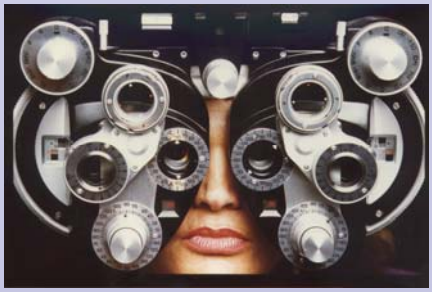
NEI Research Day 2004



October 8, 2004

Natcher Conference Center

National Eye Institute
National Institutes of Health

NEI Intramural Research Day

October 8, 2004

Agenda

8:00 a.m. **Registration and Continental Breakfast**

(Light refreshments and beverages will be served Natcher P1 level)

9:00 a.m. **Paul Sieving, MD, PhD and Sheldon Miller, PhD**

Welcome Remarks *(in Natcher's Main Auditorium)*

9:15 a.m. **William W. Li, MD,** President, Medical Director The Angiogenesis Foundation Cambridge, MA

Angiogenesis and Neurodegeneration: New Insights into Interactions between Vascular and Neuronal Systems

9:55 a.m. **Jurg Ott, MD,** Professor Rockefeller University

Genetic Epidemiology: Studies to Elucidate the Genetic Basis of Disease

10:35 a.m. **Curt I Civin, MD,** Professor of Oncology & Pediatrics Sidney Kimmel Comprehensive Cancer Center Johns Hopkins University

Stem Cells: Re-engineering the immune system

11:15 a.m. **Peter A Campochiaro,** MD, Professor of Ophthalmology & Neuroscience Johns Hopkins University

Mechanisms and Therapeutic Strategies for Retinal Diseases

11:55 a.m. **John J. Hooks, Chair NEI Research Day Committee**
Announcement of Outstanding Abstracts

12:00 p.m. **Lunch *(Cafeterias are available in Buildings 10, 31, 1, 45)***
There will be tables available in E1/E2, Natcher P1 level for anyone who would l like to meet or have their lunch there.

1:30 p.m. **Poster Session *(in Natcher Atrium and Rooms G and F)***
(Light refreshments and beverages will be served Natcher P1 level)

Poster Session

1:30–3:30 p.m.

The posters are divided into three groups: Basic, Clinical, and Translational.

Poster Category	Poster Numbers	Page Number	Poster Location
Basic	1-44	4-21	Natcher Atrium
Clinical	45-52	22-24	Room G, P1 Level
Translational	53-64	25-26	Room F, P1 Level

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

Basis Research Posters

POSTER 1

Disruption of CD40/CD40L interactions in a retinal autoimmunity model results in protection without tolerance

Agarwal, R.K., Bagenstose, L.M., Silver, P.B., *Harlan, D.M., *Hoffman, S.C., *Kampen, R.L., Chan, C.C., and Caspi, R.R.
Lab of Immunology, NEI, *Islet and Autoimmunity Branch, NIDDK, NIH, Bethesda, MD 20892.

Abrogation of CD40/CD40L interaction leads to inhibition of T cell immunity and amelioration of disease in several autoimmunity models. The role of this receptor-ligand pair in uveitis has not been investigated. In this study, we wanted to examine the role of CD40/CD40L interactions on the development of EAU, a cell-mediated, Th1-driven autoimmune disease that serves as a model for autoimmune uveitis in humans. EAU susceptible B10.RIII mice immunized with the retinal autoantigen IRBP in complete Freund's adjuvant and treated with anti-CD40L Ab (MR-1) had reduced incidence and severity of disease compared with controls (hamster-Ig treated mice). Real-time PCR analysis of draining lymph nodes collected on day 4 after immunization revealed that the innate response of MR-1-treated mice was reduced, possibly with a slight shift toward a type 2 profile, but this was not translated into a deviated adaptive response. Rather, MR-1 treated mice showed reduced Ag-specific responses to IRBP (DTH and lymphocyte proliferation) but a largely unaltered cytokine profile *in vitro* to IRBP. In contrast to some other reports, no evidence was found for regulatory cells in adoptive transfer experiments. To determine whether CD40L blockade resulted in long-term tolerance, mice protected by treatment with MR-1 Ab were re-challenged for uveitis after circulating MR-1 Ab levels dropped below the detection limit of ELISA. MR-1-treated mice developed severe EAU and strong cellular responses to IRBP, comparable to those of control Ig-treated mice. These responses were higher than in mice that had not received the primary immunization concurrently with anti-CD40L treatment. We conclude that: (i) CD40/CD40L interaction is required for EAU and its disruption prevents disease development. (ii) CD40L blockade inhibits the innate response to immunization and reduces priming, but does not result in immune deviation (iii) Protection is dependent on persistence of anti-CD40L antibodies and long-term tolerance is not induced. Furthermore, immunological memory develops under cover of CD40L blockade causing enhanced responses upon rechallenge. Taken together, our data indicate that ongoing CD40/CD40L blockade would be required to maintain a therapeutic effect against uveitis in this type of treatment.

POSTER 2

HuR Binding is Necessary, but Not Sufficient, for CD28-Meidated IL-2 mRNA Stabilization

Azmi H. Ragheb A.J.

Purpose: To show whether the HuR protein binds the 3'UTR of IL-2 mRNA *in vivo* and to determine whether deletion of this binding site has an effect on IL-2 mRNA stabilization. **Methods:** UV cross-linking with radiolabeled IL-2 probes was used to identify the HuR binding site *in vitro*. Immunoprecipitation RT-PCR (IP RT-PCR) with an anti-HuR monoclonal antibody and IL-2 mRNA specific primers was used to confirm that HuR binds the IL-2 mRNA *in vivo*. A murine T-cell clone was transfected with a IL-2 reporter deleted for the HuR binding site. Real-time PCR was performed to study the effects of this deletion. **Results:** Deletion of the HuR binding site in the 3'UTR impairs IL-2 mRNA stability in T-cells co-stimulated by the TCR & CD28 receptors, but not in cells stimulated by TCR alone. **Conclusions:** The HuR binding site of IL-2 mRNA is necessary for CD28-mediated stabilization.

POSTER 3

Chemokine/Chemokine Receptor Expression in Transformed B-Cell Lines Containing Single Nucleotide Polymorphisms (SNPs) in CX3CR1 and CCL-2

Bojanowski CM[1a], Tuo J[1a], Keller LMM[1a], Jiao X[1b], Hackett J[1a], Hejtmancik F[1b], Chan CC[1a]

[1] National Institutes of Health - National Eye Institute, Bethesda, MD., [a] Laboratory of Immunology-Immunopathology Section, [b] Ophthalmic Genetics and Visual Function Branch-Ophthalmic Molecular Genetics Section

Purpose: The immune system is involved in AMD. Recently, we reported an association between AMD and 2 SNPs in CX3CR1, a chemokine receptor. These SNPs have been associated with decreased receptor expression and ligand binding affinity. In this study, we compared the expression profiles of 9 resting and LPS-stimulated transformed B-cell lines carrying either the CX3CR1 T280M or CCL-2 A⟶G 2518 SNP. **Methods:** Total RNA extracted from nine resting and LPS-stimulated immortalized human cell lines (5 wt CX3CR1/SNP CCL-2, 3 SNP CX3CR1/wt CCL-2, 1 wt CX3CR1/ wt CCL-2) were subjected to human chemokine and receptor cDNA microarray, Immunohistochemistry was also performed to determine CX3CR1, CD19/CD20, CD3, and CD 68 expression. **Results:** 95% of these cells expressed B-cell markers. All cell lines stained positively for CX3CR1. For cell lines bearing the CX3CR1 SNP, CX3CR1 transcripts were below detectable levels and protein expression was reduced. CCL-2 SNP bearing cell lines exhibited slightly lower CX3CR1 expression than the wild-type cell line. **Conclusions:** Our data provides evidence for decreased CX3CR1 expression in cells bearing the CX3CR1 SNP. The CCL-2 SNP may effect CX3CR1 protein expression. Further analysis is needed to examine multiple cytokine/chemokine interactions in the presence of these SNPs.

POSTER 4

Fenretinide induced neuronal differentiation of ARPE-19 cells: mediation by retinoid receptors and potential neuronal function.

Chen S, Fariss R, Kutty RK, Nelson R, Wiggert BN.

Purpose: RPE and retinal neurons are derived from the same sheet of neuroepithelium during development. We have previously reported differentiation of human RPE cells (ARPE-19) into neuronal cells by fenretinide (Chen et al., 2003), a synthetic retinoid, which might have the potential function to regulate transcription of target genes. The purpose of this study was to determine the possible mechanism of fenretinide induced neuronal differentiation and whether the differentiated cells acquire neuronal cell function. **Methods:** ARPE-19 cells were transformed into neuronal phenotype by 1μM fenretinide as previously described (Chen et al., 2003). Total RNA was extracted from control and treated cells for RT-PCR analysis. Immunohistochemical analysis using antibodies against retinoid receptors (RAR-α, RAR-β or RXR-β) were applied. The neuronal function of differentiated cells was tested using a voltage probe (oxonol, $DiBaC_4(5)$) physiological recordings. **Results:** Fenretinide treatment changed RAR and RXR mRNA and protein expression in ARPE-19 cells. RT-PCR analysis showed that RAR-γ and RXR-β mRNA are up-regulated after 3 hours of fenretinide treatment. Immunocytochemical analysis revealed that RXR-β receptor protein in ARPE-19 cells was increased after 5 days of fenretinide treatment. Voltage probe recordings showed that fenretinide treated ARPE-19 cells had cell membrane responses different from that of the control cells. We observed that some treated cells showed either a delayed GABA response, or a delayed glutamate response. **Conclusions:** The regulation of RAR and RXR receptors by fenretinide suggests that the neuronal differentiation of ARPE-19 cells is mediated by RAR and RXR receptor pathways. The presence of GABA and glutamate responses on treated cells suggests that the fenretinide induced differentiated cells may possess a neuronal cell function.

POSTER 5

Principal Expression of Two mRNA Isoforms (*ABCB5*α and *ABCB5*β of the ATP-Binding Cassette Transporter *ABCB5*, In Melanoma Cells and In Melanocytes.

Chen KG, Szakacs G, Jean-Philippe Annereau J-P, Rouzaud F, Liang X-J, Valencia JC, Nagineni CN[1], Hooks JJ[1], Hearing VJ, and Michael M. Gottesman MM

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ATP-binding cassette (ABC) transporters play a pivotal role in physiological and in pathological processes of organisms. We identified and cloned two novel isoforms (*ABCB5*α and *ABCB5*β) of the ABC transporter *ABCB5* in human melanoma cells. The deduced *ABCB5*α protein appears to be an altered splice variant containing only a partial ABC, whereas the *ABCB5*β isoform shares approximately 70% similarity with *ABCB1* (*MDR1*) and has a deduced topological arrangement similar to that of the whole carboxyl terminal half of P-glycoprotein (P-gp) including an intact ABC. Northern blot, real-time PCR, and conventional RT-PCR were used to verify the expression profiles of *ABCB5*α/β. Both *ABCB5*α and *ABCB5*β were preferentially expressed in the melanomas among the NCI-60 panel cell lines. *ABCB5*α/β expression was undetectable in two amelanotic melanomas (M14 and LOX-IMVI). The expression profile of *ABCB5* α/β in the melanomas of the NCI-60 panel has been confirmed both by RT-PCR and by sequencing. Neither *ABCB5*α nor *ABCB5*β expression has been found in normal tissues such as liver, spleen, thymus, kidney, heart, lung, colon, small intestines or placenta. *ABCB5*α/β mRNAs were also expressed in normal melanocytes and in retinal pigment epithelial cells (RPEs), suggesting that *ABCB5*α/β expression is pigment cell-specific. In addition ABCB5β-expressing cells, as opposed to two multidrug resistant cell lines which overexpress *ABCB1* (*MDR1*) (KB-V1 and MES-SA/Dx5), have a distinct drug response pattern, suggesting that *ABCB5*β might have a distinct transport function, compared to P-gp, that has not yet been determined. Expression of *ABCB5*α/β provides two molecular markers for differential diagnosis of melanomas as well as potential molecular targets for therapy of melanomas

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POSTER 6

Identification of retinal autoantigens in Experimental Coronavirus Retinopathy (ECOR)

Chin MS[1], Hooper LC[1], Detrick B[2], Hooks JJ[1]

[1]Immunology & Virology Section, Laboratory of Immunology, NEI, NIH, Bethesda, MD; [2]Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD.

Purpose: Experimental coronavirus retinopathy, an animal model of a progressive retinal degenerative disease, is characterized by the presence of anti-retinal/anti-RPE autoantibodies in genetically susceptible mice. We propose to identify autoantigens in mice susceptible to the retinal degenerative disease. Methods: Sera from virus-infected mice were collected 20 dpi and evaluated for anti-retinal/anti-RPE antibodies by immunohistochemistry on rat eyes. Protein blots of retina and RPE extracts were examined with pooled sera, and a rat retina cDNA expression library was screened. Reactive clones were purified, insert cDNA sequenced and compared to sequences in GenBank to identify the protein(s). Results: Sera from virus-infected mice were immunoreactive to RPE and the neural retina. Western blot analyses of (1) rat RPE proteins showed reactivity to three species, 21 kDa, 26 kDA and 80 kDa proteins, (2) bovine RPE proteins showed reactivity to two proteins, 65 kDa and >188 kDa and (3) mouse and bovine retina proteins showed reactivity to proteins between 33-40 kDa and >188 kDa. A positive clone was identified from a rat retina cDNA expression library with sera from virus-infected mice. Conclusions: These studies identify multiple retinal and RPE autoantigens in ECOR. One of these proteins was identified as a serine/threonine kinase.

POSTER 7

Altered Peptide Ligands Derived from a Major Uveitogenic Epitope Protect from Experimental Autoimmune Uveitis

Cortes LM, Avichezer D, Silver PB, Chan CC, and Caspi RR.
Laboratory of Immunology, NEI, NIH, Bethesda, MD

In this study we identified altered peptide ligands (APL), capable of immunomodulating experimental autoimmune uveitis (EAU) induced by the retinal antigen IRBP. Alanine-substituted peptides were tested for immunogenicity, crossreactivity and pathogenicity with the major pathogenic epitope of IRBP (residues 161-180). Two peptides, 169A and 171A, did not elicit disease when used in a uveitogenic immunization protocol, but crossreacted with the native sequence by lymphocyte proliferation, suggesting that they might serve as APL. They were tested for their ability to prevent EAU induced by the 161-180 sequence. Mice were pre-treated with 169A or 171A in incomplete Freund's adjuvant (IFA). Two weeks later they were challenged according to a uveitogenic protocol using the native peptide. Disease was monitored by fundus examination and histopathology. Antigen-specific responses were evaluated by lymphocyte proliferation and delayed hypersensitivity. Mice pre-treated with either of the putative APL in IFA were protected from EAU compared to controls pre-treated with IFA alone. Protected mice were not rendered unresponsive, as their lymphocyte proliferation to p161-180 was not reduced compared to controls. We hypothesize that APL pretreatment protects from EAU by directing the response to a subsequent uveitogenic challenge with the native peptide towards a non-pathogenic phenotype.

POSTER 8

Proteomic analysis of mouse melanoma tumor progression

Culp WD[1, 2], Neal R[1], Massey RD[2], Pisa P[2], Garland D[1]
[1]NEI, NIH, Bethesda, MD 20892, USA. [2]Department of Oncology and Pathology, Karolinska Institute, Stockholm, Sweden.

Purpose: Melanoma tumor progression is a dynamic biological process involving a wide spectrum of host and tumor proteins, and we hypothesized that many of these proteins contribute to tumor immune evasion. In wild type mice, melanoma has been treated successfully using immunotherapy three and four days post tumor challenge, but after one week of established tumor growth, immunotherapy generally fails. It appears that the tumor is controlling a host's immune response against the tumor. Method: Our approach was first to perform a comparative proteome analysis on the aggressive, B16-F10 mouse melanoma and the syngeneic, non-tumorigenic, melan-a cell lines. We then challenged C57Bl/6 mice with B16-F10, and resected the tumors on day 3, 5,7,10, and 14. The soluble proteins from the tumor and cell samples were separated using 2D gel electrophoresis, and spots were identified using mass spectrometry. Results: Our data indicate significant alterations in the tumor proteome *in vivo*, as well as differences in the base proteomes of the cell lines. The majority of the proteins identified were associated with physiological properties; however, antioxidant and chaperone protein expression was increased as the tumor progressed *in vivo*. Conclusion: This work identifies proteins involved in melanoma tumor progression and potential proteins associated with tumor immune evasion.

POSTER 9

Phosphorylation of Interphotoreceptor Retinoid-Binding Protein: Characterization of Phosphorylation Sites

Duncan T, Wiggert B. NEI, Bethesda, Maryland.

Purpose: Protein phosphorylation / dephosphorylation are a common and important regulatory modification of proteins. The phosphorylation of bovine interphotoreceptor retinoid-binding protein (IRBP) has been examined in crude bovine interphotoreceptor matrix (IPM) washes using [γ-^{32}P]ATP (Wiggert et al., 1988). In that study, it was found that the phosphorylated IRBP was bound tightly to Con-A sepharose and not eluted by 50 mM α-methyl-D-mannoside. During the purification of IRBP from crude bovine IPM preparations, approximately 18% of the IRBP in the initial wash is tightly bound to Con-A. The purpose of these experiments was to characterize the phosphorylation sites of the two fractions of IRBP, fraction I that is eluted from Con-A with 50 mM α-methyl-D-mannoside and fraction II that is bound tightly to Con-A. **Methods:** Interphotoreceptor retinoid-binding protein, fractions I and II, was subjected to SDS polyacrylamide gel electrophoresis. In-gel detection of phosphate groups was achieved using the Pro-Q® Diamond phosphoprotein gel stain. Total protein was stained with Sypro® Ruby. Immobilized metal ion affinity chromatography was used for the isolation of phosphorylated peptides from proteolytic digests of bovine IRBP, fractions I and II. An aliquot of the sample, containing either phosphorylated or non-phosphorylated peptides, was spotted onto a target plate. An equal volume of α-cyano-4-hydroxycinnamic acid (10 mg/ml in 50% acetonitrile/0.1% TFA) was applied on top of the sample. Linear and reflectron MALDI mass spectra were recorded on an Applied BioSystems Voyager DE sSTR mass spectrometer. Samples were also subjected to β-elimination followed by alkylation with methylamine prior to mass spectrometry to distinguish between phosphorylated serine, threonine, and tyrosine residues. **Results:** Both IRBP fractions, I and II, were stained with the phosphoprotein specific fluorescent stain, an indication that IRBP is phosphorylated. Mass spectrometric analysis of the phosphopeptides isolated from proteolytic digests revealed that the sites of phosphorylation were different for IRBP fractions I and II. In the case IRBP fraction I, the phosphorylated peptides are localized toward the C-terminus, whereas, the phosphorylated peptides isolated from IRBP fraction II are localized closer to the N-terminus. **Conclusions:** Both IRBP fractions I and II are phosphorylated. IRBP fraction I contains phosphopeptides localized toward the C-terminus, whereas, IRBP fraction II contains phosphopeptides localized toward the N-terminus. Phosphorylation of IRBP may be important in the function of IRBP in the IPM.

POSTER 10

Plasma Membrane Translocation of Lens MIP/Aquaporin 0 Requires Ca^{2+}/Calmodulin Signaling

Fan J, Fariss R, and Chepelinsky AB
Laboratory of Molecular and Developmental Biology, NEI, NIH, Bethesda, MD

Purpose: Major Intrinsic Protein (MIP)/Aquaporin 0, specifically expressed in lens fiber membranes, is required for lens transparency. Our goal is to examine the roles of Ca^{2+}/calmodulin signaling on MIP plasma membrane translocation. **Method:** Rabbit kidney epithelial (RK13) cells stably expressing MIP, S235D mutant, or Enhanced Green Fluorescent Protein (EGFP)-tagged MIP and differentiated rat lens epithelial explants expressing MIP were established. The effect of Ca^{2+}/calmodulin specific inhibitors on MIP plasma membrane translocation was assessed by confocal fluorescence microscopy. **Results:** MIP translocated from plasma membrane to cytoplasm in RK13 cells expressing MIP or EGFP-MIP upon 3-hour treatment with BAPTA (Ca^{2+} chelator), KN62 (Ca^{2+}/calmodulin-dependent Kinase inhibitor), MLCK (calmodulin inhibitory peptide), or W7 or trifluoperazine (calmodulin antagonists). Inhibitors of other major signaling pathways showed no effect. Confocal fluorescence microscopy under live culture conditions showed that treatment with Ca^{2+}/calmodulin inhibitors led to disappearance of MIP from plasma membrane in <5 minutes. The effect of Ca^{2+}/calmodulin inhibitors on MIP plasma membrane translocation was abolished when MIP C-terminal was fused to EGFP. MIP S235D mutant was unable to insert into plasma membrane. Endogenous MIP expressed in differentiated lens epithelial explants translocated from plasma membrane to cytoplasm upon treatment with Ca^{2+}/calmodulin inhibitors. **Conclusions:** Our results demonstrate that Ca^{2+}/calmodulin signaling regulates plasma membrane translocation (thereby biological functions) of MIP. The calmodulin binding site and serine-235 on MIP C-terminal domain may play critical roles in the effect of Ca^{2+}/calmodulin signaling on MIP plasma membrane translocation.

POSTER 11

Suppressive DNA inhibits immune-mediated ocular inflammation in mice

Fujimoto C[1], Klinman DM[2], Vistica BP[1], Takase H[1], Chan C-C[1], and Gery I[1]
[1]Laboratory of immunology, NEI, [2]Section of Retroviral Immunology, Center for Biologics Evaluation and Research, Food and Drug Administration

Objective: To examine whether systemic treatment of suppressive oligodeoxynucleotides (ODNs) inhibit immune-mediated ocular inflammation in mice. **Methods:** Suppressive ODN was tested in two models: (1) In the experimental autoimmune uveitis (EAU), B10.A mice were immunized with a retinal antigen, interphotoreceptor retinoid-binding protein (IRBP) emulsified in complete Freund's adjuvant (CFA) and treated systemically with suppressive ODN on day 0, 3, 7 and 10. (2) In the second system, activated Th1 cells specific against hen egg lysozyme (HEL) were adoptively transferred into mice expressing HEL in their eyes, followed by the treatment of suppressive ODN on day 0 and 3. Eyes of mice developing EAU were examined on day 14, whereas eyes of recipient mice were tested on day 7. **Results:** Treatment with suppressive ODN significantly inhibited both the actively induced EAU and the adoptively transferred inflammation. Lymphocytes from mice treated with suppressive ODN demonstrated lower proliferative response and production of proinflammatory cytokines such as IFN-g, tumor necrosis factor α and IL-6.
Conclusion: Systemic treatment of suppressive ODN inhibited both EAU development and the adoptively transferred ocular inflammation. These results raise the possibility that suppressive ODN can inhibit the pathogenic process of ocular inflammation and may be useful in the future treatment of immune-mediated ocular inflammation.

POSTER 12

Cdk5 phosphorylated on Tyrosine 15 co-localizes with E-cadherin along cell-cell borders in human corneal epithelial cells

Gao CY, Qiao FY, and Zelenka PS
Laboratory of Molecular and Developmental Biology, NEI, NIH

Purpose: Previous studies have shown that Cdk5 promotes cell to matrix and cell to cell adhesion in corneal epithelial cells. In the present study we have used an immortalized Human Corneal-Limbal Epithelial Cell line (HCLE) to examine the role of Cdk5 in cell-cell adhesion under conditions where endogenous Src or Cdk5 kinase activity has been inhibited. **Methods:** HCLE cells were either incubated overnight with 10μM PP1, a Src family kinase inhibitor, or with 15μM Olomoucine, a Cdk5 inhibitor. Cells were then briefly exposed to lysophosphatidic acid (LPA) at 200ng/ml. For immunofluorescence, cells were fixed with 4% paraformaldehyde and incubated with anti-(pY15)Cdk5, anti-E-cadherin, and Rhodamine Phalloidin. For immunoblotting, whole cell extracts were immunoprecipitated with anti-E-cadherin or anti-c-Src. Immunoprecipitated proteins were immunoblotted for E-cadherin, β-catenin, and/or pY15-Cdk5 and Src. **Results:** pY15-Cdk5 largely colocalized with E-cadherins along the cell-cell border. Treatment with Cytochalasin D or Olomoucine completely shifted the localization of pY15-Cdk5 to the cytoplasm. Src, β-catenin, and pY15-Cdk5 all co-immunoprecipitated with E-cadherin. Treatment with PP1 enhanced cell-cell adhesion and reduced pY15-Cdk5 phosphorylation. **Conclusions:** Src suppresses cell-cell adhesion and partially phosphorylates pY15-Cdk5. Co-localization of pY15-Cdk5 with E-cadherin at cell-cell border requires Cdk5 kinase activity and an intact actin cytoskeleton.

POSTER 13

Subcellular Localization of Oxysterol Binding Proteins (OSBPs) in Cultured Human RPE Cells

Gordiyenko NV, Lee JW, Fariss RN, Alam S, Rodriguez IR

Purpose: To determine the intracellular localization of OSBPs present in RPE cells and their translocation in response to internalization of LDL, oxLDL and oxysterols. **Methods:** Full-length and N-terminal domains of OSBPs were cloned into the Invitrogen pcDNA3.1/CT-GFP-TOPO vector and transfected into ARPE19 cells. Endogenous OSBPs were localized by indirect immunofluorescent using specific antibodies to OSBP1, OSBP2, OSBPL9 and OSBPL2. The cells were examined by confocal and light fluorescent microscopy.
Results: The OSBP1-GFP localized predominantly in the Golgi apparatus. The OSBP2- GFP concentrated around some vesicular structures. The OSBPL9-GFP construct showed targeting to the perinuclear region and vesicular structures, but did not significantly co-localize with any lysosome and peroxisome markers tested, or with Caveolin1 and 2. OSBPL2-GFP protein showed diffuse cytosolic staining. The endogenous OSBPs analyzed by indirect immunostaining showed similar subcellular distribution. OSBP1 demonstrated clear translocation from cytosolic-vesicular compartments to the Golgi apparatus in response to 25-hydroxycholesterol (25HCh), but not to other oxysterols. No ligand-dependant translocation has been identified for other OSBPs. None of the OSBPs showed clear translocation in response to LDL or oxLDL internalization. **Conclusions:** All of the examined OSBPs showed distinct intracellular localizations. Only OSBP1 undergoes translocation in response to 25HCh. No obvious translocation of OSBPs was detected after internalization of LDL or oxLDL.

POSTER 14

CD4+CD25+ regulatory T cells set the threshold of autoimmunity to an immunologically privileged retinal antigen

Grajewski RS, Silver PB, Su S-B, Agarwal RK, Chan C-C, Caspi RR
Laboratory of Immunology, NEI, NIH, Bethesda, MD 20892

Purpose: To investigate the role of CD4+CD25+ regulatory T cells in experimental autoimmune uveitis (EAU). **Methods:** EAU-susceptible or resistant mice were depleted of CD25+ cells by monoclonal anti-CD25 antibody treatment and were immunized for EAU with the retinal antigen IRBP. **Results:** CD25-depleted B10.RIII mice (highly susceptible) had an enhanced disease incidence and severity compared to non-depleted controls ($p<0.009$) and exhibited higher DTH and proliferative responses to IRBP. Depletion of CD25+ T cells from C57BL/6 mice (moderately susceptible) resulted in elevated EAU scores, and depletion in BALB/c mice (resistant) permitted severe disease to develop. Furthermore, T cells of CD25-depleted B10.RIII-IRBP KO donors exhibited enhanced proliferation to IRBP and transferred more severe EAU to naïve wild type recipients than did parallel cells from nondepleted donors, suggesting that a regulatory cell had been removed. **Conclusion:** Our data indicate that CD4+CD25+ regulatory T cells play an important role in setting the threshold of susceptibility to EAU and may help to explain the resistance to EAU of some poorly susceptible strains. Finally, EAU-relevant regulatory T cells of this type do not appear to require endogenous expression of the cognate uveitis target antigen.

POSTER 15

Alterations in TNF-α receptor signaling in Experimental Coronavirus Retinopathy

Hooper LC[1], Chin MS[1], Detrick B[2], Hooks JJ[1]
[1]Immunology & Virology Section, Laboratory of Immunology, NEI, NIH; [2]Department of Pathology, Johns Hopkins Medical Institutions, Baltimore, MD

Purpose: Experimental coronavirus retinopathy (ECOR) is a model of retinal degeneration triggered by a virus and composed of genetic and autoimmune components. Preliminary studies indicated that both TNF-α and soluble TNFRs are elevated in virus-infected retinal degeneration susceptible BALB/c mice. The purpose of this study was to characterize alterations in downstream signaling molecules, such as NO (nitric oxide). **Methods**: Mice were injected intravitreally with mouse hepatitis virus and retinas and mononuclear cells isolated. Gene expression of iNOS (inducible nitric oxide synthase), release of TNFR2 and concentration of NO was evaluated by RT-PCR, ELISA, and nitric oxide assay, respectively. **Results**: Gene expression of iNOS decreased from day 4 to 8 in BALB/c mice, while it increased in CD-1 degeneration resistant mice. The adherent population of mononuclear cells was determined to be the source of sTNFR2 and release from BALB/c adherent cells was significantly higher than that of CD-1 mice ($p< 0.0005$). Incubation of mouse macrophages with TNF-α and IFN-γ augmented NO production while the presence of sTNFR2 significantly ($p<0.01$) inhibited this production. **Conclusion**: These studies demonstrate that high levels of sTNFR2 released by invading macrophages of infected BALB/c mice can decrease NO production within the retina and hence alter Th1 responses.

POSTER 16

Osmotic and Hydrostatic Hydraulic Conductivities (Lp) Unraveled in Bovine Retinal Pigment Epithelium (BRPE) and Bruch's Membrane-Choroid Complex

Jalickee S, Banzon T, Tripathi S, Miller SS

The bovine retinal pigment epithelium (BRPE) drives solute-coupled water transport from the retinal side of the RPE towards the choroid through Bruch's membrane. Individual hydraulic conductivities (Lp) were determined using a refined capacitance probe technique. Retina-to-choroid hydrostatic steps of 1-5 cmH_2O failed to drive transepithelial water flow. Application of osmotic steps (70 mOsm), retinal hypo-osmotic and choroidal hyper-osmotic produced a sustained increment of 16.9±3.3 microliter/cm^2/hr (n=7) over spontaneous volume flow. Application of this gradient in the reverse direction attenuated spontaneous flows by 15.2±2.6 microliter/cm^2/hr (n=6). This non-rectifying osmotic Lp was 0.016 microliter/cm^2/hr/cmH_2O; approximately 35-fold lower than previously reported for mammalian RPE. Choroid-to-retina hydrostatic steps of 1-6 cmH_2O reversibly opened the tight junctions, eliminating the BRPE as a significant barrier. The minimum residual hydrostatic Lp of the Bruch's membrane-choroid permitting these high flows is 17.1±3.1 microliter/cm^2/hr/cmH_2O, (n=5). Spontaneous absorption by the BRPE is approximately 6 microliter/cm^2/hr and is driven by transepithelial osmotic gradients of approximately 24 mOsm. Pressures of <1 cmH_2O are needed to drive fluid across the highly permeable Bruch's membrane-choroid. This new method could serve as a sensitive tool to detect small changes in water permeability of Bruch's membrane-choroid that occur in disease (age related macular degeneration) or senescence.

POSTER 17

Autosomal Recessive Familial Exudative Vitreoretinopathy Is Associated with Mutations in *LRP5*

Jiao X,[1] Ventruto V,[2] Trese MT,[3] Shastry BS,[4] and Hejtmancik JF[1]

Purpose: The goal of this study is to identify the gene causing familial exudative vitreoretinopathy (FEVR), a hereditary eye disorder affecting both the retina and vitreous body. **Methods:** Isolated autosomal recessive FEVR was diagnosed in individuals from three consanguineous families of European origin. A candidate-oriented genomewide scan was carried out and analyzed using the LINKAGE program package. LRP5 was screened by sequencing PCR amplified exons. **Results:** arFEVR maps to the 22-cM (311-Mb) region of chromosome 11q flanked by markers *D11S905* and *D11S1314* with a maximum LOD score of 3.6 at = 0 is with *D11S987* and confirmed by haplotype analysis. This region contains *LRP5* but not *FZD4*. mutations in both of which can cause autosomal dominant FEVR. All three families show homozygous nonconservative mutations in LRP5: R570Q, R752G, and E1367K, not seen in 100 controls tested using PCR amplification and restriction enzyme digestion. **Conclusions:** arFEVR maps to chromosome 11q and is associated with mutations in the LRP5 gene. This is the first report identifying the genetic cause of arFEVR, and demonstrates that mutations in this gene can cause autosomal recessive as well as autosomal dominant FEVR and osteoporosis-pseudoglioma syndrome.

POSTER 18

The Cdk5 activating protein, p39, interacts with myosin light chain

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Purpose: To explore Cdk5-dependent regulation of epithelial cell adhesion and migration by identifying proteins that interact with Cdk5, p35, and p39. **Methods:** A yeast two-hybrid E18 rat lens cDNA library was screened using Cdk5, p35, and p39 as baits. Clones that grew without histidine and expressed beta-galactosidase were sequenced. Interactions were confirmed by glutathione-S-transferase affinity chromatography (GST-pull down assay). N/N1003a lens epithelial cells were co-transfected with green fluorescent protein (GFP)-myosin light chain (MLC) and hemagluttin (HA)-tagged p39, p35, or Cdk5. Cell extracts were immunoprecipitated with anti-GFP antibody and immunoblotted with anti-HA antibody. Subcellular localization was determined by confocal fluorescence microscopy. **Results:** Sequencing a p39-interacting clone identified MLC. In pull-down assays,GST-MLC interacted with radiolabeled p39, but not p35 or Cdk5. GST-pull-down assays with p39 deletions mapped the MLC binding site to the p39 N-terminus. Both p39 and Cdk5 co-immunoprecipitated with MLC following co-transfection. Confocal fluorescence microscopy showed co-localization of p39 and MLC along cytoplasmic filaments and along cell boundaries. **Conclusion:** The Cdk5 activating protein, p39, interacts specifically with MLC. Cdk5 does not directly interact with MLC but may form an intracellular complex via p39. This interaction may target Cdk5/p39 kinase activity to cytoskeletal sites involved in adhesion and migration.

POSTER 19

Effects of Optimedin on the Differentiation of PC12 Cells

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Purpose: To study possible biological functions of optimedin. **Methods:** PC 12 cells were transfected with a tetracycline-inducible vector containing optimedin cDNA. Stable cell lines, expressing variable amounts of optimedin, were treated with nerve growth factor (NGF) to induce neuronal differentiation. Changes in the gene expression pattern of control versus optimedin in PC 12 cells were assessed using rat Affymetrix arrays. Interaction of optimedin with other proteins was investigated by immunoprecipitation and immunostaining. **Results:** In the presence of NGF, control cells differentiate and produce neurites, while optimedin-expressing cells form large aggregates with a reduced number of neurites. After 6 hrs of NGF-treatment, mRNA levels of about 100 genes were changed by $\geq$2 fold in optimedin-expressing cells versus control cells. Dramatic reductions were observed for initiation factor 2 gamma chain (13.7) and delta-like homolog (15.8) mRNAs. In contrast, significant increases were detected for neuronatin (23.1), corticotrophin releasing hormone (7.2) and retinol-binding protein 4 (6.0). At the protein level, optimedin may interact with another olfactomedin domain-containing protein, noelin-2. **Conclusion:** Optimedin is a biologically active secreted protein which may play an important role in the normal function of the retina as well as in the retina pathology.

POSTER 20

Changes in the eyes of transgenic mice expressing mutant mouse myocilin.

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Purpose: To evaluate the effects of mutant myocilin, with the Tyr423His point mutation, on gene expression in the eyes of transgenic mice. **Methods:** A point mutation was introduced into Bacterial artificial chromosome containing the full-length *myocilin* gene, using oligonucleotide-based mutagenesis in *E. coli.* The *myocilin* gene copy number in transgenic mice was estimated by real-time PCR. The distribution of myocilin was investigated by western blotting and immunohistochemistry. Gene expression was analyzed by affimetrix microarray. Whole-mount retinas and optic nerve sections were immunostained for GFAP. **Results:** Several lines of transgenic mice, containing the mutated *myocilin* gene, were produced. One line carrying 2-3 copies of the mutated *myocilin* transgene was analyzed in more detail. In the eye angle, the level of mutated *myocilin* represented about 25% of the total *myocilin* gene expression. Mutated myocilin was not properly secreted, and concentrated in the trabecular meshwork and sclera of transgenic mice. Microarray analysis demonstrated changes in the pattern of gene expression in the total eye of transgenic mice. Moderate astrogliosis was observed in the peripheral retina and proximal optic nerve. **Conclusions:** Expression of mutated myocilin results in differential gene expression in the eye of transgenic mice, and astrogliosis in the specific regions of the retina and optic nerve.

POSTER 21

Fetal Human Retinal Pigment Epithelial Cell Cultures: Physiology and Fluid Transport

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Eye diseases such as age-related macular degeneration or diabetes affect retinal pigment epithelium (RPE) function and lead to retinal degeneration, vision loss and blindness. To study RPE function, physiology and pathology many laboratories have attempted to culture RPE as a more accessible alternative to native tissue. This goal has been accomplished with varying degrees of success due to the functional and morphological complexity of the RPE and its neighboring cells in the retina and the choroid. We have developed techniques for culturing confluent monolayers of human fetal RPE cells that exhibit morphology, physiology, and patterns of protein expression similar to native human fetal RPE. One of the goals of this work was to identify a set of commercially available ingredients to create stable and reproducible RPE cell cultures. We been able to produce confluent pigmented RPE cell cultures with classic epithelial morphology, transepithelial potential of 1 - 3mV, and transepithelial resistance greater than 400 $W \times cm^2$. Epithelial polarity and function in these cell cultures closely resemble that previously shown in native fetal human RPE as measured by electrophysiology, fluorescence microscopy, and fluid transport responses to pharmacological perturbations at the apical and basolateral membrane of the RPE.

POSTER 22

Experimental Choroidal Neovascularization in Perlecan-null Mice

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Purpose: Choroidal neovascularization (CNV) is a main cause of blindness in adults. Perlecan is a major heparan sulfatrte proteoglycan (HSPG) of basement membranes (BMs) and connective tissue. Perlecan has been reported to possess angiogenic and endothelial growth promoting properties primarily by acting as a coreceptor for basic fibroblast growth factor (FGF-2). The aim of this study is to examine the role for perlecan in the development of experimental choroidal neovascualrization (CNV). **Methods:** CNV was induced in C57BL/6 (wild-type) and perlecan-null mice by making four separate choroidal burns in each eye with a diode red laser (650nm). The time –course of distribution of perlecan and glial fibrillary acidic protein (GFAP) in both mice after laser treatment was determined by immunohistochemistry. At 2 weeks, surface area of CNV was determined by using FITC-dextran fluorescence and the margins of the lesion were outlined with image-analysis software. **Results:** Immunostaining for perlecan confirmed the presence of perlecan in CNV lesions and the blood vessels of wild type. Immunostaining for GFAP was upregulated from 1 day through 14 days. Perlecan-null mice demonstrated a smaller area of CNV than did wild type at 2 weeks ($P<0.01$). **Conclusions:** Perlecan plays a role in laser-induced CNV development.

POSTER 23

Differential regulation of TGF-β1 and β 2 Expression in Human Retinal Pigment epithelial cells by Interferon-γ

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Purpose: To evaluate the role of inflammatory cytokines on the expression of TGF-β and its receptors in human RPE cells (HRPE). **Methods:** HRPE cultures derived from donor eyes were used. The levels of TGF-β1, -β2 and -β3 secreted were determined by ELISA. TGF-β and TGF-β receptor mRNA levels were analyzed by conventional RT-PCR or Real time PCR. **Results:** TNF-α and IL-1β increased mRNA levels of both TGF-β1 and TGF-β2. IFN-γ enhanced constitutively expressed as well as TNF-α and IL-1β induced TGF-β1 mRNA levels but decreased TGF-β2 mRNA. IFN-γ enhanced constitutive, and TNF-α and IL-1β induced TGF-β1secretion but inhibited TGF-β2 secretion. TGF-β1 was secreted in a latent form while 10-40 % of the secreted TGF-β2 is in a mature form. TGF-β3 secretion was not detectable under any of these conditions. Real time PCR analyses confirmed the results, and correlates with the TGF-β1, β2, and β3 secretion data. Real Time PCR showed predominant expression of TGF-β receptor-1 and-2 while receptor-3 is not detectable. IFN-γ down regulated TGF-β receptor-1 without any significant effect on receptor-2. Conclusions: The contrasting effects of IFN-γ on TGF-β1 and TGF-β2 expression in HRPE suggest differential roles for TGF-β1 and TGF-β2 in the inflammatory diseases of the retina and choroid.

POSTER 24

The Relative Concentration of Retinoschisin and the Degree of Post-translational Modification of Retinoid Binding Proteins are Altered with Age in Human Vitreous Humour.

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Purpose: To determine whether the distribution and post-translational modification of proteins in human vitreous humor alters with age. **Methods:** Donor eyes (< 20 years and >60 years) were frozen on dry ice within 6 hours post-mortem and stored at -70 C until analysis. The frozen vitreous was separated into anterior and posterior sections, centrifuged, and dialyzed extensively to remove salts. Following two-dimensional gel electrophoresis, the gel spot patterns were analyzed by Progenesis image analysis software and selected protein spots were identified by MALDI-TOF-MS. **Results:** The relative abundance of approximately 30 protein spots, including retinoschisin, in the molecular weight range of 10-50 kDa alters significantly dependent upon anterior or posterior vitreous humor location and donor age. Significant differences, as a function of age, in the relative spatial abundance and post-translational modification of proteins involved in retinoid transport and storage including retinoic acid binding protein and transthyretin have been noted. **Conclusions:** The relative abundance, degree of post-translational modification, and location within the vitreous of retinoschisin and retinoid binding/transport proteins alters with age. This data further supports the hypothesis that alterations in the vitreous humor biochemical composition occur with age and may be associated with retinal dysfunction.

POSTER 25

Pigment epithelium-derived factor (PEDF) and Erythropoietin Share Protective Properties on Retinal Neuronal Cell Lines.

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Purpose: PEDF and Erythropoietin (Epo) protect photoreceptors against light-induced damage. Epo is a survival and neurite-outgrowth factor for retinal ganglion cells (RGCs). Photoreceptors and RGCs contain receptors for both factors. The purpose of this work was to compare the effects of the two cytokines on immortalized retinal cell lines. **Methods:** R28, RGC-5, and HER-10 are immortalized retinal cell lines. Cell death was induced by serum deprivation or membrane depolarization and cell viability was determined. Cell-surface binding assays were performed with radiolabeled ligand in solution. Binding to soluble Epo-receptor (sEpoR) was performed by ultrafiltration or BIAcore analysis. Hematopoietic assays were performed in erythroid progenitors, and glycophorin A-positivity used as indication of differentiation. **Results:** PEDF and Epo increased the viability of serum-deprived R28 and RGC-5 cells and depolarized HER-10 cells in a dose-dependent fashion, being Epo more potent of PEDF. ^{125}I-PEDF specifically bound to R28 and HER-10 cells. PEDF and sEpoR formed complexes when preincubated in solution, but they did not bind in real-time BIAcore analysis. Unlike Epo, PEDF did not promote erythroid differentiation and showed only marginal survival activity. **Conclusions:** Like Epo, PEDF acted as a survival factor for retinal cells. However, PEDF did not share hematopoietic properties or affinity for EpoR with Epo. These results demonstrate that Epo and PEDF share protective activities, but suggest that they might have distinct mechanisms of action in the retina.

POSTER 26

PEDF-R1: A Potential Cell-surface Receptor for Pigment Epithelium-derived Factor (PEDF) with Phospholipase-A Activity.

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Purpose: PEDF binds with high affinity to cell-surface receptors yet unidentified. A yeast 2-hybrid system identified a PEDF interactant gene with similarity to an unknown liver orphan receptor. The purpose of this study was to characterize the product of such gene as a potential receptor for PEDF. **Methods:** Bioinformatics was performed using online databases and softwares. R1 overexpression and recombinant His-tagged protein purification were performed using Gateway system and Ni-NTA resins. PEDF-R1 binding was performed in solution, solid-phase, and SPR assays. Phospholipase (PLA) activity was measured by spectrophotometric assays. Subcellular localization was by confocal microscopy or Western blot. **Results:** Sequence alignments of the PEDF interactant gene with DNA databases identified a novel gene from RPE, named *PEDF-R1.* Bioinformatics of *PEDF-R1* predicted a polypeptide product having potential transmembrane regions, N-glycosylation sites, and a PLA domain. His-tagged-R1 polypeptide bound specifically to PEDF with high affinity (K_D = 2.4 nM), and exhibited PLA enzymatic activity. R1 localized to membranes of COS-7 cells transfected with lumio-tagged R1. **Conclusions:** A novel RPE gene codes for a protein that binds to PEDF with similar affinity to the one found previously. Subcellular localization, biophysical binding parameters and PLA activity suggest that PEDF-R1 is an enzyme-linked cell-surface receptor that could be involved in PEDF biological activities.

POSTER 27

A new locus for autosomal dominant posterior polar cataract on chromosome 14q

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Background: Posterior polar cataract is a clinically distinctive opacity located at the back of the lens. It is commonly acquired in age related cataract, and may infrequently occur in pedigrees with congenital cataract. To date, four loci for autosomal dominant congenital cataract have been identified. These include one gene CRYAB on chromosome 11, and three loci with as yet unknown genes on chromosomes 1p, 16q and 20p. **Purpose:** To find the chromosomal location of a gene causing autosomal dominant congenital posterior polar cataract in three Morocco-Jewish families. **Methods:** A whole genome scan was performed using microsatellite markers spaced at approximately 10cM intervals. For fine mapping 4 additional microsatellite markers were genotyped. Two-point lod scores were calculated using MLINK of the LINKAGE program package. **Results:** The new cataract locus was mapped to an 11.2 cM interval between D14S980 and D14S1069 on chromosome 14q. A maximum two-point lod score of 3.67 at theta=0, was obtained with the markersD14S274. **Conclusion:** A gene associated with posterior polar cataract maps to the long arm of chromosome 14.

POSTER 28

Association of Cdk5 with Src is independent of Cdk5 activity and phosphorylation status

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Purpose: Cdk5 and Src are potential substrates for each other. In corneal epithelial cells, Cdk5 complexes with Src, which may facilitate reciprocal phosphorylation. This study investigates whether Cdk5 activity and phosphorylation status affect complex formation. **Methods:** Known phosphorylation sites of Cdk5 were altered by site-directed mutagenesis. Cos7 cells were transiently transfected with wild type and mutated Src, and Cdk5, or green fluorescent protein (GFP) fusions of these proteins. Proteins were immunoprecipitated using anti-GFP antibody and immunoblotted with antibodies to Cdk5, Src, and Cdk5(pY15). **Results:** GFP-Cdk5 co-immunoprecipitated with Src from transfected Cos7 cells. Site specific mutation of Cdk5 at known phosphorylation sites, (Y15F and S159A), did not prevent association with Src, indicating that prior phosphorylation of Cdk5 is not required for association with Src. Similarly, Cdk5 mutations that abolish kinase activity maintained the ability to complex with Src. Loss of Cdk5 kinase activity did not prevent phosphorylation of Cdk5 at Y15, a site associated with increased Cdk5 activity. **Conclusions:** Mutations which block the phosphorylation and activity of Cdk5 do not prevent its association with Src. These mutations will therefore be useful for determining whether reciprocal phosphorylation of Cdk5 and Src are required for previously observed effects of Cdk5 on Src activation and localization.

POSTER 29

Gene expression profile studies of human Keratoconus cornea for NEIBank: A novel gene and the absence of transcripts for Aquaporin 5.

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Purpose: To develop a database of genes expressed in human cornea and to gain insights into the molecular basis of keratoconus (KC). **Methods:** A cDNA library was constructed from seven KC corneas and used for expressed sequence tag analysis. **Results:** 7680 clones were sequenced. After analysis by GRIST, 4090 clusters of clones, each potentially representing individual genes, were identified. The five most abundant transcripts were Keratin 12, BIGH3, decorin, ALDH3, and enolase 1, all known markers for cornea. 6 clones came from KC6 a novel gene on chromosome 18p12.3. RT-PCR of several human tissues detected KC6 transcripts only in cornea. The only other ESTs for KC6 in GenBank come from ES cells. In addition, no clones were observed for the usually prominent corneal epithelial cell marker aquaporin 5 (AQP5), a water channel protein. RT-PCR confirmed that expression of AQP5 is suppressed in KC cornea. **Conclusions:** This analysis provides important resources for studies of normal and KC cornea. KC6, a gene of unknown function, seems to be cornea-specific and may be related to stem cells. The suppression of transcripts for AQP5 in KC suggests the possibility of defects in water transport in the affected corneal epithelium.

POSTER 30

RPE65 interacts with multiple domains of Caveolin-1

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Purpose: RPE65 contains conserved caveolin-binding motifs and can bind to caveolin-1. We wished to determine which of the protein interaction domains of caveolin-1 associate with RPE65. **Methods:** An expression construct encoding GST-caveolin 1 (GST-Cav1) was subjected to site directed mutagenesis to generate a panel of GST-Cav1 deletion fusion proteins. Mutated fusion proteins were expressed and purified by binding onto glutathione sepharose. GST pulldown assay was used to assess interaction of the various GST-Cav1 fusion proteins with RPE65. **Results:** It was seen that RPE65 bound to the GST-Cav1 deletion lacking either the aa82-101 scaffolding domain or the C-terminal 134-182 just as well as to full-length GST-Cav1. Surprisingly, when both these domains were deleted (construct GST-Cav1/del82-101/Cterm) there was still significant binding of RPE65. All these constructs contain the aa102-133 caveolin-1 transmembrane domain, also implicating this region in RPE65 binding. When the scaffolding domain, the transmembrane domain and the C-terminal domain were all deleted (construct GST-Cav1/del82-178), binding of RPE65 was reduced to the background level. **Conclusions:** Both the scaffolding and the C-terminal domains of caveolin-1 are implicated in the binding of RPE65. In addition, the transmembrane domain also contributes to RPE65 binding. However, it is not clear whether this is a specific interaction of the RPE65 caveolin-binding motif or if it is a generalized hydrophobic interaction.

POSTER 31

Overexpression of Myocilin in the Eyes of Transgenic Mice

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Purpose: To obtain experimental *in vivo* information on the functional properties of myocilin for aqueous humor (AH) outflow, and to determine if increased expression of myocilin might increase outflow resistance. **Methods:** The chicken βB1-crystallin promoter was used to overexpress normal human myocilin in the lenses of transgenic mice. Expression of transgenic mRNA was monitored by northern blot analysis and *in situ* hybridization. The localization and secretion of transgenic myocilin was investigated by western blotting, light and electron microscopy. Intraocular pressure was measured by anterior chamber cannulation. **Results:** Transgenic expression resulted in a 4.7 ± 1.8-fold increase of secreted normal myocilin in mouse AH, as compared to its concentration in human AH. Immunoreactivity for transgenic myocilin was observed along the surfaces of lens and corneal endothelium, and in the chamber angle. At 12 weeks of age, the ultrastructure of the trabecular meshwork in mice expressing normal myocilin was not different from that of control eyes, and the intraocular pressure of transgenic animals did not significantly differ from that of control littermates. **Conclusions:** Our results do not support the concept that increasing amounts of myocilin in the outflow tissues obstruct the system and directly cause an increase in outflow resistance.

POSTER 32

Role of Reactive Oxygen Species (ROS) in Fenretiinide-Induced Apoptosis of Human Retinal Pigment Epithelial (RPE) Cells

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Purpose: Oxidative stress in RPE cells is thought to play a major role in the development of age-related macular degeneration. The present study is aimed at investigating whether the apoptosis in human RPE cells treated with fenretinide, a retinoic acid derivative, is mediated by oxidative stress. **Methods:** Apoptosis in ARPE-19 cells treated with fenretinide in the presence or absence of retinoid receptor antagonists was estimated using ELISA. The intracellular generation of ROS was measured fluorimetrically after pretreating cells with carboxydichlorodihydrofluorescein diacetate. RARα mRNA expression was silenced using specific siRNA duplexes. **Results:** Fenretinide induced apoptosis in ARPE-19 cells in a dose- and time-dependent manner. Silencing of RAR αmRNA expression in cells effectively blocked the fenretinide-induced apoptosis. A marked increase in ROS generation in RPE cells was noticed prior to the apoptosis. Also, pyrrolidinedithiocarbamate (PDTC), a free radical scavenger, inhibited the fenretinide-induced apoptosis. AGN194301, a RARα receptor antagonist, effectively blocked the generation of ROS as well as the apoptosis. **Conclusion:** These results suggest that fenretinide-induced apoptosis in RPE cells is mediated by ROS. Interestingly, both apoptosis and ROS generation are linked to the RARα-dependent signaling pathway. Thus, the ROS generation mediated by retinoid signaling could be an important regulator of RPE pathophysiology.

POSTER 33

Degeneration of ganglion cells in the peripheral retina of transgenic animals containing the mutated mouse *myocilin* gene.

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Purpose: To examine the retina of transgenic (Tg) mouse line #8, containing the mouse *myocilin* gene with the Tyr423His point mutation, analogous to the Tyr437His mutation in human myocilin causing juvenile open-angle. **Methods:** Whole-mount retinas and eye sec glaucoma tions of wild-type (WT) and Tg mice expressing the mutated mouse *myocilin* gene were immunostained for retinal ganglion cell (RGC) markers and assessed for apoptosis by TUNEL. Thy-1 expression was estimated using *in situ* hybridization and real-time PCR. Intraocular pressure (IOP) was measured using a non-invasive fiber-optic pressure transducer. **Results:** Neurodegeneration was found in the periphery of Tg retinas. Immunostaining for Brn3b or Nf68 showed the loss of ~20% of RGC in 6-12 months old Tg animals compared with WT littermates. Apoptotic cells were detected in the retinas of Tg mice, but not in WT. Real-time PCR analysis of Thy-1 showed a 2.0-2.9 fold decrease in Thy1 mRNA in Tg retinas as compared with WT. IOP in Tg mice was higher than that in WT (13.3±0.4 vs 11.2±0.3 mmHg, $P<0.05$). **Conclusions:** Expression of mutated mouse myocilin in the eyes of transgenic mice leads to a moderate elevation of intraocular pressure and induces selective degeneration of RGC in peripheral retina, resembling that observed in human glaucoma patients.

POSTER 34

Establishment of Stably Transfected Cell Lines over-expressing Oxysterol Binding Proteins (OSBPs).

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Purpose: To generate stably transfected cultured human RPE cell lines over-expressing different oxysterol binding proteins. The cell lines will be used to study the biological properties of OSBP over-expression *in vitro*. The cell lines will also serve as a source of biologically active OSBPs for biochemical studies. **Methods:** In this study, the ARPE19 cell line was used to establish the stably transfected cell lines. A stable host cell line containing one integrated FRT site was initially generated using Invitrogen's Flp-In technology. The parental or host cell line was then transfected with pcDNA5-FRT-based constructs containing the OSBP genes of interest fused to the V5 epitope. **Results:** Several cell lines derived from ARPE19 cells were generated for OSBP2, OSBPL-9 and OSBPL-2. Western blot analyses using anti-V5 epitope antibodies demonstrated the expression of the transgenes. Southern blot analyses confirmed insertion of the OSBP genes at the FRT site. Treatment of these cell lines with lethal concentrations of 7-ketocholesterol and 20α-hydroxycholesterol showed a significant reduction in cytotoxicity versus host and control ARPE19 cells. **Conclusions**: OSBP2, OSBPL-9 and OSBPL-2 were successfully over-expressed in stably transfected cell lines derived from ARPE19 cells. The expression of OSBPs significantly protects these cells from oxysterol cytotoxicity. Biochemical studies are in progress.

POSTER 35

Murine Model of Primary Intraocular Lymphoma

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Purpose: We report the utilization of Rev-2-T-6 cells, a T-cell lymphoma of Balb/c origin, in developing a model for primary intraocular lymphoma. **Methods:** Rev-2-T-6 cells were injected through the pars plana into the left vitreous of 6-8 weeks old Balb/c mice. Eight mice were injected with 0.5 x 10^5 cells and 20 were injected with 1.0 x 10^5 cells. Mice received fundoscopic examination weekly and euthanized on week 3, 5, 6, 7 or 8 post inoculations. All eyes were processed for histology or Immunohistochemistry. Serum from each mouse was collected to detect expression of the anti-Rev-2-T-6 cell protein using Western Blotting. **Results:** Vitreous haze and retinal lesions appeared one-week post inoculation. By the 3rd week, marked vitreous opacity was observed clinically in some mice. Histology confirmed 15/28 of injected mice were positive for intraocular lymphoma in the vitreous and/or subretinal space. Rare lymphoma cells were found in the retina. The right eyes and both optic nerves were free of tumor in all mice. **Conclusions:** Intravitreal injection of Rev-2-T-6 cells is a novel model for primary intraocular lymphoma in immune competent hosts. This model will aid in understanding the molecular mechanisms and the role of RPE cells in lymphoma invasion.

POSTER 36

Hydrodynamic Vaccination with DNA Encoding an Immunologically Privileged Retinal Antigen Protects from Anti-Retinal Autoimmunity Through Induction of Regulatory T cells

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Purpose: We wanted to examine whether a DNA vaccine which induces the expression of IRBP in the periphery can revoke its immune privileged status and protect mice from EAU. **Methods:** We used a hydrodynamic intravenous injection of naked IRBP-DNA to express the uveitogenic retinal antigen IRBP in the periphery. **Results:** IRBP was expressed in the liver within hours of administration of as little as 10 µg of IRBP-DNA. Vaccinated mice were highly protected from EAU induced by immunization with IRBP for at least 10 weeks after vaccination. Protection was partial in a reversal protocol, suggesting that regulatory mechanisms induced by vaccination target mainly new effector cell generation. Mechanistic studies revealed specific hyporesponsiveness to IRBP without evidence for immune deviation. Development of protection was not dependent on apoptosis either by the Fas or the Bcl-2 regulated (mitochondrial) pathway. Depletion of $CD8^+$ cells had no effect. In contrast, depletion of $CD25^+$ cells after vaccination and prior to challenge markedly abrogated protection. **Conclusion:** These findings suggest that expression of IRBP in the periphery by DNA vaccination induces tolerance through a mechanism that is in part dependent on $CD4^+/CD25^+$ regulatory T cells. DNA vaccination may offer a new approach to Ag-specific therapy of uveitis.

POSTER 37

A Homozygous Splice Mutation in the *HSF4* Gene is Associated with an Autosomal Recessive Congenital Cataract

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Purpose: To map the locus and identify the gene causing autosomal recessive congenital cataracts in a large consanguineous Tunisian family. **Method:** DNA was extracted from blood samples from a large Tunisian family with an autosomal recessive, congenital, total white cataract. A genome-wide scan was performed with microsatellite markers. All exons and the splice sites of the *HSF4*gene were sequenced in all members of the Tunisian family and in control individuals. RT-PCR was used to detect different transcripts of the *HSF4* gene in the human lens. **Results:** Two-point linkage analyses showed linkage to markers on 16q22 with a maximum lod score of 17.78 at θ=0.01 with *D16S3043*. Haplotype analysis refined the critical region to a 1.8-cM (4.8-Mb) interval, flanked by *D16S3031* and *D16S3095*. This region contains *HSF4*, some mutations of which cause the autosomal dominant Marner cataract. Sequencing of *HSF4* showed a homozygous mutation in the 5 'splice site of intron 12 (c.1327+4A>G), which causes the skipping of exon 12. A more detailed study of the transcripts resulting from alternative splicing of the *HSF4* gene in the lens is also reported, showing the major transcript HSF4b. **Conclusions:** This is the first report describing association of an autosomal recessive cataract with the *HSF4* locus on 16q21-q22.1 and the first description of *HSF4* splice variants in the lens showing that HSF4b is the major transcript.

POSTER 38

Effect of Th1 or Th2 cytokines on late phase CD40L expression on naïve and memory human CD4$^+$ T cells.

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CD40L expression on TCR activated human CD4$^+$ T cells consists of an early CD28-independent phase and a later CD28-dependent phase. **Purpose(s):** To determine whether IL-2R signaling directly affects the later, CD28-dependent, phase of CD40L expression or acts indirectly through IL-2 dependent Th1 and/or Th2 cytokines. To determine whether biphasic expression of CD40L reflects differential expression of CD40L on naïve and memory human CD4$^+$ T cells. **Methods:** Four-color flow cytometric analysis was carried out on resting or anti-CD3 (OKT3) and anti-CD28 activated CD4$^+$ T cells, using CD45RA (naïve) and CD45RO (memory) markers on CD4$^+$ T cells. Indirect effects of Th1 or Th2 cytokines were measured by activation in the presence of cytokine blocking antibodies or high dose IL-4. **Results:** CD45RA and CD45RO CD4$^+$ T cells are equally capable of biphasic CD40L expression. Neither IL-4 nor IL-12 had any appreciable effect on the early or late expression of CD40L, but IL-12 independent IFN-g positively regulated CD40L expression at 48 hr. Recombinant IL-2 could largely substitute for CD28 costimulation in PBMC cultures. **Conclusion:** Regulation of late CD40L expression is mediated directly through IL-2R signaling.

POSTER 39

Essential Role of the Myeloid Differentiation Factor 88 (MyD88) Pathway, but Nonessential Roles of Toll Receptors 2, 4 and 9, in the Adjuvant Effect Promoting Th1 Mediated-Experimental Autoimmune Uveitis (EAU)

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Purpose: Induction of tissue-specific EAU involves an obligatory adjuvant effect to trigger an innate response of a type that will drive a Th1-biased adaptive response. This is achieved by use of complete Freund's adjuvant (CFA) containing mycobacteria (MTB), whose recognition by cells of the innate immune system depends on Toll-like receptors (TLRs) that signal through the adaptor molecule MyD88. The authors examined the role of selected components of the MyD88 pathway in promoting EAU. **Methods:** Mice deficient in MyD88, TLR2, TLR4, TLR9, IL-1R or IL-18, were immunized with the retinal antigen IRBP in CFA, and their EAU scores and associated immunological responses were examined. **Results:** MyD88$^{-/-}$ and IL-1R$^{-/-}$ mice were completely resistant to EAU and had a profound defect in Th1 response to autoantigen challenge. Surprisingly, TLR2$^{-/-}$, TLR4$^{-/-}$, TLR9$^{-/-}$ and IL-18$^{-/-}$ mice were fully susceptible to EAU and had unaltered adaptive responses to IRBP. **Conclusions:** TLR 9, TLR4 and TLR2 signaling is either not needed, or, more likely, redundant in the adjuvant effect needed to induce EAU. In contrast, signaling through the IL-1 receptor plays a necessary and nonredundant role in EAU and can by itself account for the lack of EAU development in MyD88 mice.

POSTER 40

Gene expression profiles in eyes with inflammation induced by TCR engagement (EAU), or by local expression of cytokines (IL-1, IL-7)

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Purpose: Experimental autoimmune uveitis (EAU) is triggered by engagement of the T-cell receptor (TCR) on T-lymphocytes with their ocular-specific target antigens, followed by activation of the T-cells and expression of cytokines, chemokines, chemokine receptors and adhesion molecules. We found that inflammation also develops in eyes of transgenic (Tg) mice expressing interleukin (IL)-1 or IL-7. Here, we compared gene expression profiles of immune-related molecules in eyes with EAU or with cytokine-induced ocular inflammation. **Methods:** Total RNA was obtained from the experimental eyes and the mRNA levels of the tested molecules were measured by real-time PCR. **Results:** The majority of 6 chemokines, 6 chemokine receptors, 8 adhesion molecules and 5 transcription factors involved in CD4 cell activation were found to be up-regulated in all tested three forms of eye disease. Yet, remarkable variations were observed among the three eye diseases in their expression of the individual molecules, with the most conspicuous differences being noted between eyes with EAU and those from the Tg mice. **Conclusions:** The presented data suggest that inflammatory reactions triggered by different mechanisms, TCR engagement or local expression of cytokines, use the same battery of inflammatory-related molecules, but with considerable variations.

POSTER 41

Flt-3 ligand elicited, activated dendritic cells pulsed with a retinal antigen induce autoimmune pathology in the retina

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Purpose: Experimental Autoimmune Uveitis (EAU) in mice induced by immunization with retinal antigens in complete Freund's adjuvant serves as a model for human uveitis. We are developing an alternate EAU model where disease is induced with *in vitro*-matured, antigen-pulsed DC. **Methods:** DCs were expanded in vivo by injection of a plasmid encoding Flt-3 ligand using a hydrodynamic injection protocol. Splenic DCs were purified by using CD11c labeled magnetic microbeads. IRBP (interphotoreceptor retinoid-bind protein) peptide loading DCs were matured in the presence of LPS together with anti-CD40 and subcutaneously injected into B10RIII mice. **Results:** Five days after Flt-3 ligand encoding plasmid induction, the percent of CD11c+ splenocytes in the population increased from an average of 5% to 16%, and total yield of splenocytes per mouse was doubled. Combined stimulation of DCs with LPS and anti-CD40 resulted in enhanced expression of costimulatory molecules on the DC and in release of IL-1,IL-6,IL-12 and TNF-a into the supernatant. DCs pulsed with an uveitogenic peptide elicited vigorous immune responses in B10RIII mice. Two administrations of these DC over a four-day interval elicited a typical EAU-like ocular inflammation in susceptible B10RIII mice, with an incidence of 60% to 87%. **Conclusions:** In this study, activated lymphoid DCs primed mice peripherally and induced EAU. This is a new EAU model that is not dependent on complete Freund's adjuvant, and may in some ways be closer to a clinical situation. This model may also provide a new platform to dissect the pathogenesis of autoimmune uveitis and to examine interventional strategies.

POSTER 42

In search of retinoschisin interacting proteins: Towards understanding the molecular basis of macular degeneration in retinoschisis

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Purpose: To identify and characterize the proteins that interact with Retinoschisin (RS), a retina specific 24 kDa protein and to (b) explore and examine whether collagens serve as true ligands for RS in maintaining the cytoarchitecture and normal physiology of the retina. **Methods:** Blue-Native Gel Electrophoresis and immunoblot analysis to resolve and identify RS in multiprotein complexes; ELISA and collagen affinity chromatography to explore RS binding to collagens. **Results:** On a Blue Native Gel, the 24 kDa RS migrates as a high molecular weight (> 700 kDa) multi-protein complex. Two dimensional gel analysis of the complex, reveals the presence of RS as a multimer ranging from dimer to octamer. Purified recombinant RS binds to Type I collagen more avidly and shows saturation kinetics. Retinal membrane fractions enriched in RS show distinct binding to Type I collagen. The failure to pull down RS by collagen III affinity chromatography correlates well with the poor binding characteristics of RS to Type III collagen. **Conclusion:** In retina, the 24 kDa RS exists as a multimer in a multiprotein complex. Both under *in vitro* and *in vivo* conditions RS selectively binds to Type I collagen.

POSTER 43

Experimental Autoimmune Uveitis (EAU) Does Not Always Resemble Other Autoimmune Disease Models.

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Purpose: Many aspects of EAU are similar to other autoimmune disease models such as experimental autoimmune encephalomyelitis (EAE). However, here we report on four systems in which EAU exhibited different features. **Methods:** Osteopontin -/- mice were created at Rutgers University, while CD1d -/- mice were generated at Vanderbilt University. These mice, their +/+ controls, B10.A , B10.RIII, and C57BL/6 mice were immunized with IRBP for EAU induction. Lewis rats were immunized with S-Ag peptide 35. Treatment with atorvastatin or peroxisome proliferator-activated receptorα agonists (PPARα) was given daily by gavage. Disease severity was scored by histological examination. **Results:** EAU induction was examined in four experimental systems in which development of other autoimmune diseases was modified: (i) Osteopontin -/- mice showed reduced capacity to develop EAE (Chabas et al.); however, they resembled their controls in developing EAU. (ii) CD1d -/- mice lack NKT cells and exhibited enhanced susceptibility to autoimmune diabetes (Godfrey et al.); yet, they developed EAU similarly to their controls. (iii) Atorvastatin (Youssef et al.) and (iv) PPARα (Racke et al) treatment inhibited EAE in mice. Surprisingly, they had minimal effect on EAU development. **Conclusion:** Information collected on other experimental autoimmune diseases cannot always be applied to EAU.

POSTER 44

Angiogenic and Anti-Angiogenic Factors in Cultured Human Fetal Retinal Pigment Epithelium (hfRPE): changes in mRNA Expression and Protein Level

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Purpose: These experiments were designed to evaluate the interactions of vascular endothelial growth factor (VEGF), pigment-epithelium derived factor (PEDF), and sFlt1, each overexpressed in hfRPE, on their mRNA and protein levels. **Methods:** Confluent primary human fetal RPE cells were infected with AAV-VEGF, AAV-PEDF or AAV-sFlt1 with AAV-GFP as control. **Results:** VEGF mRNA increased 2, 4, or 270 fold in hfRPE infected with AAV-PEDF, AAV-sFlt1, or AAV-VEGF, respectively. Apical VEGF decreased 20%-40% ($p<0.05$) in AAV-sFlt1 infected cells and increased 2-3 times ($p<0.05$) in AAV-VEGF infected cells while basolateral VEGF did not change significantly in both groups. In AAV-PEDF infected cells, VEGF protein did not significantly change in either the apical or basal compartments. In hfRPE infected with AAV-PEDF, AAV-sFlt1, or AAV-VEGF, PEDF mRNA increased 6, 2, or 2 fold, respectively. In hfRPE infected with AAV-sFlt1, the sFlt1 protein increased more than 100-fold in the apical compartment and more than 10-fold in the basal compartment ($n= 2$). The sFlt1 protein increased in AAV-VEGF infected samples and decreased in AAV-PEDF infected samples. **Conclusion:** These results suggest that in disease states angiogenic and anti-angiogenic factors secreted by the RPE may differentially affect blood vessel proliferation in the extracellular spaces on the retinal and choroidal sides of the tissue.

Clinical Research Posters

POSTER 45

Ophthalmologic Features of Patients with DNA Repair Defects

Brooks BP, Chan CC, Smith J, DiGiovanna JJ, Schmidt D, Blain D, Kraemer KH

Background: Xeroderma pigmentosum (XP) is an autosomal recessive disorder characterized by defective DNA repair mechanisms and an increased risk for ultraviolet (UV) light-induced malignancies on sun-exposed areas such as the skin and conjunctiva. Trichothiodystrophy (TTD) is a related, autosomal recessive disease characterized by sulfur-deficient brittle hair and nails and a broad spectrum of skin, neurologic, immunologic, and developmental abnormalities. TTD patients do not typically develop skin cancer. It is not well-appreciated that many of these patients—including children—suffer from clinically significant ocular surface disease. **Purposes:** 1) To characterize the ocular surface disease in patients with DNA repair disorders. 2) To describe a non-invasive technique early detection of conjunctival pre-malignancies in these patients. **Methods:** Clinical examination and conjunctival cytology. **Results:** 14 XP and 5 TTD patients underwent age-appropriate, complete ophthalmologic examinations (7 females, 12 males). Schirmer testing for basal tear secretion was reduced (<10mm wetting) in 4 out of 10 XP patients and 3 of 3 TTD patients. Superficial punctate keratopathy was present in 3 of 12 XP patients and 2 of 3 TTD patients. Corneal pannus--ranging in severity from 1mm corneal neovascularization to complete opacification of the cornea--was observed in 5 of 14 XP and 1 of 5 TTD patients. 4 of 5 conjunctival cytology specimens showed evidence of inflammation (neutrophils and lymphocytes); one specimen showed epithelial dysplasia with mitotic figures. **Conclusions:** 1) Ocular surface disease is a frequent clinical finding in patients with DNA repair disorders—including children, in whom ocular surface problems are uncommon. 2) Conjunctival cytology is a non-invasive histologic technique that may be useful in the early detection of conjunctival pre-malignancy.

POSTER 46

Dietary Zinc and Risk for Age-related Macular Degeneration

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Purpose: To evaluate the relationship between dietary zinc intake and prevalence of age-related macular degeneration (AMD) in the Age-Related Eye Disease Study (AREDS). **Background:** Zinc is a trace mineral highly concentrated in the retinal pigment epithelium; it may protect the retina in its essential action as a cofactor for enzymes with antioxidant properties. **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 zinc with AMD status. **Results:** Compared with subjects without AMD, the likelihood of neovascular (NV) AMD was significantly decreased for the highest vs lowest quintiles of zinc intake (OR = 0.69; 95% CI, 0.49 - 0.98), after statistical adjustment for nonnutrient-based covariates. Adding nutrient-based factors to the multivariable model yielded slight decrease in the magnitude of the point estimate, and reduced the precision of this estimate to include the null value (OR = 0.75; 95% CI, 0.53 - 1.08). **Conclusion:** Higher intake of zinc was not associated with a decreased likelihood of having advanced AMD after adjusting for nonnutrient-based predictors and correlates of AMD.

POSTER 47

The NEI Histology Core

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Purpose: To present a summary of the various services provided by the NEI Histology Core in 2003-2004. **Methods:** Several staining techniques are utilized by the NEI Histology Core on frozen, methacrylate and paraffin embedded sections. The staining techniques include H&E, PAS, special staining, and immunohistochemistry. Electron microscopy (EM) and cytosine are also routinely applied in the core facility. **Results:** A total of 179 (1,595 slides/images) and 124 (1,645 slides/images) patient cases were processed in January-June of 2003 and 2004, respectively. A total of 227 (6,163 slides/images) 220 (5,793 slides/images) animal cases were processed in January-June of 2003 and 2004, respectively. Immunohistochemistry was requested the most for the patient specimens obtained during the 2003 time period. In the 2004 time period, most patient slides were paraffin embedded sections. For the animal specimens, methacrylate embedded slides were most requested in the 2003 time period. EM was used more for the animal specimens in 2004 than in 2003. **Conclusion:** The NEI Histology Core handles a large volume of specimens; particularly methacrylate embedded animal eye sections, and specializes in high quality production of ocular samples.

POSTER 48

Eye Movement Abnormalities in Hermansky-Pudlak Syndrome

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Purpose: Hermansky-Pudlak Syndrome (HPS) is a form of oculocutaneous albinism associated with a bleeding diathesis and pulmonary fibrosis. Although it is known that HPS patients exhibit nystagmus, the nature of these eye movements has not been studied. **Methods:** Twenty-seven patients with HPS underwent a systemic evaluation and complete eye exam. Twenty-five had eye movement recordings. **Results:** All patients had iris transillumination, foveal hypoplasia and variable hypopigmentation. All had bleeding tendency, and two reported bleeding during strabismus surgery. Nine patients had pulmonary fibrosis. Congenital nystagmus (CN) was clinically evident in 24 of 27 patients, and half of them exhibited periodic alternating nystagmus (PAN). In patients without clinical nystagmus, eye movement recordings revealed minimal end-gaze nystagmus, square-wave jerks, drift during fixation and saccades, and low-gain pursuit. These patients had better visual acuities and posterior pole pigmentation than the others. **Conclusions:** Most HPS patients have CN, and many have PAN. Some have no clinical nystagmus, which can obscure the diagnosis. Detection of subtle eye movement abnormalities in these patients may assist the diagnosis. Absence of clinical nystagmus in a child with HPS suggests a good visual prognosis. Patients with albinism, especially before strabismus surgery, should be tested for HPS to prevent life-threatening complications.

POSTER 49

DARK-ADAPTATION IN HUMAN ALBINISM

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Purpose: It is not known whether deficits in dark-adaptation (DA) reported in some studies on albino rodents, occur in patients with albinism. We examined DA in patients with Hermansky-Pudlak syndrome (HPS), a form of oculocutaneous albinism associated with a bleeding diathesis. **Methods:** Eleven HPS patients (diagnosed by platelet dysfunction and genetic analysis) were tested with a Haag-Streit adaptometer. **Results:** All patients showed a normal final DA threshold that was reached within a normal time period. However, all but one exhibited a delayed transition from cone- to rod-mediated part of the DA curve (the so-called rod-cone break (RCB)). In HPS patients, RBC occurred between 7 and 13.5 min, normal being 5.63 ± (SD) 1.17 min. This RCB delay was corrected by short-wavelength absorbing lenses. Some patients were aware of visual difficulties when going from bright to dark places. **Conclusions:** HPS patients have a normal DA threshold, but a delayed RCB. This delay, likely the result of light scatter due to ocular hypopigmentation, causes functional difficulties in some albino patients besides their poor vision and nystagmus. Appropiate filtering lenses as well as treatment modalities to improve ocular pigmentation should help visual performance by reducing glare in these

POSTER 50

Quantitation of HSV Genome in Recipient and Eye-Bank Donor Corneas Hayashi K[1], Deai T[2], Fukuda M[2], Higaki S[2], and Shimomura Y[2]
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Purpose: To quantitate herpes simplex virus (HSV) genome in the human corneal buttons and receipient's corneas. **Methods:** Forty-four receipient's corneas: 7 corneas with history of herpetic keratitis and 37 corneas without history of herpetic keratitis. Donor corneas:70 eye-bank eye donor corneas and 35 eye bank eye scleras. Primers for a real time PCR were selected in a HSV-1 and -2 common region of viral DNA polymerase. A conventional PCR was designed to detect HSV-1, -2 and varicella zoster virus (VZV). **Results:** HSV-1 DNA was detected in 85.7 % of the corneas (7/8) with average HSV –DNA copy number of $1.6X10^4$ /mg tissue, while 10.8% (4/37) of the corneas without herpetic history was positive for the HSV genome with average 8.7 copies/mg tissue. In the donor corneas, 5.7%(4/70) was positive for HSV-DNA with an average copy number of $4.9X10^2$/mg tissue. Donor sclera was positive for 8.6%(3/35) with average copy number of 10.6/mg tissue. Four keratoplasty patients, who had the last occurrence of herpetic keratitis in less than 20 months prior to the operation, had a recurrence of herpetic keratits. HSV-2 and VZV genome were not detected in the samples.

POSTER 51

Phenotypic and functional analysis of NK cells from an NK lymphocytosis associated with multiple autoimmune-associated disorders
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Purposes: To study characteristics of natural killer cells from an NK lymphocytosis patient associated with multiple autoimmune-associated disorders. **Methods:** Flow cytometry, cytokine stimulation and proliferation and multiplex cytokine assays, cytotoxicity assay were used to investigate characteristics of NK cells. **Results:** The patient demonstrated history of multiple autoimmune-associated disorders including thyroiditis, scleritis, peripheral neuropathy, and Sjogren's syndrome. Flow cytometry analysis revealed a markedly increased NK cell population (CD3-CD56+) in peripheral blood lymphocytes. All CD3-CD56+ NK cells were also CD94, CD122 and CX3CR1 positive. There was a significant increase of expression of CD94, Kir/NKAT2 and CXCR3 in those NK cells. But the expression of iNKT and NKB1 on CD3-CD56+ NK cells were not significantly increased. NK cells from the NK lymphocytosis patient showed less cytotoxicity when compared to those from healthy normal donors. In addition, purified CD56+ NK cells from the patient responded poorly to IL-2 stimulation by multiple-cytokine analysis compared to those from normal control donor. **Conclusion:** Several surface markers of NK cells from an NK lymphocytosis associated with multiple autoimmune-associated disorders showed consistent expression pattern as of those from healthy donors, but there appeared functional defect of NK cells from this NK lymphocytosis case.

POSTER 52

The Relationship of ω-3 Long-chain Polyunsaturated Fatty Acid (LCPUFA) Intake and Regular Aspirin (ASA) Use with Neovascular Age-Related Macular Degeneration (NV AMD)
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Purpose: To investigate the relationship of ω-3 LCPUFA intake and ASA use with NV AMD. **Background:** The likelihood of having NV AMD is lower among people consuming higher amounts of ω-3 LCPUFAs. Docosahexaenoic acid (DHA) is the major ω-3 LCPUFA of the neural and vascular retina. Eicosapentaenoic acid (EPA) is the primary ω-3 substrate of cyclooxygenase (COX) and lipoxygenase (LOX) pathways. COX- and LOX-derived eicosanoids operate as key molecules in inflammatory and vascular processes implicated in AMD pathogenesis. LCPUFAs of the retina are capable of reacting through aspirin ASA-driven pathways to generate a family of bioactive molecules (lipoxins) with potent immunoregulatory properties. **Methods:** In this case-control study of 657 people with NV AMD and 1112 people with no clinical signs of AMD we administered a validated food frequency questionnaire and a standardized drug use questionnaire to obtain estimates of habitual LCPUFA intake and ASA use. **Results:** Multivariable logistic regression analyses yielded relationships demonstrating that groups reporting highest ω-3 LCPUFA intake and the longest duration of aspirin use were also the least likely to have NV AMD. The likelihood of NV AMD associated with highest v. lowest reported levels of DHA intake was reduced by 40% among subjects who reported never regularly using ASA. The likelihood was reduced by 60% and 80% among those who reported ever regularly using ASA for at least 3 months or 5 years, respectively. For EPA, there were significant reductions among the regular ASA users only; respective values were 0%, 60%, and 80%. All decreases in the likelihood of NV AMD were statistically significant. **Conclusion:** These novel findings provide a reasonable basis for investigating putative mechanisms driving LCPUFA-ASA-NV AMD relationships

Translational Research Posters

POSTER 53

Novel technique for evaluation of laser-induced choroidal neovascularization (CNV) using confocal microscopy

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Purpose: Laser is an established modality for induction of CNV. Histology provides morphological details and flat-mounts offer limited information regarding volume. We will demonstrate a new technique, using choroid/RPE flat-mounts, to evaluate and measure lesion volume by confocal microscopy. **Methods:** Eight laser burns were given. The end point was bubble formation, indicative of Bruch's membrane break. Eyes were fixed, retinas removed, and RPE-choroid labeled with fluorescent markers for nuclei (DAPI), endothelial cells (isolectinIB4), and filamentous actin (phalloidin). Choroid-RPE was flat-mounted, and Z-series were collected using a Leica-SP2 laser confocal microscope to evaluate three-dimensional structures. **Results**: In non-lasered areas, phalloidin labeling of actin delineated the borders of intact RPE cells. Immediately after laser, no phalloidin/lectin-labeled cells were visible. Two days post-treatment, proliferation and migration of RPE cells was apparent. Phalloidin labeling allowed visualization of actin stress fibers. By day 4, lectin-positive cells formed vessels in the lesioned area. By seven days a well-defined neovascular membrane was formed, which was significantly larger by 14 days. **Conclusions:** Confocal microscopy of flat-mounts allowed evaluation of early changes after CNV induction. This technique revealed morphological details and permitted quantification of volume in 3-D reconstructions.

POSTER 54

Ophthalmologic Abnormalities in Rab 38 $^{cht/cht}$ Mice

Brooks BP, Larson D, Chan CC, Smith RS, Kjellstrom S, Huizing M, Xiaodong J,
King R, Hejtmancik F, John SWM, Sieving PA, Bush R, and Pavan W

Purpose/Methods: The spontaneous coat color mutant, *chocolate* (*cht*), is caused by homozygous mutation (G146TàGly19Val) in the small GTP-binding protein, Rab 38. In order to understand the effects of this mutation on the eye, we have performed clinical, histological, ultrastructural, and functional studies on young and aged *cht* mice. **Results:** We found that Rab38$^{cht/cht}$ mice—but not Rab38$^{cht/+}$ mice—show peripheral iris transillumination reminiscent of albinism. Similarly, in mice over one year of age, we observed clinical atrophy of the iris (30% of Rab38$^{cht/cht}$ mice) and retinal pigment epithelium (RPE)(70% of Rab38$^{cht/cht}$ mice)(n=31). Histopathologic analysis of eyes from three month old Rab38$^{cht/cht}$ mice showed reduced pigmentation compared to Rab38$^{cht/+}$ mice. Ultrastructural studies, however, showed early degeneration of the melanocytes of the iris stroma, the iris pigment epithelium, and the RPE. In mice over a year of age, degeneration of the iris and RPE were also evident at the light microscopy level (n=6). Interestingly, a milder degenerative phenotype was also observed on histologic sections of age-matched heterozygous mice (5 of 5). Electroretinographic responses in 3 month old Rab38$^{cht/cht}$ mice showed a statistically larger b-wave amplitude (ANOVA, $p<0.0001$) in the scotopic range when compared to wild-type mice, but no significant difference in the a-wave amplitudes (ANOVA, $p>0.1$), reminiscent of findings in albinism patients. Recent findings of reduced numbers of platelet dense bodies in the rat orthologue *Ruby* (carrying a Rab38 null mutation), suggests that both the *Ruby* rat and the *cht* mouse are models of human Hermansky-Pudlak-like syndromes (HPS). Mutation screening in 12, lightly-pigmented albino patients, in 8 HPS and 11 HPS-like patients, and in 17 patients with pigmentary glaucoma, no mutations were found. However, four novel polymorphisms were identified in several HPS, HPS-like, and pigmentary glaucoma subjects. **Conclusions:** We propose that Rab38$^{cht/cht}$ mice as a useful model for the study of ocular melanosome biogenesis.

POSTER 55

Detection of Single Nucleotide Polymorphism and Expression of CX3CR1 in Human Eyes with Age-Related Macular Degeneration (AMD)

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Purpose: AMD has a genetic component. The role of the immune system is involved in AMD. CX3CR1 is the receptor of chemokine CX3CL1, which contains two SNPs (V249I and T280M). Recently we reported an association of these SNPs and AMD. This study investigates T280M and CX3CR1 expression in the eye sections with AMD. **Methods:** Archived, paraffin-embedded, ocular slides from 40 patients with pathological diagnosis of AMD were collected. Non-retinal, peripheral and macular retinal cells were microdissected. The non-retinal cells were used for SNP analysis with PCR and RFLP. The retinal cells of selected cases were used for RT-PCR. Immunohistochemistry was also performed for CX3CR1 staining. **Results:** DNA was successfully extracted in 32 of the 40 AMD cases. Fourteen cases (43.8%) were found to be carriers of the T280M, with an allele frequency of 29.7%. The transcript and protein of CX3CR1 were lower in the macular lesion as compared with the normal peripheral retina in the AMD eyes. **Conclusion:** T280M allele could result in a decreased number of CX3CR1 receptor binding sites for fractalkine as well as reduce fractalkine-binding affinity. Our data suggest that insufficient interaction between CX3CR1 and fractalkine, and lower CX3CR1 expression in the macula may potentially promote AMD development.

POSTER 56

Doxycycline inhibits migration of human pterygial epithelial cells

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Purpose: Pterygium, a wing-shaped, fibrovascular lesion, is a blinding disorder of the ocular surface that can affect up to one-third of the population in countries near the equator. The disease is clearly correlated with ultraviolet light exposure. Other causal mechanisms include genetic predisposition, male sex, older age, and occupational exposures that irritate or dry the eye. Surgical removal is often not successful because of the high frequency of recurrence. Previously, we reported that matrix metalloproteinases 1, 2, 7, and 9 are upregulated at the advancing pterygium edge. The current study examines the effect of doxycycline, an MMP inhibitor, on the progression of human pterygial epithelial cells in culture. **Methods:** The migration of human pterygial cells was assessed by the Boyden Chamber Migration Assay. The level of MMP activity in these cells was assessed by gelatin zymography. **Results:** Human pterygial cell migration was inhibited in a dose-dependent manner by 125 microgram/ml and higher doxycycline. A concentration of 250 microgram/ml showed an 70% reduction in migration. MMP-9 activity was reduced by over 50% at 100 microgram/ml doxycycline and completely abolished at 1 milligram/ml. **Conclusions:** Doxycycline has been used to treat meibomian gland dysfunction, periodontal disease, and several other inflammatory diseases. This study shows that doxycycline, at physiologically-relevant concentrations, could potentially benefit humans by reducing the growth of pterygia and therefore prove to be vision-saving.

POSTER 57

Expression of Gap Junction Proteins in The Mouse Corneal Epithelium

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Purpose: Recently, mutations in connexins (gap junction proteins) have been implicated in a number of ectodermal dysplasias which frequently have associated ocular surface disease. The current study evaluated the expression of gap junction proteins in the mouse corneal epithelium under normal and wound healing conditions. **Methods:** The expression of the connexins 26, 30, and 43 were evaluated in 6 week old FVB normal mice by immunohistochemisty, western blot, and RT-PCR. For wound healing studies, a 1.5 mm diameter central corneal abrasion was created. **Results:** On immunohistochemistry connexins 26 and 30 were found to be expressed in the basal and intermediate layers of the corneal epithelium while connexin 43 was limited mostly to the basal layer. The expression of all three types of connexin was lower in the limbal region compared to the central cornea. The expression of all three connexins was increased at 12 and 24 hours after corneal abrasion. Current studies are evaluating the expression at later time points as well as the expression of other connexins, namely, connexins 31 and 31.1. **Conclusions:** These results demonstrate similarities and differences in the expression patterns of the three connexins examined. In particular, the expression of connexins 26 and 30 appear to be quite similar and distinct from connexin 43. Future studies are needed to determine whether connexins 26 and connexin 30 are co-regulated in the corneal epithelium.

POSTER 58

Shifting Paradigms in Ophthalmic Drug Delivery for Chronic Diseases: From Eye Drops to Implants

Kim H, Robinson MR, Csaky KG

Purpose: Eye drop formulations have been the principal mode of therapy for eye diseases for centuries and are often not effective due to poor ocular penetration and patient non-compliance. Furthermore, eye drops are not effective for posterior segment diseases. Since the corneal and conjunctival epithelia are the principal pharmaceutical barriers preventing ocular drug penetration, we developed a novel sustained-release implant that is placed in the subconjunctival space; a safer alternative to an intravitreal implant. We present the cyclosporine (CsA) implant as an example of how these implants can deliver therapeutic drug levels to the eye. **Methods:** Sustained-release CsA implants were fabricated and *in vitro* release rates, toxicity, and pharmacokinetics, were performed in dogs and rabbits. **Results:** The implants showed drug release for 12 months and there were no toxicities. The drug levels in all solid tissues were 2 log units higher compared with commercial topical CsA formulations. Drug levels were also present in the aqueous and vitreous humor. **Conclusions:** Sustained-release subconjunctival implants can be more effective than eye drops in delivering drugs to the eye for both anterior and posterior segment diseases. Studies are in progress to develop these implants for age-related macular degeneration and diabetic retinopathy.

POSTER 59

Detection of Cytokines, Chemokines, and Adhesion Molecules in Patients with Retinal Vasculitis

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Purpose: Retinal vasculitis (RV), major component of ocular inflammation, plays a critical role in retinal damage and subsequent vision loss. We evaluated potential markers of RV: cytokines, chemokines and adhesion molecules that may be useful in disease progression, and understanding pathogenesis. **Methods:** Sera were collected from 18 healthy individuals and 31 RV patients with Behcet's disease (18) or idiopathic uveitis (13). Sera were tested for cytokines, chemokines and adhesion molecules by ELISA. Human retinal vascular endothelial cells were stimulated with IFN-gamma, IL-1beta and TNF-alpha and tested for the secretion of sE-selectin. **Results:** Sera from Behcet's patients had elevated levels of sICAM-1 ($p<0.02$) whereas idiopathic uveitis patients had elevated levels of sE-selectin ($p<0.02$). No differences were detected for a variety of other adhesion molecules, cytokines, and chemokines. Treatment of the retinal vessel endothelial cells with IL-1beta and TNF-alpha resulted in the secretion of E-selectin. **Conclusion:** Experimental animal models and human in vitro studies have demonstrated that the expression of adhesion molecules, E-selectin and ICAM-1, in vascular endothelium correlates with leukocyte adhesion and blood-retinal barrier breakdown in vaculitis. This study identifies the differential expression of these adhesion molecules in the sera of RV patients and demonstrates that the retinal endothelial cell is a source of E-selectin.

POSTER 60

HLA DR and DQ interact to control susceptibility to Experimental Autoimmune Uveitis (EAU)

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Purpose: Linkage analysis and association studies have shown that susceptibility to autoimmune uveitis in humans is associated with genes in the HLA class II region. In this study we used several HLA Tg (Transgenic) mouse strains bearing highly (DR3) or moderately (DQ6 and DQ8) susceptible alleles, and double Tg strains bearing mixed alleles (DR3DQ6 and DR3DQ8), to investigate the role of MHC class II interactions to the uveitis-inducing autoantigens. **Methods:** The onset and severity of EAU was monitored in this humanized model after giving uveitogenic regimen of IRBP (interphotoreceptor retinoid binding protein) and SAg (Soluble antigen). T cell responses and cytokine profiles to the immunizing antigens were compared between the strains. Epitope mapping in the presence of single HLA Class II alleles like DR3, DQ6, DQ8 or in the presence of a combination of alleles like DR3DQ6 or DR3DQ8 were done using overlapping peptides of IRBP and SAg. Uveitogenesity of the immunodominant peptides were evaluated in each strain to determine the level of HLA class II interaction in the manifestation of the disease. **Results:** The double Tg mice (DR3DQ6 and DR3DQ8) developed severe EAU with hypopyon and neovascularization in contrast to moderate or mild disease in the single Tg mice. In concordance with the disease severity, the double Tg mice also showed higher T cell proliferative responses to the antigens and higher Th1 to Th2 ratio of cytokine profile compared to their single Tg counterparts. Epitope mapping of the whole IRBP and S-Antigen revealed that double Tg mice either responded to additional epitopes not recognized by either of the single Tg strains or a complete unresponsiveness to the peptides recognized by their single transgenic counterparts. Some of these immunodominant epitopes were also found to be pathogenic in an interactive fashion and are also reported to be the dominant epitopes in uveitis patients. **Conclusion:** Our findings indicate that interactions among the MHC class II molecules, in particular a combination of susceptible DR and DQ allotypes, are important in the development of uveitis and is an important factor to be considered while developing preventive or curative strategies for this autoimmune disease.

POSTER 61

Differences in Gene Expression Profiles in Dermal Fibroblasts elicited by Nonlethal Injury from Control and Patients with Age Related Macular Degeneration.(*Phase I case-control study)*

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Purpose: The pathogenesis of age related macular degeneration (AMD) is still unknown but there is growing evidence that a combination of both oxidative injury and genetic factors may play a role. One particle hypothesis proposes that dysregulation of multiple genes in response to an oxidative injury could contribute to the development of AMD. AMD appears to have a systemic component; therefore, as is the case with other central nervous diseases, peripheral sites may be used to detect underlying genetic abnormalities. **Methods:** For the present study, biopsy-derived fibroblasts from 4 patients with the early form and 4 patients with the late form of AMD and 3 age-matched control patients were grown in culture and treated with a nonlethal dose of the oxidative stimulus menadione. Gene expression patterns were quantitatively and qualitatively examined using Human Genome U95A GeneChips (Affymetrix) and verified by real-time PCR analysis. **Results:** 755 genes were found to be upregulated at least two fold in one of the patients groups but only in response to the injury. Cluster analysis of expression profiles demonstrated injury initiated gene dysregulation in the fibroblasts from early and/or late AMD patients but not in the control group. Six disease specific patterns of dysregulated genes (98 genes total) were detected. Clusters of genes dysregulated by the sublethal oxidative injury in either early and/or late AMD groups were further categorized by overrepresentation of GO "biological process" categories using Expression Analysis Systematic Explorer (EASE) software. This approach demonstrated that four major functional gene groups including inflammatory/innate immune response, transcriptional regulation, cell cycle and proliferation were significantly overrepresented (Fisher test ranging from 0.0393 to 0.00018) in both AMD patients groups. **Conclusion:** Specific biological and statistical differences in gene expression profiles between control and AMD patients were identified but only in the presence of an environmental stimulus. These results support the concept of a dysregulation of environmentally related genetic pathways in the pathogenesis of AMD.

POSTER 62

The DNA sequence variation and age-related macular degeneration

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Age-related macular degeneration (AMD) is the leading cause of irreversible central visual loss in aged people. Although the pathogenesis of the disease has yet to be elucidated, various studies have indicated genetic contribution to the risk of AMD. This study is to investigate the possible association between the DNA sequence variation, e.g. single nucleotide polymorphism (SNP, and AMD by candidate gene approach in case-control design. One hundred eighteen advanced AMD case and 40 pathologically diagnosed AMD slides were included. The control is composed of 192 subjects in older age and 194 subjects in younger age. Nineteen SNPs from 15 genes have been analyzed with PCR-restrictive fragment length polymorphism or Taqman technique. The genes selected function as DNA repair, immunological molecule, oxidative stress, extracellular matrix components and lipid metabolism. The SNPs selected are located either in coding region as nonsynonymous or in regulatory region. So far, we have identified 3 SNPs which are associated with AMD. Among those, 2 SNPs are nonsynonymous at amino acid position of 249 and 280 of CX3CR1, a CC chemokine receptor. The CX3CR1 association is in a gene dose response manner with the highest odds ration of 3.66. Another SNP is located in the promoter region of CSB, a gene functioning in DNA repair.

POSTER 63

AG13764 and AG13711 Reverses VEGF-Induced Choroidal Neovascularization in Rat Eye

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Purpose. Age-related macular degeneration (AMD) is the major cause of blindness for people over 60. In the wet form of AMD, VEGF is a major stimulator of choroidal neovascularization (CNV) in the posterior pole of the eye. We used a model previously developed in the lab to examine the efficacy of AG13764 and AG13711 (Pfizer, antiangiogenic molecules) to *reverse* VEGF–induced CNV. **Methods.** These compounds were injected intraperitoneally or intraocularly over a two week period starting at 6 weeks after AAV-VEGF injection. FITC-dextran whole-mounts of RPE-choroid-sclera were prepared after the animals were sacrificed. CNV area was quantified using Neurolucida. **Results.** In 12/16 animals, the level of CNV was reduced by 16% to 100%, following intravitreal injections of AG13764. In 11/14 animals, the level of CNV was also reduced by 16% to 100%, following intravitreal injection of AG13711. In two groups of IP injected animals (5 per group), AG13764 vs control, the mean CNV level was reduced by 35% ($p < 0.04$) in the treated eye. This reduction in CNV area is an underestimate since it does not reflect changes in volume. **Conclusion.** These data indicate that both Pfizer compounds reduce blood vessel proliferation in our AAV-VEGF model of CNV.

POSTER 64

RS-1 Gene Delivery To An Adult RS1b Knockout Mouse Model Restores ERG B-Wave With Reversal Of The Electronegative Waveform Of X-Linked Retinoschisis

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Purpose: To create and evaluate a mouse model of human X-linked juvenile retinoschisis (XLRS) and investigate whether supplementing with retinoschisin protein by gene delivery can reverse the abnormal "electronegative" retinal function. **Method:** An X-linked retinoschisis mouse (*Rs1h*-KO) model was created by conventional gene targeting method. RS protein was evaluated by immunohistochemistry and Western blot analysis. RS protein supplementation therapy was conducted using AAV-mediated gene delivery system, and Retinal function was evaluated by ERG recordings. **Results:** No RS protein was detected by Western blot analysis or immunohistochemistry in the *Rs1h*-KO mouse. Dark-adapted ERG responses showed an "electronegative" configuration. Histological examination of *Rs1h*-KO mice showed disorganization of multiple retinal layers. After intraocular administration of AAV(2/2)-CMV-*Rs1h*, immunohistochemistry showed retinoschisin expression in all retinal layers of *Rs1h*$^{/Y}$ mice, and ERG recordings showed reversal of the "electronegative" waveform. **Conclusion:** The *RS*-KO mouse mimics structural features of human X-linked juvenile retinoschisis. The *Rs1h*-KO functional deficit results in an "electronegative" ERG waveform that is highly characteristic of human retinoschisis disease. Replacement therapy by supplementing a normal *Rs1h* protein in the adult *Rs1h*-KO mouse restored the normal ERG configuration.

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