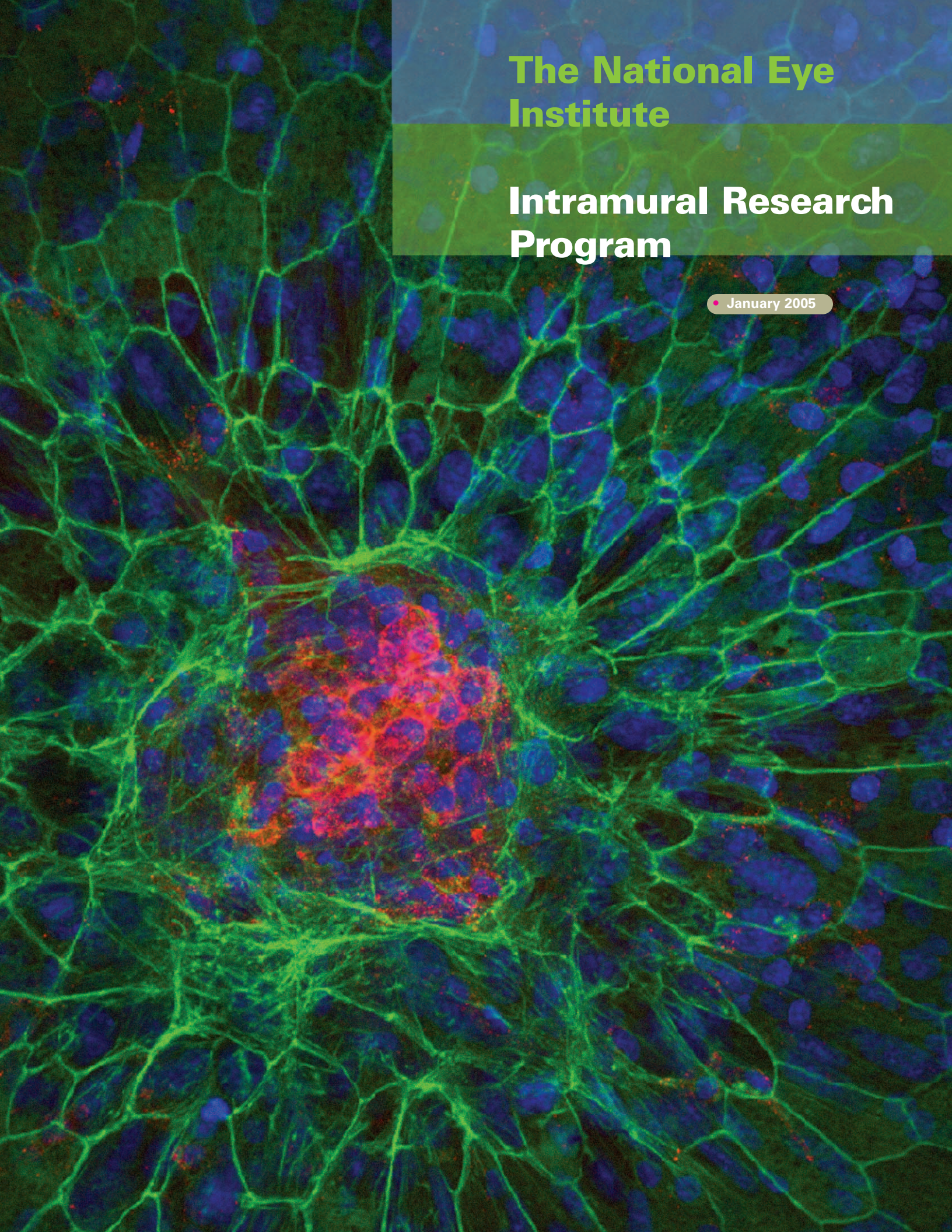


The National Eye Institute

Intramural Research Program

January 2005

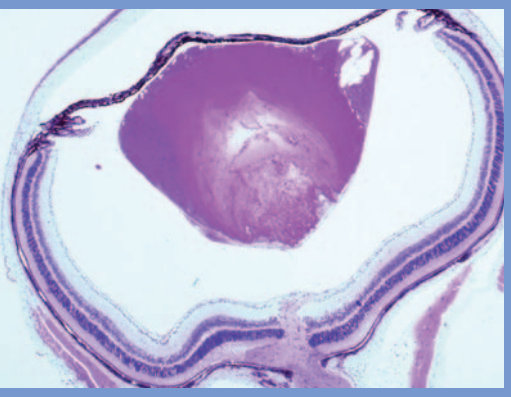

NATIONAL INSTITUTES OF HEALTH

National Institutes of Health
A World Resource

Mission

The mission of the National Institutes of Health (NIH) is to uncover new scientific knowledge that will lead to better health for everyone by conducting research in its own laboratories (Intramural Research Program) and by providing support for research conducted by scientists in universities, medical schools, hospitals, and other research institutions throughout the US and abroad (Extramural Research Program). An important part of the NIH mission is to train research investigators and foster the communication of medical information worldwide.

The NIH comprises 27 Institutes and Centers situated in 75 buildings on 322 acres in Bethesda, Maryland and in several other locations in the United States. There are approximately 7,000 researchers on the NIH campus, housed in 1246 intramural research laboratories and clinical branches. In addition to the senior investigators, there are more than 5000 scientists at the PhD/MD level and a growing population of graduate students (almost 300, currently). Information about the NIH and its various programs can be obtained from the website *http://www.nih.gov.*



For more information about the
NEI Intramural Program contact:
Belinda Davis
NEI, Office of the Scientific Director
Building 31, Room 6a22
31 Center Drive, MSC 2510
Bethesda, Maryland 20892 2510
Email: davisb@nei.nih.gov
Website: http://www.nei.nih.gov

National Eye Institute

NEI

Combating Visual Impairment and Blindess

The National Eye Institute (NEI) was established by Congress in 1968 to protect and prolong the vision of the American people. Thirty eight million Americans suffer significant vision impairment, costing an estimated $60 billion annually. This public health burden is expected to increase 40% by 2020, especially in the population of Americans over age 40.

Impaired vision and blindness is a world-wide health burden. The World Health Organization (WHO) estimates that there are greater than 160 million people worldwide who are visually disabled. Of these, approximately 40 million persons are blind and, by definition, cannot walk about unaided. Blindness represents a public health, social and economic problem, especially for developing countries, where 9 out of 10 of the world's blind population live. Approximately 60% of this population resides in sub-Saharan Africa, China and India (Global data on visual impairment in the year 2002. Serge Resnikoff, et al., Bulletin of the World Health Organization, November 2004, 844 851).

The largest proportion of blindness is related to aging. Approximately 50% of the world's blind suffer from cataracts. The majority of the remaining visually impaired individuals are blind from conditions that include glaucoma, age-related macular degeneration, corneal opacities, diabetic retinopathy, trachoma, onchocerciasis (also known as river blindness) and conditions that cause childhood blindness. Glaucoma is the second leading cause of blindness globally, followed by age-related macular degeneration.

NEI intramural and extramural supported research has advanced our knowledge of how the visual system functions in health and disease. In its extramural program, NEI supports approximately 1600 vision research grants and training awards to scientists at more than 250 medical centers, hospitals, universities, and other institutions across the US and around the world. For the inherited retinal degenerative diseases alone, more than 100 genes have been cloned and disease-causing mutations identified. This remarkable collection of genetic information highlights that significant inroads are being made into understanding the genetics of human ophthalmic diseases. Gene-based therapies are on the horizon to alleviate or circumvent errors caused by genetic mutations. Novel genetic treatments will soon be available for diseases that were once considered intractable.

NEI Intramural Research Program

The NEI Intramural Research Program is located on the Bethesda, Maryland campus of the National Institutes of Health. A major focus of the program is basic and clinical research on retinal diseases. Scientists are currently being recruited in genetics, vascular biology, cell and developmental biology and mechanisms of retinal neurodegeneration. In this stimulating environment, researchers can develop world-class multidisciplinary programs of vision research. With a greater understanding of the molecular basis of diseases, the possibility of developing successful prevention and treatment strategies of ocular diseases is greatly enhanced.

Currently, there are approximately 120 researchers (senior investigators, post-docs and other scientific staff) in the NEI intramural program involved in research in a variety of scientific disciplines including:

- Immunology
- Molecular and Developmental Biology
- Retinal Cell and Molecular Biology
- Systems Neuroscience
- Genetics and Visual Function
- Epidemiology and Clinical Trials

The NEI Intramural Program also maintains several core facilities and resources that include transgenic/knockout rodent facilities, histopathology services, an imaging core, and the NEI Eye Bank, *http://neibank.nei.nih.gov*. For more information about the NEI and its programs visit the website *http://www.nei.nih.gov*.

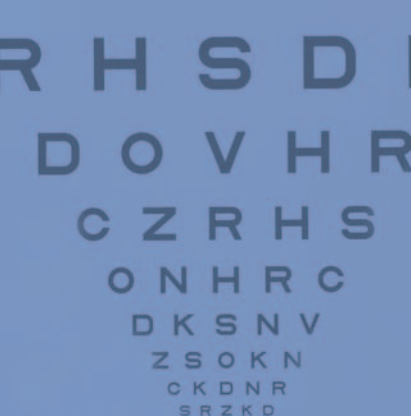
The NEI has done much to promote healthy vision and pioneering advances are currently being made in the exploration of gene replacement therapy for recessive disease, gene suppression for dominant disease, pharmaceutical and nutritional therapies, cell transplantation, stem cell work, and the development of retinal and cortical prosthetic chips. The human genome project has provided unparalleled opportunities for identifying those individuals with inherited blinding disorders who could potentially benefit from these new treatments. In response to 21st century biomedical opportunities, the NEI intramural program is committed to five goals:

- Establishing new basic and clinical research opportunities in genetics, retinal neurodegenerative disease and retinal vascular biology
- Emphasizing multidisciplinary & translational research
- Leveraging resources through trans-NIH initiatives
- Creating global scientific partnerships
- Providing exceptional training opportunities

These goals have provided the driving force for a wide range of research efforts, including the following examples of intramural and combined intra- and extramural research activities and accomplishments.

Neurodegeneration, Multidisciplinary & Translational Research

- In 1997, the gene responsible for causing Leber congenital amaurosis (LCA), a severe childhood retinal degeneration, was cloned by NEI intramural scientists. By 1998, a knockout mouse was generated and the role of the LCA RPE65 gene in Vitamin A metabolism was established. Recently, a multi-institutional group of NEI -supported vision scientists used gene therapy to restore vision in a dog model that mimics human LCA caused by mutations in the RPE65 gene. Visual function in the dog model has persisted for 4 yrs following gene transfer. These investigators are currently preparing for a human phase I gene therapy trial for RPE65 LCA.
- In the early 1990s, NEI -supported scientists investigated the protective effects of survival-promoting agents on ischemia-induced retinal injury. The agents included brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), and basic fibroblast growth factor (bFGF). Using genetically engineered neurotrophic factor-producing cells, a phase I genetic therapeutic approach is currently underway at the NEI to deliver the gene product of CNTF by an intravitreal encapsulated cell implant in patients with retinitis pigmentosa.
- The NIH is taking a pivotal role in the area of multidisciplinary science with several major Roadmap Initiatives underway to spur discoveries that will ultimately benefit the public's health, see *http://nihroadmap.nih.gov/*. One related initiative is the Neuroscience Blueprint, which involves the creation of a toolkit of scientific resources that neuroscientists can use to advance research, see *http://neuroscienceblueprint.nih.gov/*. The NIH is investing significant resources over the next five years in this initiative. The NEI has an important role in this initiative since neuroscience is a major research area for the Institute.



At NEI, The Laboratory of Sensorimotor Research, one of the most prestigious neuroscience laboratories in the world, seeks to understand higher level brain function by focusing on visual and oculomotor systems in the primate brain.

- Most recently NEI has occupied laboratory space in the newly constructed Porter Neuroscience Research Center on the main NIH campus in Bethesda, Maryland. This building houses an integrated group of investigators from a diverse set of NIH Institutes, including the National Institute of Neurological Disorders and Stroke (NINDS), the National Institute of Mental Health (NIMH), and the National Institute on Deafness and Other Communication Disorders (NIDCD). The research interests of these investigators include basic neurophysiology, developmental neurobiology, neurodegenerative disease, tumor cell biology, and stem cell biology.

There are unparalleled opportunities for NEI investigators to develop strong interdisciplinary collaborations among a diverse group of independent investigators throughout the NIH.

Leveraging Resources

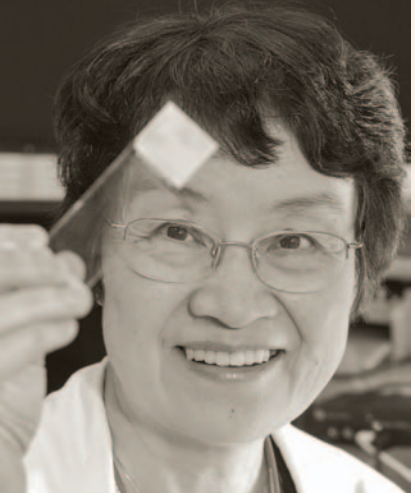
- The NEI is currently implementing a new initiative called the National Ophthalmic Disease Genotyping Network and Resource.™ The NEI will help to coordinate resources that are already available in the ophthalmology and vision community as well as aid in the building of a genetic information infrastructure. The goal of this Network is to enroll patients interested in participating in therapeutic clinical trials to prevent and treat genetic eye diseases. Accomplishing this goal will require diagnostic gene testing that will directly benefit patients and provide them with access to genetic counselors and an information clearinghouse. The benefits to clinicians and researchers include access to diagnostic genetic testing, centralized blood collection, processing and repository, standardization of phenotypic descriptors, and a shared database of genotype/phenotype information, which would allow for the analysis of larger datasets necessary to identify novel genetic risk factors for ocular diseases and eventually answers to pharmaco-genetic and epidemiologic questions of ocular disease.
- The NEI Division of Epidemiology and Clinical Research is actively involved in planning, developing, and conducting human population studies concerned with causation and prevention of eye disease and vision disorders with emphasis on the major causes of visual impairment. This includes studies of incidence and prevalence in defined populations, prospective and retrospective studies of risk factors, clinical trials, and genetic studies.

Global Partnerships & Exceptional Training Opportunities

- An ongoing international collaboration between the NEI and the Centre for Excellence in Molecular Biology (CEMB) is the International Genetic Collaboration on Consanguineous Families from Pakistan. This collaboration has yielded the identification of disease loci for both cataract and retinitis pigmentosa in 16 families. The disease-causing genes have already been identified in 5 of these families. Studies of 50 additional families are currently underway.
- The NEI is engaged in recruiting imaginative, dedicated post-doctoral fellows from the basic sciences to join a core of investigators focused on understanding the complexities of the visual system and its associated diseases. One approach to developing this new training and development paradigm is to attract the highest quality post-doctoral investigators from the increasing pool of scientific talent in developing countries. In this regard, the NEI is interested in establishing cooperative research training mechanisms with leading overseas graduate programs (NEI Overseas Scholars Program). The intent is to identify exceptionally talented individuals with clear promise of developing high impact, independent research careers and have them spend 2-4 years of post-doctoral training at the NIH in NEI intramural research laboratories.

NEI Cores and Facilities

- **NEI Histology Core**
- **NEIBank**
- **NEI Biological Imaging Core**
- **NEI Rodent Transgenic/Knockout and Veterinary Research and Resources Facility**
- **NIH Clinical Center**



NEI Histology Core

Chi-Chao Chan, MD
Head

The NEI Histology Core provides services for routine histology, transmission electron microscopy, cytology, and immunohistochemistry. This unit processed more than 33,000 specimens in the last four years. The majority of the samples are animal eyes submitted for methacrylate sections.

Techniques

Conventional Histology

- Methacrylate section
  2-3 microns, high quality and resolution, stains: H&E, PAS, toluidine blue
- Paraffin section
  6-7 microns, stains: H&E, PAS, and many other special stains
- Frozen section
  6-8 microns, low resolution, stains: H&E and immunohistochemistry, Recommended for quick surgical biopsies and molecular studies

Cytology

- Cytospin and stains:
  Giemsa and other stains including immunohistochemistry

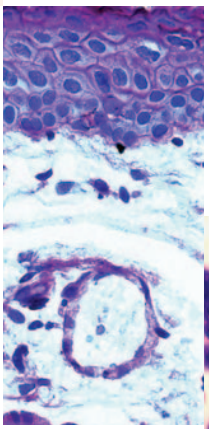
Immunohistochemistry

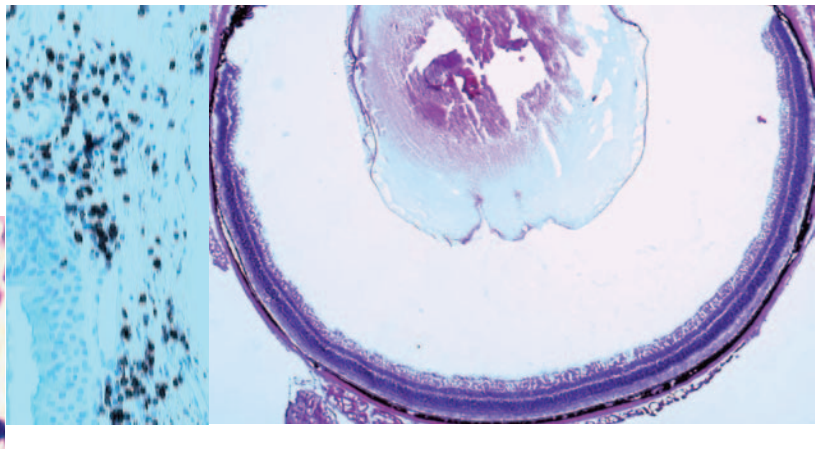
- Identifies expression of various specific antigens

Transmission Electron Microscopy

Selected Publications

1 A.M. Mansour, C.C. Chan, M.A. Crawford, Z.A. Tabbara, W.F. Haddad, H.I. Salti, N.G. Ghazi: Virus-induced chalazion. Eye 2004, in press.
2 C.C. Chan, Y.S. Lee, Z. Zhuang, J. Hackett, E.Y. Chew: von Hippel-Lindau (VHL) gene deletion and expression of hypoxia-inducible factor and ubiquitin in optic nerve hemangioma. Trans Am Ophthalmol Soc 2004, in press.
3 D. Avichezer, G.I. Liou, C.C. Chan, G.M. Lewis, B. Wiggert, L.A. Donoso, M.A. Crawford, R.R. Caspi: Interphotoreceptor retinoid-binding protein (IRBP)-deficient C57BL/6 mice have enhanced immunological and immunopathologic responses to IRBP and an altered recognition of IRBP epitopes. J Autoimmunity 21:185-194, 2003.
4 R.W. Hertle, C.C. Chan, D.A. Galita, M. Maybodi, M.A. Crawford: Neuroanatomy of the extraocular muscle tendon enthesis in macaque, normal human, and patients with congenital nystagmus. J AAPOS 6:319-327, 2002.





NEIBank

Research Interests

NEIBank is a project for ocular genomics that began as a response to a relative lack of available genomic data for genes expressed in eye tissues. It has evolved into a successful intra- and extramural collaborative effort that has produced significant resources for vision research. Initially, the major focus has been the creation and sequence analysis of high quality cDNA libraries made from freshly dissected tissues. The objective is to obtain full-length clones and a close representation of the transcriptional repertoire of the eye in humans and in important model species. This has proved to be valuable for gene discovery and for further functional genomics studies and provides wide opportunities for future developments.

Graeme Wistow, PhD
Head

Selected Publications

1 Ida, H., S.A. Boylan, et al., 2004. "EST analysis of mouse retina and RPE/choroid cDNA libraries." Mol Vis 10: 439-44.
2 Pompeia, C., B. Hurle, et al., 2004. "Gene expression profile of the mouse organ of Corti at the onset of hearing." Genomics 83(6): 1000-11.
3 Tomarev, S. I., G. Wistow, et al., 2003. "Gene expression profile of the human trabecular meshwork: NEIBank sequence tag analysis." Invest Ophthalmol Vis Sci 44(6): 2588-96.
4 Wistow, G., 2002. "A project for ocular bioinformatics: NEIBank." Mol Vis 8: 161-3.

Summary

High throughput sequencing is performed at the NIH Intramural Sequencing Center (NISC), of which the NEI was a founding member. Data are analyzed and assembled inhouse using a custom bioinformatics package called GRIST (GRouping and Identification of Sequence Tags). The results are displayed on the web site: *http://neibank.nei.nih.gov.* Clones are freely available through OpenBiosystems and can be ordered through the web site. In addition, data from eye libraries produced by other groups are collected and displayed on the NEIBank web site. An eye specific genome browser, EyeBrowse, has been created that displays known eye-expressed genes aligned on the human, mouse and rat genomes *(http://eyebrowse.cit.nih.gov/genome/)*. NEIBank also has fruitful trans-institute collaboration with investigators in NIDCD on the tissues of the inner ear.

Non-redundant sets of human and mouse eye-expressed cDNAs from NEIBank libraries have been selected and printed as microarrays. In addition, rabbit sequences have been used in a recent collaboration with groups at the University of Florida to create oligomer-based arrays, particularly for studies of rabbit cornea.

Several major libraries have been studied or are under current investigation.

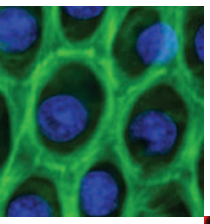
- Human Libraries: Lens, fetal lens, retina, RPE/choroid, iris, trabecular meshwork, lacrimal gland keratoconus cornea, pterygium, ocular pericytes.
- Rodent Libraries: Mouse whole eye, mouse retina, mouse RPE/choroid, mouse lacrimal gland, rat whole eye, rat retina, rat iridio-corneal angle, mouse lens yeast 2-hybrid, mouse retina yeast 2-hybrid.
- Other Species: Dog lens, dog cornea, dog rest-of-eye, rabbit cornea, rabbit rest-of-eye, zebrafish whole eye, zebrafish lens, zebrafish retina, zebrafish anterior segment, zebrafish RPE/choroid, embryonic chicken eye, adult chicken eye, guinea pig lens, guinea pig retina, guinea pig rest-of-eye.

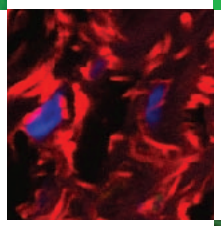
NEI Biological Imaging Core

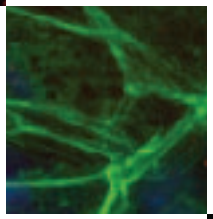
Interests

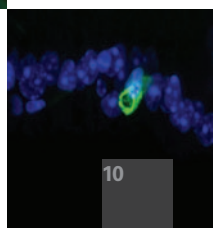
The NEI Biological Imaging Core provides a variety of high resolution imaging applications for vision research, including confocal microscopy, transmission electron microscopy, calcium imaging, and advanced image analysis. The facility provides access and training for a Leica SP2 laser scanning confocal microscope with 4 lasers (UV-argon, krypton-argon, krypton, and red HeNe). Live cell imaging capabilities permit time-lapse in vitro studies of GFP-transfected cells and explants from GFP-transgenic animals. Leica confocal software permits FRET (fluorescence resonance energy transfer) assays for evaluating protein-protein interactions, as well as FRAP (fluorescence recovery after photobleaching) for evaluating diffusion kinetics within living cells. Complex cellular structures within 3 dimensional confocal data sets can be quantified using Improvision Volocity software running on a dedicated image processing workstation. Fast ratiometric measurement of Ca+ signals in live cells can be conducted using a Hamamatsu Orca ER CCD camera and Lambda DG4 filter switcher. A JEOL 100CX transmission electron microscope is available for TEM applications. A Leica automated freeze substitution apparatus can be used for preparing TEM samples for immunolabeling.

The facility is staffed by three full time personnel with expertise in ocular anatomy, sample preparation, image acquisition and analysis. Dr. Robert Fariss, an NEI staff scientist with 20 year of experience in high resolution imaging applications, oversees operation of the facility. Dr. Jen-Yue Tsai, a molecular biologist with expertise in the areas of angiogenesis and mouse genetics, provides assistance to intramural researchers interested in immunolabeling and in situ hybridization techniques. Dr. Mercedes Campos, a clinical pathologist and visiting fellow provides advice and technical support for immunolabeling studies.

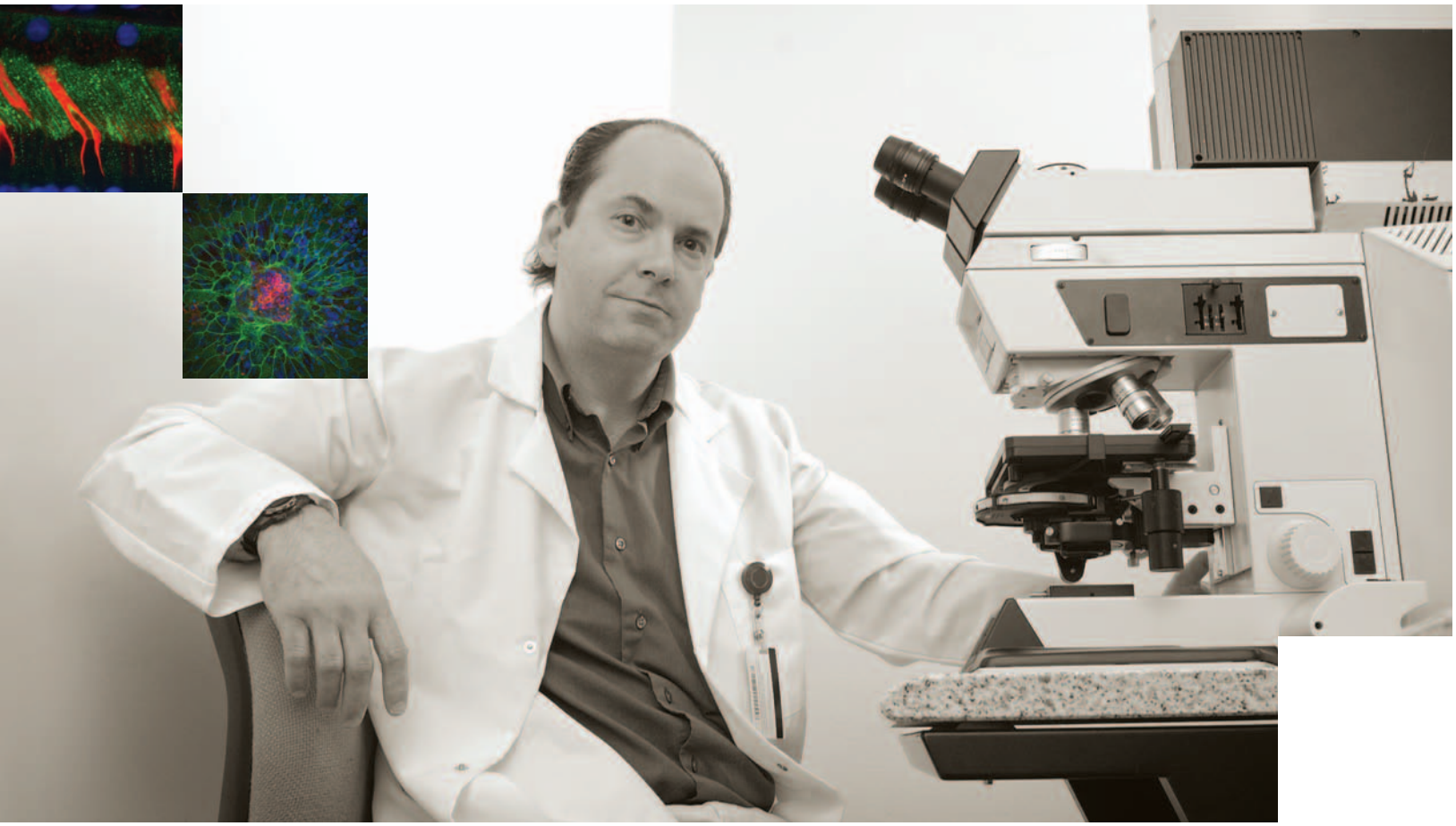


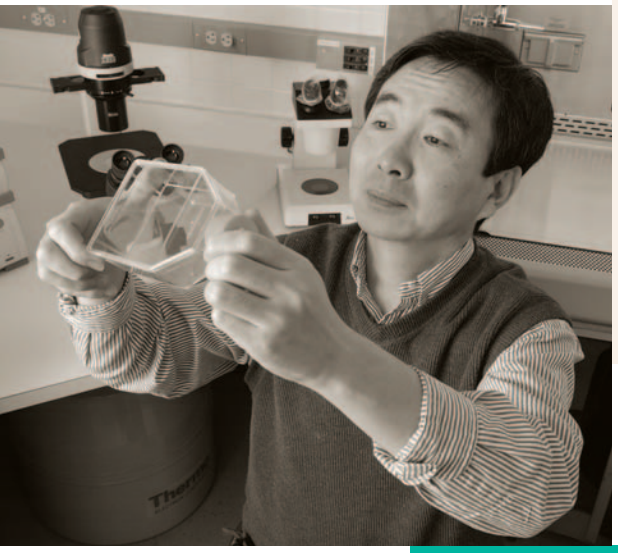






Robert Farris, PhD
Head





NEI Rodent Transgenic/Knockout and Veterinary Research and Resources Facility

Lijin Dong, PhD
Cohead

This newly established NEI facility has three components:

- gene targeting center
- transgenic animal facility
- veterinary research and resources section.

The goal of the gene targeting center is to adapt cutting edge technologies in functional genomics to perform experiments of gene knockout, knockin and gene knock down. Individual investigators from NEI and other participating institutes have access to the services and equipment provided in the center. One of the functional components is to assist in DNA engineering. ET recombination is used as the technology platform to modify large genomic clones such as BAC, P1 and YAC. Engineered genomic clones are then used for a variety of purposes such as transgenic production, gene targeting in embryonic stem (ES) cells, and cell transfection assays. Another functional component is ES cell manipulation for the purpose of gene targeting. Currently, this facility has the capability to perform gene knockout, gene knockin, and conditional allele construction in mouse ES cells. In addition, the center is actively exploring siRNA technology; our immediate goal is to make the process of siRNA sequence design, vector choice, and testing of the targeting sequence streamlined for NEI researchers. The Gene Targeting Center at NEI is currently led by Dr. Lijin Dong who recently joined NEI with extensive expertise in mammalian genetics and ES cell biology.

The NEI transgenic animal facility provides support for all NEI intramural researchers requiring the use of transgenic mice in their research programs. Our program has handled approximately 318 DNA constructs, which are at various stages of completion. DNA constructs are submitted, transgenic mice are then created by standard procedures, biopsied, and transgene positive mice identified. Facility personnel mate positive transgenic mice, wean litters, biopsy and analyze DNA from successive generations of transgenic mice, provide the transgenic animals to researchers for use in their experiments, and cryopreserve and bank embryos from important mouse lines (both transgenic and naturally occurring) for long term storage. For generating gene knockout mice, ES cell injections into blastocysts are performed. Facility staff also help researchers design transgenic projects and transgene/knockout vectors on a collaborative basis.

Eric Wawrousek, PhD
Cohead

Jim Raber, PhD, DMV
Cohead

In 2004 we have:

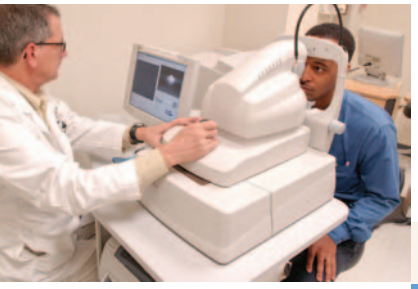
- Accepted 12 new constructs for transgenic mouse production, including 2 BAC constructs
- Generated 84 transgenic founder mice, including 7 containing BAC inserts
- Set up 414 matings of transgenic mice
- Weaned, tagged and tail biopsied more than 3,300 mice
- Isolated DNA from more than 6,000 samples
- Performed more than 8,000 PCR analyses
- Cryopreserved 5,000 embryos from 13 lines of mice.

The NEI veterinary research and resources section is responsible for the facilitation of all animal related activities within the Intramural Research program. The program is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and provides services to the NEI Intramural Research Program, as well as five other institutes at NIH. The section has a commitment to programs of excellence in animal related research, veterinary care and services, animal husbandry, technical support and program administration. Support to NEI investigators includes:

- Assistance with animal study proposal development and execution
- Veterinary services
- Assistance with animal procurement, importation and exportation
- Training

The section also provides a wide range of services designed to assist NEI investigators in the tracking and management of their animal colonies. Services include breeding consultation, technical support, genetic screening, development of genetically modified rat models, embryo rederivation, and cryopreservation of sperm, embryos, and ovaries. In addition, the section conducts research in support of the development of new animal models and refinement of current methodologies. Future services will include the development of an ocular phenotyping program.

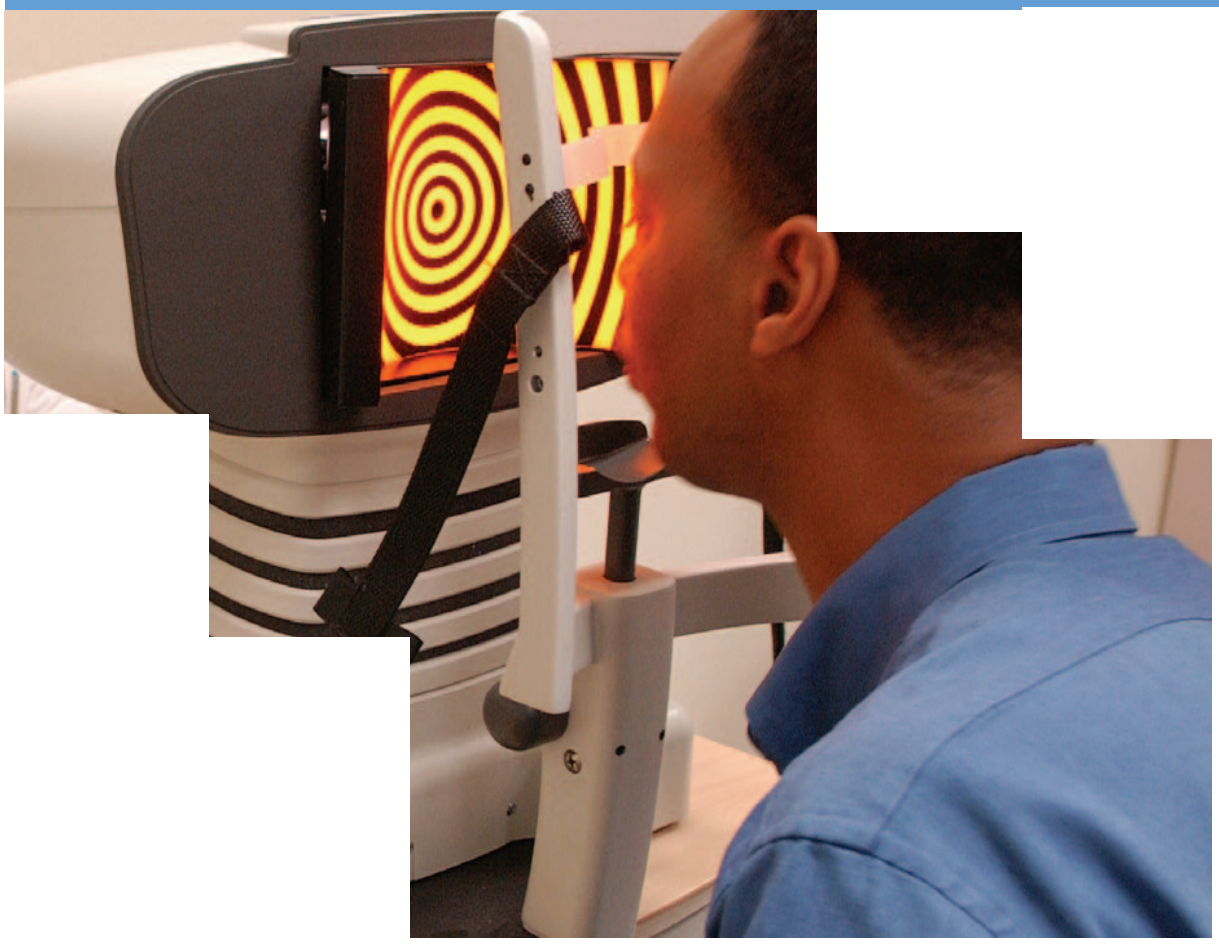
NIH Clinical Center



Frederick Ferris III, MD
Clinical Director

Mission Statement

The NIH Mark O. Hatfield Clinical Research Center is the world's largest hospital devoted exclusively to clinical research. The Center has 267 inpatient beds and 15 outpatient clinics, manages 7,000 inpatient admissions yearly, and provides state-of-the-art diagnostic, treatment, surgical, and research facilities to 1,200 credentialed clinicians, dentists and PhD researchers and clinical experts across many disciplines. The Clinical Center provides care only for patients participating in research protocols; non-research patients are not admitted to the Center. This creates an environment that is completely oriented toward clinical research and the development of translational research skills.



NEI Intramural Clinical Research

The NEI supports a number of clinically-oriented research programs at the Clinical Center/NIH campus facilities. Scientists perform research in numerous areas including angiogenesis, genetic, genomic and proteomic expression, sensory/motor coordination, and immunology and infectious diseases. A complete description of NEI's intramural scientific portfolio can be found at *http://www.nei.nih.gov/intramural/*. Specific NEI clinical trials being conducted at the Mark O. Hatfield Clinical Research Center in Bethesda, Maryland can be found at *http://clinicalstudies.info.nih.gov/cgi/protinstitute.cgi?nei.0.html* and include among others: imaging/diagnostic studies on corneal dry eye, diabetic macular edema, cataract, and vision motor disorders; nutritional, pharmacologic, and/or gene therapies for retinal vascular and genetically-inherited ocular diseases; and laser approaches and supplemental methodologies for neovascularization in age-related macular degeneration.

The NEI clinical program is centrally managed by the Office of the Clinical Director *http://www.nei.nih.gov/intramural/cbranch.asp* which consists of a state-of-the-art outpatient facility and a multidisciplinary team of ophthalmic imagers, technicians, and research coordinators, all highly skilled and trained to support clinical research. The outpatient facility sees more than 9000 outpatient-study visits annually, and over half of them are inter-institute consultations & collaborations with investigators from a wide variety of clinical programs throughout NIH. These consultations and collaborations provide an unique mix of rare pathologies such as: neurocysticercosis (NIAID), graft versus host disease with ocular involvement (NHLBI), and Wegener's granulomatosis (NIAID).

The Office of the Clinical Director also offers assistance and advice on protocol development, regulatory compliance issues, data management, and quality assurance to ensure that clinical studies are conducted safely and within regulatory requirements.

Investigators

Pat Becerra, PhD
Deborah Carper, PhD
Rachel R. Caspi, PhD
Chi-Chao Chan, PhD
Emily Y. Chew, MD
Mary Frances Cotch, PhD
Karl Csaky, MD, PhD
Bruce Cumming, MD, PhD
Charles E. Egwuagu, MPH, PhD
Leon B. Ellwein, PhD
Frederick Ferris, MD
Edmond J FitzGibbon, MD
Donita Garland, PhD
Igal Gery PhD
J. Fielding Hejtmancik, MD, PhD
Okihide Hikosaka, MD, PhD
John Hooks, PhD
Frederick A. Miles, D.Phil
Sheldon Miller, PhD
Robert Nussenblatt, MD
Lance Optican, PhD
Joram Piatigorsky, PhD
Jack Ragheb, MD, PhD
T. Michael Redmond, PhD
George F. Reed, PhD
Ignacio R. Rodriguez, PhD
Paul Russell, PhD
Paul Sieving, MD, PhD
Kirk G. Thompson, PhD
Stanislav Tomarev, PhD
Eric Wawrousek, PhD
Barbara N. Wiggert, PhD
Graeme Wistow, PhD
Robert H. Wurtz, PhD
Peggy S. Zelenka, PhD
J. Samuel Zigler, Jr., PhD

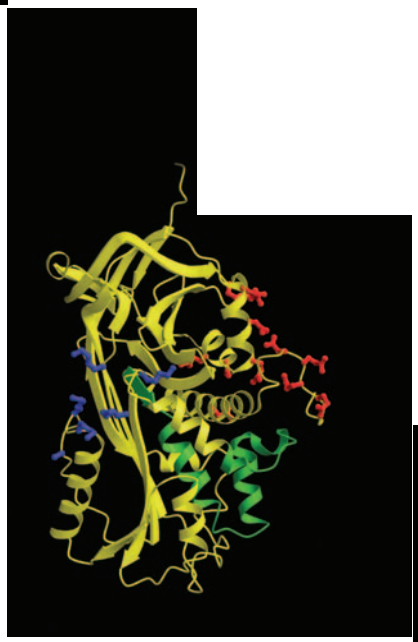
Protein Structure and Function Section

Pat Becerra, PhD

Research Interests

The interests of this section are in the area of protein structure as it relates to function, with a focus on interactions of components involved in cell differentiation and survival. Our research at NEI has applied these interests to systems in the retina.

We have been studying pigment epithelium-derived factor (PEDF), a protein that acts in neuronal differentiation and survival in the retina and CNS. PEDF inhibits angiogenesis and its expression is down-regulated with aging. This interesting factor is secreted by retinal pigment epithelial cells into the interphotoreceptor matrix, where it acts on photoreceptor cells. Its importance in the development, maintenance, zand function of the retina and CNS is evident in animal models for inherited and light-induced retinal degeneration, as well as for degeneration of spinal cord motor neurons, and for ocular neovascularization.



1 The serpin PEDF. Human recombinant PEDF structures showing putative collagen binding regions (red), glycosainoglycan binding region (blue) and neurotroohic active region (green). P2 is in the serpin exposed loop.
2 The second structure is about 180° of the first.
3 Human PEDF structure

Selected Publications

1 Becerra, S.P., Fariss, R.N., Wu, Y.Q., Montuenga, L.M., Wong, P., Pfeffer, B.A.: Pigment epithelium-derived factor in the monkey retinal pigment epithelium and interphotoreceptor matrix: apical secretion and distribution. Exp Eye Res. 2004 Feb;78(2):223-34.

2 Alberdi, E.M., Weldon, J.E., Becerra, S.P.: Glycosaminoglycans in human retinoblastoma cells: Heparan sulfate, a modulator of the pigment epithelium-derived factor-receptor interactions. BMC Biochemistry 4:1, 2003, 19 February 2003.

3 Meyer, C., Notari, L., and Becerra, S.P.: Mapping the type I collagen-binding site on pigment epithelium-derived factor. Implications for its antiangiogenic activity. J. Biol Chem. 277, 45400-45407, 2002.

4 Aymerich, M.S., Alberdi, E., Martinez, A., and Becerra, S.P.: Evidence for pigment epithelium-derived factor (PEDF) receptors in the neural retina. Invest. Ophthalmol. Vis. Sci. 42, 3287-3293, 2001

5 Alberdi, E., Aymerich, M.S., and Becerra, S.P.: Binding of pigment epithelium-derived factor (PEDF) to retinoblastoma and cerebellar granule cells: Evidence for a PEDF receptor J. Biol. Chem. 274, 31605-31612, 1999

Research Summary

Much of our progress in understanding PEDF has relied on our development of overexpression systems that yielded recombinant proteins as functionally active neurotrophic factors identical to the native protein and ideal for biochemical, biophysical, and biological studies. The cDNA sequence for PEDF predicts a unique protein with strong homology to members of the serine protease inhibitor (serpin) superfamily. Our studies on PEDF as a serpin have established that it belongs with the subgroup of noninhibitory serpins acting like substrates rather than inhibitors of serine proteases. Moreover, a region from its amino-terminus confers neurotrophic properties to the PEDF polypeptide, while its serpin reactive loop is dispensable. Thus, a mechanism independent of serine protease inhibition must mediate PEDF's neurotrophic activity. During evolution, this serpin might have lost its inhibitory activity and gained neurotrophic function. Our findings provided an example of the separation of inhibitory and other activities in a serpin.

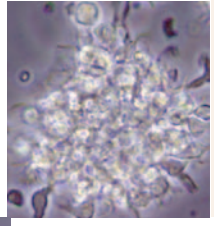
Our investigations were next directed toward the hypothesis that PEDF's neurotrophic activity is mediated by direct interactions with cell surfaces. Focusing on retinoblastoma and cerebellar granule cells, we prospected for PEDF receptors and found evidence for 1) a saturable, specific, and high-affinity class of receptors on the surface of both cells, and 2) the amino-terminal region in PEDF that interacts with the receptor. Our work demonstrated that the first step in the biological activity of PEDF is the binding to cell surface receptors, a significant advance in the elucidation of PEDF's mechanism of action.

We also investigated the association of PEDF with extracellular matrixes (ECM) and found that PEDF can interact with glycosaminoglycans and collagens. In the folded PEDF protein, the binding sites for these two ECM components are distinct and separated from each other, from the binding site for the receptor and from the serpin exposed loop. Our functional studies showed that glycosaminoglycans can act as positive modulators of the PEDF-receptor interactions. These findings are significant because association with ECMs can regulate PEDF's spatial and temporal localization and/or activities.

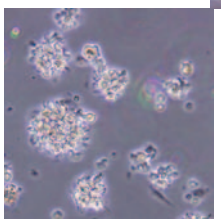
We hope our PEDF research lays the groundwork for the development of therapies for diseases involving defective neuronal differentiation or cell survival, such as retinitis pigmentosa, age-related macular degeneration, and amyotrophic lateral sclerosis, as well as for diseases in which new blood vessel formation plays a role, such as diabetic retinopathy, age-related macular degeneration, tumor growth, and rheumatoid arthritis.

The role of a cell-surface receptor in the mechanisms of action of PEDF represents a key aspect of regulation. Along these lines, our first priority is the identification of the PEDF receptor protein. Consistent with our goal of elucidating the mechanisms of action, we are interested in signal transduction and the expression of genes affected by PEDF's stimuli. We are also exploring the development of sustained delivery systems for PEDF in animal models of retinal and CNS diseases.

1



2



Immunoregulation Section

Rachel R. Caspi, PhD

Research Topics Currently Under Study Include

- **Central vs. peripheral mechanisms in tolerance to retinal antigens**
- **Pathogenesis of EAU - the role of innate immune responses**
- **Therapy of EAU - Antigen-specific and nonspecific approaches to therapy**
- **EAU model in HLA transgenic mice**
- **Analysis of genes associated with EAU susceptibility and resistance**

Research Interests

The interest of the Section on Immunoregulation centers on tolerance and immunity to retinal antigens. We want to understand how tolerance is achieved by natural mechanisms, what triggers its breakdown, the mechanisms involved in disease pathogenesis, and finally, how to restore functional tolerance by directed immunotherapy. Many of our approaches and conclusions are generalizable to other tissue-specific autoimmune diseases which have underlying immunopathogenic pathways that share common mechanisms with uveitis.

Cells Dendritic isolated and cultured by Jun Tang
1 DC 6=32X
2 DC 6=10X

Research Summary

- We demonstrated directly, using IRBP deficient mice and thymus transplantation, that central (thymus dependent) tolerance sets the threshold of reactivity to retinal antigen. Namely, EAU susceptible mice express trace amounts of IRBP in their thymus, detectable only at the single-cell level, which are nevertheless functionally relevant and serve to extinguish responses to IRBP epitopes that would otherwise be pathogenic. Furthermore, we showed that thymus-dependent "natural" CD4+CD25+ regulatory T cells have control of autoimmunity to retinal antigens, an unexpected finding, since the eye can elicit its own highly specialized regulatory circuits. Current work addresses the question whether the intact eye can elicit peripheral tolerance by using mice that have an identical thymus, but differ in their IRBP expression in the eye.
- We demonstrated that innate immunity can promote, or prevent, EAU. Pertussis toxin (PT), an adjuvant that enhances autoimmune diseases, aborts EAU if given during the time of effector cell migration, by inhibiting chemokine receptor signaling. The inhibitory activity was localized to the A-subunit of PT, and the enhancing activity to the B-subunit, that was subsequently shown to bind to TLR4 on dendritic cells. In contrast, the related cholera toxin, that targets Gs rather than Gi proteins, was shown to protect from EAU by eliciting innate IL-4 and directing the response to a nonpathogenic phenotype. Current work is aimed at defining the role of specific Toll receptors and the MyD88 pathway in EAU susceptibility, and at dissecting the role of dendritic cells in induction and regulation of EAU.
- As an immunologically privileged organ, the eye is sequestered from the immune system. This may hinder peripheral tolerance to retinal antigens. We are exploring induced expression of retinal antigens in the periphery as a potential approach to therapy. Cellular gene therapy with autologous B cells retrovirally transduced with an immunoglobulin-antigen fusion construct, protects susceptible B10.RIII mice from EAU, as does DNA vaccination with a plasmid encoding the first homologous repeat of IRBP. Other immunomodulatory protocols under study are tolerance induction using altered peptide ligands (APL) or costimulatory molecule blockade (e.g., B7, CD40). Another approach under development employs antigen- MHC class II multimers as a means to detect and modulate the antigen-specific T cells.
- We developed a "humanized" model of EAU in HLA class II transgenic mice. Importantly, HLA -DR3 transgenic mice develop severe disease with retinal S-Ag (which is thought to be involved in human uveitis, but does not elicit EAU in wild type mice) and respond to an S-Ag epitope recognized by uveitis patients. These findings validate the EAU model induced with retinal antigens as a model of human uveitis and provide a platform to identify pathogenic epitopes presented by human MHC molecules, as an approach to development of antigen-specific immunotherapies. Current studies are aimed at elucidating the uveitogenic epitopes connected to particular HLA class II haplotypes, and at defining the interactions between HLA class II alleles in determining susceptibility.
- We have defined 4 chromosomal regions (quantitative trait loci = QTL) associated with EAU susceptibility in rats, by analyzing the F2 progeny of susceptible Lewis and resistant F344 strains. We are now breeding congenic strains, isolating the regions of interest on the reciprocal background. We are at the same time characterizing the genes associated with susceptibility and resistance using microarray technology in the parental strains and in the congenic lines. A number of genes, many located within the QTL regions, are showing differential expression in the susceptible vs. the resistant strain. Current work is aimed at validating these genes by protein expression data and use of gene-manipulated mice which lack or overexpress the genes of interest.

Selected References

1 Shao Bo Su, Phyllis B. Silver, Peng Wang, Chi-Chao Chan and Rachel R. Caspi. Cholera toxin prevents Th1-mediated autoimmune disease by enhancing Th2 polarization. J. Immunol., 173: 755–761, 2004.

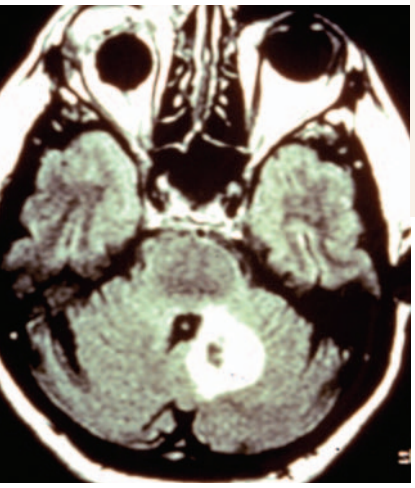
2 Su, S-B., P.B. Silver, P. Wang, C.C. Chan and R.R. Caspi. Dissociating the enhancing and inhibitory effects of pertussis toxin on autoimmune disease. J. Immunol 171:2314, 2003.

3 Avichezer, D., R.S. Grajewski, C.-C. Chan, M.J. Mattapallil, P.B. Silver, J.A. Raber, G.I. Liou, B. Wiggert, G.M. Lewis, L.A. Donoso, and R.R. Caspi. An immunologically privileged retinal antigen elicits tolerance: major role for central selection mechanisms J. Exp. Med., 198:1665-1676, 2003.

4 Pennesi, G., M.J. Mattapallil, S.H. Sun, D. Avichezer, P.B. Silver, Z. Karabekian, C.S. David, P.A. Hargrave, J.H. McDowell, W.C. Smith, B. Wiggert, L.A. Donoso, C.C. Chan and R.R. Caspi. A humanized model of experimental autoimmune uveitis in HLA class II-transgenic mice. J. Clin Invest. 111:1171-1180, 2003.

5 Silver, P.B., Hathcock, K.S., Chan, C.C., Wiggert, B. and Caspi, R.R. Costimulatory blockade inhibits a primary episode of experimental autoimmune uveoretinitis, but does not lead to long term tolerance. J. Immunol. 165:5401-5407, 2000.

6 Agarwal, R.K., Y. Kang, E. Zambidis, D.W. Scott, C.C. Chan and R.R. Caspi. Retroviral gene transfer of an IgG-antigen fusion construct protects from experimental autoimmune uveitis J. Clin. Invest. 106:245-252, 2000.

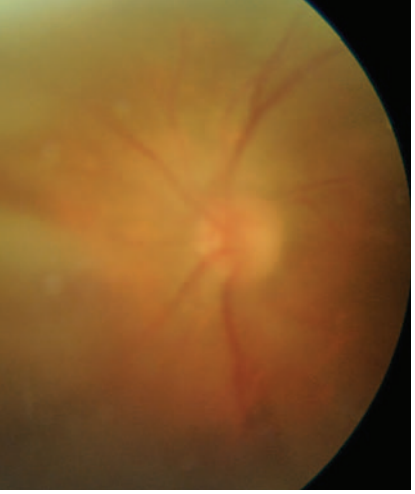


Immunopathology Section

Chi-Chao Chan, MD

Research Interests

Immunopathology is an important bridge between clinical and laboratory studies of human and experimental diseases. The main interests of the Immunopathology Section are molecular pathology and pathogenesis of ocular diseases with focus on primary intraocular lymphoma (PIOL) and age-related macular degeneration (AMD).



Selected Publications

1 C.C. Chan, J.A. Smith, D. Shen, R. Ursea, P. LeHoang, H.E. Grossniklaus: Helicobacter pylori (H. pylori) molecular signature in conjunctival mucosa-associated lymphoid tissue (MALT) lymphoma. Histol Histopathol 19:1219-1226, 2004.

2 J. Tuo, N. Tuaillon, D.F. Shen, C.C. Chan: Endotoxin-induced uveitis in cyclooxygenase 2 deficient mice. Invest Ophthalmol Vis Sci 45:2306-2313, 2004.

3 J. Tuo, B. Smith, C.M. Bojanowski, A.D. Meleth, I. Gery, K. Csaky, E.Y. Chew, C.C. Chan: The involvement of sequence variation and expression of CX3CR1 in the pathogenesis of age-related macular degeneration. FASEB J 18:1297-1299, 2004 (FASEB J express article on line, 6/18/2004).

4 J. Tuo, C.M. Bojanowski, C.C. Chan: Genetic factors of age-related macular degeneration. Prog Ret Eye Res 23:229-249, 2004.

5 C.C. Chan, D.F. Shen, J.J. Hackett, R.R. Buggage, N. Tuaillon: Expression of chemokine receptors, CXCR4 and CXCR5, and chemokines, BLC and SDF-1, in the eyes of patients with primary intraocular lymphoma. Ophthalmology 110:421-426, 2003.

6 C.C. Chan: Molecular pathology of primary intraocular lymphoma. Tranas Am Ophthalmol Soc 101: 269-286, 2003.

Research Summary

Primary Intraocular Lymphoma, PIOL

PIOL is a subset of primary CNS lymphoma. PIOL is mostly a diffuse large B cell non-Hodgkin's lymphoma that usually invades the retina, vitreous, or optic nerv with or without concomitant CNS involvement. Although PIOL remains a relatively rare malignancy, the incidence of this disease has trebled over the past 15 years. The disease is aggressive with a 5-year survival rate of less than 25%. PIOL is a masquerade syndrome whose clinical presentation closely resembles chronic uveitis. Early diagnosis and prompt treatment are the keys to reducing mortality due to this malignancy. Given its nonspecific presentation and aggressive course, PIOL provides a diagnostic and therapeutic challenge.

We are interested in improving diagnosis, understanding lymphomatogenesis, developing animal model, searching and providing optimal therapy. We identified immunoglobulin heavy chain gene rearrangement in PIOL cells. We also detected IL-10 and B-cell chemokine transcripts and expression in these cells. Some of these cells were also found to contain infectious molecular signatures, a finding that may offer insight into the pathogenesis of this malignancy.

Age-Related Macular Degeneration, AMD

AMD is a leading cause of blindness in the US and developed countries among people 65 years and older. Although the etiology and pathogenesis of AMD remain unknown, a complex interaction of genetic and environmental factors is thought to exist. The incidence and progression of AMD are known to increase significantly with age. Recently, studies have begun to provide evidence that genetic sequence variations may play important roles in the pre-symptomatic stages of AMD as well as in the specific pathogenesis of AMD. Single nucleotide polymorphism (SNP) analysis has become an attractive tool in the exploration of the genetic component of complex diseases such as AMD because these SNP s are easily visualized and typed, highly abundant, and extremely stable. Using a case control study design, we have found an association between CX3CR1 (a chemokine receptor) SNPs and AMD. Furthermore, lower CX3CR1 expression was found in the maculae of AMD patients and subjects that carried the variant allele type.

We are interested in analyzing further SNPs (e.g. of genes related to other retinal hereditary diseases, the oxidative stress pathway, and inflammatory responses) and their potential association with AMD. Once an association between AMD and the SNPs of certain specific genes has been established, we are interested in continuing our research by conducting functional studies and developing animal models. Hopefully, the data will elucidate the pathogenesis and genetic risk factors of AMD, which will in turn enable us to develop better prevention and treatment for this common complex disease.

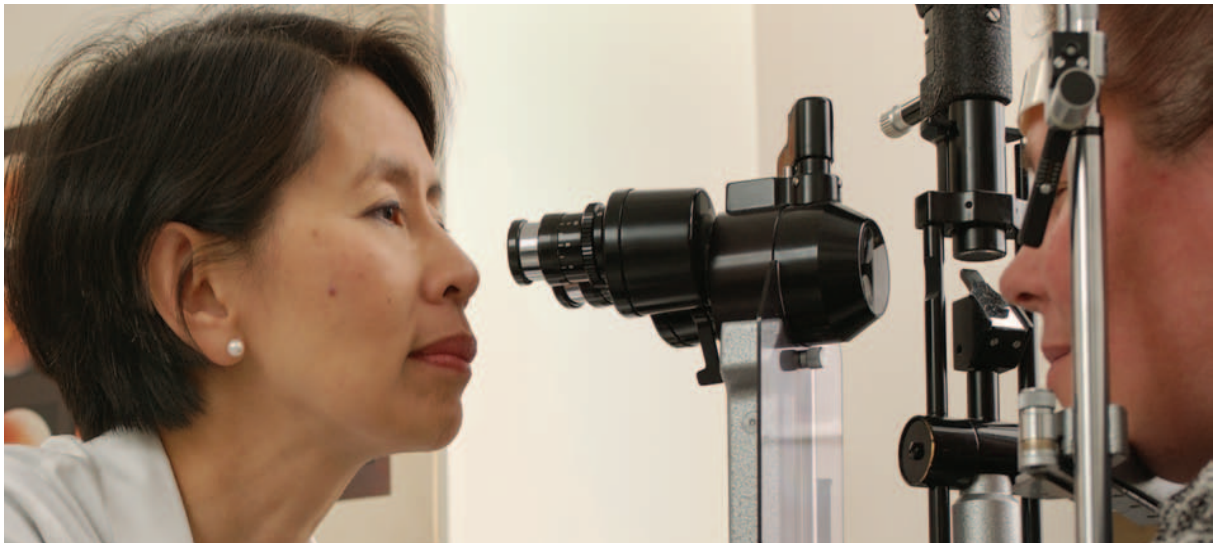
Clinical Trials Branch

Emily Y. Chew, MD

Research Summary

The mission of Clinical Trials Branch in the Division of Epidemiology and Clinical Research (DECR) is to conduct human population studies concerned with the cause, prevention and treatment of eye diseases with the major emphasis on the major causes of blindness.

The Clinical Trials Branch has 2 members, Drs. Emily Y. Chew and Frederick L. Ferris, however, these studies often involve the collaboration of the other two branches in the division. Biometry provides the expertise in statistical issues in the study designs and data analyses. Collaboration with the Epidemiology Branch is also important for the success of these studies.



The Clinical Trials Branch conducted a number of important clinical trials of treatment of diseases of major public health importance, including studies of diabetic retinopathy: The Diabetic Retinopathy Study (DRS), the Early Treatment Diabetic Retinopathy Study (ETDRS), and the Diabetes Control and Complications Trial. The Age-Related Eye Disease Study (AREDS) is a study of age-related macular degeneration and lens opacities. These studies have resulted in a number of important treatment strategies for the treatment of diabetic retinopathy and age-related macular degeneration that have become the standard of care. The Clinical Trials Branch continues to contribute to clinical trials methodology by collaborating with the extramural community on the diabetic retinopathy network and designing new clinical trials of age-related macular degeneration. In addition, a number of phase I/II trials are designed and conducted in the Clinical Center of NIH for these two major causes of vision loss.

Selected Publications

1 SanGiovanni JP. Chew EY. Long-chain polyunsaturated fatty acids in health and disease of the retina. Progress in Retinal and Eye Research 2005;24 :1, 87-138.

2 The Age-Related Eye Disease Study Group. Mortality in the Age-Related Eye Disease Study: Associations with ocular disorders and an intervention of high-dose antioxidants and zinc. AREDS Report No. 13. Arch Ophthalmol 2004: 122:716-726.

3 The Age-Related Eye Disease Study Group. Potential public health impact of the Age-Related Eye Disease Study Results. AREDS Report No. 11. Arch Ophthalmol 2003;121:1621-1624.

4 Chew EY, Ferris FL, Csaky KG, Murphy RP, Agron E, Thompson DJS, Reed GF, Schachat AP. Long-term effects of laser photocoagulation in patients with diabetic retinopathy: The Early Treatment Diabetic Retinopathy Follow-up Study. Ophthalmol 2003; 110: 1683-1689.

5 Chew EY, Klein ML, Ferris FL 3rd, Remaley NA, Murphy RP, Chantry K, Hoogwerf BJ, Miller D, the ETDRS Research Group. Association of elevated serum lipids with retinal hard exudate in diabetic retinopathy. ETDRS Report No. 22. Arch Ophthalmol 1996; 114: 1079-1084.

Epidemiology Branch

Mary Frances Cotch, PhD

Research Overview

The Epidemiology Branch (EB) plans, develops, and conducts human population studies concerned with causation and prevention of eye disease and vision disorders, with emphasis on the major causes of visual impairment, including: studies of incidence and prevalence in defined populations; prospective and retrospective studies of risk factors; genetic studies; and studies to evaluate diagnostic procedures. In addition to conducting research, the EB collaborates with other NEI programs and offices to further the Institute's efforts and activities in public awareness, policy and planning. Services include data retrieval, estimating rates of disease burden using national or geographically representative data systems, serving as a resource to link interested parties with sought after information or to facilitate partnerships, advising on study designs to answer specific questions of interest, critically evaluating data and research findings with respect to scientific validity and public health significance, and integrating information on ocular and systemic risk factors to galvanize the efforts of others to improve our collective understanding of ocular and visual health.

Refractive Error

Refractive errors are common conditions which, if undiagnosed or improperly corrected, account for a large proportion of the visual impairment experienced by the general public. Studies are underway to describe the distribution of refractive error, including the degree to which, among those visually impaired, optical correction could result in improvement of vision to normal acuity levels. Functional impairment attributable to vision loss and economic burden of refractive error are also of interest. The nature, extent, and genetic basis of specific refractive errors are under investigation in several on-going collaborative studies.

Age-related Retinal Disease

Retinal changes occur with age. Individuals with functional impairment as a result of these changes are more likely to come to clinical attention and therefore retinal pathology is ascribed. The timeframe over which retinal changes occur, as well as the nature and extent of these change among individuals without function impairment, is unclear. It is also not known whether changes occurring in the retina are correlated with those indicative of other, non-ocular, conditions or diseases. Differential expression in age-related retinal change may exist among culturally and ethnically diverse populations. Population-based and family-based studies are underway to address these issues. Genetic studies are on-going.

Vision Section

Bruce Cumming, MD, PhD

Research interests

Action potentials generated by neurons in the cerebral cortex eventually give rise to conscious sensations. To understand how this happens I study action potentials and sensation simultaneously in awake behaving monkeys. I use a simple perceptual system (stereopsis, the ability to perceive depth by combining images from the two eyes) which is sufficiently well understood that the goal of explaining our perceptions in terms of the activity of cortical neurons seems feasible:

- It seems likely that we will explain the exact neuronal mechanisms that generate signals related to binocular disparity in single neurons. This is especially true of neurons at the earliest cortical stage, known as V1. The last 10 years of quantitative modeling has successfully described how v1 neurons respond to a wide range of binocular stimuli. An ongoing experimental effort to test this model (by me and by others) has generated a growing body of evidence at odds with the original form of the model. New developments in the model, explaining such data, bring us ever closer to an exact mechanistic description of how these signals are generated in the brain.
- The psychophysical properties of stereopsis have been extensively studied in humans and monkeys. Many of these properties are not straightforwardly reflected in the activity of single neurons, at least in V1. One of my important contributions was to use our knowledge of the underlying neuronal mechanisms to devise a variety of stimuli that reliably altered the activity of disparity selective neurons without producing the corresponding depth sensation. By dissociating the activity of early cortical neurons from visual perception we gain importantinsights into the subsequent neural processing that is required from other parts of the brain. At the same time, we can identify what properties of perceptual experience are constrained by this early processing.
- Neurons in subsequent brain areas ("extrastriate" cortex) are more closely linked to the perception of stereoscopic depth than V1 neurons. Small groups of cortical neurons can be artificially activated by passing current out of recording electrodes. Such stimulation in a brain area known as MT systematically biases animals' depth reports, in the direction expected from the tuning properties of neurons recorded at those sites. I also demonstrated a close connection between the activity of single neurons and perception by exploiting ambiguous stimuli: an identical visual stimulus is seen as near on some trials but seen as far on other trials. Recording the activity of single MT neurons while animals report the perceived configuration, I showed that the neuronal activity was correlated with the animals' reported sensation. This correlation was measured between neuronal and behavioral responses to the same physical stimulus. Thus the activity of single neurons in this area carries information not only about the disparity of external stimuli, but also information about the depth sensations experienced by the animal.

This combination of experimental and theoretical work offers insights into how hierarchical processing by a series of cortical areas leads from machine-like processing of input images to conscious perception of the visual world.

Selected Publications

1 Nienborg, H, Bridge, H, Parker, AJ and Cumming, BG, 2004, Receptive field size in V1 neurons limits acuity for perceiving disparity modulation. J Neurosci 24: 2065–2076

2 Cumming, BG, 2002, An unexpected specialization for horizontal disparity in primate V1. Nature 418: 633-636

3 Dodd, JV, Krug, K, Cumming, BG and Parker, AJ, 2001, Perceptually bistable three-dimensional figures evoke high choice probabilities in cortical area MT. J. Neuroscience 21: 4809-21

4 Cumming, BG and DeAngelis, GC, 2001, The physiology of stereopsis. Annu Rev Neurosci. 24: 203-38

5 Cumming, BG and Parker, AJ (1999) Binocular neurons in V1 of awake monkeys are selective for absolute, not relative, disparity. J. Neuroscience 19: 5602-18

6 Cumming, BG and Parker, AJ (1997) Responses of primary visual cortical neurons to binocular disparity without depth perception. Nature 389: 280-83

Research Summary

The current active research projects in my group include:

- An experimental and theoretical examination of how stimulus changes over time are integrated into estimates of stereoscopic depth, using human psychophysics and recording from single neurons in striate cortex.
- Single cell recording projects in area V2 (the next level of processing) exploring how simple local measures of depth at different locations are combined to represent more complex surfaces.
- A series of experiments designed to challenge current models that attempt to explain the nature of disparity signals in striate cortex (the binocular energy model and its variants). A variety of stimuli have been devised for which a mechanism that have adapted to detect the correlations seen during natural viewing should behave differently from current models. The responses of neurons in striate cortex to such stimuli are recorded, and we are developing new models that can explain these responses.
- Two studies examine correlations between perceptual reports and neuronal firing across repeated presentations of ambiguous stimuli, in two different brain areas. Such correlations have generally been found to be weak or absent in striate cortex, but this has never been studied with a stimulus/task that produces strong correlations in other cortical areas. We are recording in striate cortex using a task for which a strong correlation has been found in MT. At the other end of the visual processing stream, we are also exploring responses in area MST to another stimulus which has previously been used in MT. Characterizing these correlations at different levels of the visual processing stream is an essential part of understanding how activity in neurons throughout the visual pathway support perception.

Molecular Immunology Section

Charles E. Egwuagu, MPH, PhD

Research Interests

Transgenic (TR) Rats with targeted expression of Interferon gamma in lens and retina results in induced lens and retinal diseases
1 Newborn TR rat lens
2 7-day old TR rat lens
3 3-month old TR rat eye

The overall thrust of research in the Section on Molecular Immunology is governed, to a large extent, by the belief that most infectious and chronic diseases result from inability of the host to mount an adequate immune response or because of an exuberant and over-reactive immune system. Consequently, our work has focused on understanding molecular and cellular mechanisms that regulate immune responses, with particular emphasis on the roles played by pro-inflammatory and anti-inflammatory cytokines elaborated by armed effector T lymphocytes.

1

2

3

Research Summary

Over the past ten years, research in this section has been oriented towards the characterization of JAK/STAT signal transduction pathways of resident ocular cells and inflammatory cells that mediate ocular diseases. These investigations have been carried out using cell lines or primary cells and validated in transgenic mouse and rat models. Earlier emphasis was on positive regulatory factors that mediate cytokine signaling in the eye and this led us to develop transgenic (TR) rats and mice with targeted over-expression of IFNγ in the lens and retina. Analyses of these rodent models have led to better appreciation of the role of this proinflammatory cytokine in ocular inflammatory diseases. An important consequence of prolonged exposure of ocular cells to proinflammatory cytokines such as IFNγ, as may occur during chronic or recurrent uveitis, is development of retinal degenerative changes and selective loss of ganglion cells by apoptosis. Thus, these transgenic rats provide a useful model for studying the role of proinflammatory cytokines in uveitis and retinal degeneration. These studies also established for the first time that the lymphoid-specific transcription factor, ICSBP (interferon consensus sequence binding protein), is constitutively expressed in the lens and that dysregulated expression of this factor altered the developmental fate of lens cells, resulting in development of cataract.

Recent work in this lab has focused on negative feedback mechanisms that regulate JAK/ STAT pathways and coordinate crosstalk between the plethora of cytokines that converge on uveitogenic lymphocytes, as well as, resident ocular cells. Particular emphasis is on a family of intracellular regulatory proteins called SOCS (suppressors of cytokine signaling). These proteins regulate the intensity and duration of cytokine signals. Four areas of specific interests are:

- Understanding the role of SOCS proteins in adaptive immunity. These studies have led to the discovery that: i)SOCS proteins are T-cell lineage markers and potential therapeutic targets for immune modulation therapy. SOCS3 has a gatekeeper function in T cells and has a role in maintaining T-cells in a quiescent state prior to activation; iii). SOCS proteins play important roles in regulating the crosstalk between Th1 and Th2 cytokines during T-cell differentiation.
- Understanding the role of SOCS proteins in innate immunity. These studies have revealed that STAT1 is required for dendritic cell (DC) maturation and that DC development is under feedback regulation by SOCS proteins.
- Understanding the role of SOCS proteins in the eye. In these studies we showed that the crosstalk between factors involved in lens differentiation is under feedback regulation by SOCS proteins.
- Translation of SOCS research to clinical application. We show that SOCS mRNA levels in PBMC can serve as prognostic marker for monitoring response to anti-uveitic drugs.

Selected Publications

1 Yu cr, Mahdi rm, Ebong S, Vistica bp, Chen J, Guo Y, Gery I and Egwuagu ce. (2004) Proliferation and stat6 signaling pathways are negatively regulated in T-helper cells by stat1 and suppressors of cytokine signaling (socs) J. Immunol. 173:737-746.

2 Jackson SH, Yu CR, Mahdi RM, Ebong S, Egwuagu CE, 2004. Dendritic cell maturation requires STAT1 and is under feedback regulation by suppressors of cytokine signaling. J Immunol. 172:2307-15.

3 Egwuagu C.E., R-C. Yu, M. Zhang, R. M. Mahdi, S. J. Kim and I. Gery. 2002. Suppressors of cytokine signaling (SOCS) proteins are constitutively and differentially expressed in Th1 and Th2 Cells: implications for Th cell Lineage commitment and maintenance. J Immunol. 168(7):3181-7.

4 Li, W., Nagineni, C.N., Ohtaka-Marayuma, C., Efiok, B., Chepelinsky, A.B. and Egwuagu, C.E. 1999. Interferon regulatory transcription factors (IRFs) are constitutively expressed and spatially regulated in the mouse lens. developmental biology 210: 44-55.

5 Egwuagu, C.E., Charukamnoetkanok, P. and Gery, I., 1997. Thymic expression of autoantigens correlates with resistance to autoimmune disease. J. Immunol. (Cutting Edge Paper) 159: 3109-3112.

Division of Epidemiology and Clinical Research

Frederick Ferris III, MD

Research Interests

The Division of Epidemiology and Clinical Research has had three main functions: research, education, and consultation. Research is the dominant function. It is the Division's mission to plan, develop, and conduct human population studies concerned with the cause, prevention, and treatment of eye disease and vision disorders, with emphasis on the major causes of blindness. This includes studies of incidence and prevalence in defined populations, prospective and retrospective studies of risk factors, natural history studies, clinical trials, genetic studies, and studies to evaluate diagnostic procedures.

The Division carries out a program of education in biometric and epidemiologic principles and methods for the vision research community. This program consists of courses, workshops, a fellowship program for ophthalmologists, publications, and consultation and collaboration on research.

Finally, the Division provides biometric and epidemiologic assistance to National Eye Institute intramural and extramural staff and to vision research workers elsewhere. The assistance ranges from consultation to collaboration as co-investigator. It now provides both scientific as well as administrative support to investigators at the NIH Clinical Center. The Division currently includes the Office of the Clinical Director, which provides the administrative support for the intramural clinical research program.

Overview of Research

Some examples of these studies completed or in progress within DECR, include:

- Clinical trials evaluating the treatment of diabetic retinopathy–Diabetic Retinopathy Study, Diabetic Retinopathy Vitrectomy Study, Sorbinil Retinopathy Trial, Krypton-Argon Regression of Neovascularization Study, Early Treatment Diabetic Retinopathy Study and the new Diabetic Retinopathy Clinical Research Network (DRCR.net)–as a collaborator rather than director
- Clinical trial and natural history studies AMD and Cataract The Age Related Eye Disease Study and the Italian-American Trial of Age-Related Cataract
- Population based studies–Framingham Eye Studies
- Case-control studies–Eye Disease Case-Control Study and the Italian-American Case-Control Study of Cataract
- Extensive Collaboration with ongoing extramural studies

Selected Publications

1 The Eye Diseases Prevalence Research Group. Causes and prevalence of visual impairment among adults in the United States. Arch Ophthalmol 2004; 122:477-485.

2 Wilkinson CP, Ferris FL 3rd, Klein RE, Lee PP, Agardh CD, Davis M, Dills D, Kampik A, Pararajasegaram R, Verdaguer JT. Global Diabetic Retinopathy Project Group. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. Ophthalmology 2003; 110(9):1677-82.

3 The Age Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age related macular degeneration and vision loss. AREDS Report No. 8. Arch Ophthalmol 2001;119:1417-1436.

4 The Diabetic Retinopathy Vitrectomy Research Group. Early vitrectomy for severe vitreous hemorrhage in diabetic retinopathy. Two-year results of a randomized trial. DRVS Report No. 2. Arch Ophthalmol 1985;103: 1644-1652.

5 Early Treatment Diabetic Retinopathy Study Research Group. Photocoagulation for diabetic macular edema. ETDRS Report No. 1. Arch Ophthalmol 1985;103:1796-1806.

6 The Diabetic Retinopathy Study Research Group. Photocoagulation treatment of proliferative diabetic retinopathy: clinical application of Diabetic Retinopathy Study (DRS) findings. DRS Report No. 8. Ophthalmology 1981; 88:583-600.

Edmond J. FitzGibbon, MD

Research Interests

This laboratory's primary interest is in studying eye movements in humans, both normals and patients with neuro-ophthalmic diseases.

Selected Publications

1 Kattah JC, FitzGibbon EJ. Superior oblique myokymia. Curr Neurol Neurosci Rep. 2003 Sep;3(5):395-400. Review.

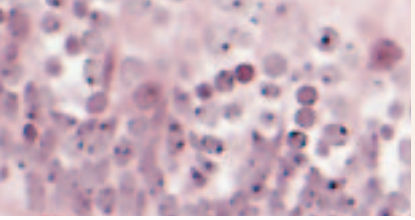
2 Yang DS, FitzGibbon EJ, Miles FA. Short-latency disparity-vergence eye movements in humans: sensitivity to simulated orthogonal tropias. Vision Res. 2003 Feb;43(4):431-43.

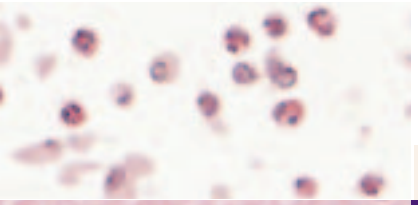
3 Hertle RW, Dell'Osso LF, FitzGibbon EJ, Thompson D, Yang D, Mellow SD. Horizontal rectus tenotomy in patients with congenital nystagmus: results in 10 adults. Ophthalmology. 2003 Nov;110(11):2097-105.

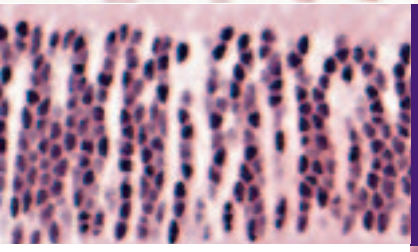
4 Lee JS, FitzGibbon E, Butman JA, Dufresne CR, Kushner H, Wientroub S, Robey PG, Collins MT. Normal vision despite narrowing of the optic canal in fibrous dysplasia. N Engl J Med. 2002 Nov 21;347(21):1670-6.

Research Summary

I am using eye movement recordings in humans to characterize, stage, determine response to treatment, and longitudinally follow neurodegenerative diseases such as Niemann Pick C, Gaucher type 3 and progressive supranuclear palsy. I am also using eye movement recordings to document nystagmus and response to interventions to ameliorate nystagmus such as tenotomy surgery. Lastly, patients with diseases of neuro-ophthalmic interest such as fibrous dysplasia are followed longitudinally in the eye clinic to help elucidate the natural history of their disease.





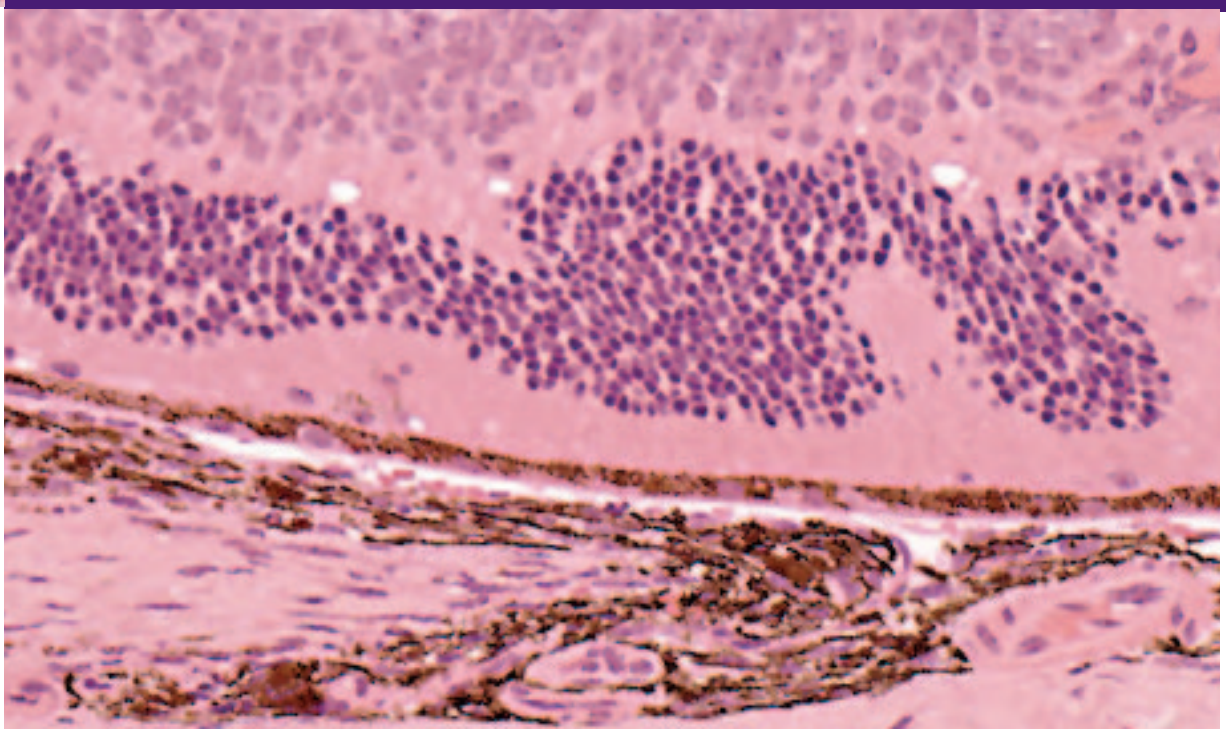


Experimental Immunology Section

Igal Gery, PhD

Research Interests

- Pathogenic autoimmunity to ocular antigens: prevention by Immunotolerance and mechanisms of induction
- Ocular inflammation: molecules and cells involved in the process.
- Involvement of inflammation-related molecules in degenerative retinal diseases



Histological changes characteristic for ocular inflammation induced by T-helper cells of type 1 or type 2. Recipient mice that express hen egg lysozyme (HEL) in their lens were injected 7 days previously with HEL-specific polarized Th1 or Th2 cells. Changes characteristic for Th1 cells-induced inflammation include edematous retina, that is folded and partially detached, as well as cellular infiltration in the retina and vitreous, consisting mainly of mononuclear cells. Inflammation induced by Th2 cells is characterized by cellular infiltration, mainly in the vitreous, consisting mostly of polymorphonuclear cells and eosinophils. Hematoxylin and eosin.

Research Summary

We have studied two opposite aspects of pathogenic autoimmunity to ocular antigens, namely, prevention by immunotolerance and mechanisms of induction. Immunotolerance against self antigens is achieved mainly by the central immunotolerance mechanism whereby lymphocytes specific to autoantigens are deleted in the thymus following exposure to these antigens, expressed by stromal thymic cells. We demonstrated that ocular antigens are expressed in the thymus and their level of expression is inversely correlated with the animal's susceptibility to develop autoimmune uveitis induced by these antigens.[6] In another study we showed that the deletion mechanism is particularly efficient when self antigens are released by thymic epithelial cells and are captured and presented by bone marrow-derived dendritic cells.[5] Finally, we demonstrated that uveitogenic retinal antigens are expressed in human thymi, with remarkable variability among individuals, suggesting that differences in susceptibility to autoimmune uveitis among individuals is related to their level of thymic expression of these molecules.

Pathogenic autoimmunity may be triggered when natural immunotolerance breaks down[4] and lymphocytes specific to self antigens are activated and acquire the capacity to induce inflammation. Microbial infection has been suggested to be a major triggering mechanism for pathogenic autoimmunity and we are testing this hypothesis by an experimental system we developed. We have identified so far two families of microbial molecules capable of initiating pathogenic ocular autoimmunity, i.e., bacterial DNA sequences containing the unmethylated CpG dinucleotide motif and poly (I:C), that represents viral double stranded RNA. The activity of additional molecules is under investigation (study in progress).

To learn about cells and molecules involved in immune-mediated ocular inflammation we developed an experimental system in which inflammation is induced in eyes of transgenic mice expressing a hen egg lysozyme (HEL) following adoptive transfer of HEL specific lymphocytes. T helper (Th) cells of both type 1 or type 2 were found capable of inducing inflammation[3] and the major cytokines, chemokines and chemokine receptors involved in these immunopathogenic processes were identified.[2] The chemokine receptor CXCR3 is strongly expressed in Th1-induced inflammation site and treatment with antibody against this chemokine receptor efficiently inhibited the disease.[1] Analysis of CXCR3 expression by Th1 cells during their activation and migration into the target eye revealed a unique pattern of up- and down-regulation of this molecule. These observations shed new light on the inflammation process.[1] We are now investigating the active participation of host cells in the pathogenic process (study in progress).

Light-damaged eyes serve as a model for retinal degenerative diseases. Microarray analysis of light-damaged mouse eyes revealed up-regulation of certain inflammation-related molecules, including the chemokine MCP-1. The importance of MCP-1 in protecting the eye was shown by the finding that light damage was much more severe in MCP-1 null mice than in their wild type controls (study in progress).

Selected References

1 Chen, J., Vistica, B.P., Takase, H., Ham, D.-I., Fariss, B.N., Wawrousek, E.F., Chan, C.-C., DeMartino, J.A., Farber, J.M. and Gery, I.: A unique pattern of up- and down-regulation of chemokine receptor CXCR3 on inflammation-inducing Th1 cells. Europ. J. Immunol. 34:2885-2894, 2004.

2 Foxman, E.F., Zhang, M., Hurst, S.D., Muchamuel, T., Shen, D., Wawrousek, E.F., Chan, C.-C., and Gery, I.: Inflammatory mediators in uveitis: Differential induction of cytokines and chemokines in Th1 vs. Th2-mediated ocular inflammation. J. Immunol. 168:2483-2492, 2002.

3 Kim, S.J., Zhang, M., Vistica, B.P., Chan, C.-C., Shen, D.-F., Wawrousek, E.F., and Gery, I.: Induction of ocular inflammation by T-helper lymphocytes type 2. Invest. Ophthalmol. Vis. Sci., 43: 758-765, 2002.

4 de Vos, A.F., Fukushima, A., Lobanoff, M.C., Vistica, B.P., Lai, J.C., Grivel, J.-C., Wawrousek, E.F., Whitcup, S.M., and Gery, I. Breakdown of tolerance to a neo-self antigen in double transgenic mice in which B cells present the antigen. J. Immunol. 164:4594-4600, 2000.

5 Zhang, M., Vacchio, M.S., Vistica, B.P., Lesage, S., Egwuagu, C.E., Yu, C.-R., Gelderman, M.P., Kennedy, M.C., Wawrousek, E.F., and Gery, I.: T cell tolerance to a neo-self antigen expressed by thymic epithelial cells: The soluble form is more effective than the membrane-bound form. J. Immunol., 170:3954-3962, 2003.

6 Egwuagu, C.E., Charukamnoetkanok, P., and Gery, I.: Thymic expression of autoantigens correlates with resistance to autoimmune disease. J. Immunol. 59: 3109-3112, 1997.

Ophthalmic Molecular Genetics Section

J. Fielding Hejtmancik
MD, PhD

Selected Publications

1 Smaoui N, Beltaief O, Benhamed S, M'Rad R, Maazoul F, Ouertani A, Chaabouni H, and Hejtmancik JF. A Homozygous Splice Mutation in the HSF4 gene is associated with an autosomal recessive congenital cataract. Invest Ophthalmol.Vis.Sci. 45:2716-2721, 2004.

2 Kantorow M, Hawse JR, Cowell TL, Benhamed S, Pizarro GO, Reddy VN, and Hejtmancik JF. Methionine sulfoxide reductase A is important for lens cell viability and resistance to oxidative stress. Proc.Natl.Acad.Sci.U.S.A2004.

3 Li A, Jiao X, Munier FL, Schorderet DF, Yao W, Iwata F, Hayakawa M, Kanai A, Shy CM, Alan LR, Heckenlively J, Weleber RG, Traboulsi EI, Zhang Q, Xiao X, Kaiser-Kupfer M, Sergeev YV, and Hejtmancik JF. Bietti crystalline corneoretinal dystrophy Is caused by mutations in the novel gene CYP4V2. Am.J.Hum.Genet. 74:817-826, 2004

4 Sergeev YV, Hejtmancik JF, and Wingfield PT. Energetics of domain-domain interactions and entropy driven association of β-crystallins. Biochemistry 43:415-424, 2004.

5 Jiao X, Ventruto V, Trese MT, Shastry BS, and Hejtmancik JF. Autosomal recessive familial exudative vitreoretinopathy is associated with mutations in LRP5. Am.J.Hum.Genet. Electronic Publication September 2, 2004.

Research Interests

The long-range objective of the Ophthalmic Molecular Genetics Section is to increase understanding of inherited visual diseases to improve their diagnosis and perhaps therapy. Elucidating the mechanisms of inherited visual diseases increases our understanding of both normal and aberrant visual processes. Three approaches are used. One is candidate gene analysis, in which proteins likely to cause a disease are picked and the genes encoding these proteins are screened for mutations. The second approach is positional cloning, in which the inheritance of a genetic disease within families is compared statistically to the inheritance pattern of alleles of genetic markers from known locations of the genome, mapping disease loci to defined regions of the genome. Genes lying within the critical region can be identified and screened for causative or associated mutations. A third approach is to identify alleles of genes or markers that are associated with the disease of interest in a population. These or nearby genes can then be screened for mutations. While all three of the above approaches have been successful independently, in practice they are complementary and are often used in conjunction, as is perhaps best seen in our studies of congenital cataracts and Bietti crystalline dystrophy.

Once the gene responsible for a disease has been identified, much scientifically and medically valuable information can be gained by delineation of the pathophysiology of the disease. Initially, the structure-function relationships of the gene product are characterized, and then the way in which malfunction of the product impacts on the normal metabolism or development of the cell and eventually the tissue are explored. In this fashion, understanding the pathophysiology of rare inherited disorders provides insights that might be crucial to understanding normal physiology and more common disease processes, clarifies relationships between disease entities, and has the potential to allow rational therapy to be designed, even though this might only be achieved in future years.

The Ophthalmic Molecular Genetics Section is currently carrying out a variety of positional cloning efforts, both independently and collaboratively. These projects typically progress through several discrete stages: sample collection, genotyping and statistical or linkage analysis, physical mapping and/or candidate gene analysis, and finally characterization of the causative mutations and their effects on the gene product and the tissues in which it is expressed. They include positional cloning projects for Mendelian diseases such as cataracts, corneal dystrophies, retinal degenerations and keratoconus. Ongoing positional cloning projects for complex diseases include age related cataracts and progressive open angle glaucoma. After identification of disease genes, we carry out structural, molecular biological and biochemical analysis of inherited diseases and provide clinical diagnostic services related to molecular genetic research carried out in the other projects and by other investigators at the NEI. The OMSG has been moving steadily towards projects involving complex as opposed to Mendelian diseases, both because of advancing technologies increasingly allow these diseases to be approached and because of their greater clinical burden on the population.

Neuronal Networks Section

Okihide Hikosaka, MD, PhD

Research Interests

My original interest in neuroscience was to discover the neural mechanisms for voluntary movement. For this purpose, I have studied neuronal mechanisms underlying the following functions:

- Brainstem and basal ganglia networks
- Procedural and motor skill learning
- Attention and response selection
- Motivational control of behavior

In the coming several years, I would like to concentrate my research on motivational control of saccadic eye movements, specifically in relation to the function of the basal ganglia. The main targets of my study will be the striatum (especially caudate nucleus, CD) and the superior colliculus (SC), since sensorimotor signals and motivational signals may be integrated in each of these structures, possibly in different ways. I plan to investigate what kinds of signals are conveyed from CD to SC through the substantia nigra pars reticulata (SNr), especially in relation to the parallel cortical inputs to SC. Although the main target is the basal ganglia, I will also compare the results from the basal ganglia with those from other brain structures, especially saccade-related areas in the cerebral cortex (called cortical eye fields). Based on these studies, I hope to find a unique role of the basal ganglia in voluntary eye movements and hope to propose a general scheme for voluntary movement.

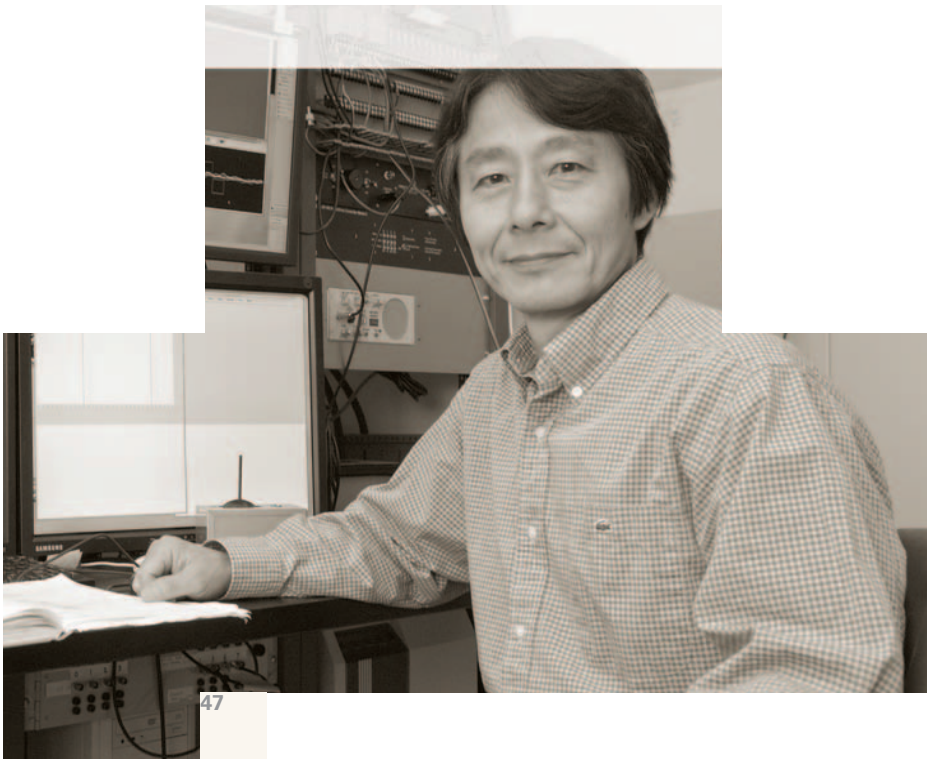
My hypothesis is that the basal ganglia are specialized for motivational control of saccadic eye movement, whereas the cortical eye fields are relatively specialized for cognitive control of saccades. This issue is related to consciousness, attention, and intention. Anatomically, this is probably a key issue on the relationship between the cerebral cortex, basal ganglia, and cerebellum.

Research Summary

In a series of studies conducted at NEI and universities in Japan in the 1980s and 1990s, we discovered that the basal ganglia control saccadic eye movement. We recently found that visual and saccade-related activity of neurons in the basal ganglia is often strikingly dependent on the behavioral context. Reward turned out to be a key factor that characterizes the information processing in the basal ganglia.

Using a reward-biased task, we found that a group of CD projection neurons are likely to be a major source of signals that create such a strong bias in saccades. In IDR task CD neurons exhibit a strong bias dependent on reward position. Such a positional bias in CD neuronal activity is translated, with a disinhibition mechanism through SNr, into a hemispheric bias in excitability in SC saccadic neurons, which effectively facilitates a saccade to a rewarded position. It is an internal and motivational process which might reflect desire or wanting. We then found that dopaminergic neurons in the midbrain mediate predictive reward signals in the basal ganglia.

Expectation of reward motivates our behaviors or influences our decisions. Our research using saccadic eye movement suggests that the basal ganglia play a key role in behaviorally expressing reward expectation. Both GABAergic and dopaminergic neurons in the basal ganglia, probably owing to their mutual interactions, would then create a bias in activity in the brain, thus guiding gaze to rewarded position.



Selected Publications

1 Kawagoe R, Takikawa Y, Hikosaka O (2004) Reward-predicting activity of dopamine and caudate neurons - a possible mechanism of motivational control of saccadic eye movement. J Neurophysiol 91:1013-1024.

2 Nakahara H, Itoh H, Kawagoe R, Takikawa Y, Hikosaka O (2004) Dopamine neurons can represent context-dependent prediction error. Neuron 41:269-280.

3 Ikeda T, Hikosaka O, 2003. Reward-dependent gain and bias of visual responses in primate superior colliculus. Neuron 39: 693-700.

4 Lauwereyns J, Watanabe K, Coe B, Hikosaka O, 2002. A neural correlate of response bias in monkey caudate nucleus. Nature 418:413-417.

5 Hikosaka O, Takikawa Y, Kawagoe R, 2000. Role of the basal ganglia in the control of purposive saccadic eye movements. Physiol. Rev. 80: 953-978.

6 Kawagoe R, Takikawa Y, Hikosaka O, 1998. Expectation of reward modulates cognitive signals in the basal ganglia. Nat Neurosci 5: 411-416.

Immunology and Virology Section

John Hooks, PhD

Research Interests

The long term goal of the Immunology & Virology Section, LI, NEI is to investigate host and viral factors involved in pathogenic processes within the eye. We hypothesize that the manner in which the host responds to an insult, such as an infectious event, will contribute, in part, to ocular tissue damage. A better understanding of host responses (e.g. genetic, immunologic) will provide a rationale for interventive strategies. In order to test this hypothesis, we are using animal model systems and a human RPE cell model system.

Research Summary

Animal Model Systems

Experimental Coronavirus Retinopathy, ECOR

We have established a model system for studying retinal degenerative diseases, experimental coronavirus retinopathy (ECOR). This is a virus triggered retinal degeneration that is controlled by genetic and immune factors. Our aim is to identify host factors which contribute to either the retinal degenerative process in BALBI/c mice or to the protection from retinal degeneration seen in CD-1 mice.

This model has potential importance for six reasons. First, it demonstrates that a virus can play a significant role in retinal degenerative diseases. Second, a retinal degeneration that may appear to be solely of genetic origin may have a viral co-factor or trigger. Third, this model allows us to dissect mechanisms of viral clearance within the retina. Fourth, this model system affords us the opportunity to dissect specific host factors that contribute to or limit the retinal degenerative process. Fifth, identification of specific retinal auto antigens may provide avenues to monitor retinal degenerations. Sixth, this model may be used to evaluate interventive therapies.

RPE Cell Model System

RPE Cell Cytokine, Chemokines and Growth Factor Interactions

The RPE cell plays a basic role in maintaining the structural and physiological integrity of the neural retina. We have studied the RPE cell extensively as an important immunoregulatory cell within the posterior pole of the eye. Cytokines exert profound influences on cellular development and on a variety of cellular functions. This project has concentrated on studying the ways in which cytokines interact with cells of the immune system and with cells in the ocular microenvironment. Our aim is to investigate the human RPE cell model system to evaluate the regulatory role of cytokines and growth factors in the retina.

RPE Cells in Infectious Diseases

Human CMV is a herpesvirus that is a major cause of blindness in children born with congenital infections and in immunocompromised individuals. It is difficult to study CMV latency in man. Therefore cell culture models of CMV replication and latency may provide insight into a rationale for alternative treatment modalities. In order to understand the retinal tissue tropism for CMV, we have extended our original studies of CMV replication in HRPE Our aim is to utilize the human RPE cell model system to investigate CMV and T. gondii host cell interactions.

We have recently identified that Toll-like receptors, TLRs on retinal pigment epithelial cells provide a rapid defense system for the retina. The body defends itself against microorganisms by turning on an innate immune response followed by an acquired immune response. The innate immune system can be activated within minutes after the invasion of a bacteria or virus. On the other hand, the acquired immune response can be activated days after the invasion. Recent studies identified that in the innate immune response, the body recognizes the invaders through TLRs that are present on the surface of selected cells within the body. When the TLRs are engaged, genes important for an effective host defense, such as pro-inflammatory cytokines, are activated. Using real time PCR analysis of TLR gene expression, we describe the presence of TLRs on the human RPE cell. We also show that an important TLR for defense against virus infection, the TLR-3, is highly expressed on these cells. When we analyzed signaling through TLR-3 in human RPE cells, we discovered that these human cells secreted pro-inflammatory cytokines, chemokines and adhesion molecules.

Learning how the body quickly turns on genes and produces molecules that can protect the retina from invading organisms is crucial to our attempts to design augmented responses to infection. This discovery identifies that the TLR system and the immediate protection mechanisms in the innate immune response can function within the retina. Now that we know that the TLR system is operational within the retina, we can search for additional regulatory functions for TLR and interferon-beta within the retina.

Bench to Bedside / Bedside to Bench Studies

Infectious Agent Diagnosis: We have developed molecular and immunologic diagnostic methods using PCR analysis to distinguish among infectious, immunopathogenic and autoimmune posterior segment intraocular inflammation. CLIA Licensed Laboratory (1999-present). Our aim is to use the expertise gained in basic studies to aid in the diagnosis and management of ocular and infectious diseases.

Retinopathies The identification of autoantibodies during the course of a disease has been shown to be useful in making a diagnosis, understanding mechanisms of pathogenesis, identifying therapeutic strategies and monitoring treatments. The concept of immune–mediated vision loss, with an emphasis on autoimmune reactivity and autoimmune disease in the eye is a rapidly expanding area of research and therapy. We have initiated studies to screen for the presence of anti-retinal antibodies in patients with progressive retinal degenerative diseases. Our aim is to evaluate patients with retinal degenerative processes in order to detect and characterize anti-retinal autoantibodies. We plan to subtype retinal disease according to autoantibody profile in order to define specific subgroups of retinopathies in terms of pathogenesis and therapy.

Retinal Vasculitis This major component of ocular inflammation plays a critical role in retinal damage and subsequent vision loss. Retinal vascultis can occur as a primary ocular disease or secondarily, as a component of a systemic vascular disease. Unfortunately, little is known about primary retinal vasculitis. In new human protocol "Identification of Biological Markers in Retinal Vasculitis" Protocol Number: 03-EI-0068. We hypothesize that there are biological markers of retinal vasculitis, such as, cytokines, chemokines, adhesion molecules, T-cell surface markers and autoantibodies which may determine disease progression, understanding mechanisms of pathogenesis, identifying therapeutic strategies and monitoring treatments. The purpose or objective of this study is to investigate selected biological markers to collect clinical and biologic information to better understand the natural history of conditions indicative of primary retinal vasculitis.

Selected Publications

1 Kumar, MV, Nagineni, CN, Chin, MS, Hooks, JJ and Detrick, B. Innate immunity in the retina: toll-like receptor (TLR) signaling in human retinal pigment epithelial cells. J Neuroimmunol 153: 7-15, 2004.

2 Hooks, JJ, Detrick, B, and Nussenblatt, RN. Infections associated with retinal autoimmunity. In Infection and Autoimmunity, Eds. Shoenfeld, Y and Rose, NR. In Press. 2004.

3 Nagineni, CN, Samuel, W, Nagineni, S, Pardhasaradhi, K, Wiggert, B, Detrick, B and Hooks, JJ. Transforming growth factor-b induces expression of vascular endothelial growth factor in human retinal pigment epithelial cells: involvement of mitogen activated protein kinases. J Cellular Physiology 197: 453-462, 2003.

4 Momma, Y, Nagineni, CN, Chin, MS, Srinivasan, K, Detrick, B and Hooks, JJ. Differential expression of chemokines by human retinal pigment epithelial cells infected with cytomegalovirus. Invest Ophthal Vis Sci 44: 2026-2033, 2003.

5 Chin, M.S., Nagineni, C.N., Hooper, L.C., Detrick, B and Hooks, J.J.: Cyclooxygenase-2 Gene Expression and regulation in human retinal pigment epithelial (HRPE) cells. Invest Ophthal Vis Sci. 42: 2338-2346, 2001.

6 Detrick, B., C. Nangineni, K.P. Anderson, S.P. Henry and J.J. Hooks: Inhibition of human cytomegalovirus replication in a human retinal epithelial cell model by antisense oligonucleotides. Invest Ophthal Vis Sci. 42: 163-169, 2001.

Oculomotor Control Section

Frederick A. Miles, D.Phil

Research Interests

I am interested in the early cortical processing of optic flow information in humans, and I use their eye movements as an objective probe. We discovered some years ago that complex motion or binocular disparity applied to large textured patterns elicits machine-like eye movements at ultra-short latencies that are very sensitive to the detailed properties of the visual stimulus that is used. We now know that these eye movements are mediated by a small region of the cerebral cortex and I am interested in using them to study the signal processing in that region. Our approach is to study the quantitative dependence of these eye movements on the various parameters of the visual stimuli and thereby uncover the underlying neural processing.

Selected Publications

1 Sheliga, B.M., and Miles, F.A. Perception can influence the vergence responses associated with open-loop gaze shifts in 3D. Journal of Vision. 3: 654-676, 2003. http://journalofvision.org/3/11/2/, doi:10.1167/3.11.2.

2 Yang, D.-S and Miles, F.A. Short-latency ocular following in humans is dependent on absolute (rather than relative) binocular disparity. Vision Research. 43, 1387-1396, 2003.

3 Yang, D.-S., Fitzgibbon, E.J. and Miles, F.A. Short-latency disparity-vergence eye movements in humans: sensitivity to simulated orthogonal tropias. Vision Research. 43: 431-443, 2003.

4 Masson, G.M., Yang, D.-S. and Miles, F.A. Version and vergence eye movements in humans: dynamics determined by monocular rather than binocular image speed. Vision Research. 42: 2853-2867, 2002.

Research Summary

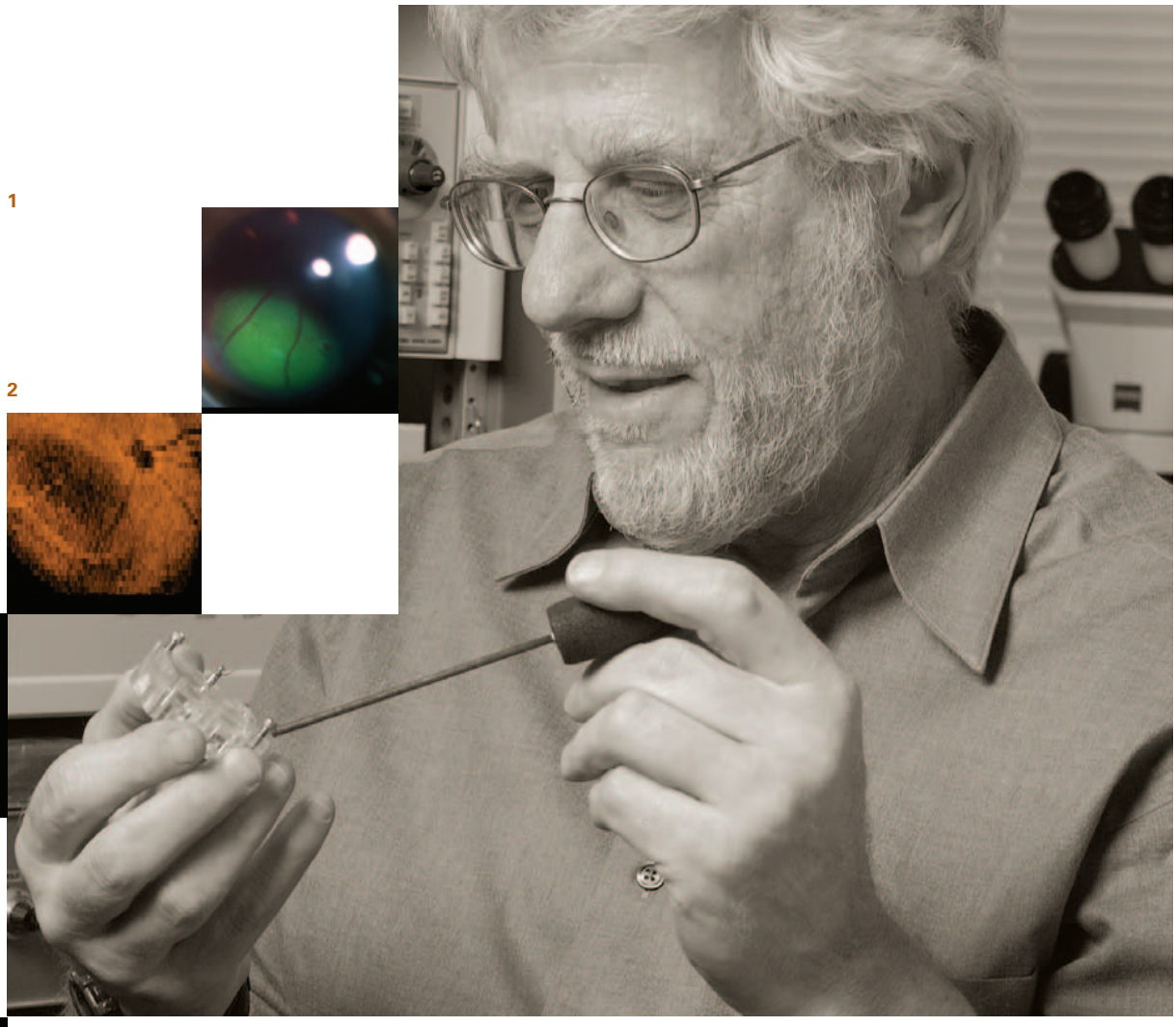
A major focus of my research has been three visual reflexes, all of which were first discovered in my laboratory, and that we have postulated help to stabilize the eyes of the moving observer. We have shown that two of these reflexes have a number of special features that we have postulated would help the observer to maintain ocular stability during translational disturbances and so operate as backups to otolith-mediated vestibulo-ocular reflexes. The third reflex would also operate in these same circumstances but we have reason to believe that this is a secondary property of this system, its primary purpose being to eliminate small (residual) binocular misalignments of the two eyes. One of the first two reflexes responds to planar motion and generates version (conjugate) eye movements that we have suggested help to stabilize gaze when the moving observer looks off to one side: ocular following. The other reflexes both generate vergence eye movements that we suggest help the moving observer to maintain binocular alignment on objects that lie ahead: one responds to the radial patterns of optic flow (radial-flow vergence) and the other to the changes in binocular parallax (disparity vergence) that occur when the moving observer looks in the direction of heading. Lesions and electrophysiology indicate that, despite their short latency, all three reflexes are mediated by the medial superior temporal area of cortex (MST), and share many features in common, leading us to suggest that they constitute a single family of reflexes. One common feature that we discovered only recently is that all rely on sensors that respond only to the first-order characteristics of the visual input. This means that their responses are all dominated by the principal Fourier components of the visual input, whether it be motion, binocular disparity or radial optic flow.

Section on Epithelial and Retinal Physiology and Disease

Sheldon Miller, PhD

Research Interests

Our interest is in the structure, regulation and function of epithelia. A distinguishing characteristic of epithelia is that their apical and basolateral membranes face different extracellular environments and contain different proteins. This asymmetry allows epithelia to direct the vectorial traffic of nutrients, ions and fluid from one extracellular space to another.



Images obtained from rat eye using polarization OCT.

1 Color CCD camera image of rat eye with artificially created retinal detachment by injection of 3μL of modified PBS solution containing fluorescein.

2 3D reconstruction of rat eye fundus from multiple 2D OCT scans. Darkened oval area in 3D reconstruction corresponds to retinal detachment.

3 Visualization of retinal detachment in rat eye using 3D reconstruction from 2D scans.

4 3D OCT scan of the rat eye with retinal detachment (highlighted with blue line) before and

5 after 30 min purinergic receptor agonist treatment. Volume calculations show 24% reduction in bleb size following treatment.

Research Summary

Three epithelial systems, in the breast, lung and eye, are under study. In the lung, the airway epithelial surface is covered by a 5µm layer of fluid and a blanket of mucus, produced by surface and submucosal gland cells. Pathology results from dehydration of this surface. In the eye, the retinal pigment epithelium (RPE) transports fluid between the neural retina and the choroidal blood supply. In this case, pathology results from abnormal fluid accumulation in the distal retina or in the subretinal (extracellular) space between the photoreceptors and the RPE apical membrane.

Pathogenesis of Cystic Fibrosis, CF

The CF gene product (CFTR) is a CI channel that can mediate ion and fluid secretion into the airway lumen. In CF the absence of CI -dependent fluid secretion is only part of the problem; we demonstrated that fluid is absorbed, not secreted across normal trachea and this absorption is mediated by apical membrane Na channels. Na-dependent fluid absorption is increased in CF, but how CFTR regulates the Na transport pathway is not well understood. The submucosal glands (serous cells) contain significantly more CFTR than the airway surface cells and secrete fluid. In CF these glands undergo a massive reversal from secretion to absorption, which would dehydrate the airways. One goal is to determine the membrane and intracellular mechanisms that could be used to mitigate these CF induced changes in fluid transport. Another goal is to understand the intra- and extracellular signals that help regulate the hydration and chemical composition of the airway surface liquid.

Mammary Cell Function

Recently we have used mouse mammary gland cultures and native tissue as a first step toward the identification and functional characterization of apical (and basolateral) membrane proteins such as CI (CFTR) and Na channels (ENaC) and P2Y purinoceptors that help mediate fluid secretion into the lumen of the mammary gland. Here we seek to understand the basis for abnormal breast fluid accumulation that occurs in [a] 40% of pre-menopausal women.

Retinal Pigment Epithelial, RPE, Function and Retinal Disease

Interactions between the retina and the RPE are mediated by light-induced changes in the extracellular activity of ions, catecholamines and neuropeptides. A diverse array of plasma membrane and intracellular signaling mechanisms integrate these input signals to regulate fluid flow between the subretinal space and choroid, thus maintaining the hydration and chemical composition of the extracellular spaces on both sides of the RPE. Gene transfer techniques have been used to introduce CFTR into human RPE to alter the magnitude and direction of fluid transport across the epithelium- as occurs in some disease processes. Recently we have developed two animal models: (1) to test pharmacologic interventions for retinal detachments and diseases that cause abnormal fluid accumulation in the distal retina; (2) to test gene therapeutic interventions against diseases such as age related macular degeneration that lead to choroidal neovascularization and blindness in the elderly (over 60) population. The goal in all of these experiments is to provide the basis for therapeutic approaches to a host of diseases that adversely affect the retina/RPE interface.

Selected Publications

1 Volobouéva, L.A., Liu, J., H. Suh, J.H., Ames, B.N., and S.S. Miller, 2004. (R)-a-Lipoic Acid Protects Retinal Pigment Epithelial Cells from Oxidative Damage. *Investigative Ophthalmology & Visual Science –in revision.*

2 Peterson, W.M., Quong, J.N., Blaug, S.A., and S.S. Miller, 2004. SERCA ATPases Regulate Epithelial Chloride Transport: A New Role For Phospholamban, 2004. submitted

3 Liou GI, Samuel S, Matragoon S, Goss KH, Santoro I, Groden J, Hunt RC, Wang F, Miller SS, Caldwell RB, Rustgi AK, Singh H, Marcus DM., 200). Alternative Splicing of the APC gene in the Neural Retina and Retinal Pigment Epithelium. *Molecular Vision* 10: 383-391.

4 Wang, F., Rendahl, K.G., Manning, W.C., Quiroz, D., Coyne, M. and S.S. Miller (2003). Adeno-Associated Virus Mediated Expression of Vascular Endothelial Growth Factor Induced Choroidal Neovascularization in Rat. *Investigative Ophthalmology & Visual Science* 44: 781-790.

5 Blaug, S., Quinn, R., Quong, J., Stephen Jalickee, and S.S. Miller (2003). Retinal Pigment Epithelial Function: a Role for CFTR? *Documenta Ophthalmologica* 106: 43-50.

6 Blaug, S. Rymer, J., Jalickee, S. and S.S. Miller, 2003. P2 purinoceptors regulate calcium-activated chloride and fluid transport in 31EG4 mammary epithelia. *American Journal of Physiology* 284: Cell Physiol. 50 C897–C909.

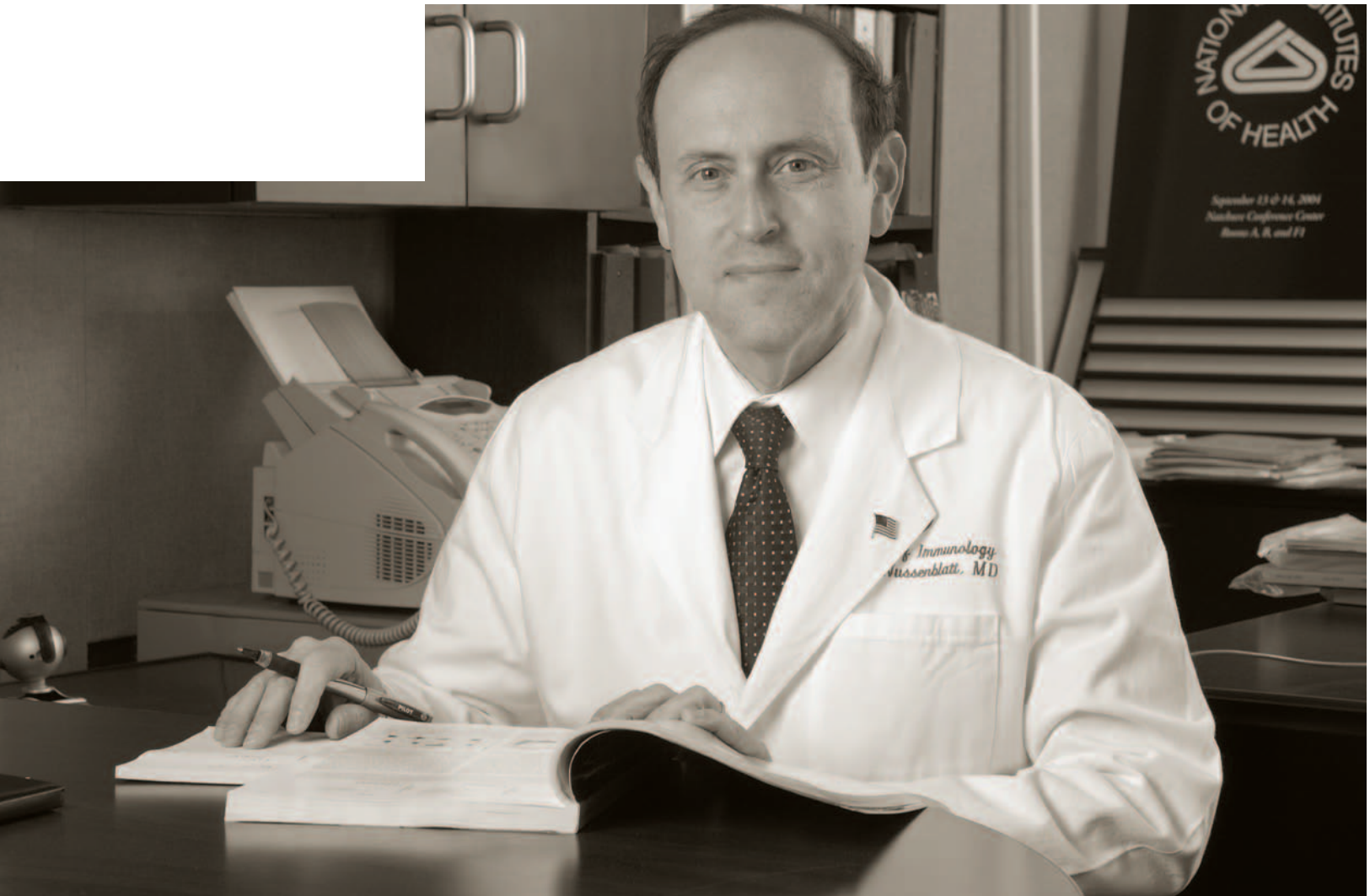
7 Maminishkis, A., Blaug, S.A., Rymer, J., Peterson, W.M., and S.S. Miller, 2002. Purinoceptor agonists increase fluid transport across RPE in vitro and fluid clearance out of subretinal blebs in vivo. *Investigative Ophthalmology & Visual Science* 43: 3555-3566.

Clinical Immunology Section

Robert Nussenblatt, MD

Research Interests

The Clinical Immunology Section of the Laboratory of Immunology has as its main function to better understand and treat human ocular inflammatory disease. This goal is in consonance with others in the Laboratory of Immunology whose work tends to be mostly laboratory based. However, as mentioned in the review of the Laboratory, all of those in the LI are dedicated to translational research. Therefore, two major goals of the Clinical Immunology Section are to bring observations from the bench to the bedside and to concentrate on the immune characteristics of patients, mainly, using animal models to help in that specific endeavor. Clinical activities are integrated into the activities of the section and the majority of the clinical protocols are centered in this section. This includes mentoring and training physicians in the care of patients with ocular inflammatory disease, learning how to do clinical trials, and acquiring the expertise needed to interact with bench scientists or to work there, as well, if they wish.



Selected Publications

1 Kim BJ, Li Z, Fariss RN, Shen DF, Mahesh SP, Egwuagu C, Yu CR, Nagineni CN, Chan CC, Nussenblatt RB: Constitutive and cytokine-induced GITR ligand expression on human retinal pigment epithelium and photoreceptors. Invest Ophthalmol Vis Sci (In press 2004)

2 Nussenblatt RB, Thompson D, Li Z, Chan CC, Velez G, Roy C, Levy-Clarke GA, Suhler E, Djalilian A, Sen N, Al-Khatib S,. Goldman CK, Bamji A, Sran P, Jack Ragheb1, Waldmann TA, Ronald R. Buggage RA. Humanized anti-IL2 receptor alpha therapy: Long term results in uveitis patients. Journal of Autoimmunity (2003) 21:283-293.

3 Zhuqing Li, Sankaranarayana P Mahesh, Ronald Buggage, Robert B Nussenblatt. Expression of Glucocorticoid Induced TNF Receptor Family Related Protein (GITR) on peripheral T cells from normal human donors and non-infectious uveitis patients. Journal of Auto-immunity., 21: 81-90, 2003.

4 Chan CC, Buggage R, Nussenblatt RB. Ocular Lymphoma. Curr Opin Ophthalmol 13: 411-418, 2002

5 Nussenblatt RB, Fortin E, Schiffman R, Rizzo L, Smith J, Van Veldhuisen P, Sran P, Yaffe A, Goldman CK, Waldmann TA, Whitcup SM: Treatment of non-infectious intermediate and posterior uveitis with the humanized anti-tac monoclonal Antibody: A phase I/II clinical trial. Proc Natl Acad Sci USA. 1999 Jun22;96 (13):7462-6.

Major Themes

Immunomodulation

This includes the evaluation of the interleukin-2 (IL-2) receptor system, a well characterized lymphokine receptor system that plays a central role in the induction of immune responses. IL-2 mediates its biologic effects through binding to specific surface receptors on activated T and B lymphocytes. This work is in conjunction with Dr. Thomas Waldmann of the NCI and has led to exciting observations that we plan to translate into a Phase III trial.

Measuring the markers of Ocular Inflammatory Disease

We are establishing a clinical immunology center for the evaluation of patient material. This emphasizes the use of techniques using small samples.

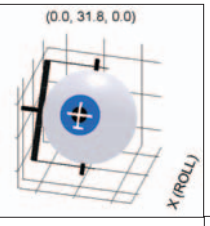
Intraocular Lymphoma, *Masquerade syndrome*

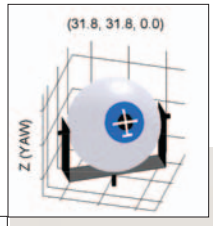
We have received a bench to bedside award from the NIH Clinical Center. With Dr. Ira Pastan of the NCI, we are developing a new therapy for ocular lymphoma. We just completed a workshop on the subject, which we hope will promote continued interaction in many areas - mechanisms as well as therapy.

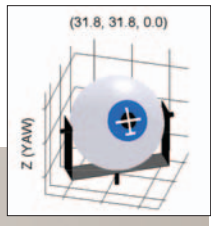
Dr. Nussenblatt received training in both Internal Medicine and Ophthalmology. He did an immunology fellowship at the NIH with Dr. Igal Gery. Over the years at NIH, he established a section on Ocular Immunology, which then became the Laboratory of Immunology in the National Eye Institute. He has directed a clinical fellowship program for ophthalmologists for over 20 years, and as well residents from both the National Naval Medical Center and the Walter Reed Medical Center have rotated through his service. Many of his fellows have gone on to assume leadership positions in academia and in industry. He has served as Clinical Director and Scientific Director of the National Eye Institute. Currently, in addition to being the Chief of the Laboratory of Immunology, he is also the Chief of the Office of Protocol Services, CC, NIH. This unit is responsible for the administrative handling of the 1100 protocols on the NIH campus. He is also a senior advisor to the Deputy Director of Intramural Research, NIH. His major area of interest remains immunology and the eye, trying to unlock the mechanisms of uveitis and applying these observations to the treatment of patients with blinding diseases. He is author or coauthor of over 400 publications and several books, including a standard text on uveitis entitled Uveitis: Fundamentals and Clinical Practice. He has received multiple awards including the Alcon Research Institute Meritorious Award Medal, the Public Health Service Senior Achievement Award, awards from the American Academy of Ophthalmology, and the Proctor Award from ARVO, where he also served as President. He is listed in "Best Doctors in America" and the "Top Ophthalmologists in the United States". He has been granted two honorary degrees. He was given the Gold Medal for lifetime achievement in the field of ocular inflammation by the International Uveitis Study Group.

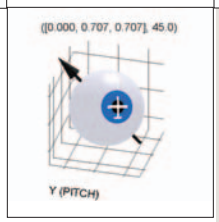
Neural Modeling Section

Lance Optican, PhD









Research Interest

Brain functions arise through the complex interactions of many neurons. During behavior, neurophysiological techniques can observe the responses of some neurons, but they cannot reveal directly the nature of their functional interactions. The Neural Modeling Section was established to provide insight into the nature of neuronal interactions underlying sensorimotor behavior through theoretical and mathematical modeling. Without good models, it is impossible to explain what signals are intrinsic to the brain, what behaviors to expect, or how to interpret the effects of brain lesions. My goal is to construct and test models of sensory and motor functions that are based on experimental observations.

One of the most important brain functions supporting vision is the ability to coordinate the movements of the eyes and head to control gaze, where we look in space. The section on neural modeling is devoted to understanding the nature of neuronal interactions underlying the neural control of gaze. Neurophysiological techniques can observe the responses of some neurons, but they can not reveal directly the nature of their functional interactions. This project constructs and tests mathematical models of sensory and motor functions involved in the control of gaze based on experimentally observed neuronal activity. In prior models of saccades (rapid, voluntary eye movements), the key role of controlling the movement's goal and speed was given to the superior colliculus (SC). The role of the cerebellum (C), in contrast, was assumed to involve the long-term regulation of saccadic accuracy. Analysis of neuronal responses from the SC and the C has led us to postulate a new model of how the brain controls visually guided saccades. The new model has two branches, one through the SC and one through the C, operating in parallel. This helps explain one of the earliest lesion studies in SC: even after bilateral SC ablations, the brain can still make saccades. Under normal conditions, the model uses the SC to control saccade beginnings, and the C to control saccade endings. More importantly, it lets us form a new interpretation of the role of each area. Thus, the SC is now believed to be generating a signal, in retinotopic coordinates, that represents where the selected target is. Thus, it is a sensory, and not a motor signal (as has been previously thought). Several recent studies have lent further support to this reinterpretation. The model also suggests a new, dual role for the cerebellum. The first, and more basic, role is to update the distribution of output activity during a movement. This is the role that corresponds to feedback in a classical model. The second, and more subtle, role is to recognize the constellation of inputs (i.e., sensory, motor, behavioral), or context, before the movement. This context causes the C to inhibit activity at a specific locus in the cerebellar vermis. This in turn disinhibits activity on specific fastigial nuclear cells, which in combination with the first role of the cerebellum drives and steers the eye to its final position. These two roles lead us to describe the cerebellum as having a "pilot map", which controls the eye movement. Although considerable evidence supports the spatial integration part of this theory, the context-dependent aspect of the theory has only recently begun to be studied experimentally. Currently, this theoretical approach is also being extended to the study of vergence eye movements. Vergence movements are the disconjugate movements made when the eyes look at objects at different distances. Closer objects require convergence of the two eyes, and farther objects require divergence of the eyes. Importantly, failures of the vergence mechanism may be related to the inappropriate ocular alignment seen in infantile strabismus (esotropias result from too much convergence, and exotropias result from too much divergence). Surprisingly, how the brain maintains the alignment of the two eyes is still unknown. A theoretical study may elucidate much of the existing clinical data on strabismus and its surgical treatment, and may suggest improved methods for characterizing and treating strabismus.

Selected Publications

1 Miura K, Hertle RW, FitzGibbon EJ and Optican LM: Effects of tenotomy surgery on congenital nystagmus waveforms in adult patients. II. Dynamical systems analysis. Vision Res, 43: 2357-2362, 2003.

2 Quaia C and Optican LM: Dynamic eye plant models and the control of eye movements. Strabismus, 11:17-31, 2003.

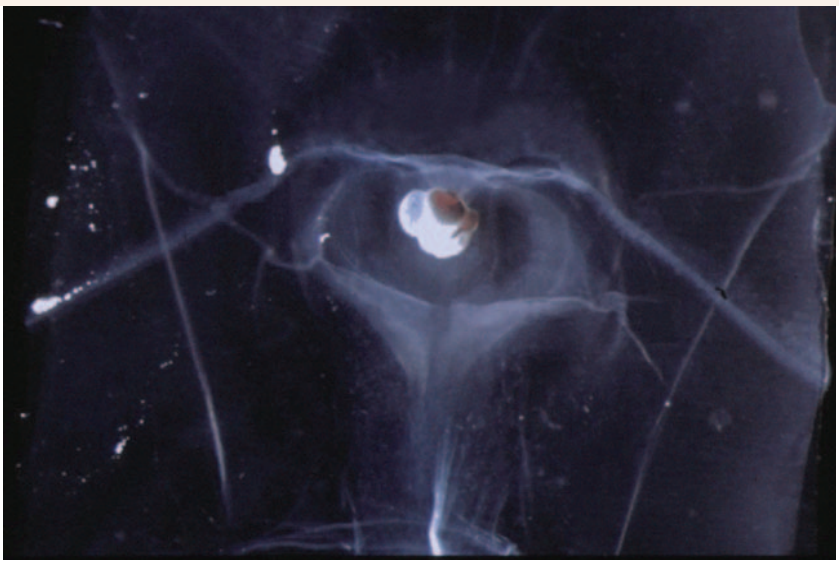
3 Miura K and Optican LM: Membrane properties of medium-lead burst neurons may contribute to dynamical properties of saccades. Proceedings of the first International IEEE EMBS Conference on Neural Engineering, Pages 20-23, 2003.

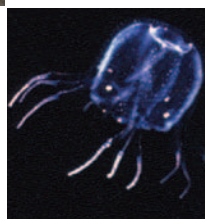
4 Optican LM and Quaia C: Distributed model of collicular and cerebellar function during saccades. Annals of the New York Academy of Sciences, Kaminski HJ and Leigh RJ, eds. vol. 956, pgs. 164-177, 2002

5 Optican LM and Quaia C: From sensory space to motor commands: lessons from saccades. Engineering in Medicine and Biology Society, 2001. Proceedings of the 23rd Annual International Conference of the IEEE , Volume: 1, Page(s): 820-823, 2001.

Section on Molecular Genetics

Joram Piatigorsky, PhD





1

1 Photograph of a living jellyfish, Tridpedalia cystophora. The 4 refractive spots indicated by the arrows are the rhopalia, sensory structures that contain the ocelli (eyes) and statocyst (balancing organ). The statocyst has been considered as an ancient form of an inner ear in more advanced species. This photograph was taken by the late Mr. Charles Cutress (Marine Station, Department of Marine Sciences, University of Puerto Rico, Mayaguez, PR).

2 Photograph of a rhopalium of a living jellyfish, Tripedalia cystophora. The rhopalia are structures in jellyfish that dangle from stalks and are set within a notch on the surface of the umbrella.. Each of the four rhopalia contains a large and small ocellus (eye) set at right angles to one another, and a statocyst (balancing organ). This photograph of a single rhopalium was taken by the late Dr. Toichiro Kuwabara (NEI, NIH).

2

Selected Publications

1 Hough, R.B. and Piatigorsky, J., 2004. Preferential transcription of rabbit Aldh1a1 in the cornea: implication of hypoxia-related pathways. Mol. Cell. Biol. 24: 1324-1340.

2 Nees, D.W., Fariss, R.N. and Piatigorsky, J., 200). Serum albumin in mammalian cornea: implications for clinical application. Invest. Ophthalmol. Vis. Sci. 44: 3339-3345.

3 Swamynathan, S.K., Crawford, M.A., Robison, Jr., W.G., Kanungo, J. and Piatigorsky, J., 2003. Adaptive differences in the structure and macromolecular compositions of the air and water corneas of the "four-eyed fish (Anableps anableps). FASEB J. 17: 1996-2005.

4 Kanungo, J., Kozmik, Z., Swamynathan, S.K. and Piatigorsky, J., 2003. Gelsolin is a dorsalizing factor in zebrafish. Proc. Natl. Acad. Sci. 100: 3287-3292.

5 Kozmik, Z., Daube, M., Frei, E., Norman, B., Kos, L. Dishaw, L.J., Noll, M. and Piatigorsky, J., 2003. Role of Pax genes in eye evolution: A cnidarian PaxB gene uniting Pax2 and Pax6 functions. Dev. Cell 5: 773-785.

6 Swamynathan, S.K. and Piatigorsky, J., 2002. Orientation-dependent influence of an intergenic enhancer on the promoter activity of the divergently transcribed mouse Shsp/αB-crystallin and Mkbp/HspB2 genes. J. Biol. Chem. 277: 49700-49706.

Research Summary

The Section of Molecular Genetics performs research on gene expression in the lens and cornea. Lens crystallins differ among taxonomic groups and are often related or identical to metabolic enzymes in both vertebrates and invertebrates. We coined the term 'gene sharing' to emphasize the dual refractive and metabolic (or stress protective) functions of crystallins. We extended the gene sharing concept to the cornea which also expresses taxon-specific, unexpectedly abundant proteins (often enzymes) in the cornea.

Evolution: Jellyfish are the most ancient species known to have complex eyes with lens and cornea, Figuress 1 and 2. Despite their sophisticated eyes, however, we showed that they lack Pax6, the transcription factor implicated in the evolutionary origin of eyes and important for crystallin gene expression. Instead, jellyfish have PaxB, which recognizes Pax2 DNA regulatory regions. Nonetheless, jellyfish PaxB stimulates jellyfish crystallin promoter activity and can experimentally induce small ectopic eyes in the fly. Both the jellyfish PaxB and crystallin genes are expressed in the eyes and statocysts. We have proposed that PaxB was the primordial Pax protein in eye evolution and crystallin gene expression. Moreover, since statocysts may be considered as precursors to ears, we suggested that eyes and ears may have an ancient, common origin.

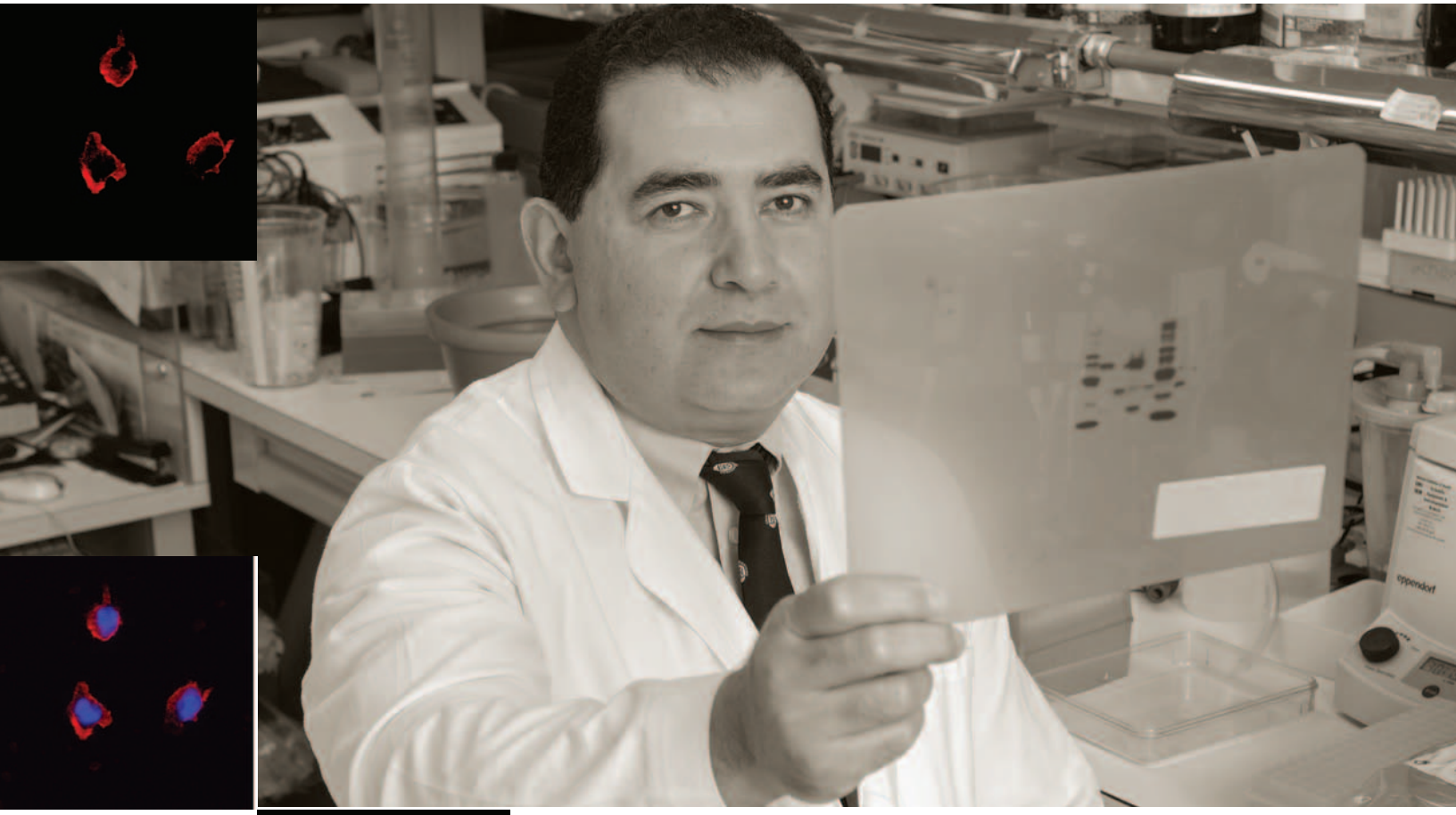
αB-crystallin gene expression: The small heat shock protein/αB-crystallin is very abundant in the vertebrate lens and is also present in lesser amounts in other tissues. We identified lens-specific regulatory elements in the proximal promoter and an orientation-dependent distal enhancer that modulates lens expression and is responsible for tissue-specific expression of the mouse αB-crystallin gene. The enhancer is also associated with an upstream silencing/insulator element that appears to further modulate gene expression. Transgenic mouse tests revealed that the blind mole rat αB-crystallin promoter/enhancer has lost lens activity and gained muscle activity. Thus, specific molecular changes in small heat shock protein/αB-crystallin promoter/enhancer structure and function have accompanied the subterranean adaptations of the mole rat. Together these findings begin to create a molecular model for lens specialization of a ubiquitously expressed gene.

Corneal gene expression: Various enzymes are over-expressed in mammalian corneas. In zebrafish, however, gelsolin comprises half of the water-soluble corneal proteins. The corneal gelsolin also participates in the dorsal/ventral signaling cascade during embryogenesis. A second zebrafish gelsolin gene, not highly expressed in cornea, is expressed ubiquitously but is not involved in developmental signaling. These experiments show that abundant corneal proteins, like lens crystallins, can participate in multiple functions by a gene sharing mechanism. We are also mapping the complexity of gene expression by SAGE (serial analysis of gene expression) in the developing mouse cornea. Expression of ~20,000 genes was identified in the mouse cornea. One-third of these are expressed before eye opening, one-third at 6 weeks, and one-third at both developmental times. These data illustrate a surprising dynamism of gene expression during postnatal corneal development and provide new approaches to study the normal and diseased cornea.

Clinical and Molecular Immunology Section

Jack Ragheb, MD, PhD

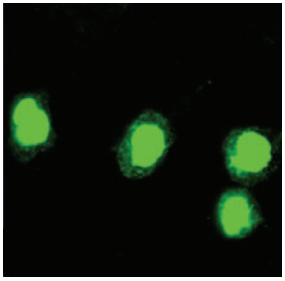
1



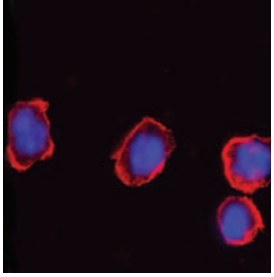
2

3

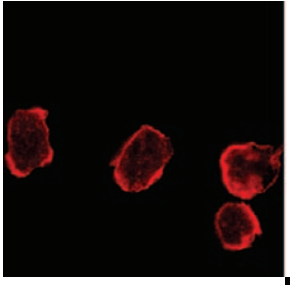
1, 2, 3 Resting T cells
4, 5, 6 and stimulated T cells were triple labeled with an antibody to HuR green, the actin-specific probe phalloidin Alexa 568 red and the DNA-specific fluorophore DAPI blue. In resting cells, HuR green is largely confined to nuclei. In stimulated cells, HuR is visible in both the nuclei and cytoplasm demonstrating translocation of this protein
7 as shown by the orange color in the overlaid image 7 of 5 and 6.



4

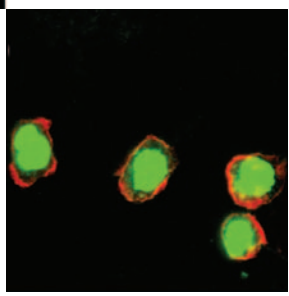


5



6

7



Research Summary

My group conducts basic and clinical research that has the long-term goal of reprogramming the immune system to develop or reestablish tolerance to self-antigens. In the absence of a costimulatory signal, T cell receptor (TCR) ligation results in apoptosis or an unresponsive state termed anergy, both of which contribute to the normal state of immune tolerance. In my lab, we investigate the molecular mechanisms underlying the costimulatory component of T cell activation. By doing so, we aim to develop novel agents capable of specifically blocking these pathways and thus inducing immune tolerance in patients. Clinically, we also use novel combinations of existing pharmacological agents in an attempt to induce immune tolerance in patients.

With few exceptions, the same drugs used to treat autoimmune disease and transplant graft rejection also block the induction of immune tolerance; a prerequisite for graft acceptance in the absence of continued immunosuppression, or for an autoimmune disease cure. The observation that immunosuppressive monotherapy with anti-IL-2R Ab, an agent that does not interfere with the induction of tolerance, can control autoimmune disease presents us with a unique opportunity to clinically test the hypothesis that rapamycin, a drug shown to induce anergy, can produce clinical immune tolerance. Such a clinical trial is presently underway.

The principal immunomodulatory drugs in clinical use today act to block TCR signaling, which also prevents the induction of anergy and T cell apoptosis. Elucidation of the CD28 costimulatory pathway may lead to the development of novel agents that inhibit T cell activation through their effects on CD28 signaling without interfering with TCR signaling. We study CD28 signaling by examining its effects on the stability of the IL-2 mRNA. We hypothesized that sequences outside of the IL-2 mRNA 3'UTR are required for CD28 -mediated stabilization. Having identified such CD28 responsive elements, we're using these sequences as probes to isolate the final transducers of the CD28 signal pathway; the RNA binding proteins that act to stabilize the IL-2 mRNA. One such protein is HuR, which is translocated to the cytoplasm and binds the IL-2 mRNA in response to TCR signals.

Besides CD28, the cytokines IL-2 and interferon (IFN)-γ, and the costimulatory molecule CD40 ligand (CD40L), are of central importance in T cell activation. We were the first to report that CD40L expression can be separated into an early CD28-independent phase and a later, CD28-depedent phase. Furthermore, we reported that an IL-2 receptor (IL-2) blocking antibody (Ab) completely inhibits late CD40L expression and consequentially IFN-γ production. The most effective means shown of inducing immune tolerance in a primate allotransplant model is Ab blockade of CD40L. Unfortunately, in clinical trials the anti- CD40L Ab was associated with intolerable side effects. While anti-IL-2 Ab completely inhibits the late phase of CD40L expression in vitro, it is an ineffective means of controlling allotransplant rejection in vivo. We hypothesize that dissecting the cellular and molecular mechanisms that control the IL-2 independent early phase of CD40L expression may lead to novel means of blocking its expression and new therapeutic strategies to induce immune tolerance and treat immune-mediated disorders.

Selected Publications

1 Shen J, Snyder J, Azmi H, Ragheb JA. IL-2, but not Th1 or Th2 Cytokines Regulate the Biphasic Expression of CD40 Ligand: Evidence that IL-2 Receptor Blockade Directly Inhibits Late CD40 Ligand Expression. Submitted.

2 Seko Y, Azmi H, Fariss R, Ragheb JA. Selective cytoplasmic translocation of HuR and site-specific binding to the interleukin-2 mRNA are not sufficient for CD28-mediated stabilization of the mRNA. J Biol Chem. 2004 Aug 6;279(32):33359-67. Epub 2004 Mar 12.

3 McDyer JF, Li Z, John S, Yu X, Wu CY, Ragheb JA. IL-2 receptor blockade inhibits late, but not early, IFN-gamma and CD40 ligand expression in human T cells: disruption of both IL-12-dependent and -independent pathways of IFN-gamma production. J Immunol. 2002 Sep 1;169(5):2736-46

4 Ragheb JA, Deen M, Schwartz RH. CD28-Mediated regulation of mRNA stability requires sequences within the coding region of the IL-2 mRNA. J Immunol. 1999 Jul 1;163 (1):120-9.

5 Miller C, Ragheb JA, Schwartz RH. Anergy and cytokine-mediated suppression as distinct superantigen-induced tolerance mechanisms in vivo. J Exp Med. 1999 Jul 5;190 (1):53-64.

Molecular Mechanisms Section

T. Michael Redmond, PhD

Research Summary

The retinal pigment epithelium (RPE) plays a key role in the development and function of the outer retina. Without the RPE, the photoreceptors, and vision itself, could not function. In the RPE, all-trans retinol is enzymatically isomerized to 11-cis retinol, oxidized to 11-cis retinal and secreted to the photoreceptors to regenerate visual pigment. When light is absorbed, rhodopsin-bound 11-cis retinal is isomerized back to all-trans and returned to the RPE for re-isomerization. This process is called the visual cycle. We are studying the role of RPE65, a highly specific RPE protein, in this process. Evidence from biochemical and molecular genetics studies in mouse models and human genetic eye disease show that RPE65 is essential to the visual cycle. The techniques employed in these studies include molecular biology, molecular genetics, transgenic/knockout animal models, biochemistry, and protein chemistry.

The crucial nature of RPE65, discovered by this lab in 1993, is demonstrated by its role in genetic blindness. Mutations in the human RPE65 gene (see also: *http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?180069*) result in Leber congenital amaurosis (LCA; see: *http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?204100*) with about 60 mutations identified since 1997. Common features of these include severe loss of vision from birth or early childhood, complete night-blindness, and severely reduced rod and cone responses. To define the role of RPE65, we made an RPE65 knockout mouse. Its phenotype showed lack of rhodopsin in the rod photoreceptors, though it expresses opsin apoprotein. As a result, the rod and cone electroretinograms are essentially abolished. The almost complete lack (>99.9% absent) of 11-cis retinal coincides with accumulation in the RPE of all-trans retinyl esters, the precursor to 11-cis retinol. Thus, RPE65 is necessary for the production of 11-cis retinoids by the RPE. Recently, the Briard dog model of LCA that harbors a RPE65 mutation, was treated by somatic gene therapy to successfully restore functional vision. This provides hope for future treatment of patients with RPE65 retinal dystrophy. Our lab is cooperating with the groups involved in this important endeavor.

We have identified transcriptional and translational regulatory regions involved in the expression of RPE65. We found that a 700 bp upstream region of the mouse RPE65 gene conferred RPE-specific expression in transgenic mice and in a human RPE cell line. Mutations of potential transcription sites abolished binding and activation and indicated that octamer and E-box transcription factors synergistically regulate the RPE65 promoter function. Current work in our group is directed towards identifying the transcription factors binding to the RPE65 promoter.

RPE65 is a member of an ancient, family of enzymes that primarily cleave carotenoids, including β-carotene 15,15'-monooxygenase (BCM), a crucial enzyme that forms vitamin A from plant derived pro-vitamin A precursors. Our group has cloned and characterized mouse BCM. We have studied the transcriptional regulation of the BCM gene and how it is integrated into the overall regulation of vitamin A metabolism. Recently, we have identified residues in BCM, conserved in all members, that are required for metal binding and thus for enzymatic activity of this enzyme. Consequently, the conservation of a common mechanism of action between β-carotene 15,15'-monooxygenase and RPE65 is a possibility that is under study.

Selected Publications

1 Boulanger, A., McLemore, P., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Gentleman, S., and Redmond, T.M.: Beta-carotene 15,15'-monooxygenase is a peroxisome proliferator activated receptor target gene. FASEB J. 10.1096/fj.02-0690fje, 2003.

2 Redmond, T.M., Gentleman, S., Duncan, T., Yu, S., Wiggert, B., Gannt, E., and Cunningham, F.X., Jr.: Identification, expression and substrate specificity of a mammalian β-carotene 15,15'-dioxygenase. J.Biol. Chem., 276: 6560-6565, 2001.

3 Boulanger, A., Liu, S., Henningsgaard, A.A., Yu, S., and Redmond, T.M.: The upstream region of the RPE65 gene confers retinal pigment epithelium-specific expression in vivo and in vitro and contains critical octamer and E-box binding sites. J. Biol. Chem. 275: 31274-31282, 2000.

4 Redmond, T.M., Yu, S., Lee, E., Bok, D., Hamasaki, D., Chen, N., Goletz, P., Ma, J.-X., Crouch, R.K. and Pfeiffer, K.: Rpe65 is necessary for production of 11-cis-Vitamin A in the retinal visual cycle. Nature Genetics 20: 344-350, 1998.

5 Hamel, C.P., Tsilou, E., Pfeffer, B.A., Hooks, J.J., Detrick, B. and Redmond, T.M.: Molecular cloning and expression of RPE65, a novel retinal pigment epithelium-specific microsomal protein that is post-transcriptionally regulated in vitro. J. Biol. Chem. 268: 15751-15757, 1993.

George F. Reed, PhD

Selected Publications

1 Chan, C.-C., G.F. Reed, E. Agrón, and R.R. Buggage, 2004. A correlation of pregnancy term, disease activity, serum female hormones, and cytokines in uveitis. British Journal of Ophthalmology, 88, 1506-1509.

2 Reed, G.F., F. Lynn, and B.D. Meade, 2002. Use of coefficient of variation in assessing variability of quantitative assays. Clinical and Diagnostic Laboratory Immunology, 9, 1235-1239.

3 Reed, G.F., B.D. Meade, and M.C. Steinhoff, 1995. The reverse cumulative distribution plot: a graphic method for exploratory analysis of antibody data. Pediatrics, 96, 600-603.

Research Summary

The Biometry Branch specializes in the methodology of design, conduct, and data analysis of vision and ophthalmological research. The Branch is engaged, independently and in vigorous collaboration with NEI intramural scientists, in developing and employing the appropriate techniques for articulating testable hypotheses, collecting data pertinent to formation and evaluation of hypotheses, and analyzing data in ways that minimize variability and bias. Current issues of interest are methods for controlling multiple testing error in microarray assays in genetic research, design strategies for small clinical trials, development and evaluation of ophthalmic outcome measures, and methods that account for the correlation between paired eyes. Examination and resolution of these issues is by way of graphical analysis and exploration, resampling methods, Bayesian approaches, Monte Carlo simulation methods and statistical computing, generalized estimating equations, mixed models, and other recent and emerging statistical methodologies.

Outcomes Measures: One of the most active areas of research is outcome measures in ophthalmology. We are currently developing a method for quantifying the severity of uveitis, based on the array of clinical indices that characterize uveitis, and have initiated an examination, in collaboration with NEI clinical scientists, of the method's properties. In another collaboration, with NEI and NASA scientists, we are searching for the best scalar outcome from Dynamic Light Scattering technology for tracking the development and growth of lens opacities; the technique itself produces a bimodal distribution of particle sizes that needs to be summarized in a single number that precisely and accurately measures cataracts and nascent cataracts. Another outcomes issue under scrutiny by Branch personnel is how best to define improvement, or deterioration, in visual acuity for use in clinical trials given the presence of measurement and classification errors.

Biography

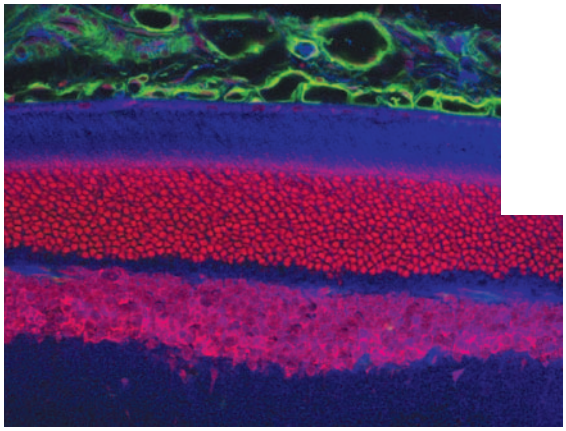
Dr. Reed has a B.S. in mathematics from Morgan State College and, from the University of Minnesota, an M.A. in Statistics and PhD in Biometry. His career began in data analysis and statistical methods development in toxicology studies at the U.S. Food and Drug Administration. He later joined the National Institute of Child Health and Human Development and extended his collaborative and methodological activities to multicenter clinical trials and epidemiological studies. In 1989, he turned to research in the prevention and treatment of infectious diseases, with emphasis on vaccine clinical trials, at the National Institute of Allergy and Infectious Diseases. In 1996, he became Chief of the Biometry Branch of the National Eye Institute.

Mechanisms of Retinal Deseases Section

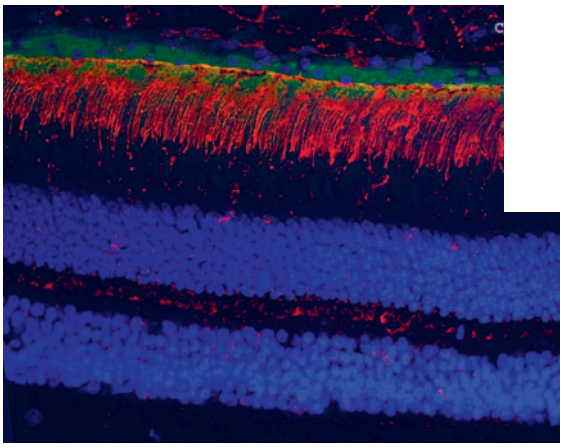
Ignacio R. Rodriguez, PhD

1 Retina taken from a rat 4 hrs after an intravenous injection of human LDL containing the blue fluorescent cholesterol analog cholestatrienol. The nuclei were stained with propidium iodide, red, and the capillaries were stained with Alexa 488, green, labeled isolectin B. LDL deposits can be observed in the choriocapillaris and Bruch's membrane.

2 Localization of the SR-BI-2 scavenger receptor in monkey RPE and photoreceptors. The SR-BI-2 receptor was detected using an anti-rabbit Cy5, red, antibody. The nuclei were stained with DAPI, blue. SR-BI-2 was found in the apical side of the RPE and in the photoreceptor outer segment.



1



2

Research Summary

Age-related macular degeneration is a complex disease affected first and foremost by the aging process but also by genetics and environmental factors. The disease is characterized by the loss of central vision. The loss of vision is suspected to be caused by the loss of function of the retinal pigment epithelium (RPE) cells that support the macular photoreceptors which lead to the death of the photoreceptor cells. My research program is focused on finding the early molecular events that lead to the RPE malfunction.

One of the events associated with aging is the gradual accumulation of cholesterol and other lipid deposits in Bruch's membrane especially around the macular region. The hypothesis is that this age-related accumulation of these lipids leads to their oxidation during the highly oxidative exchanges between the neural macula and the RPE-choroid. The oxidation of these lipids then leads to direct cytotoxicity on the RPE as well as to an inflammatory response via the scavenging choroidal macrophages. The age of onset, the type (wet or dry) and the severity of the disease are likely influenced by the genetic and environmental factors.

Using fluorescently labeled low density lipoprotein (LDL) we have demonstrated that the RPE will internalize LDL in vitro and in vivo (IOVS, 2004). We have further demonstrated this LDL transport into the retina by incorporating a blue fluorescent cholesterol analog (cholestatrienol) into purified human LDL and intravenously injecting it into rats. We observed incorporation of the LDL into the RPE within 2 hr. The cholestatrienol can be visualized in the photoreceptors by 4 hrs (Figure 1) and it is still present in the retina 24 hrs later. We also observed LDL deposits in the choroid and Bruch's membrane (Figure 1) which form and disappear with time. Some LDL deposits can be observed in Bruch's membrane after 24 hr. We also examined the effects of oxidized LDL (oxLDL) on cultured RPE cells and found that RPE cells like macrophages can internalize oxLDL via the CD36 and other scavenging receptors. Depending on the degree of oxidation of the LDL, this internalization may be lethal. After analyzing individual oxysterols present in the oxLDL we found one component, 7-ketocholesterol could account for most of the cytotoxicity associated with oxLDL.

We are systematically analyzing the retina for the presence of scavenger receptors capable of moving lipid in and out of the retina. One of these receptors SR-BI-2 is present in the apical side of the RPE and in the tips of the rod outer segments (Figure 2). This receptor may be responsible for moving lipid from the RPE to the photoreceptors. Our overall data suggests that the accumulation and oxidation of LDL in Bruch's membrane and the choroid may be the initial events that lead to age-related macular degeneration.

Selected Publications

1 Tserentsoodol, N., Gordiyenko, N., Lee, J.W., and Rodriguez, I.R. Localization of scavenger receptors responsible for lipid transport in the retina. (in preparation)

2 Rodriguez, I.R., Tserentsoodol, N., Fliesler, S.J., and Lee J.W. Measurement of low density lipoprotein internalization by the rat retina using the fluorescent cholesterol analog cholestatrienol. (in preparation)

3 Alam, S., Gordiyenko, N., Lee, J.W., and Rodriguez, I.R. Overexpression of oxysterol binding proteins increases resistance to 7-ketocholesterol cytotoxicity in cultured retinal pigment epithelium cells. (in preparation)

4 Rodriguez, I.R., Alam, S., Lee, J.W. Cytotoxicity of oxidized low density in cultured RPE cells is dependent on the formation of 7-ketocholesterol. Invest. Ophthalmol. & Vis. Sci. 45(8): 2830-2836, 2004.

5 Gordiyenko, N., Campos, M., Lee, J.W., Farris, R.N., Sztein J., and Rodriguez, I.R. RPE cells internalize low density lipoprotein (LDL) and oxidized LDL in large quantitites in vitro and in vivo. Invest. Ophthalmol. & Vis. Sci. 45(8): 2822-2829, 2004

6 Rodriguez, I.R. Rapid analysis of oxysterols by HPLC and UV spectroscopy. Biotechniques, 36(6): 952-958, 2004

7 Jaworski, C.J., Moreira, E.F., Li, A., Lee, R., and Rodriguez, I.R. Family of twelve genes containing oxysterol-binding domains in the human genome. Genomics 78(3): 185-196, 2001

8 Moreira, E.F., Jaworski, C., Li, A. and Rodriguez, I.R. Molecular and biochemical characterization of a novel oxysterol binding protein (OSBP2) highly expressed in retina. J. Biol. Chem 276:18570-18578, 2001

Aging and Ocular Disease Section

Paul Russell, PhD

Research Interests

The research of the Aging and Ocular Disease Section is centered on the examination of changes, particularly in the anterior segment in the eye, that occur with age and how some of these alterations may cause ocular disease. We are interested in glaucoma, particularly primary open angle glaucoma. In a large number of cases of primary open angle glaucoma, the ability of the aqueous humor to flow out of the eye is impaired and the pressure within the eye is increased. Biochemical and molecular biological changes occur in the trabecular meshwork, the primary site for aqueous humor outflow in humans, with this increase in pressure. We are attempting to understand these changes. We are working to develop therapeutic interventions using approaches that involve inhibition of certain biochemical pathways in the trabecular meshwork to improve outflow of the aqueous humor. We hope to develop modalities to treat both genetically related glaucomas as well as other glaucomas that individuals develop.

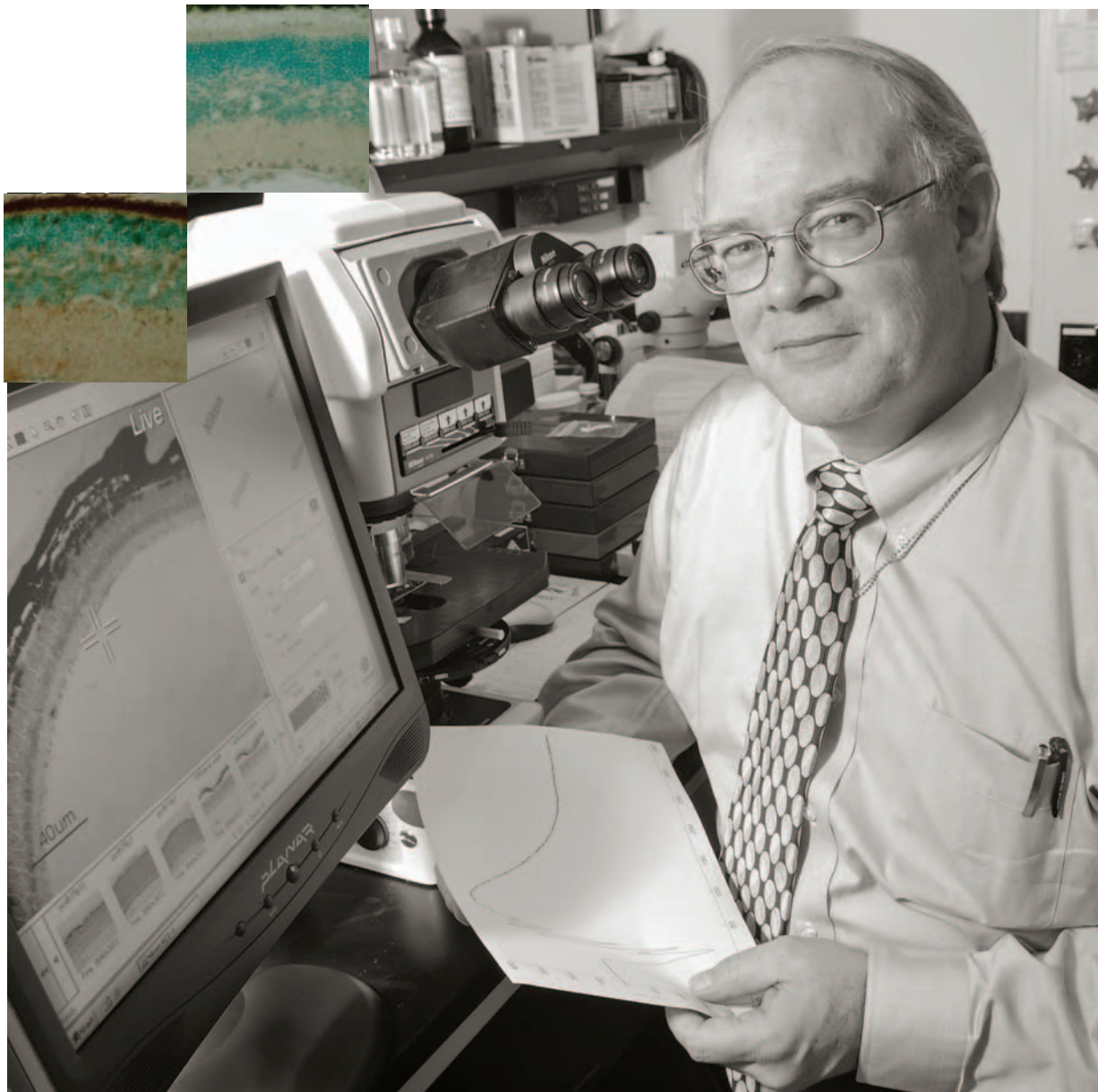
Section on Translational Research for Retinal and Macular Degeneration

Paul Sieving, MD, PhD

Research Interests

Our laboratory studies retinal neurodegeneration in animal models, with the intent of identifying disease mechanisms that will allow us to develop interventions to explore in human clinical trials. The laboratory has expertise in retinal electrophysiology, retinal cell biology, biochemistry and histology, genetic manipulation, and whole animal ocular biology. This multifaceted approach allows us to evaluate rodent models of retinal degeneration, both naturally occurring and animals made through laboratory genetic manipulation.

1 2



XLRS rescue by RS gene transfer using adeno-associated virus AAV(2/2)-CMV-Rs1h delivered by intraocular injection to the
1 right eye of Rs1h-/Y mouse number 554,
2 with PBS injected into left eye; both eyes were injected at 13 weeks of age and were evaluated 9-11 weeks later (i.e., at 6 months of age). Immunohistochemistry using polyclonal RS antibody shows RS protein (reddish brown staining) in multiple retinal layers of right eye whereas no RS is evident in the PBS-injected contralateral left eye.

Juvenile X-linked Retinoschisis

Juvenile X-linked Retinoschisis (XLRS) is of particular interest in the laboratory. Our recent studies of the cell biology of retinoschisin protein expression and the creation of a retinoschisin (RS) gene knockout mouse model provide a starting point toward an eventual clinical trial for human subjects. This protein is expressed developmentally in all retinal neurons, first in ganglion cells and then in an expression pattern moving posterior through the retina, as a "developmental wave of retinoschisin expression." The XLRS mouse gene knockout model recapitulated the ERG changes in XLRS human patients, in which the b-wave is selectively reduced. We transferred the RS gene into the RS knockout mouse using AAV and AV vectors and could restore the b-wave amplitude, thereby providing a possible noninvasive tool to monitor an eventual human clinical trial.

Neurotrophic Factors

Neurotrophic factors slow the natural rate of degeneration in animal models. LEDGF (lens epithelium derived growth factor) and CNTF (ciliary neurotrophic factor) rescue neurons and may provide a promising generalized therapy for inherited retinal degenerative conditions. We are currently investigating the mechanism of LEDGF and whether it works through heat shock proteins (HSPs). HSPs are generally assumed to play protective roles in a wide range of cell types. They enable cells to survive and recover from a variety of stresses. Our results indicate that LEDGF plays a significant role in protecting photoreceptor cells against environmental stress in normal rats and from hereditary degeneration in RCS rats. We are also conducting phase I clinical trials to evaluate CNTF treatment in retinitis pigmentosa patients. We deliver CNTF by encapsulated cell technology (ECT), which provides delivery of CNTF through continual and stable intraocular release at known doses via a device implanted in the vitreous.

Vitamin A Metabolism in Retinal Disease

Vitamin A is a retinoid important for visual process. 11-cis retinal is critical for regeneration of rhodopsin after a bleaching light exposure. We have identified that accutane targets the RDH5 enzyme in the retinal pigment epithelium and impedes retinoid metabolism in the retinal pigment epithelium. We proposed a therapeutic strategy in which other retinoid analogs of accutane could be used to retard rhodopsin cycling after bleaching light exposure and thereby reduce the effective retinoid load required for RPE processing, thereby possibly retarding retinal degeneration. These concepts continue to be explored.

Electroretinogram (ERG) Basic Studies

We have published extensively on the origins of the ERG in primates and rodents. We continue to probe the cellular and mechanistic origins of the ERG components. We currently are working on the photopic negative response (PhNR) to learn whether the mechanism involves amacrine cells and potassium circuits of the proximal retina.

Working in this laboratory requires an interactive mindset for people who must be willing to collaborate with others in the laboratory, as no one has the full set of skills to study all aspects of any problem. The strength of the laboratory is the collaboration among the laboratory investigators and a willingness to share information and gain expertise from other laboratory investigators.

Selected Publications

1 Bush RA, Lei B, Tao W, et al. Encapsulated cell-based intraocular delivery of ciliary neurotrophic factor in normal rabbit: dose-dependent effects on ERG and retinal histology. Invest Ophthalmol Vis Sci. 2004;45:2420-2430.

2 Khan NW, Kondo M, Hiriyanna KT, Jamison JA, Bush RA, Sieving PA. Primate retinal signaling pathways: Suppressing ON-pathway activity in monkey with glutamate analogs mimics human genetic CSNB1-NYX night blindness. J Neurophysiol. E-pub Aug 25, 2004.

3 Takada Y, Fariss RN, Tanikawa A, et al. A retinal neuronal developmental wave of retinoschisin expression begins in ganglion cells during layer formation. Invest Ophthalmol Vis Sci. 2004; 45:3302-3312.

4 Zeng Y, Takada Y, Kjellstrom S, et al. RS-1 Gene delivery to an adult Rs1h knockout mouse model restores RG b-wave with reversal of the Electronegative Waveform of X-Linked Retinoschisis. Invest Ophthalmol Vis Sci. 2004; 45:3279-3285.

5 Ayyagari R, Demirci FY, Liu J, Bingham EL, Stringham H, Kakuk LE, Boehnke M, Gorin MB, Richards JE, Sieving PA. X-linked recessive atrophic macular degeneration from RPGR mutation. Genomics. 80:166-171, 2002.

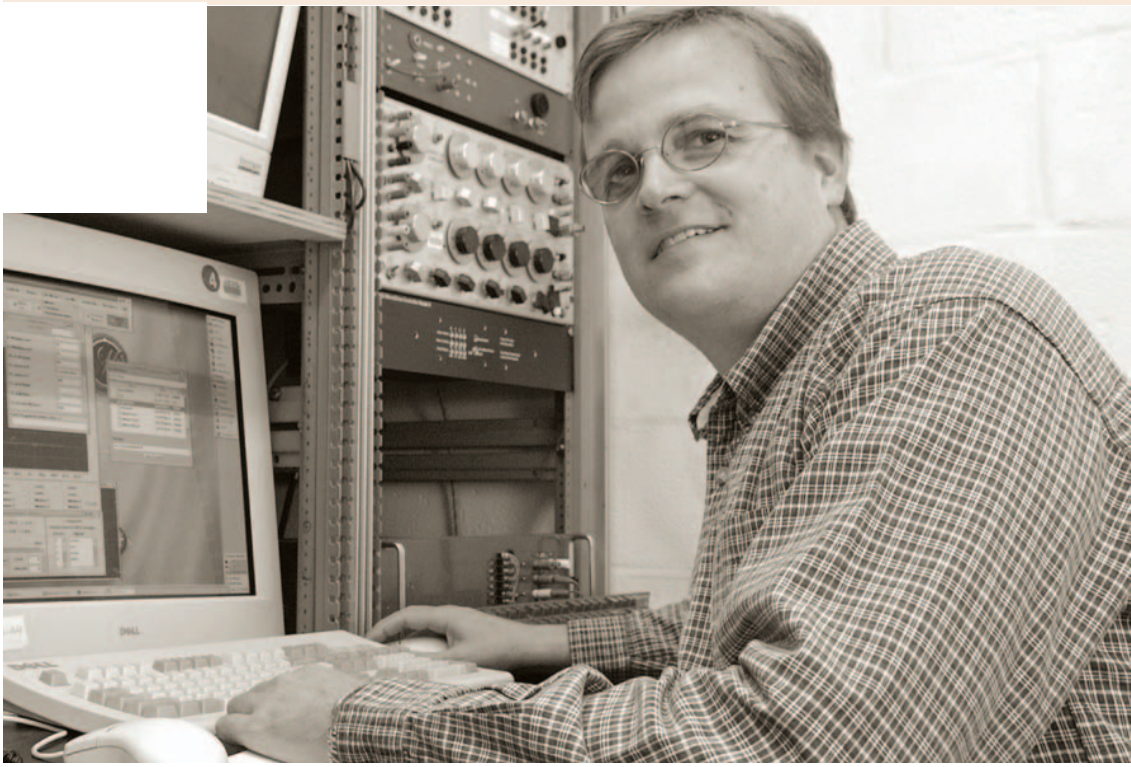
6 Sieving PA, Chaudhry P, Kondo M, et al. Inhibition of the visual cycle in vivo by 13-cis retinoic acid protects from light damage and provides a mechanism for night blindness in isotretinoin therapy. Proceedings of the National Academy of Sciences of the United States of America. 2001; 98:1835-1840.

Perception and Action Section

Kirk G. Thompson, PhD

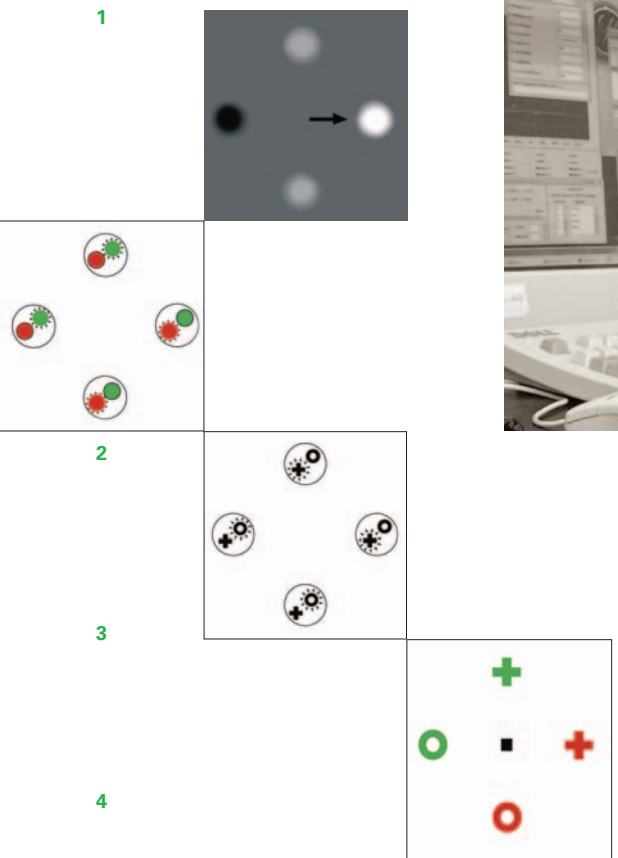
Research Interests

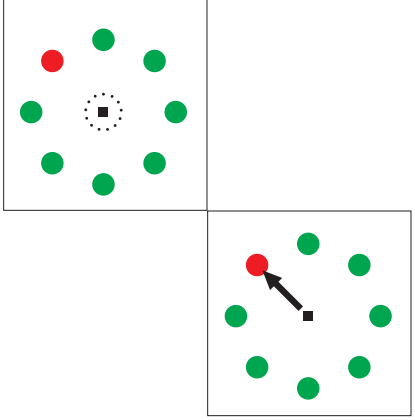
My research interest is to understand mysteries of cognition. How do we make perceptual decisions? How do we choose what to look at? How are voluntary movements initiated? What is the neural basis of directed visual attention? And how do our individual experiences, memories and goals affect these processes? To understand how subjective perception and voluntary motor actions emerge from brain activity, I record neural activity in the brains of monkeys performing visual search and forced-choice discrimination tasks. Most of my research has focused on the frontal eye field, which is located in the prefrontal cortex and participates in the transformation of visual information into commands to move the eyes.



Frontal eye field as a salience map. Knowledge of the target interacts with representations of motion, color, and shape within the frontal eye fields to form a salience map. It is hypothesized that the peak activation within this map directs attention and eye movements.

1 Frontal eye field salience map
2 Color
3 Shape
4 Input image
5 Visual selection by FEF neuron
6 Gaze shifts made by a monkey searching for a randomly oriented T among L's. Beginning at the central fixation spot, the monkey made a sequence of eye movements before fixating the target. Notice that the monkey looked away from the target before returning; it seems he looked at the target but did not see it.





5

Research Summary

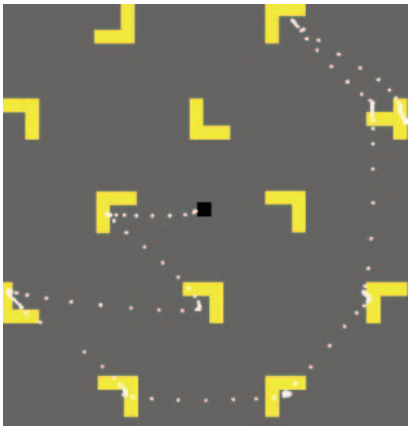
Visual attention and eye movements

Directed visual attention is necessary for normal vision and eye movements. However, we still do not understand how attention is controlled. Theoretical models of visual attention propose that within the brain exists a topographic map that represents the relative importance of every object in the visual scene. It is proposed that this 'salience map' guides both attention and eye movements. My research has shown that the frontal eye field contains a salience map derived from both bottom-up visual representations and top-down cognitive influences (Sato et al., 2003; Thompson and Bichot, 2005). The spatial location represented by the highest activity in frontal eye field guides saccades in a probabilistic manner (Bichot et al., 2001; Thompson et al., 2004). However, the selective activity in the frontal eye field is independent of eye movements and likely corresponds to the spotlight of covert attention that, in addition to guiding eye movements, modulates ongoing visual processing (Thompson et al., 1996; Thompson et al., 1997; Murthy et al., 2001). Further understanding of the role that the frontal eye field plays in attentional processes requires more direct manipulations of frontal eye field activity. This will be the focus of future work.

Perceptual decisions

How do we decide if something is there or not? To answer this question I used the phenomenon of backward masking to make the visibility of a target unreliable. I found that small variations in the visual responses of single neurons in the frontal cortex predict very accurately whether or not monkeys will report the presence or absence of a target, even when the target was absent (Thompson and Schall, 1999). These small initial variations lead to larger differences in neural activity that correspond to the monkeys' report of their perceptual state (Thompson and Schall, 2000). Currently we are conducting experiments that are probing perceptual and motor decision processes in monkeys trained to perform near-threshold luminance judgments. We are interested in how the brain represents visual information, uses past experience to influence real-time perceptual judgments, and how perceptual decisions are then transformed into appropriate motor commands; and all of this within 200 milliseconds! We are looking for answers in the frontal eye field and in the lateral intraparietal cortex.

6



Selected Publications

1 Thompson KG, Bichot NP (2005) A visual salience map in the primate frontal eye field. Progress in Brain Research 147. In Press.
2 Thompson KG, Bichot NP, Sato TR (2004) Frontal eye field activity before visual search errors reveals the integration of bottom-up and top-down salience. J Neurophysiol. In Press.
3 Sato TR, Watanabe K, Thompson KG, Schall JD (2003) Effect of target-distractor similarity on FEF visual selection in the absence of the target. Experimental Brain Research 151:356-363.
4 Bichot NP, Thompson KG, Chenchal Rao S, Schall JD (2001) Reliability of macaque frontal eye field neurons signaling saccade targets during visual search. J Neurosci 21:713-725.
5 Murthy A, Thompson KG, Schall JD (2001) Dynamic dissociation of visual selection from saccade programming in frontal eye field. J Neurophysiol 86:2634-2637.
6 Thompson KG, Schall JD (2000) Antecedents and correlates of visual detection and awareness in macaque prefrontal cortex. Vision Res 40:1523-1538.
7 Thompson KG, Schall JD (1999) The detection of visual signals by macaque frontal eye field during masking. Nat Neurosci 2:283-288.
8 Thompson KG, Bichot NP, Schall JD (1997) Dissociation of visual discrimination from saccade programming in macaque frontal eye field. J
9 Neurophysiol 77:1046-1050. Thompson KG, Hanes DP, Bichot NP, Schall JD (1996) Perceptual and motor processing stages identified in the activity of macaque frontal eye field neurons during visual search. J Neurophysiol 76:4040-4055.

Molecular Mechanisms of Glaucoma Section

Stanislav Tomarev, PhD

Research Interests

This section conducts basic research on glaucoma, one of the leading causes of blindness in the world. Recent studies have focused on the development of novel mouse models of glaucoma. Another main area of research is the identification of new genes responsible for glaucoma as well as those important for normal eye development and function. Several novel genes were identified that are expressed in different eye structures. One encodes a novel olfactomedin domain-containing protein that was named optimedin.

Research Summary

Recent studies have focused on the development of novel genetic models of glaucoma using a transgenic approach. Properties of the myocilin gene were used to develop a mouse model of glaucoma. It is well documented that mutations in this gene may lead to glaucoma in humans. Several lines of transgenic mice containing BAC DNA with a point mutation in the myocilin gene were produced. Transgenic mice demonstrated changes in the retina similar to those observed in glaucoma in humans. These transgenic animals will be used to address several fundamental questions in glaucoma including identification of the signaling pathways involved in the disease, effects of modifier genes, and neuroprotection.

The existing rat models of pressure-induced optic nerve degeneration are used to study molecular changes in the retina. It has been demonstrated that prolonged (5 weeks) exposure to elevated IOP modifies expression levels of at least 80 genes in the retina. Future experiments will include short exposure to elevated IOP, identification of signaling pathways activated by elevated IOP, and comparison of changes in the retina in different models of glaucoma.

We are looking for new genes involved in glaucoma, as well as for genes that might be important for normal eye development and function. Several novel genes were identified that are expressed in different eye structures. One of such genes encodes a novel olfactomedin domain-containing protein that we named optimedin. Optimedin is a secreted protein that interacts with myocilin. Functions of optimedin and other olfactomedin-containing genes in the eye are under study.

Selected Publications

1 Ahmed, F., Brown, K.M., Stephan, D.A., Morrison, J., Johnson, E., and Tomarev, S.I., 2004, Microarray analysis of changes in mRNA levels in the rat retina after experimental elevation of intraocular pressure. Invest. Ophthamol. Vis. Sci. 45, 1247-1258.

2 Ahmed, F., Torrado, M., Zinovieva, R.D., Senatorov, V.V., Wistow, G., and Tomarev, S.I., 2004, Gene expression profile of the rat eye irido-corneal angle. NEIBank expressed sequence tag analysis. Invest. Ophthamol. Vis. Sci. 45, 3081-3090.

3 Gould, D.B., Miceli-Libby, L., Savinova, O.V., Torrado, M., Tomarev, S.I., Smith, R.S., and John, S.W.M., 2004, Genetically increasing Myoc expression supports a necessary pathological role of abnormal proteins in glaucoma. Mol. Cell. Biol. 24, 9019-9025.

4 Tomarev, S.I., Wistow, G., Raymond, V., Dubois, S., and Malyukova, I. (2003) Gene expression profile of the human trabecular meshwork. Invest. Ophthamol. Vis. Sci. 44, 2588-2596.

5 Torrado, M., Trivedi, R., Zinovieva, R., Karavanova, I., and Tomarev, S.I., 2002, Optimedin: a novel olfactomedin-related protein that interacts with myocilin. Hum. Mol. Genet. 11, 1291-1301.

Transgenic Animals and Genome Manipulation Section

Eric Wawrousek, PhD

Research Interests

The Transgenic Animal and Genome Manipulation Section is focused on two major topics:

- using transgenic and gene knockout technology to study the roles of ocular proteins in the normal development and function of the eye, and in pathological conditions; and
- development of novel techniques to introduce point mutations into the germ lines of mice.

Research Summary

Most of our work has concentrated on the multifunctional small heat shock proteins αA-and αB-crystallin, which were originally discovered in lens, but have since been shown to be expressed, at lower amounts in other parts of the eye. αB is expressed almost ubiquitously throughout the body and is a bonafide small heat shock protein. Deletion of αA results in severe cataract early in life, and allows the remaining αB in the lens to become insoluble, forming large spherical inclusion bodies. Deletion of αB and HSPB2 (encoded by an adjacent gene) has little or no affect on the lens, however, severe degradation of some skeletal muscles develop in these mice. They develop severe spinal curvature, lose body mass and fat reserves starting at 40 weeks of age, and die at about half their normal life expectancy. Although the heart is structurally normal and functions normally under standard conditions, these hearts are significantly less able to recover from hypoxic stress in ischemia/reperfusion models of heart attack. We currently have many collaborations with researchers around the world who are using these mice to study the many functions of the small heat shock proteins in a variety of tissues and physiological pathways.

Recently we have begun to investigate the role of the α-crystallins in controlling apoptosis via interactions with executioner caspases, and concomitantly their role in modulating the apoptosis-like program of lens cell differentiation and maturation. We have also recently begun a project using large BAC clones and recombineering technology to produce a 50 kb deletion, spanning 5 related genes expressed in the eye, in the mouse genome.

Our research to develop efficient, novel techniques of introducing point mutations into the germ lines of mice involves microinjecting modified single stranded DNA (ssDNA), alone, or in combination with other factors, into pronuclei of one celled mouse embryos, incubating them to the blastula stage, and genotyping them. Several experiments yielded promising results. Addition of proteins involved in the homologous recombination pathway of DNA repair increase the frequency of directed mutation, and inactivaiton of proteins involved in the non-homologous end-joining pathway, appear to increase the induced mutation frequency.

We are currently developing a fluorophore system for assessing mutation rate, which should be much more efficient for high throughput screening, and provide more definitive data than the current laborious nested PCR assays.

Selected Publications

1 Bai F, Xi JH, Wawrousek EF, Fleming TP, Andley UP. Hyperproliferation and p53 status of lens epithelial cells derived from alpha B-crystallin knockout mice. J Biol Chem. 2003 Sep 19;278(38):36876-86. Epub 2003 Jun 25.

2 Morrison LE, Whittaker RJ, Klepper RE, Wawrousek EF, Glembotski CC. Roles for alphaB-crystallin and HSPB2 in protecting the myocardium from ischemia-reperfusion-induced damage in a KO mouse model. Am J Physiol Heart Circ Physiol. 2004 Mar;286(3):H847-55. Epub 2003 Oct 30.

3 Brady JP, Garland DL, Green DE, Tamm ER, Giblin FJ, Wawrousek EF. AlphaB-crystallin in lens development and muscle integrity: a gene knockout approach. Invest Ophthalmol Vis Sci. 2001 Nov;42(12):2924-34.

4 Brady JP, Garland D, Duglas-Tabor Y, Robison WG Jr, Groome A and Wawrousek EF, 1997, Targeted Disruption of the Mouse αA-Crystallin gene induces cataract and cytoplasmic inclusion bodies containing the small heat shock protein αB-crystallin, Proc. Natl. Acad. Sci. USA. 94, 884.

Molecular Structure and Functional Genomics Section

Graeme Wistow, PhD

Research Summary

Genome projects and other new technologies give us the opportunity to build an integrated view of molecular systems from gene sequence, to protein structure, to biological function. This group has a major interest in the NEIBank project which applies the techniques of genomics and bioinformatics to explore the transcriptional repertoire of eye tissues from many species of biomedical interest. This has given us tremendous opportunities for gene discovery; identification of novel alternative transcripts and splice variants. It has also provided essential resources for functional studies using recombinant proteins; probes for in situ localization of expression; and clones for the construction of cDNA microarrays of eye expressed genes.

- Several novel genes with key roles in the eye and in eye disease are under further investigation, in-house and in various collaborations.
- Lengsin is a tissue-specific protein associated with terminal differentiation in the lens and studies underway range from construction of a knock-out mouse model and morpholino knock-down in zebrafish to expression of recombinant protein and cryo-EM structure determination.
- Analysis of corneal gene expression has revealed a novel tissue-specific gene transcript (currently named "KC6") that is a potential maker for corneal epithelial stem cells. The same studies have also uncovered the first molecular defect identified in keratoconus, an inherited disease of the human cornea.
- In the human retina we have discovered a novel, tissue-specific protein, retbindin, that may be involved in binding of carotenoids, a topic of considerable current interest for ocular health, particularly in the area of macular degeneration.
- Gene discovery has also led us to PDGFD, a novel member of the PDGFD family of growth factors. PDGFD has a key role in the coordinated growth and development of the lens and surrounding tissues of the anterior segment and is currently under investigation for another important role in the retina.

In addition we have identified several novel proteins that are candidates for "missing" enzymes of the visual cycle and these too are being followed up in collaborative studies with extramural colleagues.

We also have a long standing interest in the structure, function and evolution of the crystallins and other characteristic proteins of the lens. This relates to cataract and also to the distinctive way in which the lens has been adapted to fit the particular visual requirements of humans and other species. Furthermore, it provides important insights into the development of multigene families, a key feature of complex genomes.

Among other projects, we are currently investigating the role of γ-crystallins in the lens and other parts of the eye. We have created a knockout mouse for γS-crystallin, a protein that is highly conserved from fish to humans. Loss of this protein results in remarkable defects in the characteristic cellular organization of the lens, apparently related to loss of normal organization of junctional protein complexes. We are also pursuing a collaborative NMR protein structure determination of γS and also of a mutant version of the protein from a cataract. The mutant is unstable at body temperature and forms amyloid-like plaques. Understanding the unfolding process could have important implications for many age-related neurological diseases.

Selected Publications

1 Evans, P., K. Wyatt, et al. 2004. "The P23T Cataract Mutation Causes Loss of Solubility of Folded γD-Crystallin." J Mol Biol 343(2): 435-44.

2 Bateman, O. A., A. G. Purkiss, et al. 2003. "Crystal structure of η-crystallin: adaptation of a class 1 aldehyde dehydrogenase for a new role in the eye lens." Biochemistry 42(15): 4349-56.

3 Wistow, G., S. L. Bernstein, et al. 2002. "Expressed sequence tag analysis of human RPE/choroid for the NEIBank Project: over 6000 non-redundant transcripts, novel genes and splice variants." Mol Vis 8: 205-20.

4 Wistow, G., S. L. Bernstein, et al. 2002. "Expressed sequence tag analysis of human retina for the NEIBank Project: retbindin, an abundant, novel retinal cDNA and alternative splicing of other retina-preferred gene transcripts." Mol Vis 8: 196-204.

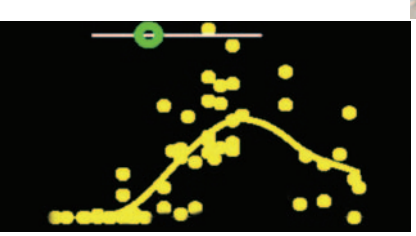
5 Sinha, D., M. K. Wyatt, et al. 2001. "A temperature-sensitive mutation of Crygs in the murine Opj cataract." J Biol Chem 276(12): 9308-15.

Visuomotor Integration Section

Robert H. Wurtz, PhD

Research Interests

The Section on Visual Motor Integration studies visual processing for the control of movement, particularly eye movements. Experiments are directed toward understanding the systems within the brain for the generation of saccadic and smooth pursuit eye movements, the use of visual motion for the control of movement, the alteration of visual processing by movement, and the selection of visual stimuli for the control of movement. Current experiments are primarily on the saccadic system and are centered on the superior colliculus but extend to the relevant areas of parietal and frontal cortex, and use the Rhesus monkey as the animal model for the study of these systems.



Selected Publications

1 Wurtz, R.H. and Sommer, M.A. Identifying corollary discharges for movement in the primate brain. Progress in Brain Research, 144: 47–60, 2004.

2 Sommer, M.A. and Wurtz, R.H. A pathway in the primate brain for the internal monitoring of movements. Science, 296: 1480-1482, 2002.

3 Wurtz, R.H., Sommer, M.A., Paré, M. and Ferraina, S. Signal transformations from cerebral cortex to superior colliculus for the generation of saccades. Vision Res. 41: 3399-3412, 2001.

4 Paré, M. and Wurtz, R.H. Progression in neuronal processing for saccadic eye movements from parietal cortex area LIP to superior colliculus. J. Neurophysiol. 85: 2545-2562, 2001.

5 Wurtz, R.H., Basso, M.A., Paré, M., and Sommer, M.A. The superior colliculus and the cognitive control of movement. In: The New Cognitive Neurosciences, 2nd Edition, Gazzaniga, M.S. (Ed), MIT Press, Cambridge, MA, 2000, p. 573-587.

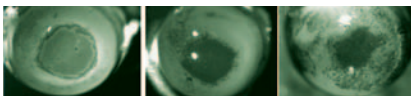
Research Summary

The work of Robert H. Wurtz PhD is centered on the organization of the brain underlying visual perception and the generation of eye movements. He uses the visual and oculomotor system of the old world monkey as a model for studying systems in the brain. The visual and oculomotor systems are among the best understood systems in the primate brain and this understanding allows him to address higher level questions related to cognitive function.

His current experiments have concentrated on the inputs to the cerebral cortex that underlie the stability of our visual perception in spite of frequent eye movements; a corollary discharge of eye movement preparation has been identified that projects from the superior colliculus in the brainstem to the frontal cortex and indicates to cortical visual processing that an eye movement is about to occur. Another set of current experiments investigates the mechanism of visual spatial attention. In these experiments, monkeys are trained in an attention task and neurons are recorded or artificially activated in order to determine the relation of brain regions to the shift of attention.

A curriculum vita, bibliography, and complete set of reprint PDF files can be found at *http;//lsr-web.net/*

Section of Cellular Differentiation



Effect of olomoucine on debridement wound healing in organ culture.

A 1.5 mm debridement wound was made in corneas of wild type mice and enucleated eyes were organ-cultured for 12 hours in defined media in the presence or absence of olomoucine (15 mM). Initial wound areas were measured immediately after wounding (20 eyes). To determine the effect of olomoucine, one eye of each animal was cultured with olomoucine and the contralateral eye was cultured without, as a paired control (10 pairs). After 12 hours, eyes were stained and photo-graphed. Each wound area was measured using ImagePro Plus image analysis software.

1 Corneal debridement wound immediately after wounding (0).
2 Debrided cornea after 12 hours in organ culture.
3 Debrided cornea after 12 hours organ culture in the presence of 15mM olomoucine.

Peggy S. Zelenka, PhD

Research Interests

The Section on Cellular Differentiation investigates signal transduction pathways that regulate adhesion and migration of lens and corneal epithelial cells, with the goal of identifying enzymes that may be therapeutically targeted in pathological conditions. Our primary focus is the role of the proline-directed kinase, Cdk5, in these tissues. However, this section also explores other regulatory pathways that affect adhesion and migration, particularly PKC isoforms and endogenous metabolites of arachidonic acid.

Research Summary

Until recently, Cdk5 was thought to be a neuron specific enzyme. However, work from this and other laboratories has established that it is expressed in active form in a variety of cell types and regulates multiple cellular functions. Previous work from this laboratory has established that Cdk5 activity regulates cell-matrix adhesion, cell-cell adhesion, and cell migration in epithelial cells of the cornea and lens. Recently, our work has used a variety of cellular and molecular approaches to explore the mechanism underlying these functions. An important advance was the discovery that Cdk5 regulates the localization and activation of the tyrosine kinase Src in migrating corneal epithelial cells. Src is a well known regulator of cell adhesion and migration, through its ability to regulate Rho family GTPases. Results from this laboratory have shown that in corneal epithelial cells Src-family kinases also activate Cdk5. This in turn, retards the transport of activated Src to peripheral cell sites and decreases the total amount of activated Src in the cell, thus counteracting the effects of Src. Consequently, inhibiting Cdk5 activity promotes accumulation of activated Src at the leading edge of a scratch wound in cultured mouse corneal epithelial cells and accelerates wound closure.
The relationship between Src and Cdk5 is being probed in greater detail by site-directed mutagenesis of potential phosphorylation sites on both kinases, in conjunction with functional and biochemical studies.

Studies are also in progress to explore the possible clinical application of Cdk5 inhibitors to promote corneal wound healing, especially in cases where wound closure is impaired. We previously found that a transgenic mouse line which overexpresses Cdk5 in the corneal epithelium has a markedly reduced rate of wound healing. Moreover, in vitro tests have shown that culturing eyes in the presence of a Cdk5 inhibitor significantly increases the rate of wound closure. We have now begun animal studies to test the safety and efficacy of Cdk5 inhibitors in promoting corneal wound closure in vivo.

Other ongoing studies are exploring the role of Cdk5 in the lens. Since our previous work implicated Cdk5 in both cell-to-matrix and cell-to-cell adhesion in this tissue, we are now investigating possible mechanisms that may mediate these effects. One experimental approach has been to identify lens proteins that interact with Cdk5 or its activating proteins, p35 and p39, using the yeast two-hybrid technique. These studies have identified two proteins, muskelin and myosin light chain, as specific interactors of p39. Both of these proteins are involved in cytoskeletal regulation, and may play a role in Cdk5-dependent regulation of cell-matrix or cell-cell adhesion. We are now examining the functional consequences of these interactions on migration and adhesion.

Selected Publications

1 Wurtz, R.H. and Sommer, M.A. Gao, C.Y, Stepp, M.A., Fariss, R., and Zelenka, P. Cdk5 regulates activation and localization of Src during corneal epithelial wound closure. J. Cell Sci. 117, 4089-4098, 2004.

2 Lin, D., Zhou, J., Zelenka, P.S. and Takemoto, D.J. Protein kinase Cgamma regulation of gap junction activity through caveolin-1-containing lipid rafts. Invest Ophthalmol Vis Sci 44: 5259-68, 2003.

3 Zhou, J., Fariss, R.N., and Zelenka, P.S. Synergy of epidermal growth factor and 12(S)Hydroxyeicosatetraenoate on protein kinase C activation in lens epithelial cells. J. Biol. Chem. 278, 5388-5398, 2003.

4 Gao, C.Y., Negash, S. Guo, H.T., Ledee, D., Wang, H.S., and Zelenka P. CDK5 Regulates cell adhesion and migration in corneal epithelial cells. Mol Cancer Res. 1, 12-24, 2002.

5 Negash, S., Wang, H.S., Gao, C. Ledee, D., and Zelenka, P. Cdk5 regulates cell adhesion in lens epithelial cells. J. Cell Science 115, 2109-2117, 2002.