RGC-5 viability. IgG from patient sera killed RGC-5 in a dose dependent manner when the cells were incubated with IgG and complement. ***Conclusions:*** Autoantibodies to retinal proteins were discovered in a patient with cone-rod degeneration. The antibodies reacted strongly to an antigen in the nuclei of retinal ganglion cells. In vitro these autoantibodies were able to cause retinal ganglion cell death by complement mediated lysis.

POSTER 13

Changes in the Predicted Secondary Structure of Interleukin-s mRNA Associated with Its CD-28 Mediated Stabilization

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Historically, mRNA secondary structure has been overlooked compared to its protein counterpart, however these structures are extremely active metabolically with numerous unique protein interactions. Signaling through the CD28 receptor stabilizes the Interlukin-2 (IL-2) mRNA. Traditional wet lab research has demonstrated that the second exon of the mRNA is necessary for this stabilization. We hypothesized that stabilization may be dependent on an interaction between the second exon and an unknown protein. A Genetic Algorithm (GA) implementation was used to generate possible secondary structures, which were further analyzed using Stem Trace, a tool from the STRUCTURELAB computer workbench, both developed at NCI's LECB. We have identified a potential hairpin-loop structure within exon 2 that may represent a binding site for such a protein. Further genetic and biochemical analyses of these sequences will be required to verify the physical existence and biological significance of this unique architecture in CD28-mediated IL-2 mRNA stabilization.

POSTER 14

Keratinization in the Conjunctiva of Patients with Keratoconjunctivitus Sicca

M.A. Crawford, .H.C. Humphrey, J.J .Hackett, I.M. Wise, J.A. Smith, C.C. Chan

Purpose: To identify abnormal keratin expression in conjunctival epithelium from patients with keratoconjunctivitis sicca (KCS). ***Methods:*** Conjunctival epithelial cells from KCS patients were collected and divided into two portions. One portion was cytospun for immunohistochemistry using avidin-biotin-complex immunoperoxidase. Primary antibodies were monoclonal CK 10- a marker of abnormal keratinization and polyclonal keratin. For immunohistochemistry, the intensity of staining was graded on a 0-4+ scale and the proportion of total cells with positive staining was also determined and expressed as a percentage. The other portion was centrifuged and the fixed pellet was processed through ascending alcohols and propylene oxide and embedded in Ladd LX-112 epoxy resin. Thin sections were then taken and stained with uranyl acetate and lead citrate and observed by Transmission Electron Microscopy. ***Results:*** Immunohistochemistry results showed abnormal conjunctival epithelial keratin expression in KCS. Specifically, cytokeratin type 3 (positive CK 10) was detected in all samples. The intensity and extent of CK 10 staining correlated with the severity of keratinization observed by using routine histology. Ultrastructural examination revealed epithelial cell degeneration with loss of cytoplasmic microorganelles in most cells. Cytokeratin or cytokeratin-like filaments were found in some epithelial cells. The number of intracellular keratin-like structures seemed to be more associated with the intensity of CK 10-positive staining rather than the proportion of positively stained cells. ***Conclusion:*** CK 10 is a specific marker for abnormal keratinization and correlates with the severity of dry eye. It may be a useful target for modulation with therapy for KCS.

POSTER 15

In vivo Dynamic Light Scattering (DLS) Studies of the Lens and Cornea

Datiles MB[1], Ansari RR[2], Suh K[2], Reed G[1], King J.[2] [1]National Eye Institute-NIH, Bethesda, MD, [2] NASA-Glenn Research Center, Cleveland, OH

Purpose: To study age related changes in the human lens and cataract formation in vivo and non-invasively using the new NASA-NEI Dynamic Light Scattering Clinical Device; and to determine if the DLS device can yield useful information about the cornea. ***Materials and Methods:*** Dynamic Light Scattering tests were performed on the lenses of normal volunteers of various age groups, as well as on patients with various types and severities of cataract using the new clinical DLS device. Static and Dynamic Light Scattering tests were performed on the corneas of animal eyes using the fiber-optic DLS probe. ***Results:*** We found that in the normal lens, there is a shift of both low molecular weight and high molecular weight proteins towards higher molecular weight with aging. In cataract, there is a more pronounced shift toward high molecular weight of both groups of lens proteins, and there is loss of low molecular weight proteins (in some cases total loss). In the normal cornea, there were differences in particle fluctuations in the anterior versus middle and posterior stroma. In HCL and Ethanol treated corneas, DLS detected molecular changes, observed in spite of the presence of clouding anteriorly. ***Conclusion:*** We detected molecular changes in vivo in the human lens in normal aging as well as in cataract formation with the new DLS device. The DLS can also be used to study molecular interactions in the cornea.

POSTER 16

Postnatal Gene Expression in the Normal Mouse Cornea: A SAGE Analysis

Janine Davis, Barbara Norman, and Joram Piatigorsky.

Purpose: To provide a detailed gene expression profile of the normal postnatal mouse cornea. ***Methods:*** Serial analysis of gene expression (SAGE) was performed on PN9 and adult mouse total corneas. Expression of selected genes was analyzed by in situ hybridization. ***Results:*** 64,272 (PN9) and 62,206 (adult) tags were sequenced. Mouse corneal transcriptomes are composed of at least 19,544 and 18,509 unique mRNAs, respectively. One third of the unique tags were expressed at both stages whereas a third was identified exclusively in PN9 or adult corneas. Abundant transcripts were associated with metabolic functions, redox activities, and barrier integrity. Three members of the Ly-6/uPAR family whose functions are unknown in the cornea constitute >1% of the total mRNA. Aquaporin 5, epithelial membrane protein, glutathione-S-transferase omega-1 and -alpha-4 mRNAs were preferentially expressed in distinct corneal epithelial layers, providing new markers for stratification. >200 tags were differentially expressed, of which 25 mediate transcription. ***Conclusions:*** The present SAGE data demonstrate dynamic changes in gene expression after eye opening, and provide new probes for exploring corneal epithelial cell stratification, development and function, and for exploring the intricate relationship between programmed and environmentally induced gene expression in the cornea.

POSTER 17

The Potential for Trans-differntiation of Bone Marrow Stem Cells into Ocular Surface Epithelial Cells

A.R. Djalilian, Q.Y. Fan, J.A. Smith, R.B. Nussenblatt, J.C. Vogel, E.J. Holland, C.C. Chan. National Eye Institute, National Cancer Institute, National Institute of Health, Bethesda, MD; Cincinnati Eye Institute, University of Cincinnati, Cincinnati, OH.

Purpose: Recent studies have demonstrated a remarkable ability of adult bone marrow stem cells to trans-differentiate into a number of different tissues. This prompted us to examine the ocular surface epithelium after bone marrow transplantation to determine if donor derived cells can be detected. ***Methods:*** Eight week old female mice were irradiated and then transplanted with the whole marrow of B6129S-Gtrosa26 (Rosa 26) male mice (transgenic mice expressing beta galactosidase). The ocular surface was examined for the presence of donor derived cells using FISH (X and Y chromosomes), beta-galactosidase staining (X-gal and immunostaining), and PCR (Y chromosome). ***Results:*** Examination of the peripheral blood revealed successful engraftment by the donor marrow. The recipient mice were examined at 2 weeks and at 7 months after bone marrow transplantation. Donor derived surface epithelial cells could not be conclusively identified. ***Conclusions:*** Plasticity of adult stem cells can have significant clinical implications for the repair of damaged organ. The results of this study did not show any conclusive evidence for in vivo trans-differentiation of bone marrow stem cells into ocular surface epithelium in a murine model. An upcoming clinical study will also examine the same question by analyzing samples of the ocular surface epithelium from patients that have previously undergone allograft bone marrow transplantation.

POSTER 18

Specific interaction between lens MIP/Aquaporin-0 and gamma-E-crystallin results in gamma-E-crystallin recruitment to the plasma membrane.

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Laboratory of Molecular and Developmental Biology, National Eye Institute, National Institutes of Health, Bethesda, MD.

Purpose: Major Intrinsic Protein (MIP)/Aquaporin 0 is required for lens transparency. Our goal is to identify and characterize proteins that interact with MIP and to elucidate the role of these interactions in MIP functions. ***Methods:*** A MIP C-terminal peptide was used as bait to screen a rat lens cDNA yeast two-hybrid library. Gamma-E-crystallin, a water soluble lens fiber-specific protein, was identified as a binding protein to MIP.
Results: By using co-immunoprecipitation assays, we demonstrate that gamma-E-crystallin interacts specifically with full-length MIP in mammalian cells. Interestingly, MIP does not interact with gamma-D-crystallin, another member of the highly conserved gamma-crystallin gene family. By using confocal fluorescence microscopy, we demonstrate that MIP interacts with gamma-E-crystallin in individual mammalian cells and that this interaction results in the recruitment of gamma-E-crystallin from the cytoplasm to the plasma membrane. Furthermore, we found that MIP does not interact with a naturally-occurring gamma-E-crystallin mutant (Elo) that is associated with dominant genetic cataract. ***Conclusions:*** This study provides the first demonstration of MIP interaction with other lens proteins at the molecular level. It suggests that MIP may play a structural role in the organization of gamma-crystallins in lens fibers and that perturbation of MIP and gamma-E crystallin interaction may be involved in genetic cataract.

POSTER 19

Cdk5 Regulates Corneal Epithelial Cell Migration and Peripheral Localization of Active Src during Wound Healing

[1]C.Y. Gao, [2]M.A. Stepp, [1]Robert Fariss, and [1]P.S. Zelenka, National Eye Institute, NIH, and [2]George Washington University

Purpose: Cdk5 retards corneal epithelial cell migration in scratch wounded cultures. This study investigates the relationship between Cdk5 and Src, a known regulator of cell migration. ***Methods:*** Mouse A(6) corneal epithelial cells were used for in vitro studies. Transfection experiments used GFP-Cdk5 or a kinase inactive mutation, GFP-Cdk5T33. Antibodies to Src, active Src (pY416) and Cdk5 were used for immunodetection. Scratch wounded cultures were incubated 12hr with or without the Cdk5 inhibitor, olomoucin (15mM). For organ culture studies, euthanized mice were subjected to corneal debridement wounding (1.5mm) and eyes were cultured 12hr. Images from confocal microscopy were analyzed by image processing. ***Results:*** Cdk5 and Src(pY416) co-immunoprecipitated from A(6) cells. Olomoucin increased overall Src activation. GFP-Cdk5 overexpression reduced active Src(pY416) concentration at the cell periphery. In scratch wounded cultures, Src was activated in cells along the leading edge. Olomoucin enhanced peripheral staining and decreased perinuclear staining of Src(pY416) in these cells. In organ cultured eyes, olomoucin enhanced peripheral staining of Src(pY416), increased the rate of corneal wound healing, and caused cells to separate from the epithelial cell sheet. ***Conclusions:*** Cdk5 regulates activation and/or localization of Src during corneal epithelial wound healing in cultured cells and organ cultured eyes.

POSTER 20

Effects of Extended Organ Culture on the Rat Lens

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Purpose: Lens organ culture has been used extensively to study cataract induction and prevention. The purpose of this study was to analyze the effects of extended organ culture on the rat lens. ***Methods:*** Lenses from Sprague-Dawley rats (~4weeks old) were cultured in modified TC-199 medium under standard conditions. Lenses were analyzed histologically, protein synthesis was assessed by ^{35}S-methionine incorporation, and cell proliferation was measured using BrdU. ***Results:*** Although remaining transparent and metabolically active , lenses placed in culture cease to grow whereas *in vivo* lenses of the same age are growing rapidly. Protein synthesis decreases with time in culture, but after 7 days is still about 2/3 of that in freshly extracted lenses. While synthesis of crystallins is clearly diminished, synthesis of most non-crystallin proteins is apparently not affected. Histological analysis of cultured lenses shows abnormal morphology in the bow region. BrdU labeling indicated normal levels of epithelial cell proliferation in the cultured lenses, but a failure of epithelial cells to migrate to the bow region and differentiate into lens fibers. ***Conclusion:*** The data at present suggest that while epithelial cells are proliferating normally they are not differentiating and elongating into fiber cells, a failure which would account for the lack of growth and the abnormal histology at the lens equator. Studies are continuing to confirm this and to determine its molecular basis.

POSTER 21

The possible role of Protein Kinase C in the trafficking of MIP/Aquaporin 0

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Purpose: MIP is specifically expressed in the ocular lens fibers, functions as a water channel and plays an important role in lens transparency. We study the role of the PKC signaling in MIP gene expression and localization in the lens epithelial explants. ***Methods:*** We used confocal microscopy, Real Time PCR, immunohistochemistry and immunoprecipitation in rat lens epithelia explants cultured with FGF2 for 3 days. A specific PKC phosphorylation inhibitor (GO-6983) was used. Point mutations in MIP putative PKC phosphorylation sites were constructed. ***Results:*** FGF2 induces MIP gene expression in a dose dependent manner. Confocal microscopy shows MIP localization in the plasma membrane. However, in the presence of the GO-6983, MIP does not integrate into the plasma membrane, remains in the cytoplasm. RT-PCR analysis did not show any significant change of MIP mRNA level in the presence or absence of GO-6983. ***Conclusions:*** Active PKC is not required to induce MIP expression by FGF2. However, it is required for MIP integration in the lens cell plasma membrane. MIP phosphorylation by PKC may be important for MIP trafficking from cytoplasm to plasma membrane. The constructions with MIP PKC putative phosphorylation sites will determine the role of these sites in the trafficking of MIP.

POSTER 22

Eye Movements in Chorea-Acanthocytosis

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Purpose: To describe the eye movement abnormalities in chorea-acanthocytosis (ChAc), a neurodegenerative disorder with aberrant erythrocyte morphology (acanthocytosis). Mutations in the chorein gene (CHAC, chromosome 9) are responsible for ChAc. In contrast to Huntington's disease (for which ChAc cases have been mistaken in the past), ocular involvement in ChAc has not been systematically studied. ***Methods:*** Three ChAc patients, ages 26–44, were included. Patients had an extensive clinical and laboratory work-up, and neuroimaging. A neuro-ophthalmological examination and eye movement recording using magnetic search coil were performed to assess fixation characteristics, saccades, pursuit and antisaccades. ***Results:*** Patients had dystonia, hyperkinetic movements, dysarthria, dysphagia, and acanthocytosis. MRI showed degeneration of the basal ganglia typical for ChAc. In two patients, CHAC mutations have been confirmed. All patients had >30 square-wave jerks per minute and fractionated saccades. A decrease of saccadic peak-velocity was more pronounced for vertical saccades. Pursuit testing done in two patients showed low gain. Antisaccade testing done in one patient was abnormal. ***Conclusions:*** Our findings may suggest brainstem involvement as another site of neurodegeneration outside the basal ganglia in ChAc. ChAc patients have pronounced oculomotor abnormalities. Eye movement recordings could assist in moni-

POSTER 23

Pax-6 as a Possible Regulator of the Optimedin Gene Activity

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Purpose: To understand the mechanisms involved in regulation of the optimedin gene activity. ***Methods:*** Promoter fragments of the mouse optimedin gene were cloned into the pGL3 vector and their activity was tested *in vitro* after transfection into COS7 cells using luciferase as a reporter. Interaction of Pax-6 with a putative binding site in the proximal optimedin gene promoter was studied by gel shift assays. Mutations in a putative Pax-6 binding site were introduced by site-directed mutagenesis. ***Results:*** Mouse optimedin promoter fragments (3kb and 0.1 kb) showed 4-5 times higher activity than the pGL3 vector alone after transfection into COS7 cells. Cotransfection experiments with several transcription factors expressed in the retinal ganglion cells (Brn3b, Math5, and NeuroD) did not show any stimulatory effect on the minimal optimedin promoter. A putative Pax-6 binding site (positions -86/-70) was identified in the optimedin promoter. Pax-6 is able to bind to this site as judged by gel shift assay, while mutation GCG⟶ttc(position -73 /-76) in this site completely eliminated Pax-6 binding. Similar mutation in 3kb and 0.1 kb promoter constructs significantly decreased stimulatory effect of Pax-6. ***Conclusion:*** Our data suggest that Pax-6 may be involved in the regulation of the optimedin promoter in the retina in vivo.

POSTER 24

Immunohistochemistry and Ultrastructure of Angioma in von Hipple-Lindau Disease

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Purpose: Retinal angioma occurs in 60% of patients with von Hipple-Lindau disease (VHL). We have previously indicated the "stromal" cells in the angiomas are the tumor cells. This study is to evaluate protein expressions and ultrastructure of the "stromal" cells. ***Methods:*** 4 cases with von Hipple-Lindau disease were evaluated using routine histology, immunohistochemistry of avidin-biotin complex immunoperoxidase technique and transmission electron microscopy. Antidodies included GFAP, NSE, factor VIII, CD34, CD117, vascular endothelial growth factor (VEGF), hypoxia-inducible factor-a (HIF-a) and ubiquitin. ***Results:*** All eyes had retinal angiomas associated with VHL. They were characterized with numerous small capillary-like vascular channels intermixed with vacuolated "stromal" cells. NSE, CD34, VEGF, and ubiquitin were expressed in all components. Factor VIII and CD34 also outlined the vascular channels. GFAP was expressed in the glial tissue mingled within the angioma. A few scatter cells were positive for CD117. Ultrastructure showed large vacuolar intracytoplasmic lipid inclusions with homogeneous, medium electron density. ***Conclusion:*** The "stromal" or tumor cells in VHL retinal angioma contain stem-cell component and release various ischemic angiogenic factors including VEGF and ubiquitin, which can be responsible for abundant neovascularization of the disease. Higher NSE expression on the angiomas suggests VHL cells may closely relate to neuronal stem cell origin.

POSTER 25

Elevated TNF-a and soluble TNF Receptor in Degeneration Susceptible Mice with Experimental Coronavirus Retinopathy

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Purpose: Experimental coronavirus retinopathy (ECOR) is a retinal degeneration model system that is triggered by a virus and is composed of a genetic and autoimmune component. Increasing evidence suggests that TNF family members play a crucial role in immune-mediated disease processes. Therefore, we compared the levels of TNF-a and soluble TNF-a receptors (sTNFRs) in BALB/c (retinal degeneration susceptible) and CD-1 (retinal degeneration resistant) mice after viral infection. ***Methods:*** Mice were injected intravitreally with mouse hepatitis virus and eyes and serum were evaluated for the presence of TNF-a and TNF-a receptors by RT-PCR and ELISA. ***Results:*** TNF-a mRNA was detected at higher levels within the retinas of BALB/c than in CD-1 mice. Serum concentrations of TNF-a and TNFR1 proteins were significantly increased, $p<0.005$ and $p< 0.0005$ respectively, in BALB/c but not in CD-1 mice. While concentrations of sTNFR2 proteins were elevated in both BALB/c ($p< 0.00005$) and CD-1 ($p< 0.005$) mice early in infection, compared to their mock-injected controls, concentrations of sTNFR2 were significantly higher in BALB/c as compared to CD-1 mice ($p< 0.0005$). ***Conclusions:*** These studies demonstrate that TNF-a and sTNFRs are elevated in virus-infected retinal degeneration susceptible BALB/c mice and that these molecules may participate in retinal degenerative processes.

POSTER 26

Microarray Gene Expression in Pterygia

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Purpose: To examine the gene expression profile of whole pterygia using DNA microarray technology. ***Methods:*** Thirteen primary and one recurrent pterygia and normal residual superior limbal conjunctival graft tissue were surgically removed. Three pterygia, one recurrent and two primary, were found suitable for individual microarray testing. RNA was extracted from the tissue samples and used as probes for the Affymetrix human gene chip U95AV2, containing 12,000 genes. Hybridization signals were normalized and filtered, and the data were analyzed using Gene Spring Analysis Software. ***Results:*** We found 41 genes with statistically significant changes. Upregulation occurred in the following gene categories: collagen (2), extracellular matrix (2), extracellular matrix protein precursor (1) and oncogenes (2). Down regulation was found in the following: cell cycle regulated (1), collagen (2), extracellular matrix (1), and oncogenes (3). Prominent among genes with altered expression levels were: fibronectin, collagen III, and protein tyrosine phosphatase. P53 was at normal levels. ***Conclusion:*** DNA microarray technology is useful in studying the genetic changes in pterygia. These studies suggest specific sites of gene regulation that may be involved in the growth and migration of pterygia; this information could potentially be translated into a clinical treatment for pterygia.

POSTER 27

Major pathogenic epitope(s) of human retinal soluble antigen (h-SAg) in a humanized model of experimental autoimmune uveitis in HLA class-II transgenic mice.

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Purpose: To investigate immunodominance and immunopathogenicity of S-Ag peptides. Human retinal soluble antigen (S-Ag) is believed to be the most relevant autoantigen for human uveitic diseases. Experimental Autoimmune Uveitis (EAU) is an animal model of human posterior uveitic diseases. Development of a humanized EAU model in HLA-transgenic mice, allows assessment of immunogenicity for clinically important human autoantigens. ***Methods:*** HLA-DR3 transgenic mice were immunized with the whole S-Ag molecule or h-SAg peptides to induce EAU and test immunogenicity of used peptides. 20 amino acid-long overlapping peptides spanning S-Ag molecule were used to recall primed T cells. ***Results and conclusions:*** HLA-DR3 transgenic mice develop severe EAU upon S-Ag immunizations, to which wild type mice are highly resistant. S-Ag primed T cells from DR3 transgenic mice proliferated in response to recall with peptides 51-70, 61-80, and 291-310. Therefore these peptides were chosen for further immunizations for EAU induction. Peptides 289-300 and 306-317 were also chosen for immunizations because of close proximity to 291-310, and because T cells from human uveitis patients were demonstrated to respond to these peptides. Peptide 291-310 induced a strong, specific immunological response, however it did not result in EAU. Interestingly, peptide 289-300 induced a strong, response and moderate EAU in HLA-DR3 mice. This is an important finding as peptide 289-300 has been shown to be immunogenic in human patients

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POSTER 28

Mitochondria permeability changes due to regulation of Bid expression in human retinal pigment epithelium (HRPE)

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Purpose: Past studies have shown that Bid can be cleaved when the C-FLIP level is knocked down by SiRNA and that some mitochondria membrane permeability changes can be associated. The present study aims to study how truncated-Bid specifically acts on mitochondria membrane permeability by using a tetracycline inducible system for expression control. The release of proapoptotic mitochondria molecules including Cytochrome C, AIF, EndoG, and Smac/Diablo was examined. ***Methods:*** A tetracycline inducible Tet-off system with a pTRE-tight response vector was used for transfection of RPE cells. A western blot analysis was performed to confirm Bid presence and cleavage and immunohistochemistry was performed to examine the localization of the molecules after induction. ***Results:*** Trucated-Bid expression in RPE cells appeared to result in condensation of nuclei and change in mitochondria potential. Immunohistochemistry showed the release of cytochrome C and Smac/Diablo. AIF and EndoG were not localized to the nuclei, and appear still within the mitochondria. ***Conclusion:*** Under truncated-Bid expression, Cytochrome C and Smac/Diablo were released. However AIF and EndoG stayed in the mitochondria. Therefore the release of AIF and EndoG appears to be controlled differently from that of Cytochrome C/Smac. Further studies using tBid expressing RPE cells will be necessary for definitive conclusions.

POSTER 29

Regulatory Elements Activating the Lens-specific MIP/ Aquaporin 0 Gene Promoter in Differentiated Lens Epithelia

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Purpose: Major Intrinsic protein (MIP)/ aquaporin 0, that is specifically expressed in the ocular lens fibers and is required for lens transparency, functions as a water channel. It may also function as an adhesion molecule and play a role in gap junction formation. We study the MIP gene regulatory elements responsible for the activation of the MIP promoter in explanted lens epithelia induced to differentiate by FGF2. ***Methods:*** Various murine MIP gene 5'-flanking sequences were inserted upstream of the luciferase reporter gene. These constructs were transfected into rat lens epithelia explants cultured with FGF2 and assayed for promoter activity. ***Results:*** Lens epithelia explants cultured with 100 ng/ml FGF2 express MIP, whereas no MIP transcripts are observed in its absence. MIP gene 5'-flanking sequence -1648/+44 and -790/+44 contain an active promoter in lens explants. However, sequence -559/+44 is not able to activate the luciferase gene. When an internal deletion -540/-461 is made, the sequence -790/+44 looses its promoter activity. DNase I footprinting analysis with lens and NIH 3T3 nuclear extracts indicates that the region -608/-592 is protected by factors present in the lens but not in the fibroblast nuclear extract. ***Conclusions:*** Our results indicate that MIP gene 5'-flanking sequence -790/+44 contains regulatory elements required for MIP gene expression in differentiating lens cells. We have identified a proximal (-540 to -461) and a distal (-790 to -540) domain. Both domains contain elements required to activate the MIP promoter in differentiated lens cells. The domain -608/-592 may interact with a lens specific factor that may be responsible for the lens specific expression of the MIP gene.

POSTER 30

Variants of CTACK (CCL27) are Expressed in Mouse Eye

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Purpose: An RT-PCR based screen of mouse eye RNA detected mRNAs related to the chemokine, CTACK (CCL27), thought to be skin-specific. This study seeks to characterize these RNAs. ***Methods:*** Primers were synthesized corresponding to the 5' and 3' ends of the reported CTACK mRNA sequence and RT-PCR was used to examine CTACK-related sequences in mouse eye RNA. 5'-RACE was used to examine sequences 5' of exon 2. ***Results:*** RT-PCR amplified three variants of CTACK: fully spliced (exons 1,2, and 3), partially spliced (retaining intron 1) and un-spliced (retaining introns 1 and 2). 5' prime RACE predominantly amplified a previously reported CTACK variant, called PESKY, which differs from CTACK at the 5' end. Comparing the reported PESKY mRNA sequence with the mouse genomic DNA sequence revealed that the 5'-end of PESKY joins 2 upstream exons that are separated by a 2kb intron in the genomic sequence. 5'-RACE also detected a PESKY variant containing this intron. ***Conclusion:*** The eye contains multiple splice products related to CTACK, some of which may be eye-specific. The predominant RNA corresponds to PESKY, a non-secreted chemokine that is targeted to the nucleus and enhances cell motility. We also detect a novel, alternatively spliced variant of PESKY.

POSTER 31

Bietti Crystalline Corneoretinal Dystrophy is caused by CYP4V2: A Novel Member of the Cytochrome P450 Family

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Purpose: Bietti crystalline corneoretinal dystrophy (BCD) is an autosomal recessive retinal degeneration. We mapped BCD locus on chromosome 4q35 previously. The goal of this study is to find and identify the gene for BCD. ***Method:*** The BCD region was refined by linkage analysis and haplotype analysis in 10 BCD families. A YAC/BAC/PAC contig was constructed in BCD region for physical map. Candidate genes in this region were screened for mutation by direct sequencing of PCR amplified exons from BCD patients. A full length cDNA of a predicted gene HCG1787816 from Celera database was obtained by direct PCR and 3' and 5' RACE from human retina and RPE cDNA. The cDNA sequence was used as a BLAST query for homology searching against the public nucleotide and protein database (NCBI). A novel gene CYP4V2 was identified by sequence similarity to the protein cytochrome P450 super family. The gene structure was assembled by alignment of the cDNA sequence to the genomic DNA sequence using BLAST and DNAStar software. Gene expression was tested by PCR of cDNA from human multiple tissues. The protein structure and mutation effects of CYP4V2 were predicted by molecular modeling. ***Result:*** The BCD region on 4q35 was refined to a 1.6 mb interval flanked by D4S2924 and D4S3051. A physical map of BCD region at 4q35.1-4qter covered contiguously by 6 overlapping YACs and 32 BACs representing the shortest path. Assembly of sequence from overlapping 3' and 5' RACE products and 3 PCR fragments amplified from human retinal cDNA provides a 2041bp cDNA sequence extending from 5'UTR through the stop codon (TAA) and 3'UTR. It consists of 11 exons, spans 19kb and encodes a 525 amino acid protein, with BLASTP showing 80% identity and 89% positive to mus musculus CYP4V3. 11 mutations were found in 18 of 21 unrelated BCD patients. Molecular modeling analysis of cyp4v2 shows most of these mutation may change the protein's structure and function. CYP4V2 is widely expressed in human tissues and might have a role in fatty acid and steroid metabolism, consistent with biochemical studies of BCD patients. ***Conclusions:*** We mapped BCD to chromosome 4q35 and identified a novel gene, CYP4V2, which shows mutations in 10 of 11 familial and 8 of 10 sporadic cases. The mutations found in BCD patients suggest BCD is caused by the mutation of CYP4V2 gene.

POSTER 32

Expression of Glucocorticoid Induced TNF Receptor Family Related Protein (GITR) on peripheral T cells from normal human donors and patients with non-infectious uveitis

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Purpose: To study the expression of the glucocorticoid induced TNF receptor family related gene (GITR) in subsets of T lymphocytes from human peripheral blood. ***Methods and Results:*** Reverse transcriptase PCR and multicolor flow cytometry were used. In normal human PBMCs, GITR expression on the resting CD4+ T cells was low but markedly increased after activation. The percentage of GITR+ T cells in the CD4+CD25+ T cell subpopulation (15.1%) was significantly higher than that in the CD4+CD25- T cell subpopulation (5.2%, $p<0.01$). In a group of patients with non-infectious uveitis, a proposed T helper cell mediated autoimmune ocular disease, the GITR expression on the CD4+ T cells in both the active patients (34.5%) and the inactive patients (19.6%) was significantly higher as compared to that in the normal donors (10.7%, $p<0.01$). This increased GITR expression was only seen in the CD4 positive T helper cell subpopulation but not in the CD4 negative subpopulation. GITR expression on CD4+ T cells decreased when the patients became clinically quiescent. ***Conclusions:*** GITR is an activation marker for the CD4+ T cells and preferentially co-expressed with CD25 on the CD4+ T cells in human peripheral blood. Its expression correlates with the clinical course of non-infectious uveitis.

POSTER 33

Alpha tropomyosin as a self-antigen in patients with Behcet's syndrome

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Purpose: To evaluate the cellular and humoral response against Tropomyosin and its possible role as self-antigen in patients with Behcet's syndrome. ***Methods:*** Peripheral blood mononuclear cells from 18 patients with Behcets syndrome as well as normal controls were isolated & co-cultured with tropomyosin and its peptides. The proliferative responses of these cells to these antigens were studied using the 3H thymidine incorporation assay. Serum samples were also screened by ELISA for auto antibodies against alpha tropomyosin. ***Results:*** Six out of eighteen patients showed an increased proliferative response to tropomyosin or its peptides compared to the normal controls. Significantly higher titers of anti-tropomyosin antibodies were seen in three out of the eighteen patients. No relationship was found between the cellular or humoral response with the duration of the disease, clinical status, HLA subtype, or the clinical manifestations. ***Conclusions:*** These results suggest that an active immune response against tropomyosin occurs in a subgroup of patients with Behcets syndrome. The occurrence of these abnormalities suggests a putative role for tropomyosin to act as a self-antigen in Behcets disease.

POSTER 34

Transgenic mice containing BAC clone with a point mutation in the *myocilin* gene as a model for glaucoma.

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Purpose: To develop a novel mouse model of glaucoma. ***Methods:*** The Tyr423His point mutation was introduced into a BAC clone containing the full length mouse *myocilin* gene. Transgenic mice containing mutated BAC DNA were generated using a standard protocol. The gene copy number was estimated by real-time PCR. Western blotting and immunohistochemistry were used for myocilin expression analysis. ***Results:*** The Tyr423His mutation disrupted a secretion of myocillin and, by this criteria, resembled the most severe glaucoma-causing mutations in the human myocilin gene. Two lines of transgenic animals containing about 30 and 2-3 copies of mutated BAC DNA were analyzed. Western blot analysis demonstrated that mutated myocilin was mostly located in the insoluble cellular fraction as compared to wild-type myocilin. Immunostaining revealed preferential expression of myocilin in the sclera and trabecular meshwork (TM) in both TG and WT with stronger signal in TG. ***:*** Transgenic mice expressing mutated myocilin in the TM may represent a novel model of glaucoma.

POSTER 35

Identification of Candidate Genes for Autoimmune Uveoretinitis Using QTL and cDNA Microarray Analysis in Rat Model

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Experimental Autoimmune Uveoretinitis (EAU) is a model of human posterior uveoretinitis induced in rodents by retinal antigens like Interphotoreceptor Retinoid Binding Protein (IRBP) and Soluble Antigen (S-Ag). Genetic contributions from both MHC and Non-MHC genes had been shown to be associated with disease susceptibility and disease resistance in mouse and rat models. We previously identified four QTL (quantitative trait loci) regions on rat chromosomes 2, 4, 10 and 12 in the F2 population of resistant (F344/N) vs susceptible (Lewis/N) strains of rats that are MHC class II haplotype matched. In this study we performed human cDNA microarray analysis to evaluate the gene expression profile that might yield insights into the uveitis associated genes and their role in conferring disease resistance and susceptibility. Relative expression levels of more than 1000 immunologically relevant genes were compared between F344 and Lewis rat strains at different time points after the induction of disease, using RNA isolated from lymph node cells. Our results revealed differential expression of several inflammatory cytokines, their regulatory genes, transcription factors, apoptosis related genes and some pituitary hormones between the susceptible and the resistant strains. Selected genes were validated by real time quantitative PCR (Taqman PCR). Localization of these genes to syntenic segments on rat chromosomes revealed that some of them are present in the previously identified QTL regions. This may be useful in prioritizing candidate QTL genes for further confirmation. These findings have important implications for the design of future microarray-based studies in EAU and for establishing functional links between genotype and phenotype of complex polygenic traits like uveitis.

POSTER 36

Differential expression of PDGF and their receptors in human RPE and choroidal cells: Potential role in choroidal neovascularization (CNV) and proliferative vitreoretinopathy (PVR)

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Purpose: We evaluated the expression of PDGF and their receptors in human RPE (HRPE) and choroid fibroblasts (HCHF) to examine their possible roles in PVR and CNV. ***Methods:*** RT-PCR and ELISA assays were used for mRNA and secreted protein analyses. Cellular proliferation and migration were measured by Celltiter (MTT) and in vitro wounding assays respectively. ***Results:*** Expression of PDGF-A and PDGF-B mRNA was enhanced significantly by TGF-b in HRPE. In HCHF, very low levels of PDGF-A and B mRNA was observed. PDGF-Ra mRNA expressed prominently in HCHF, but at very low levels in HRPE. PDGF-Rb was not detectable in either HRPE or HCHF. Secretion of PDGF-AA and AB by HRPE was increased significantly by TGF-b. In contrast, HCHF cultures did not secrete all three isoforms of PDGF (AA, AB, BB). All three rPDGF isoforms enhanced HCHF cell proliferation and migration significantly. ***Conclusions:*** Our results suggest that PDGF secreted by RPE under the influence of TGF-b acts on fibroblasts and other mesenchyme derived cells present in the surrounding choroidal and retinal tissues that express PDGF receptors. Promotion of proliferation and migration of these mesenchymal cells by PDGF may facilitate the formation of fibrovascular tissues and neovascularization associated with PVR and CNV.

POSTER 37

Interactions of pigment epithelium-derived factor with a novel polypeptide: A putative receptor

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Purpose: Pigment epithelium-derived factor (PEDF), a neurotrophic and antiangiogenic factor, binds to unidentified cell-surface receptors. Yeast-2 hybrid identified several genes of potential PEDF binding proteins. We resolved to overexpress, purify and evaluate the interactions of a polypeptide derived from one of these genes with PEDF. ***Methods:*** DNA amplification was performed by PCR. Expression DNA plasmids were constructed using Gateway Technologies. Gene overexpression was achieved in in vitro protein systems and E. coli cells. Purification of His-tagged fusion proteins was by Ni-NTA affinity column chromatography. Binding of purified PEDF and overexpressed His-tagged polypeptides was with either protein immobilized and detected by SPR and immunological techniques. ***Results:*** Sequence alignment and comparison of a DNA fragment obtained from yeast-2 hybrid, p12, to GenBank databases identified a novel human RPE gene, R1, with transmembrane domains, an extracellularly-oriented p12 region, and a phospholipase-like motif. PCR amplification confirmed *p12* expression in human RPE, RPE cell lines and retina. Purified p12-His peptide bound to PEDF in a specific manner. Increasing concentrations of NaCl did not affect the PEDF-p12 interactions. ***Conclusions:*** The *R1* gene is novel, expressed in RPE and retina, codes for a transmembrane protein and has a region with strong binding affinity for PEDF.

POSTER 38

Subcutaneously Administered Daclizumab Therapy for Uveitis

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Purpose: To evaluate subcutaneously administered daclizumab, a humanized anti-IL-2 receptor antibody, for the treatment of non-infectious intermediate and posterior uveitis. ***Methods:*** Fifteen study participants with sight-threatening uveitis quiescent on immunosuppressive therapy were enrolled at 3 sites and treated with subcutaneous daclizumab, 2 mg/kg every 2 weeks x2, then maintenance at 1 mg/kg every 2 weeks, with simultaneous tapering of the standard immunosuppressive therapy. ***Results:*** Treatments were well tolerated and 11/14 patients reached the preset outcome by eliminating 50% of their standard immunosuppressive medications by 12 weeks without recurrence of their ocular inflammatory disease. One case was withdrawn at 4 weeks due to decreased vision in one eye. ***Conclusions:*** Subcutaneous daclizumab therapy is well tolerated and has demonstrated positive clinical effects in patients with uveitis.

POSTER 39

A Central Role for Iris-Expressed Growth Factor/PDGFD in Proliferation of Lens Epithelial Cells and Control of Lens Growth

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Purpose: To examine the role of Iris-Expressed Growth Factor/PDGFD (IEGF) in the eye. ***Methods:*** Clones were obtained from EST analysis and PCR. IEGFp1 antiserum was raised against a specific peptide. IEGF expression was examined by western and northern blots and immunofluorescence (IF). Protein was purified by immunoprecipitation (IP). Activity was tested on rat lens explants and anterior segments and blocked with IEGFp1. ***Results and Conclusions:*** IEGF/PDGFD is a novel member of the PDGF/VEGF family of growth factors. IEGF localizes to the posterior regions of iris and ciliary body opposite the proliferative zone of the lens. Lens-derived N/N1003A cells express high levels of IEGF while αTN4-1 lens cells have no expression. N/N1003A media and IP- purified IEGF induce proliferation in rat lens explants, while immuno-depleted medium or αTN4-1 conditioned medium has no effect. IEGFp1 antiserum blocks lens cell proliferation in cultured rat anterior segments. IEGF seems to have a key role in lens cell proliferation and lens growth. IEGF blockage could be useful in suppressing secondary cataract. IEGF also shows remarkably specific localization to the OPL/ONL boundary in the retina, suggesting a possible role in the normal function of photoreceptors.

POSTER 40

Fenretinide Induces Apoptosis in Human Retinal Pigment Epithelial (RPE) Cells: The Role of Retinoid Receptors

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Purpose: Retinoic acid (RA) is a known regulator of many cellular functions including proliferation and differentiation. Its actions are mediated through a signaling pathway involving nuclear receptors, RAR and RXR. Fenretinide, *N*-(4-hydoxyphenyl) retinamide, a retinoic acid derivative and a potential cancer preventive agent, is known to induce apoptosis in cancer cells. Long term administration of fenretinide has been shown to lower plasma retinol levels and affect night vision. Recent studies from our laboratory have shown that low concentrations of fenretinide induce differentiation of cultured human retinal pigment epithelial cells into a neuronal phenotype (1). The present study is aimed at assessing the toxic effect of fenretinide on human retinal pigment epithelial cells. ***Methods:*** Human RPE cells (ARPE-19) in culture were treated with 1-20 mM fenretinide in the presence or absence of retinoid receptor antagonists for various time intervals. Cell lysates were used to measure the progression of apoptosis by a sandwich-enzyme immunoassay using anti-histone antibody directed against mono-and oligonucleosomes. Activities of caspases, 2, 3, 8 and 9, a family of cysteine proteases involved in apoptosis, were measured using a fluorimetric method. ***Results:*** Fenretinide induced apoptosis in ARPE-19 cells in a dose- and time-dependent manner as indicated by the generation of mono- and oligonucleosomes. AGN194301, a RARa receptor specific antagonist, effectively blocked the apoptosis. Retinoid receptor pan-antagonist, AGN194310 and AGN193109 also showed this effect. Fenretinide induced apoptosis was accompanied by a marked increases in caspase-2 and caspase-3 activities. ***Conclusion:*** We have shown for the first time that fenretinide induces apoptosis in ARPE-19 cells and that RARa is involved in this process.

POSTER 41

Dietary Omega-3 Long-chain Polyunsaturated Fatty Acids and Risk for Age-related Macular Degeneration

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Purpose: To evaluate the relationship between omega n-3 long-chain polyunsaturated fatty acid (LCPUFA) intake and prevalence of age-related macular degeneration (AMD) in the Age-Related Eye Disease Study (AREDS). ***Methods:*** AREDS participants aged 60 to 80 years at enrollment (n=4,513) were included in this case-control study. All had best-corrected visual acuity of 20/32 or better in at least one eye. Information was obtained on demographic, lifestyle, and medical characteristics. AMD was graded from stereo color fundus photographs with a standardized protocol at a reading center. Subjects completed a self-administered 90-item semi-quantitative food frequency questionnaire at enrollment. Nutrient intake estimates were energy-adjusted with the nutrient density model. We used staged model building techniques with multiple logistic regression methods to evaluate the relationship of dietary LCPUFA composition with AMD status and covariates. AREDS subjects without AMD served as controls for each of 4 AMD groups: intermediate drusen, large drusen, geographic atrophy, neovascular AMD. Multivariable models were also analyzed with categories of fish intake as the primary predictor variable, since n-3 LCPUFAs are predominantly concentrated in marine products. ***Results:*** The risk for neovascular AMD was significantly decreased for the highest vs lowest quintiles of total n-3 LCPUFA intake (OR = 0.60; 95% CI, 0.40 - 0.88), after statistical adjustment for all nutrient- and nonnutrient-based covariates. This relationship also persisted for docosahexaenoic acid (DHA, C22:6n-3), an n-3 LCPUFA that is selectively accreted and retained in the photoreceptor outer segments (OR = 0.53; 95% CI, 0.35 – 0.79). Higher total fish consumption (> 2 servings of fish/week compared to no intake) was associated with a decreased risk for neovascular AMD (OR = 0.49; 95% CI, 0.28 – 0.84). This relationship also existed for > 1 four-ounce weekly serving of broiled/baked fish (OR=0.64; 95% CI, 0.44 – 0.94) or tuna (OR=0.66; 95%CI, 0.45 – 0.98). No significant relationships were found for the other AMD groups. ***Conclusion:*** Higher intake of n-3 LCPUFAs and fish was associated with decreased risk of having neovascular AMD after adjusting for nutrient- and nonnutrient-based predictors and correlates of AMD.

POSTER 42

Intravitreal Methotrexate Resistance in a Patient with Primary Intraocular Lymphoma

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Purpose: To demonstrate a possible mechanism of drug resistance in a patient with PIOL treated with intravitreal methotrexate. ***Methods:*** A 57-year-old woman with PIOL and primary central nervous system lymphoma (PCNSL) was treated with intravitreal methotrexate injections for recurrent PIOL alone and monitored with ophthalmic examinations over a 2.5-year period. PIOL cells were evaluated for multi-drug resistance-related protein (MRP), folate binding protein (FBP) and reduced folate carrier (RFC) expression using immunocytochemistry viewed under confocal microscopy. ***Results:*** One year after the initial tumor diagnosis the patient developed multiple PIOL recurrences in the right eye with no recurrence of PCNSL over a period of 18 months. Although initially responsive to treatment, the recurrent PIOL became refractory to intravitreal methotrexate. Examination of the refractory PIOL cells revealed aberrant expression of the MRP and decreased expression of the RFC and FBP. ***Conclusions:*** Alterations in the transport of methotrexate across the cell membrane of the PIOL cells could explain the development of methotrexate resistance in this patient.

POSTER 43

Confocal analysis of retina ganglion cells retrogradely labeled with DiI in fixed mouse eyes.

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Purpose: Mouse models of retinal degenerative diseases, such as glaucoma, rely on a flat-mount retina preparation (FMR) for the quantitative analysis of degenerative changes, and require markers for retinal ganglion cells (RGC). We developed a method of *post mortem* fluorescent labeling for measurement of morphological changes in the RGC layer. ***Methods:*** Mouse eyes were fixed in 4% paraformaldehyde. The optic nerve was cut 2 mm behind the globe, and DiI was inserted into the end of the optic nerve. Eyes were incubated in 4% paraformaldehyde for 2 weeks at 37°C. The progress of RGC labeling was monitored by fluorescent imaging through the pupil. Then, the retinas were excised, counterstained with DAPI, and flat-mounted. ***Results:*** Confocal microscopy through retina revealed intense DiI labeling of RGCs, including axonal fibers. Quantification of DiI- versus DAPI stained cells showed that DiI stained about 35-40% of the total number of cells in the RGC layer, which correlates with previous studies of the total proportion of RGC in the RGC layer (Jeon et al., 1998). Z-series of optical sections were collected for volumetric analysis of single RGCs. ***Conclusion:*** Foregoing procedure for labeling of RGC and their axons is useful for 3-D morphometric analysis of RGC in mouse eye.

POSTER 44

Lack of Ocular Response to Lipopolysaccharide in CCR-1 Deficient Mice

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Purpose: To ascertain therole played by CC chemokine receptor-1 (CCR-1) in the evolution of uveitis using the murine Endotoxin Induced Uveitis (EIU) model. ***Methods:*** EIU was induced in CCR-1 deficient (CCR-$1^{-/-}$) and C57B1/6 mice by a single subcutaneous injection of 100 mg lipopolysaccharide (LPS) in 0.1 ml PBS. The control groups received PBS alone. Mice were sacrificed at 24 hours after injection. The eyes were collected for histology examination and RNA isolation. RT-PCR was performed for b-Actin, IL-6, IL-12, IFN-g, RANTES and MIP-1a. Serum IL-6, IL-12, IFN-g, MIP-1a and MIP-2 were measured using ELISA. ***Results:*** There were 5.27±6.65 (mean±SD) ocular inflammatory cells in CCR-$1^{-/-}$ and 17.00±15.73 cells in C57B1/6, $p<0.0001$. In contrast, serum ELISA showed a higher level of IL-6, IL-12, IFN-g, MIP-1a and MIP-2 in CCR-$1^{-/-}$ than that in C57B1/6. RT-PCR also showed a higher concentration of IL-6 mRNA and slightly increases in other cytokines in CCR-$1^{-/-}$ than in C57B1/6 mice. ***Conclusions:*** EIU was significantly reduced in CCR-$1^{-/-}$ compared with C57Bl/6 mice. A lack of CCR-1 may disturb the chemokine signaling and cytokine-chemokine network, cause defects in inflammatory cell recruitment and migration to infected sites, and reduce the number of ocular inflammatory cells during EIU.

POSTER 45

Complex regulation of biphasic CD40L expression in PBMC

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Purpose: To elucidate the regulation of biphasic CD40 ligand (CD40L) expression on PBMCs. ***Methods:*** The kinetics of CD40L expression were examined via cell surface staining of CD4+ PBMC activated with TCR alone or TCR & CD28. Quantitative measurements of CD40L mRNA from whole PBMC using real time RT-PCR were also performed. Transfection of primary PBMC with a CD40L promoter driven luciferase reporter allowed examination of transcriptional activity. ***Results:*** CD40L expression has two phases post-activation, a CD28-independent phase (6 hours), and a CD28-dependent phase (48 hours). CD28 mediated augmentation of late CD40L expression is entirely IL-2 dependent. Expression on resting cells is restricted to CD45RO+ CD4+ T cells. CD40L expression at 6 hrs is evenly distributed between CD45RO+ and CD45RA+ T cells. CD40L mRNA from whole PBMC examined using real time RT-PCR demonstrated little correlation between the quantity of CD40L mRNA and CD40L protein expression on the cell surface. Measurement of transcriptional activity using a CD40L promoter driven luciferase reporter indicated a progressive increase in transcriptional activity over time. However, CD28 costimulation had minimal effects on reporter activity. ***Conclusions:*** These results suggest that CD40L expression is regulated by a complex pattern of transcriptional, posttranscriptional and possibly translational mechanisms.

POSTER 46

Dry eye signs and symptoms in women with premature ovarian failure

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Purpose: To examine whether women with premature ovarian failure have abnormal findings in ocular surface or tear parameters, or reported symptoms of ocular discomfort, compared with age-matched controls. ***Methods:*** Sixty-five women with premature ovarian failure (POF) and 36 age-matched controls were examined for signs and symptoms of dry eye. The Ocular Surface Disease Index questionnaire© and the NEI-VFQ 25-item vision functioning questionnaire were administered to the participants. Assessments of ocular surface damage (Oxford and van Bijsterveld scores of vital dye staining), and tear status (Schirmer 1 and 2, tear break-up time) were performed. ***Results:*** Women with POF scored significantly worse than controls on all ocular surface damage parameters: Oxford score (3.2 vs 1.7, $p=0.001$), conjunctival lissamine green (2.1 vs 1.3, $p=0.02$), corneal fluorescein staining (1.2 vs 0.4, $p=0.005$), and van Bijsterveld score (2.1 vs. 1.3, $p=0.02$). Further, the proportion of patients with POF meeting the dry eye diagnostic criterion of van Bijsterveld score ≥ 4 was significantly greater among women with POF than among controls (20% vs. 3%, $p=0.02$). The POF group also tended to have worse scores than controls on self-reported symptoms: overall OSDI (12.5 vs. 2.1, $p=0.0006$) and the overall NEI-VFQ (94 vs. 98, $p=0.001$) after adjustment for age and race. Schirmer scores and tear breakup time did not differ. ***Conclusions:*** Women with POF were more likely to exhibit ocular surface damage and symptoms of dry eye than age-matched controls. They were not, however, more likely to have reduced tear production. This association between ocular surface disease and premature ovarian failure has not been previously reported. These data provide further evidence of the multi-faceted role of sex hormones in health and disease of the ocular surface.

POSTER 47

Specifics of the Nonllethal Injury Response In Retinal Pigment Epithelial Cells (RPE) on Cellular and Molecular Levels

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Purpose: During the lifetime retinal RPE exposed to a high degree of oxidative stress because of their location and function. The aim of the present study was to examine the specifics of cellular and molecular responses of the RPE to the nonlethal oxidative injury and to evaluate these responses in relationship to the development of AMD. ***Methods:*** Human differentiated ARPE-19 cells were exposed to different concentrations of the oxidative stimulus hydroquinone. Absence of cell death at nonlethal doses was measured by XTT conversion, ^{3}H leucine incorporation, and trypan blue exclusion. AIF localization, phosphorylation of SAPK and p38, actin reorganization was detected by immunocytochemistry and Western blot. Cell membrane blebbing was detected using GFP-membrane labeled RPE cells. Changes in gene expression were evaluated by microarray analysis. ***Results:*** At nonlethal oxidative doses morphological changes including actin rearrangement, activation of p38 and SAPK and cell membrane blebbing were observed. Genes involved in the protection and recovery of injured cells from oxidative stress were up-regulated (MGST1, TNX1, FTH1) and cell death related genes were down-regulationed (CASP9, BNIP, CASP7). Gene expression profile was normalized back to baseline levels within 48 hours after the injury. ***Conclusions:*** These results indicate that RPE cells have an elaborate death avoidance system when exposed to lower doses of oxidative stress. In addition, nonlethal injury does elicit specific sets of both cellular and molecular response, which may be involved in AMD development.

POSTER 48

Cholera toxin prevents Th1-mediated autoimmune disease by enhancing Th2 polarization

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Cholera toxin (CT), a major enterotoxin produced by *Vibrio cholerae*, is known for its properties as a mucosal adjuvant that promotes Th2 or mixed Th1+Th2 responses. Here, we explore the ability of CT to act as a systemic adjuvant to counteract the Th1 response leading to experimental autoimmune uveitis (EAU). We report that susceptible B10.RIII mice immunized with a uveitogenic regimen of the retinal antigen IRBP could be protected from disease by a single systemic injection of as little as 2 µg of CT at the time of immunization. The protected mice were not immunosuppressed, but rather displayed evidence of immune deviation. Subsequent adaptive responses to IRBP showed evidence of Th2 polarization, as indicated by reduced delayed-type hypersensitivity in the context of enhanced antigen-specific lymphocyte proliferation and IL-4 production. Antigen-specific production of several other cytokines, including IFN-g and TNF-a, was not appreciably altered. The inhibitory effect of CT was dependent on the enzymatic A subunit of CT, because the cell-binding B subunit alone could not block disease development. Mice given CT displayed detectable IL-4 levels in their serum within hours of CT administration. This innate IL-4 production was critical for protection, as infusion of neutralizing antibody against IL-4 to mice, given a uveitogenic immunization and treated with CT, counteracted immune deviation and abrogated protection. Our data indicates that systemic administration of CT inhibits EAU by skewing the response to the uveitogenic auto-antigen to a nonpathogenic phenotype.

POSTER 49

Expression and Localization of Retinoschisin (RS-1) in the Developing Mouse Retina

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Purpose: To re-evaluate the role of retinoschisin, mutations of which cause juvenile X-linked retinoschisis, in development of the normal mouse retina. ***Methods:*** RS-1 mRNA and protein expression during retinal development were studied with *in situ* hybridization, immunohistochemistry and RT-PCR on tissue obtained by laser capture microdissection. The developmental date of functional synapses was estimated using the ERG. Differentiating retinal neurons were identified using homeobox transcription factors Pax6, Brn3b and Chx10. ***Results:*** RS-1 expression was first seen in retinal neuroepithelium of prenatal mouse retina at E12, and detected in immature retinal ganglion cells (RGCs) at E16. Expression occurred in the same sequence as neuronal development: RGCs, amacrine cells (P3), and bipolar and photoreceptor inner segments (P7 to P10). RGC expression waned by P10 - P14 but did not disappear. RS-1 appeared in the outer plexiform layer with the development of the ERG b-wave, and localized to postsynaptic membranes. Nearly all adult retinal neurons, except possibly horizontal cells, expressed RS-1 mRNA. Co-localization of RS-1 with Muller cells was not seen. ***Conclusions:*** RS-1 expression occurs in a "developmental wave" across the retina that corresponds with layer formation and the establishment of functional synapses.

POSTER 50

Expression of Retinal Antigens in Human Thymi: Variability among Antigens and Individual Thymi

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Purpose: We have previously reported a correlation between expression of retinal antigens in thymus and resistance to experimental autoimmune uveoretinitis induced by these antigens in mice and rats. (Egwuagu et al. *J Immunol* 1997, 159: 3109). The present study investigated the expression of retinal antigens in human thymi. ***Methods:*** Thymic samples were collected from eighteen patients during surgery for congenital heart disease. Total RNA was extracted and RT-PCR (30 - 40 cycles) was performed with primers of five uveitogenic retinal proteins and five melanocyte-specific antigens. Antibodies against S-antigen and RPE-65 were used to detect these proteins in sections of human thymi. ***Results:*** Remarkable variabilities were seen among both the individual thymi and the retinal antigens. S-antigen, RPE65, recoverin and 4/5 melanocyte antigens were detected in almost all tested thymic samples, whereas IRBP, rhodopsin and tyrosinase were detected in only six or seven samples. Immunohistochemistry showed protein expression of S-antigen and RPE-65, predominantly in medullary areas of the thymus. ***Conclusions:*** It is assumed that central tolerance develops against the antigens expressed in the thymus, with a potential effect on the resistance/susceptibility of the individuals to specific pathogenic autoimmunity initiated by these antigens.

POSTER 51

The Cone ERG During Light Adaptation In Mice Lacking Functional Rods

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Purpose: To investigate cone ERG changes during light adaptation in mice lacking functional rods. ***Methods:*** Dark- and light-adapted full-field ERG were obtained from 5 NRL wild type, 3 NRL null and 3 rhodopsin null mice. Dark-adapted ERGs were first recorded with a 0.6 log cd·s/m² stimulus. Light-adapted ERGs were elicited 10 sec after the onset of a 42 cd/m² white background and at 1 min intervals for the subsequent 10 minutes. ***Results:*** None of the null mice had detectable dark-adapted ERG responses below cone threshold. The waveforms were identical to the light-adapted ERG, indicating that these were cone responses. During 10 minutes light adaptation, the mean relative amplitude of NRL wild type, null and rhodopsin null mice increased by 43.4 ± 21.1, 9.7 ± 7.1 and 8.3 ± 2.8 (mean ± SD) %, respectively. The mean implicit time shortened by 6.0 ± 1.6, 3.2 ± 1.1 and 3.2 ± 1.4 sec, respectively. ***Conclusions:*** Previous reports show that these two mutants lack functioning rods but are very different in numbers of cones, cone ERG amplitude and rate of degeneration. However, they show very similar deficiencies in their ERG responses to light adaptation compared to wild types, indicating that these changes require intact rod function.

POSTER 52

Eyelid Myxoma in Carney Complex: Histo-Pathology & Genetics

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Purpose: Carney complex (CNC) is a rare inherited disorder associated with spotty cutaneous and mucosal pigmentation, myxomas and endocrine overactivity; in half of the patients, mutations of the *PRKAR1A* gene, a protein kinase A regulator, have been identified. In the present study eyelid tumors from one patient were subjected to histopathologic and genetic studies after microdissection. ***Methods:*** Two large nodular lesions that were identified in the right upper eyelid of a 27-year-old with CNC, were excised and processed for routine histology and immune histochemistry against vimentin. Cells from the tumor, the normal stroma and the epithelium were microdissected. DNA was extracted and analyzed of the *PRKAR1A* gene. ***Results:*** Histological analysis of the excised tumors revealed a myxomatous area with mucinoid and fibrous elements as well as areas with hypertrophy of sebaceous glands. The myxomatous areas stained positively with vimentin. DNA analysis showed that the patient was a heterozygote for the 578delTG *PRKAR1A* inactivating mutation. When the DNA isolated from different phenotypic tumors was compared there was no loss of heterozygosity (LOH). ***Conclusions:*** Eyelid myxomas are a frequent manifestation of CNC, a multiple tumor syndrome associated with an increased risk for certain malignancies and endocrine dysfunction. Patients with eyelid myxomas, especially when those are multiple and/or recurring, may need to be screened for this genetic condition. In at least one case, LOH was not present within the tumor, suggesting that *PRKAR1A* haploinsufficiency may be sufficient for tumorigenesis in the eyelid.

POSTER 53

Endotoxin-Induced Uveitis in Cyclooxygenase 2 Deficient Mice

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Purpose: Endotoxin-induced uveitis (EIU) mimics human acute anterior uveitis. COX-2 is an enzyme that converts arachidonic acid (AA) into prostaglandins (PG). Another AA metabolism is to generate leukotrienes (LT) by 5-lipoxygenase (5-LO). This study is to delineate the role of COX-2 in acute ocular inflammation. ***Methods:*** EIU is induced in wild-type (WT), heterozygotic ($COX\text{-}2^{+/-}$) and COX-2 null mice ($COX\text{-}2^{-/-}$) with injection of lipopolysaccharide (LPS). Alternatively, mice were co-injected with LPS and IFNγ. Ocular histology, serum cytokines and AA products, and relevant ocular transcripts were compared among the groups. ***Results:*** EIU score was significantly enhanced in $COX\text{-}2^{-/-}$ in comparison to WT and $COX\text{-}2^{+/-}$. PGE_2 was increased in WT and $COX\text{-}2^{+/-}$ EIU but not in $COX\text{-}2^{-/-}$ EIU. LTB_4 in serum and ocular 5-LO transcripts were increased in $COX\text{-}2^{-/-}$ EIU in comparison with WT and $COX\text{-}2^{+/-}$ EIU mice. IL-6 was increased, whereas IFNγ was decreased both in serum and ocular transcripts in $COX\text{-}2^{-/-}$ EIU mice in comparison with WT and $COX\text{-}2^{+/-}$. Furthermore, EIU was suppressed in mice treated with recombinant IFNγ as shown by the decreased EIU scores, serum LTB_4 and IL-6, and ocular 5-LO and IL-6 mRNA; and increased serum IFNγ and ocular IFNγ, particularly in $COX\text{-}2^{-/-}$ mice.
Conclusions: Disturbance of AA pathway exacerbates EIU in COX-2 deficient mice. IFNγ could moderately reverse this exacerbation and protect against EIU.

POSTER 54

The Role of Ultrasound Biomicroscopy in Assessment of Patients with Anterior Segment Ocular Inflammatory Disease

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Purpose: Ocular inflammation affecting the anterior segment can present diagnostic problems because it often involves structures that are not readily accessible by routine examination methods. Our purpose was to assess the utility of ultrasound biomicroscopy (UBM) in the evaluation and management of patients with anterior segment inflammatory disease. ***Methods:*** Retrospective, non-comparative review of the medical records of 35 patients with ocular inflammation involving the anterior segment examined with high-resolution ultrasonography over a period of 5 years. The findings were determined and the clinical relevance of UBM information was analyzed. ***Results:*** Ophthalmic abnormalities were present in 29 / 32 eyes and failed to add additional clinical information in 3 eyes. In 11 eyes UBM demonstrated abnormalities not detected on clinical exam. The UBM findings correlated with the clinical exam in the majority of cases (14 eyes). The results provided influenced the management in 8 eyes. UBM demonstrated CB detachment in 2 patients with hypotony and cyclitic membrane in 2 patients with cataracts on their preoperative evaluation. ***Conclusions:*** The UBM is a noninvasive technique that provides critical information in the preoperative assessment and surgical planning of anterior segment inflammatory disease; it is also an objective measure for monitoring disease progression.

POSTER 55

Macrophage driven transgenic expression of IL-10 ameliorates induction of Experimental Autoimmune Uveitis (EAU)

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Purpose: Endogenous interleukin 10 (IL-10) has a regulatory role in EAU. Here we wanted to investigate the effects on EAU of transgenic IL-10 targeted to different cell types. ***Methods:*** Transgenic IL-10 epression was targeted to macrophages under control of the CD68 promoter (CD68/IL-10 TG), or to activated T cells under control of the P-mu (CD2) or the IL-2 promoters (CD2/IL-10 TG and IL-2/IL-10 TG, respectively). Hemizygous CD68/IL-10 TG, hemizygous CD2/IL-10 TG, or homozygous IL-2/IL-10 TG mice and their wild-type (WT) littermates were immunized for EAU with IRBP. Retinal pathology scores and Ag-specific immunological responses (DTH and lymphocyte proliferation) were measured. ***Results:*** Hemizygous CD68/IL-10 TG mice were protected from EAU and had reduced immunological responses. In contrast, hemizygous CD2/IL-10 TG mice showed only a trend towards protection, but did not attain a statistically significant difference in EAU pathology compared to WT ($p<0.083$). Homozygous IL-2/IL-10 TG mice were partly protected. ***Conclusions:*** Prior studies revealed that the CD68 promoter drives higher expression of the IL-10 transgene than either the IL-2 or the P-mu promoters. We therefore hypothesize that a quantitative difference in IL-10 production contributed to the enhanced protection from EAU in CD68/IL-10 transgenic mice. However, our data do not exclude that targeting of the expression to macrophages rather than activated T cells may also be a contributing factor.

POSTER 56

Localized T-lymphoid Proliferation in Eyes of Transgenic Mice Expressing IL-7 Selectively in their Lens

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Purpose: IL-7 is a viability and growth factor that acts on a variety of lymphoid cells. In this study we examined the effects of IL-7 when it is expressed transgenically in a sequestered organ, the lens. ***Methods:*** Transgenic mice were generated by placing the IL-7 gene under control of the promoter for alpha-A crystallin. The kinetics of ocular changes were evaluated by routine histological analysis from Day 1 through 3 months. Analyses of the infiltrating lymphocytes were performed by immunofluoresent and immunohistochemical staining. ***Results:*** No apparent systemic changes were observed in the transgenic mice, but their eyes demonstrated a unique pattern of cellular infiltration. Earliest ocular changes were detected on day 5 and consisted of lymphocyte infiltration, mainly in the limbus and iris surrounding the lens. The lymphocyte numbers increased rapidly, reaching their peak at 3-5 weeks and gradually declined thereafter. The infiltration was accompanied by severe morphological changes in the mouse eyes, including cataract formation and phthisis bulbi. The majority of the infiltrating cells are CD8 and CD4 T-lymphocytes, at different stages of maturation. ***Conclusions:*** This line of transgenic mice provide a new model for cellular infiltration into the eye.

POSTER 57

Comparison of a generic (NEI-VFQ) and a disease-specific (OSDI) questionnaire in patients with Sjögren's syndrome.

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Purpose: Many studies have documented a lack of association between clinically measured signs and patient-reported symptoms of dry eye. We examined associations among the Ocular Surface Disease Index (OSDI) subscales, the National Eye Institute Vision Functioning Questionnaire (NEI-VFQ) subscales, and ocular parameters in patients with Sjögren's syndrome, a systemic disorder characterized by dry eye and dry mouth. ***Methods:*** 42 patients with Sjögren's syndrome were assessed with OSDI, NEI-VFQ, Schirmer I, Oxford and van Bijsterveld (VB) staining scores, and tear film breakup time (TFB). ***Results:*** Mean OSDI-overall score was 30±20; mean overall NEI-VFQ score was 84±13. Associations of OSDI subscales with ocular parameters were modest ($r<0.22$) and not statistically significant. Associations of NEI-VFQ subscales with ocular parameters tended to be stronger for the near vision subscale with TFB ($r=0.32$, $p=.05$) and for the distance vision subscale with VB ($r=0.33$, $p=.04$). The strongest associations of OSDI with NEI-VFQ were for subscales measuring similar domains: OSDI-Discomfort with ocular pain ($r=-.60$); and OSDI-Function with general vision ($r=-0.60$), mental function ($r=-0.60$), role function ($r=-0.64$), and driving ($r=-0.57$). ***Conclusions:*** In this group of patients, the NEI-VFQ was similar to the OSDI in its ability to measure dry eye symptoms and their impact on functioning.

POSTER 58

Knock-out mice for gS-crystallin

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Purpose: gS is a major component of the adult lens. Mutation of gS in the *Opj* cataract causes severe disruption of lens due to loss-of-function and the presence of unfolded protein. To separate these effects the gS gene was deleted. ***Methods:*** The mouse *crygs* gene was knocked out by homologous recombination. *Opj* and -/- mice were compared by a variety of microscopic and biochemical techniques. ***Results:*** In gS -/- mice there is no obvious cataract, but the optical properties of the lens are degraded. Protein content is reduced but lens size is not obviously affected. Cortical fiber cells are poorly organized and dense nuclear bodies persist deep into the lens. F-actin content is reduced and the cytoskeleton appears to be disorganized. Similar but more severe effects are also seen in *Opj*. In wt lens, gS is present in cell nuclei in the elongating fiber cells. In *Opj/Opj* gS is present in dense cytoplasmic plaques, often adjacent to nuclei. ***Conclusions:*** The most severe effects in *Opj* derive from unfolded gS protein. Loss of function in -/- mouse indicates a role for gS involving lens cytoskeleton and normal processing of nuclei, in addition to its contribution to the bulk refractive structure of the lens.

POSTER 59

Lengsin: A Novel Marker for Terminal Differentiation in the Lens

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Purpose: Functional genomics of a novel gene discovered through the NEIBank project. ***Methods:*** cDNAs were obtained from EST analyses. Expression was examined by Northern and Western blot and by ISH. Recombinant proteins were produced using the pET system. Structure was examined by electron microscopy (EM). Photo-activatable ATP was used to examine cofactor binding and mass spectroscopy was used to validate peptide sequences. Antibody against recombinant protein was used for IF. ***Results:*** Lengsin is highly specific for the lens and is expressed in the ring of fiber cells undergoing terminal differentiation. Lengsin belongs to the glutamine synthetase (GS) superfamily. EM reveals a six-fold symmetrical oligomer similar to that of bacterial GS proteins. Recombinant protein will bind ATP, but no enzyme activity has been demonstrated. The mouse gene has 5 exons. In the human gene, the third exon is skipped in splicing, providing another example of a "pseudo-exon" in a human lens gene. A gene KO is in progress. ***Conclusions:*** Lens transparency depends on a highly ordered progression from undifferentiated epithelial cells to terminally differentiated fiber cells. In the final stages, the fiber cells lose nuclei and other organelles. Lengsin is a candidate for involvement this in process.

POSTER 60

Solution Structure of gS-crystallin from Residual Dipolar Couplings

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Opj, an inherited cataract in mice, is associated with a mutation in Crygs, the gene that encodes gS-crystallin. The single mutation (Phe9/Ser9) causes a marked, concentration-dependent decrease in solubility at physiological temperature. The solution structure of the wild type gS-crystallin has been determined by multidimensional NMR spectroscopy, using both heteronuclear and homonuclear 1H-1H residual dipolar couplings obtained in stretched hydro-gel and gelled Pf1 media, supplemented with a moderate number of HN-HN NOE restraints. The topology of this protein is similar to other crystallin proteins that contain two domains, each of which is comprised of two four-stranded b-sheets. Mutation of Phe9 is expected to disrupt the hydrophobic core, thereby lowering its thermodynamic stability and resulting in the observed aggregation at higher temperature. The aim of our study is to investigate the structural difference of the mutant at low temperature relative to wild type protein.

POSTER 61

gN: A novel member of the g-Crystallin family.

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Purpose: To fully characterize the family of g-crystallins. ***Methods:*** Human gN was identified by BLAST and clones were obtained from a testis cDNA library. Other cDNAs were obtained by EST analyses. Expression was examined by 2D-gel/mass spectrometry, Northern blot and PCR. A specific peptide antibody was designed. Recombinant mouse gN was expressed using the pET system. ***Results and Conclusions:*** gN is a novel member of the g-crystallin family. It has a short N-terminal arm, like gS-crystallin. Surprisingly the exon/intron structure of the gN gene resembles a hybrid of b- and g-crystallin genes. The mouse gene is expressed normally in lens and retina and gN appears to have an ancient place in vertebrate crystallin evolution, since a probable homolog was found in Iguana lens. However expression in humans seems to have changed. The stop codon in the human sequence has mutated and a variant transcript was detected in testis that skips the fourth structural motif. Another variant lacking the C-terminal domain was detected by PCR in RPE. This may be another example of significant evolutionary changes at the molecular level leading to the particular structure of the human lens. Recombinant mouse gN has been synthesized for future structural studies.

POSTER 62

Interaction of RPE65 with caveolin-1

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Purpose: Visual cycle proteins in RPE are membrane bound (LRAT, 11-cis RDH), membrane-associated (RPE65) or soluble (CRALBP) proteins. RPE65 has been proposed to be a "scaffolding" protein that might functionally organize visual cycle proteins. We wish to determine if and how RPE65 might perform such a function. ***Methods:*** Confocal microscopy was used to analyze localization of proteins in RPE. Fusion constructs for GST-caveolin-1 were generated for use in a GST-pulldown protocol. MALDI-TOF was used to confirm identity of proteins. ***Results:*** Inspection of RPE65 protein sequences shows a conserved caveolin-binding domain at residues 414-426 of all RPE65s. Immunofluorescence microscopy shows caveolin-1 to co-localize, at least in part, with the distribution of RPE65. GST-pulldown experiments employing expressed GST-caveolin-1 fusion proteins and native bovine RPE RPE65 revealed that RPE65 interacts with caveolin-1 in vitro. In addition to RPE65, LRAT and 11-cis RDH were detected in the pull-down assemblage, perhaps through interaction with RPE65. RPE65 was found to interact with both scaffolding and C-terminal domains of caveolin-1. ***Conclusions:*** RPE65 contains a caveolin-interaction domain that is conserved in all species and is functionally active. This interaction may allow for association of RPE65 with membrane anchored visual cycle components and provides a new focus for study of the visual cycle complex.

POSTER 63

Adenovirus-associated virus (AAV) mediated delivery of lens epithelium derived growth factor (LEDGF) protects rat photoreceptors from light-damage

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Purpose: To investigate photoreceptor protection by AAV-LEDGF in light-damaged rats. ***Methods:*** Rat LEDGF cDNA was cloned by RT-PCR. A silent mutation was introduced into the ORF to allow viral and endogenous LEDGF to be distinguished. Rat LEDGF was inserted into the vector of pZac2.1 to make Cis AAV in which LEDGF was driven by a CMV promoter. Recombinant AAV-LEDGF was generated by triple transfection. Five 9-week old Sprague-Dawley rats received subretinal injections of AAV-LEDGF. Fellow eyes received PBS. Four to 7 weeks after injection, the rats were exposed to light (350 lux, 24 hours). Ganzfeld electroretinograms (ERG) were recorded 7 days later. RT-PCR was done on 2 rats to verify viral LEDGF expression. ***Results:*** The ERG b-wave amplitudes were larger in the AAV-LEDGF treated eyes of 4 rats. The average LEDGF/PBS ratio across flash intensities was 2.02 versus 0.97 in control rats either without light damage or without injections ($p<0.0001$, Student's t-test). The RT-PCR confirmed that viral LEDGF was expressed in the AAV-LEDGF injected eyes, but in the not control eyes. ***Conclusion:*** AAV-mediated LEDGF demonstrates photoreceptor protection in light-damaged rats.

POSTER 64

Regulation of C-Flip sensitizes Human Retinal Pigment Epithelium (HRPE) To Tumor Necrosis Factor – a (TNF-a) Induced programmed cell death.

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Purpose: Cell death in HRPE, seen clinically as geographic atrophy, occurs in the later stages of age-related macular degeneration. Previous studies have demonstrated that resistance of hRPE to TNF-μ induced cell death is associated with an increased ratio of c-FLIP to procaspase-8. The present study aims to directly determine the role of c-FLIP in regulating TNF-μ signaling in HRPE. ***Methods:*** Cell viability of hRPE cells was determined by XTT assay. Suppression of c-FLIP was achieved using SiRNA-mediated RNA interference. Proapoptotic factors was immuno-localized in organelle fractionation and whole cell extract. Mitochondrial morphology was monitored by mitotracker and electron microscopy. Expression of truncated bid (tBid) was achieved by tetracycline inducible system. ***Results:*** In ARPE-19 cells, exogenous TNF-μ induced an increase in c-FLIP expression. This increase was coordinate with an upregulation PI3 kinase activity. Specific reduction of c-FLIP led to Bid cleavage, release of mitochondrial cytochrome C and nuclear shrinkage following TNF-μ addition. Over-expression of tBid resulted in the release of cytochrome C without nuclear changes. ***Conclusion:*** TNF-μ modulates hRPE survival/death by affecting c-FLIP expression via signaling cross-talk with the PI3 kinase and that c-FLIP may be a critical determinant in governing TNF-μ pathways.

***POSTER** 65*

Effects of TGFβ on Human Trabecular Meshwork Cells Open a New Window for Understanding Glaucoma Pathophysiology and Potential Glaucoma Therapy

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***Purpose**:* Concentrations of TGFβ are elevated in the aqueous humor of Primary Open Angle Glaucoma (POAG) patients. This study investigated the effects of TGFβ1 and TGFβ2 on human trabecular meshwork (HTM) cells. ***Methods**:* Cultures of HTM cells were established from five donors and were treated for 72 hours with 1 ng/ml of either activated hrTGFβ1 or TGFβ2, or with vehicle alone. The mRNA was analyzed by Affymetrix microarrays and proteomic analyses were done with 2D gel electrophoresis. Gene expression changes were confirmed by real-time PCR and proteins by MALDI mass spectrometry. ***Results**:* The data showed that TGFβ1 and TGFβ2 had similar effects on HTM cells, but TGFβ1 has a greater impact. Currently, the upregulation of tropomyosin and the down regulation of aldose reductase have been demonstrated with both methods. Proteomic analyses are continuing with several genes whose changes in mRNA have been confirmed with real-time PCR. ***Conclusions**:* The increased expression of mRNAs associated with extracellullar matrix proteins and structural proteins would be consistent with decreasing outflow facility. The leptin receptor knockout animals have increased IOP, and the reduction of its mRNA in our study is intriguing. This is the first analysis combining gene transcript and protein data on HTM cells.

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