Fighting blindness by developing new technologies, therapies and knowledge to retain and restore vision in an environment of excellence in training
Schepens Eye Research Institute

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About Schepens Eye Research Institute

Schepens Eye Research Institute (The Institute) is the largest independent vision research organization in the United States. It has been affiliated since 1991 with the Department of Ophthalmology of Harvard Medical School. Located in downtown Boston, Massachusetts, The Institute was founded in 1950 by Charles L. Schepens, M.D. (1912 - 2006), who is considered by many to be “the father of modern retinal surgery”. From, 1994 - 2004 the Institute continued to prosper under the leadership of J. Wayne Streilein, M.D (1935 - 2004).

In 2004, Michael S. Gilmore, Ph.D., became our new Ankeny Director of Research and currently also serves as President and C.E.O. Studies conducted at the Institute have historically contributed greatly to the body of knowledge comprising all the visual sciences, and have had a major impact on ophthalmic practice internationally.

The research faculty presently consists of 31 full-time scientists (Ph.D., M.D. or O.D.) and 35 affiliated clinical scientists (M.D.). All of the principal investigators have academic appointments in appropriate departments at Harvard Medical School (HMS); academic titles can be found on each scientist’s web site. Six Senior Scientists are full professors at HMS. The research programs of the faculty include both basic and clinical studies, focused into several areas, described in the section of this brochure entitled “Research Areas”. Members of the faculty, 56 postdoctoral fellows and a technical staff of 45 conduct this research; seven graduate students provide additional research effort. Last year, research at the Institute resulted in nearly 120 publications. Collaborative and interdisciplinary research is encouraged both within The Institute and with other organizations. Dissemination of knowledge is also encouraged. For example, when several faculty members recently learned that two-thirds of the blind people in the world and in America are women, they formed the Women’s Eye Health Task Force to educate people how to take care of their eyes.

The scientists here enjoy about 120,000 square feet of newly renovated, modern, well equipped laboratory and office space in two adjacent buildings connected by an indoor bridge. Training the next generation of vision investigators is an important component of Schepens’ mission. The Institute has the excellent record of having provided training in vision research to more than 600 young physicians and scientists from over 40 countries around the world who wished to develop laboratory careers in the visual sciences and as clinical scientists.

The Institute is situated two blocks from the Massachusetts Eye and Ear Infirmary, Massachusetts General Hospital (with its excellent lecture series and Treadwell Library), and the Shriners Hospital for Children (formerly Shriners Burns Institute). The Institute is contiguous with the Simches Center, MGH’s new center for interdisciplinary research. Individual faculty computers are linked with Harvard Medical School. The Institute is also linked via computer with the superb Harvard libraries. The Schepens’ affiliations and excellent location promote close, productive collaborations with the faculty of the entire Harvard Medical System and its associated hospitals, as well as with Harvard College, the Massachusetts Institute of Technology, and the Boston University and Tufts University Schools of Medicine.

Available Resources

In addition to its laboratory and office space, the Institute’s facilities include: a fully staffed computer unit which supports all computer hardware, software and server needs; a gene micro-array work station that has access to the Harvard databases; a fully staffed animal facility of about 8000 square feet; a staffed morphology unit with light, transmission electron, scanning electron, and confocal microscopes; a staffed flow cytometry facility; and a staffed facility for photography, graphic arts, web sites, and medical illustration. Recently, the Institute established a totally equipped proteomics laboratory (the first and only so equipped in the Boston area), a new real-time PCR laboratory and a renovated flow cytometry laboratory with entirely new instrumentation. Other shared equipment located throughout the Institute includes several dark rooms, high- and low-speed centrifuges, PCR cyclers, image analysis equipment, and several radioisotope-counting instruments.

Funding Sources

The Institute had an operating budget of about $26.3 million in fiscal 2006. Over two thirds of this money is derived from federal grants and contracts, primarily from the National Eye Institute of the National Institutes of Health and from the Department of Defense. Faculty members currently hold 32 federal grants. The remainder of the funding comes from endowment income, patent revenue, and private donations from individuals, foundations, corporations, and other organizations. Postdoctoral fellows training at the Institute have been successful at obtaining small research grants from a wide variety of sources. The Schepens has been awarded several multi-project, interdisciplinary grants: for blindness eye diseases, for research on the aging eye, the etiology of diabetic retinopathy, and engineering approaches to low-vision rehabilitation. A core grant from the National Eye Institute supports the morphology, laboratory computer, flow cytometry, and animal resources unit.
About the Boston Area

Schepens Eye Research Institute is centrally located in Boston close to public transportation and to some of the most exciting areas of town, including the Faneuil Hall marketplace and the waterfront. Boston is an ethnically diverse and culturally rich but compact city. Various types of entertainment are available including many forms of music, dance, theater and the visual arts; a wide assortment of museums; and several professional sports teams. Recreational opportunities are diverse. Within one-half mile of the Institute, one can sail, boat, and rollerblade along the Charles River and take boat tours of Boston Harbor and the Harbor Islands. Within a half-day's drive are the beaches of Cape Cod and the North Shore and the mountains and lakes of Western Massachusetts, Maine, New Hampshire, and Vermont.

Housing is located a short walk from The Schepens on charming Beacon Hill and in the adjacent Charles River Park. Trainees also live in Cambridge, Brookline, Somerville, or Brighton, all of which are accessible by subway, and in the northern and western suburbs of Boston, some of which are serviced by commuter trains originating at North Station, a few blocks from the Institute.

Administration

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Training Programs

There are many opportunities for undergraduates, graduate students, and postdoctoral training at Schepens Eye Research Institute. Potential applicants are encouraged to visit the Institute’s website at www.schepens.harvard.edu for detailed information about the Institute, its faculty, and its programs. Access is provided there to Faculty e-mail addresses (and, in some cases, individual web pages) and to an electronic copy of this brochure.

Training opportunities exist at several levels:

Short-term Training

Students at all levels of training will be considered for summer research projects or for part-time projects during the school year. Interested students should contact a faculty member directly to arrange for training.

Predoctoral Training

Although Schepens Eye Research Institute itself does not grant degrees, several of its faculty members hold appointments in Biological and Biomedical Sciences, Immunology, Neuroscience, or Pathology at the Harvard Graduate School of Arts and Sciences; some others are on the faculty of the Sackler School of Graduate Studies at Tufts University. Predoctoral fellows can earn a Ph.D. degree from these universities under the auspices of these faculty members. Information about the Harvard program can be obtained at: www.hms.harvard.edu/dms. Interested students should contact a faculty member directly. In addition, new opportunities for graduate students, as well as for some postdoctoral fellows, are available through a recently instituted, Harvard-wide program in ocular immunology. This program is funded by the National Institutes of Health. For information, contact Dr. Joan Stein-Streilein, Schepens Eye Research Institute, 20 Staniford Street, Boston, MA 02114, telephone (617) 912-7489, e-mail: joan.stein@schepens.harvard.edu.

Postdoctoral Training

An extensive program of postdoctoral training for candidates who hold the Ph.D., M.D., or related, advanced degree is available at the Schepens Eye Research Institute, in conjunction with the Departments of Ophthalmology and Pulmonary Medicine at Harvard Medical School. Complete descriptions of the program and of the application procedures follow.

Training of Vision Scientists

The major goal of the postdoctoral training program at the Institute is to prepare qualified individuals to start an independent career of significant funded research in the basic or clinical aspects of vision and eye diseases. The Institute provides opportunities for trainees to increase their knowledge of the eye in such areas as biology of the retina and its diseases; ocular surface diseases; infection; immunity, inflammation, and transplantation; vision and visual optics; and ocular gene therapy. Trainees conduct full-time research under the guidance of their chosen mentor. In addition, a personal advisor and members of the Institute’s Training Committee (chaired by Dr. Patricia D’Amore) follow the progress of the fellow in order to optimize the training experience. Several common activities such as weekly Fellows’ Seminars, monthly Fellows’ Journal Club sessions, and Fellows’ Luncheons, bring trainees together on a regular basis, and are forums for presentation of work in progress and discussion of major new advances that have appeared in the literature. Trainees are encouraged to participate in the meetings of relevant interest groups (Focus Groups, Programs, and Centers), and attend the weekly seminar series at the Institute, as well as seminars of interest presented at neighboring universities, hospitals, and research institutions. The Training Program here also organizes sessions on topics of common interest like grant writing. Upon completion of training at The Institute, trainees should be capable of independently formulating an hypothesis; designing, carrying out, and interpreting experiments to test that hypothesis; critically evaluating the results; and writing up the work for publication. In addition, trainees will have an opportunity to develop a command of the relevant literature and an ability to evaluate published articles critically in order to recognize important, well-documented findings.

Requirements for Completion of The Training Program

The trainee must complete at least one year of training in a laboratory- or clinic-based research project. The trainee is expected to make at least one oral presentation regarding his/her research project at the weekly Fellows’ Seminars, and write a short progress report to be reviewed with a Training Committee member after six months of training and again just prior to leaving the Institute. The trainee also must attend some of the Institutes and/or Harvard Medical School’s seminars concerning the responsible conduct of research.

Application Process

Schepens Eye Research Institute

Any individual who has completed a Ph.D., M.D., or similar degree program and has an interest in a vision-science career is welcome to apply. The applicant should contact one or more faculty members in his/her area of interest to identify an appropriate mentor. For more information about the Schepens and its faculty please visit us on-line at www.schepens.harvard.edu. Information about the training program and our current training fellows is also available at www.schepens.harvard.edu/trainingprograms.htm. For more-detailed information, contact Karen Jarvis, Schepens Eye Research Institute, 20 Staniford Street, Boston, MA 02114; telephone (617) 912-2568, E-mail: vision.training@schepens.harvard.edu.

Harvard Department of Ophthalmology Training Program in the Molecular Basis of Eye Disease

In addition, several members of Schepens Eye Research Institute faculty participate with other faculty members of the Harvard Department of Ophthalmology in the Postdoctoral Training Program in the Molecular Bases of Eye Diseases, sponsored by the National Eye Institute. This program is designed to provide expertise in molecular approaches and techniques applied to ophthalmology and vision research. The trainees are supported by the program for the first year, and are expected to apply for individual funding to support the remaining period. Positions are available for individuals immediately after the awarding of their terminal degree as well as for those who have completed their postdoctoral clinical training. Candidates must have a Ph.D or M.D. degree, and be citizens or permanent residents of the United States. Minority applicants are encouraged to identify themselves voluntarily. Applicants for this program can obtain information about the program, the mentors, and their research at: http://www.eri.harvard.edu/training/index.html. More-detailed information about this program or the application process is available from Karen Jarvis, Schepens Eye Research Institute, 20 Staniford St., Boston, MA 02114. Tel. (617) 912 - 2568, e-mail: vision.training@schepens.harvard.edu.
Research Areas

Schepens Eye Research Institute is devoted to the study of the eye and the visual system, with an emphasis on understanding the mechanisms of multifactorial diseases that cause visual dysfunction and blindness. The ultimate goal of the research is to retain and restore vision. The focus is on basic research and its applications to clinical problems; strong basic-science research programs are meshed with close collaborations between laboratory and clinical scientists. There are multiple mechanisms for collaborative research at the Institute, including focus groups, research programs, and centers; these scientific, collaborative groups are described below. Many of the faculty are members of more than one of these groups. The entire scientific staff, including trainees, is encouraged to join in these activities.

Basic and clinical scientists with Ph.D., M.D., O.D., and D.V.M. degrees conduct collaborative research on the structure, function, and diseases of the eye and related tissues. An excellent administrative and technical staff supports the research programs of the scientists. In addition postdoctoral fellows from the United States and around the world contribute to the research effort. The scientists and staff work under the leadership of Michael S. Gilmore, Ph.D., Ankeny Director of Research and Professor of Ophthalmology and Darlene A. Darst, Ph.D., Director of Scientific Affairs and Associate Professor of Ophthalmology. The Institute’s research programs focus on the causes and prevention of selected multifactorial, blinding eye diseases.

Focus Groups and Centers

Collaborative research at the Institute in the form of focus groups, centers, and programs provides the cornerstone of our research effort. Faculty members and other scientific staff participate in these programs, which meet regularly to share research work in progress and to organize scientific retreats and symposia. These groups, with their leaders, are as follows:

**Neural and Vascular Biology Focus Group**

**Leader:** Patricia D’Amore, Ph.D.

This group focuses on neural and vascular processes, specifically as they relate to ocular pathologies such as retinal degenerations, glaucoma, diabetic retinopathy and macular degeneration, and possible therapeutic interventions. Projects include investigations of: the role of VEGF in atrophic macular degeneration; signal transduction pathways controlling the formation, stabilization and regression of vessels; retinal repair by neural regeneration and stem cell transplantation; tissue engineering and stem cell biology; neuroprotection and cell replacement therapies for retinal degenerations; role of inflammatory cytokines in diabetic retinopathy; regulation of immunity; mechanisms responsible for the failure of retinal vessels in diabetes and identification of drugs for prevention; and a search for early markers of vascular abnormalities in diabetic patients.

**Neural and Vascular Biology (NVB) Focus Group**

**Leader:** Joan Stein-Streilein, Ph.D.

The Ocular Immunology (OI) group seeks to advance understanding of the nature and regulation of the immune system of the eye, and to unravel the underlying mechanisms of ocular inflammation due to infection, autoimmune disease, tumors, and corneal and retinal transplantation. This group is composed of faculty members who share a common interest in basic and clinical research concerning the role of growth factors, cytokines, hormones, neurotransmitters, and proto-oncogenes in the control of ocular immunity in both health and disease. Some of the goals of this group are to investigate the mechanisms that allow for immune privilege within the eye and the immune tolerance in the periphery, develop cytokine neutralization strategies that lead to the suppression of corneal inflammation, examine the activation of tumor-specific T-cells involved in the elimination of intraocular tumor cells and their metastases, and assess the efficacy of antiviral agents in protecting against herpes virus induced destruction of ocular and neuronal tissues in vivo. Experimental approaches in these areas should provide unique insight into the complex physiological circuitry that governs immune protection of the eye. In addition, research discoveries from this group may well lead to the development of unique therapeutic strategies for the treatment of ocular inflammatory disorders and for successful transplantation of corneal and retinal tissues into the eyes of visually impaired or high-risk patients.

**Ocular Immunology Focus Group**

**Minda De Gunzburg Research Center For Retinal Transplantation**

**Leader:** Michael Young, Ph.D.

This Center was established with the goal of using basic research to overcome barriers to successful retinal transplantation, and thereby hastening the realization of our goal: to make the restoration of sight to the blinded eye a reality. The Center has assembled a multidisciplinary team of investigators, with expertise in molecular and cellular immunology, neurobiology and neural development, as well as ophthalmic surgery and low-vision rehabilitation.

Blinding diseases of the eye, such as age-related macular degeneration, retinitis pigmentosa, diabetic retinopathy, and glaucoma, share one tragic feature: damage to neurons in the neurosensory retina, which (as part of the central nervous system, or CNS) do not regrow. Transplanting new cells presents a pathway to repairing the damage. The CNS, perhaps more so than any other system of the body, may be treatable with new therapies aimed at replacing cells lost to disease or traumatic injury. Parkinson’s Disease, Huntington’s chorea, spinal cord injury, and retinal disease are now able to be treated successfully in animal models. However, a great deal of work is needed before we can successfully apply this technology to the eye clinic. Two main problems that must be overcome are the inability of grafted cells to make connections with the host retina, and immunological rejection of donor tissue by the host. We plan to gain new knowledge concerning the roles of astrocytes, microglia, and immune cells in the success or failure of retinal transplantation, and to describe the potential roles of these cells in the pathogenesis of retinal degenerations.
Lids to Lens Focus Group

Leader: James D. Zieske, Ph.D.

Disease and injury to the anterior surface of the eye are the leading causes of visits to physicians for medical eye care in the United States; they rank among the most painful of eye conditions and can lead to disability and blindness. Major clinical problems of the surface of the eye include ocular surface drying, tear film abnormalities, and related sequelae; ocular surface wounds with resultant pathology and scarring; corneal dysfunction dystrophies and inherited disease; inflammatory disease; and external ocular infections. The research goals of this focus group are to:

1. increase our understanding of the composition, structure, and regulation of the tear film and its relation to the ocular surface in health, disease, and aging, and develop better methods of evaluating, diagnosing, and treating patients with dry eye and tear film abnormalities;
2. deepen our knowledge of the healing and regeneration of all layers of the cornea, particularly the ocular surface epithelium in relation to the subjacent stroma, and the molecular basis of tissue destruction and scarring in the cornea;
3. reveal the mechanisms and control of corneal endothelial cell turnover and replacement in order to develop alternatives to whole grafts from donor eyes;
4. more fully describe the effects of corneal inflammation and develop methods for its control; and
5. understand the protective antipathogen activity of ocular surface mucins in order to improve prevention and treatment of ocular infections.

Vision and Visual Optics Focus Group

Leader: Francois Delori, Ph.D.

The VIVO group brings together researchers from diverse academic backgrounds who are interested in a systems approach to vision and the eye. This group is united by the concept that understanding the normal, in vivo function of the eye and visual system is critical for further developments in the diagnosis and treatment of eye diseases. There are three main research directions within the group. The first area relates to advanced optical approaches to understanding the health of the eye and the initial stages of vision. In this area, the group has a strong history of developing new and unique methods to improve the understanding of ocular function in health and disease. The second research area relates to visual processing in normal and diseased eyes; this strong program uses psychophysical, electrophysiological, and optical approaches in both humans and animals. The third area is in the study of low vision and low vision rehabilitation. Our program in these latter areas brings advanced basic research and engineering approaches to improving the quality of life of the visually disabled.

Retinal Laser Injury Focus Group

Leader: Dong-Feng Chen, Ph.D.

This interdisciplinary research group brings together scientists in neurobiology, immunology, stem cell research, and clinical ophthalmology to study laser-induced eye injuries with a goal of developing therapeutic strategies for such injuries. Laser retinal damage can occur by intentional exposure from an enemy weapon or as a side effect of iatrogenic use of the medical laser when treating age-related macular degeneration, diabetic retinopathy, and uveitis. Currently, there is crucial need to develop treatments for retinal laser burns. The Retinal Laser Injury Focus Group is interested in using innovative approaches and combined expertise in the group to understand the pathology and mechanisms of retinal damage caused by lasers. By interrupting molecular signals causing neuronal death after laser burn with drug therapies, working with stem cell therapies to promote neural repair, and by controlling inflammation and wound healing, the group is addressing laser damage to the retina from various angles. These studies will expand our arsenal in the fight against age-related macular degeneration, diabetic retinopathy and other diseases that threaten the vision of human beings.

Glaucoma Interest Focus Group

Leader: Mara Lorenzi, M.D.

The Glaucoma Interest Group is a new group that came together in July 2006. The goal is to make glaucoma a focus of interdisciplinary interests at the Institute, with a declared commitment to scientific pursuits that are clinically relevant.

Toward this goal, the membership (see below) includes The Institute’s scientists from different disciplines and fields of interest –neuroscience, immunology, aging, diabetes, low-vision,-- a clinical expert in glaucoma (Louis Pasquale, MD, is Co-Director of the Glaucoma Service at MEEI as well as an investigator of genetic and environmental risk factors for glaucoma), and a liaison to pharmaceutical companies. In this building phase, membership is limited to investigators and other selected participants with a declared interest in glaucoma. It is anticipated that, as the activities of the group become better established, the meetings will be opened to postdoctoral fellows and lab members.

Currently, Dr. Lorenzi bears responsibility for organizing meetings and activities. The group meets monthly for two hours. Each meeting is organized around the steps of (i) gaining new knowledge through presentations by The Institute and outside investigators, (ii) brainstorming to elaborate on the presentations, and extract concepts for potential projects, and (iii) serving the translational commitment of the focus group by generating practical plans –from collaborations among specific investigators, to inquiries with pharmaceutical companies, to identification of topics for new grant applications.

Dry Eye Focus Group

Leaders: Reza Dana, M.D. M.Sc., M.P.H.; Ilene K. Gipson, Ph.D.; David Sullivan, Ph.D.

The goal of this Group is to advance our understanding of the pathogenesis and treatment of dry eye syndromes, with a particular emphasis on:

• determining the mechanisms involved in the regulation of the tear film, lacrimal gland, meibomian gland, and mucin-producing cells of the ocular surface; and
• unraveling the processes and risk factors that contribute to the dysfunction of these tissues and ultimately lead to dry eye syndromes.

In establishing this Group, we seek to promote collaborative research interactions among basic scientists and clinical researchers throughout the Boston environs. The frequency of meetings, and their format and location, is be determined by the Group. At present, David A. Sullivan bears organizational responsibility for the Group.

Many of the Group members currently collaborate with each other, and seek to increase these interactions. The Group plans to sponsor a series of seminars and/or a Symposium on Dry Eye Syndromes.
Schepens Eye Research Institute has several core research facilities available to all investigators. These facilities are supported on a fee-for-service basis, and are funded by a National Eye Institute Core Grant for Vision Research.

**Flow Cytometry**

The Flow Cytometry Facility provides the service of cell analysis and sorting to Institute and Boston-area research scientists. The facility utilizes state-of-art equipment to offer excellent services under the management of technician Randy Huang. The instruments include a slidebased Compucyte laser scanning cytometer, a Cytomation MoFlo cell sorter and a Coulter XL flow analyzer.

**Web & Graphic Services**

Peter Mallen provides a wide range of professional quality products for the research community to use in presentations, in publications and on the internet.
The Schepens Proteomics Facility has available for use equipment purchased from Bio-Rad Corp. for the 2-dimensional separation of protein extracts. These include equipment for isoelectric focusing using IPG strips and several sizes of apparatus for the second dimension SDS-PAGE separation of proteins according to molecular weight. SyproRuby fluorescent protein stain is available for visualization of separated protein spots using ProX-PRESS, the highly sensitive image analyzer from Perkin-Elmer. This instrument is also capable of detecting spots on Coomassie blue or silver-stained gels. Images obtained on the ProXPRESS can be downloaded to the Proteomics Workstation and analyzed using a sophisticated image analysis software system (Phoretix Evolution, Non-Linear Dynamics). A list of individual spots of interest is then sent electronically to the Perkin-Elmer ProXCI-SION robotic spot-cutter, which precisely excises each spot and places it in a designated well of a 96-well microtiter plate. Protein within each gel spot is digested with trypsin and the tryptic peptides from each sample are then spotted onto MALDI target plates using a MultiProbe liquid-handling system. The tryptic peptide profile is analyzed by a MALDI-proTOF mass spectrometer and the unknown protein identified using sophisticated database analysis. The Proteomics Facility operates under the aegis of Senior Scientist Nancy C. Joyce, Ph.D. and her associate Investigator Ian Rawe, Ph.D.

A JEOL 7401F field emission scanning electron microscope (SEM), which uses very low voltage to generate much higher resolution images than traditional SEMs (making it useful for immunogold labeling at the SEM level), is located at The Institute and is available to scientists in and around the Boston area. Also available is adjunct sample preparation equipment—a Tousimis critical point dryer and Gatan ion beam coater, as well as training and optional sample preparation.
ABI Prism 6100 Nucleic Acid PrepStation (Applied Biosystems) isolates highly pure nucleic acids free of cross-contamination, thus providing reliable results. Up to 96 samples of total RNA or genomic DNA from a variety of biological sources can be purified in 30 minutes. Agilent 2100 Bioanalyzer (Agilent Technologies), a chip-based nucleic acid analysis system, allows for the determination of RNA or DNA sample quality and integrity as well as accurate quantitation using as little as 25 µg of nucleic acid sample. ABI Prism 7900HT Sequence Detection System (Applied Biosystems) is a high-throughput real-time PCR system that detects and quantitates nucleic acid sequences. Currently, up to 96 samples can be examined in a 2-hour run. The 7900HT can be upgraded to include a 384-well capability as well as an automation accessory.

The Massachusetts Lions Eye Research Fund Inc. and The Lions Clubs International Foundation recently provided funds for purchasing our new Leica TCS – SP2 Confocal Laser Scanning Microscope.

Donald Pottle, CME (Confocal Microscopy Educator) is in charge of training our scientists on the Confocal and other microscopes.
Schepens Eye Research Institute • Current Faculty
Michael S. Gilmore, Ph.D., President & CEO, Ankeny Director of Research
Darlene A. D’Amore, Ph.D., Director of Scientific Affairs
Patricia A. D’Amore, Ph.D., M.B.A., Associate Director of Research (Elected)

Senior Scientists Emeriti
Alice J. Adler, Ph.D.
Charles Cintron, Ph.D.
Marshall G. Doane, Ph.D.
Miguel F. Refojo, Sc.D.

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François C. Delori, Ph.D.
Michael S. Gilmore, Ph.D.
Ilene K. Gipson, Ph.D.
Nancy C. Joyce, Ph.D.
Andrius Kazlauskas, Ph.D.
Mara Lorenzi, M.D.
Eliezer Peli, O.D.
Joan Stein-Streilein, Ph.D.
David A. Sullivan, Ph.D.
Robert H. Webb, Ph.D.
James Zieske, Ph.D.

Associate Scientists
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Dong Feng Chen, M.D., Ph.D.
Bruce R. Ksander, Ph.D.
Andrew W. Taylor, Ph.D.
Michael J. Young, Ph.D.

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Chiara Gerhardinger, M.D., Ph.D.
Meredith Gregory-Ksander, Ph.D.
Shuji Kishi, M.D., Ph.D.
Kameran Lashkari, M.D.
Sharmila Masli, Ph.D.

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Tat Fong Ng, Ph.D.
Ian Rawe, Ph.D.
Jose David Rios, Ph.D.
Giulio R. Romeo, M.D.
Russell Woods, Ph.D.

Adjunct Associate Scientist
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Jeffrey Ruberti, Ph.D.

Emeritus Clinical Senior Scientist
H. MacKenzie Freeman, M.D.
Felipe I. Tolentino, M.D.

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Mark Abelson, M.D.
Claes H. Dohlman, M.D.
Tatsuo Hirose, M.D.
J. Wallace McMeel, M.D.
Joan Miller, M.D.
Clement L. Trempe, M.D.
John Weiter, M.D.

Clinical Scientists
Mark A. Abelson, M.D.
Claes H. Dohlman, M.D.
Tatsuo Hirose, M.D.
J. Wallace McMeel, M.D.
Joan Miller, M.D.
Clement L. Trempe, M.D.
John Weiter, M.D.

Visiting Scientists
Susana Marcos, Ph.D.
Miguel Angel Garcia Perez, Ph.D.
Conan Young, Ph.D.

March, 2007
Schepens Eye Research Institute • Current Training Fellows

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Christian Antolik, Ph.D.
Rita Barcia, Ph.D.
David Biss, Ph.D.
Timothy Blalock, Ph.D.
Brad Bryan, Ph.D.
Sunil Chauhan, M.V.Sc., Ph.D.
Eui-Sang Chung, M.D., Ph.D.
Mohammad Dastjerdi, M.D.
Tatiana Ecoiffier, M.S.
Jaafar El Annan, M.D.
Eric Finkelstein, Ph.D.
Densen Hayashi, M.D.
Susan Heimer, Ph.D.
Koji Hirai, M.D.
Junji Inoue, M.D.
Caihui Jiang, M.D., Ph.D.
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Masataka Kasaoka, M.D.
Nanako Kasaoka, M.D.
Norikuni Kawanaka, M.D., Ph.D.
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Chun Lau, M.D., F.R.C.S.
Hetian Lei, Ph.D.
Dayu Li, M.D., Ph.D.
Shaohui Liu, M.D., Ph.D.
Kenyata Lucas Ph.D.
Janet Manson, Ph.D.
Flavio Mantelli, M.D.
Mariko Matsuba, M.D., Ph.D.
Shonna McBride, Ph.D.
Dianne Mitchell, Ph.D.
Fanny Fong-Ming Mo, Ph.D.
Ruta Motiejunaite, M.S.
Hong Qiao, M.D., Ph.D.
Marcus Rauch, Ph.D.
Stephen Redenti, Ph.D.
David Rubaltelli, M.D.
Daniel R. Saban, Ph.D.
Magali Saint-Geniez, Ph.D.
Lin Ling Shen, M.D., Ph.D.
Faramarz Taheri D.V.M., Ph.D.
Akira Takamiya, M.D., Ph.D.
Thang-Long To, Ph.D.
Budd Tucker, Ph.D.
Gisela Velez, M.D.
Fuensanta Vera Diaz, Ph.D.
Tony Walshe, Ph.D.
Ai Yamada, M.D., Ph.D.
Xian Zhang, M.D., Ph.D.

March, 2007
Current Faculty Research Projects
Ocular Allergy and Dry Eye Conditions (Clinical Research)

The focus of our research is the identification, screening, and development of novel therapies for treating external ocular diseases. Specifically, my interest lies in ocular allergy and the dry eye conditions. We select potential new agents from the literature by matching the mechanism of action of the drugs with the clinically relevant pathophysiology of the diseases. To assist in the screening process, our lab has developed a number of animal and human models for allergy, dry eye, and inflammation. By understanding and applying the parameters of the diseases, we have designed and refined clinical scales for evaluating the efficacy and safety of the drugs in these models. The histamine, Compound 48/80, and human conjunctival antigen challenge models have been adapted not only for ophthalmic anti-allergic compounds, but also for screening systemic anti-allergic agents as well, and the conjunctival antigen challenge model has been accepted by the FDA, EMEA, and Japanese Regulatory authorities as a significant component of the drug approval process. Similarly, in dry eye, we have developed an environmental challenge model to screen new classes of dry eye agents. This model has been accepted by the National Eye Institute and FDA, and is currently being used to evaluate novel mucomimetics, secretagogues, anti-inflammatory drugs, anti-epidravatives, and polymers as new treatments for dry eye. We are continuing to refine these models, scales, endpoints, and time courses to improve their clinically predictability.

By using these clinical models, and collaborating with other leading investigators in the field and many pharmaceutical and bio-tech companies throughout the world, we have brought numerous new drugs to market, fifteen for allergy alone. In the past seven years, we have been involved in the development and approval of seven new drugs, including: Patanol, Zaditor, Emadine, Almast, Livostin, Alrex, and Elestat.

Currently we are examining novel pathways for potential vasoconstrictor agents which may offer superior efficacy in terms of reduction of redness, swelling, and longer duration of action than currently available drugs. We are also examining similar pathways for potential use as mydriatic agents, for new dilating drops with shorter duration of action than current drugs, that can be used diagnostically in the physician’s office.

Summary of Research Interests:
1. Clinical ocular pharmacology
2. Modulation of mast cell mediators in the eye and evaluation of novel anti-inflammatory agents
3. Development of stressed environment model for dry eye therapeutics
4. Definition of clinical endpoints for dry eye
5. The role of novel vasoactive amines and their intracellular pathways in the modulation of external ocular conditions.

Recent Publications


Glycosylation of Mucins by the Human Ocular Surface Epithelia (Glycobiology)

Epithelial mucins are a heterogeneous group of heavily O-glycosylated glycoproteins found as major components of all mucous secretions derived from wet-surfaced epithelia. Their main function is to protect the underlying epithelium by forming a gel on the cell surface that acts as a selective barrier to molecules and pathogens from the external environment. The major focus of my research is (i) to characterize the carbohydrate portion of the different mucins expressed by the ocular surface epithelia as well as the enzymes involved with their synthesis and (ii) to determine whether the alteration of mucin glycosylation is associated with ocular surface disease (dry eye) and pathogen infection. The experimental procedures to achieve these objectives include cell culture, immunoassays, real time RT-PCR, DNA hybridization arrays, fluorometric HPLC and mass spectrometry.

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Recent Publications


Islands of rose bengal negative cells \( \star \) appear on human corneal epithelial cell cultures producing the glycosylated membrane-associated mucin MUC16 (Argüeso et al., IOVS 2006). Rose bengal (pink) is an organic anionic dye used to assess damage of the ocular surface epithelium in ocular surface disease. 

Binding of FITC-cationized ferritin (FITC-CF, green) to cultured human corneal epithelial cells at different stages of differentiation (Argüeso et al., IOVS 2006). In the absence of glycosylated mucins (A), confluent cultures display a homogeneous FITC-CF binding on the cell surface, indicating an overall negative charge of cells. Induction of mucin biosynthesis and glycosylation results in lack of FITC-CF binding to islands of stratified cells (B), indicating a decrease in anionic sites in these areas. The nuclei of the cells are counterstained with propidium iodide (red).
My research employs behavioral and imaging techniques to study the human visual system in normal and abnormal development, in normal ageing and in neurodegenerative diseases. Particular emphasis is placed on the use of natural stimuli and tasks to examine the underlying mechanisms of visual processing.

**Equivalent Noise Analysis in Glaucoma**

Glaucoma is the second leading cause of blindness in the developed world and causes steady loss of peripheral visual field. Increases in life expectancy mean that around 80 million people worldwide will have glaucoma by 2020. While visual loss caused by glaucoma is currently irreversible, clinical intervention can significantly reduce its progression and is most effective when the disease is caught in its early stages, when catastrophic field loss can be averted.

However, existing screening techniques can only detect glaucoma once significant visual impairment from gross loss of retinal ganglion cells (RGC) has occurred. The most sensitive screening for glaucoma must therefore identify early signs of RGC dysfunction before irreversible cell death. We are currently developing screening tests, based on visual sensitivity to moving images that should be able to detect early signs of glaucoma before existing techniques can. We use a technique known as equivalent noise analysis that estimates the level of internal noise in the visual system by measuring sensitivity to the direction of motion as more and more external noise is added to the stimulus, see Figure 1 for illustration. The rate at which external noise impairs performance can be used to compare the level of dysfunctional and non-functional sensors in the normal and glaucomatous visual system.

**Recent Publications**


**Age-Related Macular Degeneration**

Approximately 12 million people suffer central vision loss caused by Age-related Macular Degeneration (AMD - www.eyesight.org), a figure that is set to rise as our population ages. Treatment of macular disease with conventional ophthalmic techniques is of limited benefit in the majority of cases, forcing people to depend on their poor resolution peripheral vision and severely impairing essential tasks such as mobility, face recognition and reading. We are developing new ways to present information, images and text that may maximise residual vision and independence in people with AMD. In principle it should be possible to use magnification to compensate for the loss of resolution in the peripheral visual field. However, even magnified images can be completely masked by nearby features, an effect known as crowding, and this is particularly problematic in the peripheral visual field. Figure 2 illustrates how difficult it can be to disambiguate features in the periphery, especially under crowded conditions. We are studying the visual processes that cause crowding in an effort to develop novel ways to present information that are not vulnerable to its effects.

**Figure 1** Equivalent noise analysis can be used to estimate the level of internal noise in the visual system and the sampling efficiency of visual sensors. It measures how quickly performance, in this case a direction of movement task, declines as more external noise is added to the movie. We attribute elevated levels of internal noise to dysfunctional motion sensors and a loss in efficiency to non-functional motion sensors. Image: Bex Laboratory
Recent Publications


Rapid Perimetry

Diseases such as glaucoma or diabetic retinopathy and other causes of retinal insult, such as laser injury, cause blind spots that often go undetected. This is because the scotomas are often in different locations in the two eyes and because perceptual processes fill-in the scotomas and render them invisible, as they do in normal vision at the blind spot where the optic nerve leaves the eye. The occurrence of filling-in means that simple charts, known as campimetry, cannot be used reliably to help people locate their own blind spots. Instead, point-wise testing across the visual field, called micro-perimetry, is required, but this can be painstakingly slow. We have developed a new behavioral technique that can identify blind spots quickly and easily.

Figure 3 a) simulates a blind spot in a dynamic noise pattern and is often referred to as an artificial scotoma. After a short while, the blank patch disappears and the whole screen appears to contain noise. This effect can be easily demonstrated by sticking a small piece of paper in the centre of a detuned TV set and fixating the edge of the screen, so the square is in peripheral visual field. The paper gradually appears to be replaced by the TV noise. When the noise is switched off and replaced by a blank screen, paradoxically, the areas that did contain noise now appear blank, while the areas that were blank now appear to contain dynamic noise. We have found that this twinkling afterimage occurs for real scotomas caused by eye disease as well as for artificial scoto-
We have developed this observation into a simple test for undetected blind spots. Patients view a dynamic noise image alternated with a blank screen for several ten-second cycles. During the noise periods, the patients passively watch the screen and are free to move their eyes. During the blank periods they are asked to fixate a steady cross and trace around any anomalous areas on a touch-sensitive screen. The afterimages collected in this way in a few minutes closely correspond to those collected with micropentrimetry over 30 minutes or more. This test can be run at home on a TV or on a computer so that anyone can test themselves and self-refer at the first signs of a problem and secure the most effective early clinical intervention.

Recent Publications


Amblyopia

Abnormal binocular vision in infancy can lead to the development of amblyopia, commonly known as ‘lazy eye’, in which vision in the affected eye can be severely impaired. Amblyopia is the leading cause of visual impairment in childhood.

Treatment for amblyopia usually involves patching or blurring vision in the unaffected eye and this intervention is effective in around 75% of juvenile cases, but less than half of adult cases. We have previously studied the perception of form and motion in amblyopia and found that visual distortions, like those illustrated in Figure 4, rather than blur are a key feature of amblyopia. We are developing new computer-based treatment and assessment techniques for amblyopia. We aim to monitor the effects of treatment on the sound eye and to restore binocular vision in juvenile and adult amblyopes who are not responsive to conventional treatment.

Recent Publications


Natural Scenes

A great deal of sensory research has attended to the problem of how we detect and encode elementary components of visual images (such as sine gratings) that are experimentally presented in isolation and under conditions that render them barely visible (e.g. at contrast detection threshold). However, relatively little is known about how these components are integrated for the perception of natural images, which contain many visual elements at a range of contrasts. We have been studying the statistics of static and dynamic natural images and relating the properties that characterize natural images to the sensitivity of the human visual system. In many studies, we have found that the visual system is optimized to process natural scenes.

Recent Publications


Mareschal, I., S.C. Dakin, and P.J. Bex, Dynamic properties of orientation discrimination assessed by using classification images. Proceedings of the National Academy of Sciences of the United States of America,
Injury to the adult central nervous system, such as the retina and optic nerve, is devastating because of the inability of these neurons and their nerve fibers to regenerate. The objectives of our research are to elucidate the molecular mechanisms causing this regenerative failure in adult mammals and to develop therapeutic strategies to restore vision by retinal transplantation or promotion of nerve regeneration. This study may also shed light on treating damage and degenerative disorders in the brain and the spinal cord.

First, our lab is devoted to unraveling the molecular mechanisms, acting inside retinal ganglion cells, that control optic nerve regeneration. Our previous research has demonstrated a central role of Bcl-2 in supporting this intrinsic growth mechanism for retinal ganglion cell axons. Next, we will delineate the signaling pathways by which Bcl-2 mediates optic-nerve regeneration by taking advantage of the available genetic mouse models and neuronal culture systems.

The second part of the on-going research in our laboratory is to identify the inhibitory components that inhibit nerve regeneration. Using transgenic and knockout mouse models carrying defects in genes of various glial cells, as well as the technology of DNA microarrays, we will define the glial cell components that inhibit nerve regeneration.

In addition, we are also developing a method for using retinal transplantation or replacement therapy to treat retinal damage or diseases. Retinal transplantation has proven difficult. Recently, we provided evidence that retinal glial cells act as a barrier to prevent transplanted cells from migrating and forming connections with the host retina. By searching for a drug that will break down this glial barrier to allow a transplant in mice, we hope that our work will eventually be applied to cure blindness and other neurodegenerative diseases in human.

Recent Publications


**Neural and Glial Contributions to Early Diabetic Retinopathy (Neuroscience)**

Diabetic retinopathy is the most common complication of diabetes and affects 90% of diabetic patients. Early diagnosis and reversion of abnormalities in retinal vascular damage are challenges for the treatment of diabetic retinopathy. Neurons and glial cells are major cell populations of the retina, and they reveal abnormalities during early diabetes that precede vascular abnormalities. However, the roles of these cellular changes in diabetic retinopathy are largely unexplored.

Our objective is to determine whether the early onset of neuronal apoptosis and the appearance of reactive glial cells in the diabetic retina contribute to the later development of vascular damage of diabetic retinopathy.

Our first goal is to develop an experimental model of diabetic retinopathy in mice. The advances in the technology of mouse genetic engineering lead to a unique opportunity for exploring the molecular mechanisms underlying disease development and identifying therapeutic targets for drug development. However, little has been available for the study of diabetic retinopathy in mice. Therefore, we will begin by developing a disease model in mice and apply this model to examine the relationship between the onset of neuronal and glial cell changes and the further blockade of optic-nerve regeneration.
the development of vascular abnormalities during the disease process. We will then use transgenic and knockout mouse models to manipulate neuronal and glial cell responses and observe their impact on the vascular pathology of diabetic retinopathy.

We hope that the results of this work will eventually be brought to the clinical setting, provide potent indicators and measures for disease development, and lay the groundwork for developing treatment of diabetic retinopathy.

**Recent Publications**

Retinal progenitors isolated from adult mice develop into retinal specific neurons.

Epifluorescence photomicrographs reveal that following the removal of glial barrier, transplanted retinal progenitors (green) integrate robustly into the outer nuclear layer, where photoreceptor cells are normally located, in the adult mouse retina. The transplanted cells differentiate and display typical morphology of mature photoreceptor cells.

Images: D-F. Chen Laboratory
Molecular Regulation of Lymphatic Vessel Development and its Applications to Corneal Inflammation and Immunity

Lymphatic research represents an expansive field of new discovery owing to the recent identification of several lymphatic specific markers. The cornea provides an ideal tissue for lymphatic studies due to its accessible location, transparent nature, and lymphatic-free and -inducible character. Additionally, corneal transplantation offers an excellent model for lymphatic research because it allows for (i) functional lymphatic cell trafficking studies; and (ii) identification of cellular contributions (donor or recipient source) to the process of lymphangiogenesis (LG).

Once induced, corneal LG enhances high volume delivery of antigens and antigen presenting cells, and accelerates corneal inflammation and transplant rejection. Our long-term goal is to elucidate the molecular mechanisms of LG using in vitro and in vivo corneal models, a necessary prerequisite to the development of new therapeutic protocols. Briefly, pertinent work we have accomplished includes: 1) determination of the critical role of VEGFR-3 (vascular endothelial growth factor receptor-3) in corneal LG, lymphatic cell trafficking, and transplant rejection; 2) discovery of LYVE-1 (lymphatic vessel endothelial hyaluronan receptor-1) positive macrophage lineage cells in the normal conjunctiva, and their possible roles in supplying functional LG in the cornea during inflammation; 3) identification of the important roles of alpha 1 integrin in corneal LG, innate and immune cell infiltration, and transplant rejection; and 4) development of a new system to isolate and culture lymphatic endothelial cells for functional studies in vitro.

Research on corneal LG will have broader clinical implications beyond the treatment of ocular diseases alone, since the lymphatic network penetrates most tissues in the body, and its dysfunctions are involved in a diverse array of disorders including cancer metastasis, diabetics, delayed wound healing, AIDS, arthritis, and lymphedema, among many others.

Recent Publications


Representative micrographs showing newly developed lymphatic vessels detected in the inflamed corneas during suture-induced neovascularization (A and B) or after transplantation (C). Double staining of LVYE-1 and VEGFR-3 indicates lymphatic vessels. Images: L.Chen Laboratory

A. Normal  
B. Double staining  
C. After grafting

LVYE-1 (green)  
VEGFR-3 (red)  
Merged (yellow)  
(inner line: graft-host junction)
Role of Vascular Endothelial Growth Factor (VEGF) in the Adult

Although there is an undisputed role for VEGF in vascular development and in many vascular pathologies, including diabetic retinopathy, wet macular degeneration and tumor vascularization, the function of VEGF in the adult is less investigated. We hypothesize that VEGF in the adult is important for the maintenance of microvascular integrity and function. Toward this end, we have demonstrated that all vascularized adult tissues express VEGF, and that the VEGF is most often produced by cells in close proximity to the microvasculature. In addition, we have shown that VEGF receptor 2 (VEGFR2) is constitutively phosphorylated in adult tissues. Studies are underway in our lab using a variety of approaches to neutralize VEGF in the adult, including systemic adenoviral expression of sFlt-1, inducible endothelial-specific expression of dominant negative VEGFR2, and inducible tissue-specific deletion of VEGF. Preliminary data indicate a trophic role for VEGF not only for microvascular stability but also for non-vascular cells that express VEGFR2.

Differential Roles of VEGF Isoforms

VEGF is produced from a single gene as multiple alternatively spliced isoforms (3 isoforms in mice and 5 in humans). The isoforms differ in their binding to heparan sulfate proteoglycan (HSPG) and thus in their extracellular localization upon secretion; VEGF120 does not bind HSPG and is fully diffusible, VEGF188 exhibits strong HSPG binding, remaining cell- and matrix-associated, and VEGF164 with intermediate properties is both cell-associated and diffusible. We have shown that the isoforms are differentially expressed in mouse both during development as well as in the adult. For instance, whereas 50% of the VEGF produced by the lung is VEGF188, the retinal pigment epithelium makes virtually no VEGF188. These expression levels reflect the nature of the association between the VEGF-producing cells and the VEGF target cells. In the lung, the type 2 pneumocytes, which produce VEGF, are closely apposed to the pulmonary vasculature. In this situation, matrix-associated VEGF188 would seem to be an effective means of local VEGF delivery. In contrast, the production of diffusible VEGF by retinal pigment epithelial cells is consistent with the separation of the pigment epithelium from the target choriocapillaris by a thick elastic lamina called Bruch’s membrane. Current studies are aimed at comparing the biological effects of the isoforms in various physiologic models, including ischemia-induced retinal neovascularization, laser-induced chorioidal neovascularization and tumor vascularization.

Choroid plexus: choroid plexus and ventricle showing perfused blood vessels [green], chorioidal epithelial cells stained with podoplanin [red], and nuclei [blue]. Image: D’Amore Laboratory
Role of TGFβ Signaling in the Adult

We have previously shown, using in vitro coculture models, that contact between endothelial cells and mesenchymal cells leads to the activation TGFβ that is produced by both cell types in a latent form. The activated TGFβ acts on the undifferentiated mesenchymal cells to induce their differentiation to pericytes/smooth muscle cells, and on endothelial cells to inhibit their migration, suppress their proliferation and induce their synthesis of VEGF. However, whether a similar interaction occurs in vivo is not known.

We hypothesize that constitutive activation of TGFβ and its signaling in the endothelial cells and pericytes is central to the maintenance of vascular integrity. We are taking two approaches to address this question: (i) administration of soluble endoglin via systemic adenoviral injection to sequester TGFβ and (ii) generation of a mouse model in which a dominant negative form of the TGFβ receptor II will be expressed within the pericytes/smooth muscle or in the endothelium. These systems will allow us to assess the role of TGFβ in the adult vasculature.

Regulation of VEGF

VEGF is critical to vascular development, plays a central role in pathologic neovascularization and appears to play a survival role in the adult. In spite of its importance, little is known about the molecular basis of its control, outside of its regulation by hypoxia. We are investigating the role of insulin-like growth factor, a molecule important both in development and pathology, in the regulation of VEGF expression. Additionally, we are studying the mechanisms by which VEGF expression is controlled in the adult. Using VEGF promoter analysis, we are elucidating the molecular regulation of constitutive VEGF expression in skeletal muscle. Furthermore, most endothelium, with the exception of aortic endothelial cells, does not express VEGF. We are working to understand the mechanism by which VEGF expression is suppressed in most endothelium and the factor(s) that lead to VEGF expression in aortic endothelium. Results of these analyses may provide a novel and more precise means for therapeutic inhibition in various pathologies in which neovascularization plays a central role.
Director, Cornea Service, Massachusetts Eye & Ear Infirmary

Reza Dana
Senior Scientist
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Investigator
Lu Chen, M.D., Ph.D.

Technical Support
Qiang Zhang, M.D., Ph.D.

Molecular & Cellular
Mechanisms of Corneal Inflammation
(Immunology)

Currently available methods to suppress corneal inflammation rely on utilizing toxic pharmaceuticals that are variably effective at best, and which carry with them significant risks of side-effects to the eye and patient such as infection, cataracts, and glaucoma. The aim of our laboratory program is to (1) unravel the molecular and cellular processes that are responsible for initiating and sustaining immuno-inflammatory responses in the cornea and anterior segment, and thereby (2) develop specific molecular targeting strategies to promote mechanisms that engender immune unresponsiveness without placing the eye or the patient at risk of toxicity or secondary infections. Our work has identified interleukin-1 and tumor necrosis factor-alpha (TNF-alpha) as critical initiators of inflammation. Our lab has shown that these cytokines promote: (a) expression of specific adhesion factors such as ICAM-1, at the level of the limbal (pericorneal) vasculature, and (b) activation of antigen presenting cells including dendritic and Langerhans cells that can in turn stimulate T cells to respond to corneal antigens.

We have developed a number of molecular strategies that target important mediators of these processes including topical formulations that specifically inhibit ligation of specific membrane-bound receptors that promote ocular inflammation. These strategies have shown promise in the lab in down modulating corneal and ocular surface inflammation in allergy, neovascularization, and transplantation. A recent promising area of investigation in our lab is our determination of the role of VLA-1 (alpha-1, beta-1) integrin in mediating corneal inflammation. Suppression of this collagen-binding integrin has a profound effect on suppressing corneal inflammation. Finally, we are interested in apoptotic cell death as it has become increasingly clear that apoptosis of both inflammatory cells and target tissue cells is a common feature of many forms of immune and inflammatory responses. From a technology application standpoint, we are attempting to unravel the role of specific pro-angiogenic and anti-angiogenic factors by using gene therapy approaches in both in vivo and in vitro settings to promote cell survival and minimize tissue damage in the inhospitable microenvironment of inflammation.

Recent Publications


Dana MR, Qian Y, Hamrah P. Twenty-five year panorama of corneal immunology: emerging concepts in the immunopathogenesis of microbial keratitis, peripheral ulcerative keratitis, and corneal transplant rejection. Cornea 2000; 19: 625-43


Role of Angiogenesis In Corneal Inflammation And Immunity
(Vascular Biology, Immunology & Cornea)

Most of the work to date on corneal neovascularization has focused on vasculogenic events in terms of corneal scarring, and attendant changes in the matrix, rather than on the mechanistic role of lymph and blood vessel growth as they affect induction (via lymphatics) and expression (via blood vessels) of immunity to corneal antigens. It is exactly this area, namely, the interface of corneal vasulogenic events and immunity, that is the focus of our research. In addition to work focusing on the regulation of vascular growth factors in the cornea, we have recently focused on the expression by corneal antigen-presenting cells (APC) including dendritic cells of vascular endothelial growth factor receptor-3 (VEGFR-3) in inflammation. We have shown that this receptor is functional, and its ligation on corneal APC mediates their chemotactic egress out of the cornea into adjacent lymphatics and draining lymph nodes. When this receptor is blocked, we can suppress this trafficking and likewise down modulate induction of immunity, providing evidence for the intimate link between growth factor regulation of vasculogenic events and trafficking of immune cells. Finally, our work has led to a determination of a novel mechanism by which the cornea retains its clarity and avascular character. We have shown that high constitutive expression of ‘ectopic’ VEGF-R3 by the epithelial cells of the normal cornea acts as a “sink” mechanism to bind VEGF-C and VEGF-D that would otherwise bind VEGF-R2 to drive pathological angiogenesis. This is the reason why the threshold for angiogenesis in the cornea is as high as compared to many other tissues, and why in spite of constant or recurrent exposure of the ocular surface to noxious substances, the cornea typically retains its clarity, thereby allowing for good

Training and Research Programs in Vision Science

Reza Dana, M.D., M.P.H., M.Sc.

Current Faculty Research Projects

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vision. Continued work in this area is being done in collaboration with investigators in Erlangen, Germany.

Recent Publications


Chen L, Cursiefen C, Barabino S, Zhang Q, Dana MR. Novel expression and characterization of lymphatic vessel endothelial hyaluronate receptor 1 (LYVE-1) by conjunctival vascularized. Our principal goal is to decipher the molecular mechanisms of T cell costimulation. Additionally, we have been very interested to phenotype the diverse population of APCs that mediate alloimmunity. Of interest, is our recent finding that the cornea contains a diverse population of myeloid resident APCs which have the unusual characteristic of not expressing MHC antigens or costimulatory B7 (CD80/CD86) molecules unless activated, in which case they can migrate to draining lymph nodes and stimulate T cells upon upregulation of specific chemokine receptors including CCR7. The exact role of these resident donor APC, as compared to those host-derived APC that migrate into the grafted tissue from the limbus, is an area of intense investigation in our laboratory. In particular we are interested in determining what factors in the ocular microenvironment retain the corneal APCs in such a highly immature or precursor phenotype. Finally, our work has expanded recently in evaluating various regulatory mechanisms in transplantation tolerance such as the role of Programmed Death-Ligand 1 (PD-L1) and T regulatory cells. An active program of collaboration with colleagues at Brigham and Women’s Hospital and Harvard Medical School facilitate these investigations and provide venues for ongoing dialogue and exchange of ideas.

Recent Publications


Mechanisms Of Sensitization And Tolerance In Corneal Transplantation

(Immunology)

Corneal transplantation is by far the most frequent form of tissue transplantation. Unfortunately, immune rejection represents the principal cause of corneal graft loss. The burden of graft rejection in corneal transplantation is immense, particularly in “high-risk” cases where the host bed is inflamed and vascularized. Our principal goal is to decipher the cellular and molecular processes involved in sensitizing the host immune cells to the graft. Our work has shown that induction of immunity to graft antigens requires sequential (a) activation of antigen-presenting cells (APC), (b) migration of antigen-borne APCs into afferent lymphatics, (c) and stimulation of CD4+ host naive T cells for induction of an allospecific T helper 1-type response. Much of our work has focused on the molecular mechanisms of T cell costimulation. Additionally, we have been very interested to phenotype the diverse population of APCs that mediate alloimmunity. Of interest, is our recent finding that the cornea contains a diverse population of myeloid resident APCs which have the unusual characteristic of not expressing MHC antigens or costimulatory B7 (CD80/CD86) molecules unless activated, in which case they can migrate to draining lymph nodes and stimulate T cells upon upregulation of specific chemokine receptors including CCR7. The exact role of these resident donor APC, as compared to those host-derived APC that migrate into the grafted tissue from the limbus, is an area of intense investigation in our laboratory. In particular we are interested in determining what factors in the ocular microenvironment retain the corneal APC in such a highly immature or precursor phenotype. Finally, our work has expanded recently in evaluating various regulatory mechanisms in transplantation tolerance such as the role of Programmed Death-Ligand 1 (PD-L1) and T regulatory cells. An active program of collaboration with colleagues at Brigham and Women’s Hospital and Harvard Medical School facilitate these investigations and provide venues for ongoing dialogue and exchange of ideas.

Recent Publications


Pathogenesis of Dry Eye Syndromes and Dry Eye-related Ocular Surface Inflammation (Ocular Surface Immunology)

There are a myriad of immune-mediated inflammatory disorders that affect the cornea, sclera, and ocular surface. These include very common disorders such as dry eye and allergic conjunctivitis, and rare disorders including graft-versus-host disease after allogeneic bone marrow transplantation. In their mild form, these disorders are treatable but become sight-threatening in their severe and uncontrolled stages. There is a large literature on various treatment options but the immunopathogenesis of these disorders is poorly understood. Specific questions that we are interested in unraveling include: What are the early initiating events, at the level of the ocular surface, that lead to specific disorders? What is the role of T cell-mediated adaptive immunity in dry eye syndrome, and what cells mediate this response? What are the cytokine/chemokine factors that recruit and activate antigen-presenting cells in chronic dry eye and graft-versus-host disease (GVHD)? What are the important prognosticators of disease and how are they related to molecular and clinical levels? What are the reliable indicators of disease severity that can be used to assess the response to therapy? We are actively pursuing defining the molecular mechanisms of (chemokine receptors, toll-like receptors) that mediate the early immunopathogenesis of ocular surface disease in chronic dry eye, and have recently identified expression of CCR5 by the ocular surface epithelium (in clinic patients) as playing an important role in this regard. Our ability to study the fundamental processes that mediate dry eye disease has recently been enhanced significantly by development of a novel mouse model of dry eye that produces signs of dry eye in mice that closely mimic those seen in the clinic. This is accomplished by placement of mice in a controlled environment chamber where temperature, humidity, and airflow are tightly regulated and monitored. By varying these parameters we can induce a “hyper-evaporative” state that reliably induces dry eye (characterized by ocular surface staining, loss of goblet cells, disturbance in lacrimal function, and over-expression of pro-inflammatory cytokines and activation of ocular surface monocytes). This model system allows for mechanistic studies as well as preclinical testing of potential therapeutic agents.

In addition to these basic investigations in our laboratory, we are involved in a number of clinical research studies evaluating optimization of dry eye diagnosis and treatment strategies. Studies subject seen at the Mass. Eye and Ear Infirmary may be enrolled in studies involving harvesting of tear film and ocular surface cells for laboratory analyses. Finally, trainees who work with us on these projects additionally benefit the possibility of interacting closely with other faculty members on collaborative projects evaluating various facets of dry eye pathogenesis.
Angiogenesis means growth or development of blood vessels. It is required for life, and yet it also is a major confounder of pathologies in the eye and elsewhere. One of the remarkable things about it is that the cornea is resistant to growth of blood vessels. Why? The reason behind this is that the corneal surface (epithelium) normally expresses high levels of a receptor, called VEGFR-3, that binds factors that normally drive angiogenesis (VEGF-C and VEGF-D), preventing them from binding the principal receptor, VEGF-R2, that drives pathological angiogenesis. This is the reason why there is such a dramatic break between the blood vessel-rich conjunctiva and the clear cornea. If the cornea becomes vascularized, as it can in pathological states (accounting for the #2 cause of blindness worldwide), then vision is impaired because light cannot travel through the cornea unperturbed.

Illustration: Peter Mallen
Regulation of Conjunctival Goblet Cell Mucin Production
(Cell Biology, Biochemistry)

Goblet cells are major producers of the mucous layer, the innermost layer of the tear film. This laboratory is investigating the regulation of goblet cell mucin secretion and goblet cell proliferation. Our studies have shown that parasympathetic and sympathetic nerves are located near goblet cells in rats, mice and humans, and that activating these nerves causes goblet cell mucin secretion. Using in vivo and in vitro preparations developed in our laboratory, we have found that the parasympathetic neurotransmitters -- acetylcholine and vasoactive intestinal peptide (VIP) -- but not the sensory neurotransmitter, substance P, stimulate secretion from goblet cells. Cholinergic agonists stimulate secretion by activating muscarinic M2 and M3 receptors, which are located subjacent to the secretory granules. VIP interacts with VIP2 receptors located in an area similar to those of muscarinic receptors.

The signaling pathways used by cholinergic agonists and VIP to activate these cells are being investigated using biochemical and immunohistochemical techniques. Cholinergic agonists stimulate conjunctival goblet cell mucin secretion by increasing the intracellular Ca2+ concentration and perhaps by activating protein kinase C. Cholinergic agonists also activate p42/p44 mitogen-activated protein kinase (MAPK) to stimulate goblet cell mucin secretion. These agonists work by transactivating the EGF receptor that in turn activates GRB2/Sos/Ras pathway to stimulate MAPK.

Although sympathetic nerves also surround goblet cells in the conjunctiva and there are adrenergic receptors on these cells, adrenergic agonists do not appear to stimulate secretion.

We have cultured a purified population of goblet cells using rat and human conjunctival tissue. We are using these cells to study which neurotransmitters and growth factors regulate goblet cell proliferation.

Our studies show that ocular surface nerves respond to injury by secretion of a protective layer of mucus from the goblet cells. Neural regulation of goblet cell secretion could play an important role in diseases of the ocular surface. We hypothesize that decreased neural stimulation could lead to a depletion in mucus production, for example, in dry eye. In contrast, increased neural stimulation could lead to an overproduction of mucus, for example, in ocular allergy. Our future studies will determine the role that goblet cell proliferation plays in controlling the mucous layer of the tear film.

Recent Publications


Identification of Factors That Predict Dry Eye Complications of Lasik Surgery

The cornea is densely innervated by sensory nerves that mediate corneal sensitivity. Through a neural reflex, activation of the afferent sensory nerves in the cornea activates the efferent parasympathetic and sympathetic nerves in the glands and ocular surface epithelial tissues that produce tears. During laser in situ keratomileusis (LASIK) surgery, a flap is made through the cornea by a laser severing the sensory nerves. Thus a major complication resulting from LASIK is the development of dry eye symptoms and ocular surface disease. We are investigating whether there are factors that can identify patients who would develop dry eye symptoms, by examining the number and size of goblet cells along with the presence or absence of the mucins normally found in goblet cells before and after LASIK surgery.

Regulation of Conjunctival Goblet Cell Mucin Production

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www.schepens.harvard.edu/faculty/dartt

 regulates goblet cell proliferation. We have cultured a purified population of goblet cells using rat and human conjunctival tissue. We are using these cells to study which neurotransmitters and growth factors regulate goblet cell proliferation.

Our studies show that ocular surface nerves respond to injury by secretion of a protective layer of mucus from the goblet cells. Neural regulation of goblet cell secretion could play an important role in diseases of the ocular surface. We hypothesize that decreased neural stimulation could lead to a depletion in mucus production, for example, in dry eye. In contrast, increased neural stimulation could lead to an overproduction of mucus, for example, in ocular allergy. Our future studies will determine the role that goblet cell proliferation plays in controlling the mucous layer of the tear film.

Recent Publications


Identification of Factors That Predict Dry Eye Complications of Lasik Surgery

The cornea is densely innervated by sensory nerves that mediate corneal sensitivity. Through a neural reflex, activation of the afferent sensory nerves in the cornea activates the efferent parasympathetic and sympathetic nerves in the glands and ocular surface epithelium that produce tears. During laser in situ keratomileusis (LASIK) surgery, a flap is made through the cornea by a laser severing the sensory nerves. Thus a major complication resulting from LASIK is the development of dry eye symptoms and ocular surface disease. We are investigating whether there are factors that can identify patients who would develop dry eye symptoms, by examining the number and size of goblet cells along with the presence or absence of the mucins normally found in goblet cells before and after LASIK surgery.
Signal Transduction Pathways Regulating Lacrimal Gland Secretion (Biochemistry)

The exocrine lacrimal gland is the primary producer of the aqueous layer of the tear film. Lacrimal gland protein, electrolyte, and water secretion are neurally regulated by parasympathetic and sympathetic nerves. These nerves activate cholinergic, VIPergic, and α1-adrenergic receptors. This laboratory has been characterizing the signal transduction pathways activated by these receptors since, in the lacrimal gland, cholinergic and α1-adrenergic agonists activate different signal transduction pathways. We have used biochemical and immunohistochemical techniques to characterize the changes in inositol phosphates, Ca\(^{2+}\), and protein kinase C (PKC) isozymes induced by these stimuli. In particular, we have developed specific inhibitors of several of the protein kinase C isozymes or adenoviral vectors containing constitutively active forms of the PKC isozymes to determine the role of each isozyme in the steps of the signal transduction cascade in the lacrimal gland and other tissues. In addition, we have found that α1-adrenergic agonists, but not cholinergic agonists, transactivate the receptor for the growth factor, epidermal growth factor (EGF) leading to activation of p42/p44 MAP kinase (MAPK). Cholinergic agonists increase intracellular Ca\(^{2+}\) and activate protein kinase C to stimulate MAPK activity. Activation of MAPK negatively modulates protein secretion. We have also investigated the effects of growth factors on protein secretion from the lacrimal gland. EGF stimulates protein secretion by increasing intracellular Ca\(^{2+}\) and activating PKC, but not by activating MAPK or phosphatidylinositol-3 kinase (PI-3K). We have also shown that cholinergic and α1-adrenergic agonists stimulate EGF mRNA production and ectodomain shedding of EGF from the lacrimal gland. We are currently determining the role of metalloproteinases in this shedding. We have also determined that α-adrenergic, but not cholinergic agonists activate endothelial nitric oxide synthase (eNOS) that activates guanyl cyclase to cause protein secretion. We are investigating the role of phospholipase D in lacrimal gland secretion.

In aqueous-deficient dry eye, lacrimal gland secretion is decreased. Investigation of the regulation of secretion in normal and diseased lacrimal glands will provide a basis to develop treatments to increase tear production in patients with dry eye, thus improving the health of the ocular surface.

Recent Publications


Localization of P2X7 receptors (green) and actin (red) in the rat lacrimal gland. Image: Joanna Vrouvlianis and Marie Shatos
Excessive accumulation of lipofuscin in the lysosomes of retinal pigmented epithelium (RPE) cells may impede the metabolic activity of these cells and has been shown to act as a photosensitizer, generating free radicals. Furthermore, one of the components of lipofuscin, the fluorophore A2-E, has been shown to inhibit lysosomal digestion of protein, cause disruptions of lysosomal membranes, and initiate blue-light-induced apoptosis of RPE cells. These mechanisms are thought to play a role in pathogenesis of age-related macular degeneration (AMD) and juvenile macular degeneration. Noninvasive fluorospectrometry and fluorescence imaging have been used to study the biological consequences of lipofuscin accumulation in normal subjects during aging, and in patients with AMD or juvenile macular degeneration. Our results showed that lipofuscin increases with age in normal subjects, reaching highly variable levels at mid-life. Factors associated with increased (age, smoking) and decreased (vitamin supplementation, dark iris) risk for AMD were also significantly correlated with increased or decreased accumulation of RPE lipofuscin. These results suggest that lipofuscin measurements provide a cumulative index of oxidative damage in the eye.

In patients with AMD we observed a significant decrease in lipofuscin levels. The decrease may be caused by chemophysical changes in lipofuscin at high concentrations, causing it to lyse the lysosomes in which it is trapped and/or by partial atrophy of RPE cells. These findings are not inconsistent with lipofuscin reaching high levels before disease is initiated, and then falling. We are presently investigating lipofuscin levels in patients with the preclinical, earliest signs of AMD. In patients with Stargardt’s disease (fundus flavimaculatus), lipofuscin levels are significantly higher than normal, confirming histopathologic findings. We use autofluorescence imaging to study the lipofuscin distribution at pathological sites such as drusen, hyperpigmentation, and geographic atrophy. In parallel to these clinical studies, we are studying biophysical aspects of light interactions in tissues and are investigating, by spectroscopy and time-resolved techniques, the various fluorophores, absorbers, and scattering media that are present in the retina.

Recent Publications


Inflammatory Cytokines in Diabetic Retinopathy
(Vascular Cell Biology, Glial Cell Biology)
The focus of my laboratory is the pathogenesis of diabetic retinopathy one of the leading causes of blindness in the adult population of the United States. Future projections indicate that diabetic retinopathy will only worsen as a public health problem, as the prevalence of diabetes increases due to the current obesity epidemics and population aging. The only therapeutic intervention proven to delay the onset and progression of diabetic retinopathy is the primary treatment of diabetes to achieve near-euglycemia. However, tight metabolic control is not easy to achieve, and intensive insulin therapy is not always practical and free of complications. To preserve visual function in diabetes, we need to develop adjunct treatments that target the consequences of suboptimal metabolic control at the early stages of disease development before the occurrence of irreversible vascular damage. In order to develop such treatments, there is a crucial need for a better understanding of the mechanisms linking the diabetic milieu to retinal vascular damage.

To identify the pathogenetic pathways that could become therapeutic targets to prevent diabetic retinopathy, we have recently studied the gene expression profile of retinal Müller glial cells—an early target of diabetes in the retina—and retinal microvessels—the clinically relevant retinal target of diabetes. The results of these studies indicate that a common effect of diabetes on retinal glial and vascular tissue is the upregulation of several acute-phase response proteins and other inflammation-related genes, a process accompanied by the induction of the proinflammatory cytokine interleukin-1β (IL-1β) in the retina. Upregulation of IL-1β and acute-phase proteins in the retina occurs early in the course of diabetes and precedes the development of vascular cell apoptosis. Because many of the molecular, cellular, and functional abnormalities known to occur in the retina in diabetes correspond to known biological effects of IL-1β, I hypothesize that the retinal upregulation of IL-1β-- leading to glial activation and endothelial dysfunction -- is a major contributor to the development of diabetic retinopathy.

The current goals are to understand how diabetes leads to the inflammatory response of the glial and vascular components of the retina, and to determine whether inflammatory changes and especially IL-1β have a causal role in the development of retinopathy. Identifying IL-1β as a mediator of diabetes-induced retinal damage would have a profound impact on the field of diabetic retinopathy, opening new avenues for the prevention and treatment of this complication of diabetes.

Recent Publications
Antibiotic resistance in *Enterococcus* *faecalis* and *staphylococcus* has rendered many common and severe infections treatable only with last line drugs. The emergence in the 1980's of vancomycin resistant Enterococci among leading causes of hospital acquired infection, raised the specter of untreatable bacterial infection. Since then, Enterococci have transferred vancomycin resistance to methicillin-resistant *S. aureus*, creating the potential for untreatable community acquired infection as well. With a view toward developing new therapeutics, a main arm of our research focuses on the exchange among *Enterococcus* (and between *Enterococci* and other genera) of auxiliary elements encoding antibiotic resistance and virulence traits; and determination of how these variable traits impact the virulence and ecology of the organism. In addition to being leading causes of antibiotic resistant infection, the *Enterococci*, *streptococci* and *staphylococci* are also highly adapted members of the human commensal flora. As a result, these organisms possess sophisticated mechanisms for colonizing human mucosal surfaces and skin, and interaction with the host immune system is finely balanced. A second arm of our research has focused on identifying factors that undermine the host/microbe dynamic, leading to disease. The overarching goal of this research is to develop new strategies for preventing, mitigating or otherwise treating infections that are increasingly refractory to available antibiotics. Toward that end, we examine the pathogenesis of infection as a process, and work to identify the critical points on which outcome depends. The approaches used to identify critical points range from genomic studies to developing new models to examine the biology of infection.

Finally, *enterococci* and *staphylococci* mainly exist as commensal organisms causing little harm to the host, and potentially making a positive contribution (at the very least by excluding more pathogenic organisms from the human ecology). Because they are not obligate pathogens, the process by which these organisms infect is subtle and involves both host and bacterial factors. The eye represents a unique model for study of infectious diseases. First, the ocular surface shares many properties with other wet mucosal surfaces of the body. However, it is the most accessible for direct observation. Second, the retina provides by far the best view of the mammalian vasculature. Many of the effects of infection on the host are manifest in the circulation. Finally, the vitreous, as Robert Koch new, was highly conducive to bacterial growth – even opportunistic organisms like *enterococci* and *staphylococci*. Therefore a third arm of our research focuses on infections of the eye and developing state-of-the-art tools to study them.

**Recent Publications**


Color enhanced electron micrograph of antibiotic resistant cells of *Enterococcus faecalis* (X 30,000), a cause of post operative infections that is extremely difficult to treat. The overarching goal of the laboratory is to develop new ways of treating and preventing infections of the eye and other sites caused by *enterococci* and *staphylococci*. Image: Gilmore laboratory
Mucins of the Ocular Surface

The stratified epithelium over the surface of the eye is the first line of defense for the visual system, protecting it from invasion by pathogens, from desiccation, and from injury and abrasion. The epithelium over the cornea is also specialized to provide an extraordinarily smooth surface that serves as the major refractive power of the eye. To perform these varied functions, the epithelium has specialized surfaces at its tear film interface. This laboratory has discovered that the entire ocular surface epithelium, not just conjunctival goblet cells, produces mucin for the epithelial surface. We have determined that 4 of the 20 human mucin genes cloned to date are expressed by the ocular surface epithelium. We have shown that one specific mucin gene is expressed by conjunctival goblet cells and three membrane-associated mucins are expressed by the apical cells along the entire ocular surface epithelium. We currently study the specific functions and the regulation of expression of these mucins. To study function of mucins, siRNA methods are used to knockdown specific mucin genes, and function assays for the mucins have been developed.

We have developed methods to quantitate mucin mRNA from the human ocular surface epithelium and mucin glycoproteins from the tear film in normal subjects and dry eye patients. We have shown that in Sjögren’s syndrome dry eye, there is a decrease in goblet cell mucin mRNA and goblet cell mucin in the tear film. Understanding the functions of specific mucins, regulation of expression of ocular surface mucins, factors that induce goblet cell differentiation, and the molecular nature of the mucin layer of the tear film will yield information for treating disorders of the ocular surface including dry eye syndromes.

Recent Publications


Mucin Genes Expressed by Human Reproductive Tract Epithelia

Mucins are highly glycosylated glycoproteins that are important for protection of all wet-surfaced epithelia including that of the reproductive tract epithelia. In the reproductive tract they are also believed to be important for reproductive success. Using molecular techniques, we screened female and male reproductive epithelia and determined their mucin gene expression profiles. Current studies are focused on the relationship of membrane-associated mucins to implantation. Since dry eye syndromes are most prevalent in post-menopausal women, this project has relevance to the ocular surface.

Recent Publications


Fas Ligand and Ocular Immune Privilege

It has been proposed that the constitutive expression of Fas Ligand (FasL) in the eye maintains immune privilege, in part, through the induction of apoptosis in infiltrating Fas+ T cells. However, the role of FasL in immune privilege remains controversial due to studies that indicate FasL exists in either a membrane-bound form that is pro-inflammatory, or a soluble form that is anti-inflammatory. We hypothesize that there is a high ratio of soluble to membrane FasL that is critical in maintaining ocular immune privilege. In addition, we have identified modified forms of soluble FasL (sFasL) that are unique to the eye and we believe play a central role in maintaining ocular immune privilege. The experiments currently underway will: (i) determine where the different forms of sFasL are expressed in normal eyes and how they are modified, (ii) determine how the modified forms of sFasL stimulate innate immunity, using in vitro and ex vivo models, and (iii) determine if the modified forms of sFasL control immune privilege in the eye using a novel in vivo model. We believe our analysis of the modified forms of sFasL will significantly advance our understanding of how FasL maintains immune privilege and will help explain the controversy over the physiological role of FasL in the immune privileged eye, as well as transplants, and tumors.

Recent Publications


Fas ligand is constitutively expressed within the immune privileged eye. However, metalloproteinases are also expressed within the eye that can cleave Fas ligand and release the soluble form. Therefore, both the proinflammatory, membrane-bound Fas ligand and the anti-inflammatory, soluble Fas ligand can be expressed within the eye. We believe that the ratio of membrane to soluble Fas ligand is critical in maintaining the immune privileged environment of the eye. Image: Peter Mallen
Host Response to Endophthalmitis
Endophthalmitis is an infection of the posterior segment of the eye that can destroy the eye within 72 hours. The ultimate goal of this project is to understand the innate immune response to endophthalmitis and determine how host factors may contribute to the pathogenesis of the infection. We believe that a complete understanding of the host immune response to endophthalmitis and how this response can be modulated is critical in the development of new therapies to treat this devastating infection and prevent loss of vision.

Recent Publications


Immunology of Glaucoma
Pigmentary glaucoma is one of the most common forms of secondary glaucoma. However, the underlying cause remains unclear. The DBA/2J mouse is an animal model of pigmentary glaucoma and provides us with a unique tool to study the causes of glaucoma in order to design improved and targeted treatments. We previously demonstrated that immune abnormalities that terminate ocular immune privilege are critical in the development of glaucoma. Our preliminary results demonstrate that membrane Fas ligand is highly expressed in the trabecular meshwork of DBA/2J mice that develop pigmentary glaucoma. We hypothesize that upregulation of membrane Fas ligand in the trabecular meshwork contributes to the pathogenesis of pigmentary glaucoma by either (i) amplifying inflammation, causing termination of immune privilege and/or (ii) inducing apoptosis of trabecular meshwork endothelial cells, causing increased intraocular pressure. The ultimate goal of this project is to understand the cause of the immune abnormalities in pigmentary glaucoma and identify new targets for treatment.

Recent Publication

500 CFU
S. aureus cleared infection

5000 CFU
S. aureus destructive endophthalmitis

At 96 hours, an intravitreal injection of 500 CFU of S. aureus is cleared, while a 5000 CFU injection induces a destructive endophthalmitis resulting in extensive retinal damage. Clinical pictures were taken at 96 hours post infection of eyes that clear the infection (A) and eyes that are destroyed (E). H&E staining revealed no signs of infection (B) and no retinal damage (C) at 96 hours in eyes that cleared the infection. By contrast, a fulminant infection (F) and extensive retinal damage (G) was observed at 96 hours in eyes that could not clear the infection. Tunel staining revealed no apoptosis in eyes that cleared the infection (DI, while significant retinal apoptosis occurred in eyes that could not clear the infection (H). (Figures D and H are 100X pictures of the retina, where all nucleated cells are stained blue with DAPI and apoptotic cells are stained red with Tunel.)

Image: Emily Whiston and Norito Sugi, Gregory-Ksander Laboratory
Cell Cycle of the Corneal Endothelium
(Corneal Endothelial Cell Biology)
The corneal endothelium is responsible for maintaining corneal clarity. To function properly, corneal endothelial cell density (ECD) must be kept at or exceed a threshold cell number. The fact that ECD decreases with age and as a result of trauma, indicates that the rate of cell division does not keep pace with the rate of cell loss. Our laboratory is using cell and molecular biology techniques to identify the molecular basis for this apparent lack of cell division and to discover methods to stimulate division of these cells to increase ECD. Increasing ECD would prevent the corneal blindness that results from endothelial cell loss. Increasing ECD would prevent the corneal blindness that results from endothelial cell loss. We have developed a method to consistently grow human corneal endothelial cells (HCEC) in culture and to transplant these cells at high density to recipient human corneas in vivo. This culture system could be used to develop methods to transplant HCEC in vivo or for use in artificial corneas. Cultured HCEC are currently being used as a model to study the role of protein tyrosine phosphatases (PTPs) in regulation of cell division. In addition, we are using molecular biology methods to bypass G1-phase arrest and induce transient proliferation in HCEC. We have shown that over-expression of the transcription factor, E2F-2, which is required for G1/S-phase progression, induces cell cycle progression and increases ECD in ex vivo human corneas. We are also testing whether treatment of HCEC with siRNA for the cell cycle inhibitors, p21Cip1 and/or p16INK4a will promote proliferation. In addition, we are initiating studies to identify the underlying cause for the observed age- and topographical differences in proliferative capacity.

Recent Publications


Under permissive conditions, human corneal endothelial cells are capable of cell division. These 4 images are of normal donor corneal endothelium stained with specific antibodies to show cell borders (red) and the chromosomes of dividing cells (green). The final stages of cell division are pictured from different areas of the endothelium. In Anaphase, complete pairs of chromosomes begin to separate from each other. In Telophase, the chromosomes have almost completely separated. Note that during Anaphase and Telophase, the cell borders are less distinct and the membranes are ruffled. During Cytokinesis, the daughter cells begin to form. The cells develop a membrane between the two newly-formed nuclei to help separate the cells. Daughter Cells show two newly divided cells completely separated by their respective cell membranes. *Images: Deshea Harris, Joyce Laboratory*

Although human corneal endothelial cells are capable of dividing, they do not usually divide in vivo to replace dead or injured cells. When cell density falls below a threshold number, the endothelium cannot maintain its important “pump” and barrier functions. This loss of function results in corneal edema and permanent loss of transparency. Currently, only corneal transplantation can restore normal vision. Studies from this lab indicated that ectopic expression of the transcription factor, E2F2—which is required for S-phase entry—can induce cell division in human corneal endothelium. This figure shows nuclei of endothelial cells from a 70 year old donor that are in the process of cell division. The arrowhead shows chromosomes of cells in late Prophase, while the arrow clearly shows Telophase chromosomes in an actively dividing cell. Results indicate that even endothelial cells from older donors are capable of being induced to divide by over-expressing E2F2.
We developed a novel proteomics-based approach to identify proteins that are either contributing to, or indicate dysfunction of the vascular endothelium prior to the onset of complications. Using this screen we identified numerous previously unappreciated players. This information has been the starting point of our ongoing research to understand how diabetes-induced changes compromise the health of endothelial cells and precipitate complications such as atherosclerosis and retinopathy.

Recent Publications


The Role of Growth Factors in PVR

We are investigating the mechanism by which platelet derived growth factor (PDGF) drives fibroproliferative diseases such as proliferative vitreoretinopathy (PVR). One of the receptors for PDGF is particularly capable of inducing PVR in an experimental setting. Our strategy to identify events that promote PVR is to investigate the mechanistic basis for the unequal PVR potential of different PDGF receptors.

Recent Publications


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Recent Publications


Phases of the Angiogenic Program
Functional Genomics to Phenomics

Studies of Aging in Vertebrates

Aging is one of the most important aspects of both biology and biomedical science, but the underlying mechanisms remain largely unknown. Our research is focused on increasing our understanding of the molecular basis of aging using zebrafish (Danio rerio), which has now emerged as a powerful vertebrate model system that allows for large-scale genetic and genome-wide approaches to the study of senescence in multiple tissues and organs.

The successful whole genome sequencing of both biology and biomedical science, but the underlying mechanisms remain largely unknown. Our research is focused on increasing our understanding of the molecular basis of aging using zebrafish (Danio rerio), which has now emerged as a powerful vertebrate model system that allows for large-scale genetic and genome-wide approaches to the study of senescence in multiple tissues and organs.

The success of whole genome sequencing of many species is a major accomplishment in the field of genome science. Undoubtedly, however, the next challenge will be to elucidate the functions of every gene within these genomes and discern the genetic pathways underlying the complex traits such as those in aging that manifest at the whole-body level. An essential part of this endeavor will involve linking changes in the genome structure with the corresponding phenotypes.

Current technologies facilitate the precise determination of changes within genomes at the DNA sequence level, but measurement of the associated phenotypes remains problematic. In addition, the range and depth of the phenotypes that will need to be assayed (including both their morphological and behavioral aspects) are diverse and complex. Moreover, phenotypes are further influenced by both environmental effects and epigenetic elements. One of the fundamental goals of our research is the development of robust and reproducible phenotyping of aging in higher organisms by utilizing advanced bioinformatics and systems biology. Hence, a major objective of our aging research in zebrafish is to develop a high-throughput processing system for gene identification and to then successfully pursue phenomics projects that involve phenotype-based genomic analysis of the organismal aging process.

The Aging Eye Research in Zebrafish

The eye is one of the most sensitive organs to aging symptoms as the retina is susceptible to a variety of chronic diseases associated with aging that are accompanied by a decline in regenerative capability. In higher vertebrates, retinal neurons as well as other parts of central nervous system have a limited ability to regenerate. In lower vertebrates such as zebrafish, however, acute neural damage stimulates intrinsic photoreceptor progenitors to migrate to the outer nuclear layer, and then generate fully functional photoreceptors and all types of retinal neurons. Intriguingly, as zebrafish grow throughout their adult life, new neurons are continuously added to the retina and increase the size of the eye. Due to their remarkable growth and regenerative abilities, fish might therefore have the capacity to avoid the aging-associated dysfunctions and disabilities that occur in mammals. If this is indeed the case, it will be very worthwhile to explore the aging process in a simple vertebrate model system such as zebrafish that does not present some of the aging phenotypes of mammals. It would likely then be possible to apply the perpetual growth, regenerative ability, and slow onset of senescence of these fish to the mammalian system. Although zebrafish have proven to be a useful animal model for biomedical science as well as developmental biology, the biology of their aging has not been fully elucidated. Since many human diseases are related to aging and senescence, it is important to understand how zebrafish age in order to develop a rational model of human disease, compared with higher vertebrates. Thus, our current research seeks to identify new markers of aging in zebrafish and to determine whether they can be eventually linked to age-related eye diseases. To assess the aging eye phenotypes during the functional and systemic aging process in zebrafish, we are also undertaking large-scale mutant screens using several plausible aging biomarkers. These studies will assist us in elucidating the possible roles and potential of zebrafish in the field of ocular regenerative medicine, as well as aging eye research. Our first goal is to develop experimental models of aging eye diseases in zebrafish. The recent advances in the technologies underlying zebrafish genetics and genomics present us with a unique opportunity to explore the molecular mechanisms underlying aging versus disease development. It will then be possible to identify putative therapeutic targets for the development of clinical interventions. However, little information is currently available for the study of eye aging and age-associated eye diseases in zebrafish. Therefore, we have begun to develop a disease model in this species and apply this to the study of the relationship between late age-onset eye phenotypes (caused by the decrease-of-function of a gene with a haploinsufficiency) and early...
A wide variety of human diseases can be studied comparatively using zebrafish model systems. Zebrafish have a number of very favorable and powerful characteristics in terms of both genetics and genomics. These allow for the rapid isolation and/or characterization of genes involved in complex human diseases such as age-related macular degeneration (AMD) and glaucoma. Once we have recapitulated human age-associated eye disease phenotypes in the mutant fish, drug-based chemical genetic approaches can be readily performed to ameliorate disease states. These studies will thus open up new avenues of research into the underlying causes of human ocular and systemic diseases associated with aging, and will establish whether the small but powerful zebrafish model can be used to rapidly and inexpensively test new drugs and clinical protocols for their potential as future novel therapeutic interventions.

Recent Publications


Molecular Solutions to Low Vision Resulting from Battlefield Injuries

This research project will determine the efficacy of using soluble Fas Ligand (sFasL) to prevent and/or treat sight-threatening corneal inflammation and scarring induced by trauma. It does not include any research on corneal transplants. The major goals of this grant are:

(i) Determine the capacity of corneas treated with adenoviral vectors containing the cDNA for sFasL to suppress corneal inflammation and scarring following burns (chemical or thermal); (ii) Determine if sFasL treatment prevents the infiltration and/or activation of innate inflammatory cells within corneas following burns; (iii) Determine if sFasL treatment prevents the infiltration and/or activation of innate inflammatory cells within corneas following burns; (iv) Develop a purified recombinant sFasL protein that is easily delivered via “eye-drops” to the cornea. Determine the effectiveness of these eye-drops in preventing corneal inflammation and scarring following burns.

The Immunobiology of Corneal Transplants

The long-term goals of this project are to understand: (a) why primary orthotopic corneal allografts are so well tolerated (display immune privilege), and (b) why grafts in “high-risk” eyes fare so poorly. Two hypotheses are tested: Hypothesis 1. Atypical expression of alloantigens and expression of immunomodulatory molecules on corneal cells contribute to the cornea’s immune privileged status. Hypothesis 2. MHC class II and/or minor H alloantigen expression rob the corneal epithelium of its graft-acceptance promoting capacity. Our experiments will enable us to discover novel cellular and molecular mechanisms with which to create protocols with greater power to promote graft acceptance in clinical situations where graft failure is common.

Recent Publications


Thompson JA, Dissanayake SK, Ksander BR, Knutson KL, Disis ML, and Ostrand-Rosenberg S. Tumor cells transduced with the MHC Class II transactivator and CD80 activate tumor-specific CD4+ T cells whether or not they are silenced for invariant chain. *Cancer Res.* 66: 1147 – 1154, 2006.


Persistent fetal vasculature (PFV) is described as a phenomenon of failed regression of the vascular system (HVS) nourishing the developing eye. In general, the hyaloid vascular bed in the eye is characterized by intense retinal neovascularization, resulting in traction detachment and formation of fibrovascular membranes that lead to total retinal detachment.

Clinical evidence shows that the process of retinal neovascularization occurs on the border between vascularized and peripheral, avascular retina. The latter is the putative source for the release of VEGF, which is closely involved with this process. It is not clear why in some patients advanced ROP develops, while in others it may regress spontaneously. We have shown that there are distinct endothelial cell compartments within the RNMs, including VEGFR-2 and hMet/HGFR, as we have found that these markers are expressed very early in the lineage. We have also studied the vascular compartment of RNMs by double-fluorescence imaging. We have shown that there are distinct endothelial cell compartments within the RNMs, including VEGFR-2 and hMet/HGFR, as we have found that these markers are expressed very early in the lineage. We have also studied the vascular compartment of RNMs by double-fluorescence imaging. We have shown that there are distinct endothelial cell compartments within the RNMs, including VEGFR-2 and hMet/HGFR, as we have found that these markers are expressed very early in the lineage.
highly elevated, in excess of HGF on a weight basis, in SRF from stage 4 ROP and equally elevated in stage 5 ROP. We have further size-fractionated SRF and subjected these fractions to an in vitro angiogenesis assay using capillary endothelial cells placed in three-dimensional fibrin clots. This procedure allows us to determine which fraction exhibits the most robust pro-angiogenic activity. We are currently performing proteomic techniques including 2-D gel analysis with mass spectroscopy and luminex analysis on selected albumin and globulin-depleted SRF fractions.

Role of Hepatocyte Growth Factor in RPE Cell Biology, in Response to Wound Healing and Laser Injury

The human eye is extremely vulnerable to direct laser injury and maintaining good vision is an important determinant in success of military operations, and even survival of military personnel in theatre. Laser injury resulting from laser use as a weapon or through inadvertent retinal exposure (range finder, etc.) can immediately and potentially severely impact vision. There is increasing use of Nd-YAG lasers in military operations resulting in increased risk of potentially blinding exposure by military personnel. As a result there has been congressional appeal and military interest to develop a treatment modality that could be applied to immediately counteract acute retinal laser injury in the battlefield before debilitating injury results. Injury from laser irradiation comprises a partial disruption of the retinal pigment epithelium (RPE), remodeling of the monolayer into more motile cell types, formation of scar tissue, and eventual loss of vision. The hepatocyte growth factor (HGF) and its receptor (HGFR) have been implicated in wound healing responses. As part of our current department of defense project, we are studying the role of HGF and its receptor in retinal / RPE injury and wound healing in a mouse model of laser injury. Our current results indicate that HGF itself becomes upregulated following laser injury and activates its receptor, HGFR. Anatomically, this process is closely correlated with enhanced RPE motility. In the current study, we hypothesize that HGFR activation is closely associated with RPE responses to laser injury and abrogation of HGFR activity can taper RPE motility and the detrimental wound healing response that ensues. We will employ a transgenic mouse approach to directly manipulate the activity of HGFR following Nd-YAG laser-induced retinal injury. We expect that abrogation of HGFR activity will result in decreased wound healing responses and reduced disruption of retinal anatomy following laser injury.

This project directly addresses the mechanisms of retinal injury and wound healing caused by Nd-YAG lasers in combat and non-combat military conditions. We plan to design selective inhibitors for HGFR in order to taper wound healing responses.

Patents


Recent Publications


Current Faculty Research Projects

Kameran Lashkari, M.D.

Training and Research Programs in Vision Science

H & E section of mouse retina 5 days after diode laser photocoagulation. The laser induces local disruption of RPE/Bruch’s layers as well as loss of outer photoreceptor nuclei. Pigmented round RPE cells are seen migrating into the subretinal space as part of a wound healing response to laser injury.

Images: Lashkari laboratory

Nestin expression in retinal progenitor cells. Cultured human retinal progenitor cells were incubated with an anti-nestin antibody (red) and nuclei were stained with Dapi (blue). Undifferentiated retinal progenitor cells homogeneously express nestin in culture and lose its expression after differentiation into more mature retinal elements.

Stem cell niche within fibrovascular membranes associated with persistent fetal vasculature. Frozen sections of fibrovascular membranes were labeled with anti-CD31 (PECAM) antibody (green) and anti-nestin antibody (red). The niche is comprised of nestin-positive progenitor cells surrounded by an envelope of CD31+ cells. These progenitor cells give rise to mature neurons (not shown).
Mara Lorenzi, M.D.

Pathogenesis and Prevention of Nonproliferative Diabetic Retinopathy
Vascular Cell Biology (Clinical Investigation)

This program has two arms. The laboratory-based arm aims to reconstruct the cellular and molecular events that lead to the known histological lesions of diabetic retinopathy, and define their respective pathogenic role. In particular, we target microvascular obliteration, the main cause of retinal ischemia and neovascularization in diabetes. Studies in this laboratory have identified processes that may be operative in the causation of microvascular occlusion and obliteration in the diabetic retina: apoptosis of endothelial cells and pericytes, microthrombosis, complement activation, and activation of wound-healing pathways. We are in the process of defining their roles by using pharmacological interventions and evaluating outcomes both at the level of cellular-histological processes and programs of gene expression in retinal microvessels. Such pharmacological studies are also intended to identify candidate drug strategies for the prevention of diabetic retinopathy. The experimental approaches include studies in retinas and retinal microvessels isolated from diabetic patients and animal models of diabetic retinopathy.

The clinical investigation arm of the program aims to build practical bridges to human diabetic retinopathy. One project aims to learn the role of the polyol pathway in the early neuroretinal apoptosis and glial changes induced by diabetes in the rat. Diabetes 2003;52:506-511.

Recent Collaborators
Linda Piter, RN, CCRC
Gilbert Feke, Ph.D
Marco Songini, MD
Maurizio Fossarello, MD

Recent Publications


Aldose reductase in human retinal vessel endothelial cells. In dissociated cell preparations from human retinas immunostained for aldose reductase (green), von Willebrand factor (vWF, red), and nuclei (DAPI, blue) endothelial cells show aldose reductase immunoreactivity overlapping the characteristic perinuclear and granular pattern of vWF in Weibel-Palade bodies. Image: Zeina Dagher, Lorenzi Laboratory
It is now known that various cells and molecules in the eye contribute to the unique ocular microenvironment, which directs the immune response by influencing antigen-presenting cells (APCs). However, the molecular mechanisms that allow eye-derived APCs to bring about such a carefully regulated immune response are not clearly understood. In the current research project successfully employed DNA microarray technology has helped identify genes uniquely expressed (up or down regulated) when conventional APCs are exposed to molecules likely to be found in the ocular environment. This method has allowed a comprehensive analysis of the transcriptional program of cells that represent eye-derived APCs, and has provided information on expression of various genes so far not known to be directly related to anterior chamber associated immune deviation (ACAI). These include thrombospondin (TSP), TNFR2 and IkBa. Determining the significance of each of these genes in inducing the unique ocular immune response has revealed novel immunologic mechanisms utilized by eye-derived APCs. These studies contribute to our understanding of the ocular immune and inflammatory processes and further provide the basis for potential therapeutic strategies to treat ocular inflammatory diseases.

**Molecular Mechanisms Utilized by Ocular Antigen Presenting Cells to Induce ACAID (Immunology)**

Sharmila Masli, Ph.D.

**Current Faculty Research Projects**

**Recent Publications**


**Lacrimal Gland Inflammation and Autoimmune Sjögren’s Syndrome**

Our experiments addressing significance of thrombospondin in the regulation of inflammatory immune responses by eye-derived APCs have attributed an immunoregulatory role to this extracellular matrix protein-TSP. These results have broader implications for the regulation of ocular inflammatory processes by this molecule, as many ocular cells synthesize thrombospondin. The ability of thrombospondin to activate latent TGFβ extends its regulatory potential to the inflammation in the lacrimal gland as TGFβ deficiency in mice results in severe inflammatory infiltrates that lead to ocular surface inflammation and Sjögren’s syndrome. Role of thrombospondin in regulating immune responses to constitutively presented lacrimal gland autoantigens and resulting autoimmune Sjögren’s syndrome is being examined in a collaborative project to develop inducible mouse model of this disease.

**Lacrimal Gland Inflammation and Autoimmune Sjögren’s Syndrome**

**Thrombospondin and Blood-Retina-Barrier**

In the absence of thrombospondin mice immunized with retinal antigen developed severe inflammation in the retina that led to irreversible damage of the tissue highlighting an important role of thrombospondin in the immune privilege in the retina. The role thrombospondin plays in regulating leukocyte recruitment in the retina is being examined to understand the inflammatory responses in the retina.

**Recent Publications**


Model-based Image Enhancement for the Visually Impaired (Psychophysics)

This project is aimed at designing and evaluating practical image-enhancement methods. The first approach is to develop systematic (model-driven) methods for optimal image enhancement for the visually impaired. The second aspect of the investigation is basic research of the perception of contrast by normal and impaired observers. This project led to the development of a valid metric for contrast in complex images, which is now being used in simulation of image appearance and as a tool for image quality metrics. Current efforts are to investigate changes in form perception in the peripheral retina following adaptation to central field loss. The third aspect of the investigation involves the evaluation of developed enhancement technologies in improving the visibility of details from color motion video. These studies will lead to the development of effective image-enhancement devices for the visually impaired.

Recent Publications

Head-Mounted Display as a Low Vision Aid (Psychophysics)

As part of the effort to adapt image enhancement technologies to the visually impaired, we are working on the development of augmented vision systems for use by visually impaired. A newly developed image enhancement technique enables the projection of enhancement outline on the natural view of the environment using see-through, head-mounted display (HMD). An open peripheral design for such a system permits the use of the full peripheral field while the enhancement is limited to the central HMD field. This HMD approach is a significant advance in low vision aids. Studies on the visual effects of HMD in low vision and in normally sighted observers are part of this program. In this direction we have developed (with NASA support) a new stereo display system that is free from the problem of conflict between accommodation and convergence. We are also working with a number of the HMD manufacturers on testing comfort and safety of their new devices.

Recent Publications
Luo G, Rensing N, Weststrate E, Peli E. Registration of an on-axis see-through head-mounted display and camera system. Optical Engineering, 2005;44(2), 024002

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ners to determine the effects on function and on the quality of life.

We are developing and testing both optical and electronic devices that implement three specific engineering approaches aimed at restoring (at least in part) the important interplay of central (high-resolution) and peripheral (wide-field) vision. The three engineering approaches that we are exploring are multiplexing, dynamic control of display, and image enhancement. Also, we show that various combinations of these approaches are possible and likely to be beneficial. In our assessment and testing we emphasize two approaches: a virtual environment for controlled and quantitative testing in the laboratory (virtual reality walk on a treadmill and driving simulator), and on-the-street evaluation for real-life determination of the effect and usefulness of the devices and techniques. Specifically we are developing and evaluating: prismatic correction to assist mobility of patients with hemianopia; prismatic correction to assist mobility of patients with binocular tunnel-vision; a minified augmented viewing system (see-through HMD) to assist mobility of patients with monocular tunnelvision; dynamic control of magnified images to assist patients with central field loss (CFL) view TV; image enhancement of various types to assist patients with CFL view TV; and combinations of both magnification and enhancement to assist patients with CFL view TV.

Recent Publications


Aging is a fundamental process that mediates significant structural and functional changes in the lacrimal glands. One possible consequence of these changes is decreased secretion from the gland, resulting in a clinical condition known as “dry eye”. The mechanisms contributing to this age-associated dysfunction have yet to be defined. We hypothesize that the decreased secretory response of lacrimal glands with aging is caused by increases in oxidative stress. Oxidative stress is considered to be a major cause of aging and many age-related diseases. It describes situations in which the organism’s production of oxidants exceeds the capacity to neutralize them. In this regard, the oxidation and nitration of intracellular proteins and the formation of protein aggregates have been suggested to underlie the loss of cellular function and the reduced ability of senescent animals to withstand physiological stresses. It is, therefore, our objectives to identify the mechanisms leading to age-related deterioration, and determine if experimental manipulation of oxidative stress affects progressive changes in the lacrimal gland. Our current investigation is based on histopathological, biochemical and molecular analyses. We assess changes in oxidative stress by quantifying the progressive accumulation and/or activity of lipofuscin-like inclusions, lipid peroxidation, reduced glutathione, inducible nitric oxide synthase (iNOS), reactive oxygen species (ROS), and stress response signaling pathway in several murine models. We have recently found that changes in the levels of these indicators of cumulative oxidative stress are correlates with intracellular alterations in lacrimal acinar cell responsiveness to autonomic signaling pathways that stimulate protein secretion. This investigation will provide a new insight into the mechanism underlying the reduction in lacrimal gland secretion that occurs with aging, and also to address a clinically relevant topic. Recent Publications


Mechanisms of Injury in Diabetic Endothelial Dysfunction and Atherosclerosis (Diabetes, Cardiovascular Disease)

Vascular complications are a major cause of morbidity and mortality in diabetic individuals. A growing body of evidence indicates that impaired function of endothelial cells (EC), the cell type that lines the vessels, may participate in diabetic vascular disease. EC dysfunction may play a role in the accelerated atherosclerosis observed in diabetic individuals. However, the molecular bases underlying EC injury are poorly characterized in vivo. The goal of this project is to unravel regulatory mechanisms critical for EC injury in the context of diabetic vascular disease. Specifically, we identified several proteins, including profilin-1 (pfn), which may contribute to dysfunction of EC in the course of diabetes. Moreover, we found that pfn gene ablation largely attenuates lesion burden in a mouse model of atherosclerosis.

Our current efforts focus on two specific aspects of pfn-mediated vascular injury: 1) the mechanisms responsible for the anti-atherogenic effects observed in pfn knockout mice, and 2) the transcriptional machinery that drives the abnormal expression of pfn occurring in diabetic vessels and in the presence of oxidized lipids.

Taken together, these studies will clarify the mechanisms for pfn-mediated injury and may translate into efficient novel approaches for early diagnosis and/or management for diabetic vascular disease.

Current Collaborations
Karen S. Moulton, Assistant Professor of Surgery, Children's Hospital, HMS

Recent Publications
Romeo G., Liu W-H, Asnaghi V, Kern TS, Lorenzi M. Activation of nuclear factor-kB induced by diabetes and high glucose regulates a proapoptotic program in retinal pericytes. *Diabetes* 2002;51:2241-2248


Role of Innate Cells in Regulation of Immunity in the Eye, Lung and Gut (Immunology)
The current projects in my laboratory study the cellular and cytokine regulation of local immune responses in the lung and in the eye. It is known that while adaptive immune cells, like T lymphocytes, respond to antigen-specific signals by differentiating into effector cells, innate immune cells, like macrophages, NK and NKT cells, respond immediately by producing cytokines that bias the kind of adaptive immune response that follows. Our particular interest is the role of NKT cells [identified by surface expression of both natural killer (NK) cell markers and T cell receptors] and macrophages called “antigen presenting cells” (APC) in their ability to deviate the differentiation of T lymphocytes from effector cells toward regulatory cells. We study this mechanism in a model of ocular tolerance called “Anterior Chamber Associated Immune Deviation” (ACAID) and a model of gut tolerance called low dose oral tolerance.

Based on the observation that NKT-deficient mice are unable to develop the CD8+ regulatory T cells toward oral tolerance. We study this mechanism in a model of ocular tolerance called “Anterior Chamber Associated Immune Deviation” (ACAID) and a model of gut tolerance called low dose oral tolerance.

Selected Recent Publications


Patent
We have shown that androgens regulate the meibomian gland, which is the primary tissue involved in maintaining tear film stability and preventing tear film evaporation. This finding is also very significant, given that almost no other information exists concerning the physiological control of this tissue, and that meibomian gland dysfunction is the major cause of evaporative dry eye syndromes throughout the world. Based upon our research, we hypothesize that: [a] androgens regulate meibomian gland function, improve the quality and/or quantity of lipids produced by this tissue and promote the formation of the tear film’s lipid layer; and [b] androgen deficiency, such as occurs during menopause, aging, Sjögren’s syndrome, complete androgen insensitivity syndrome and the use of anti-androgen medications, leads to meibomian gland dysfunction, altered lipid profiles in meibomian gland secretions, decreased tear film stability and evaporative dry eye. Our data support these hypotheses, and have significantly increased our understanding of the physiological mechanisms controlling the meibomian gland in both health and disease;

- In collaboration with Drs. Debra A. Schumberg, M. Reza Dana and Julie E. Buring, we have shown that dry eye syndromes occur predominantly in women and that estrogen replacement therapy increases the prevalence of dry eye signs and symptoms in postmenopausal women. This latter finding is extraordinary, given that many millions of women worldwide are prescribed estrogen to alleviate menopausal symptoms and are therefore at heightened risk of developing dry eye. The precise mechanism(s) underlying the sex-related difference in, and the estrogen effect on, dry eye prevalence is unclear. However, we hypothesize that: [a] estrogen deficiency and estrogen use are key factors in the predominance of dry eye syndromes in women; and [b] sex, androgen and estrogen effects are mediated through the regulation of gene expression in the cornea and the lacrimal and meibomian glands. Our research supports these hypotheses, which, if correct, may be translated into new insights into how estrogens influence ocular tissues, and how these hormones may contribute to the etiology of dry eye syndromes.

In summary, our research addresses the sex steroid regulation of the ocular surface and adnexa, as well as the interrelationships between sex, sex steroids and dry eye syndromes. Our studies have involved basic, clinical and epidemiological aspects, and have required the establishment of new and unique experimental approaches (e.g. lipid analytical, proteomic and molecular biological methods). Of particular interest, our research findings have led to the development of a topical androgen treatment that is currently in clinical trials and may serve as a potential therapy for both aqueous-deficient and evaporative dry eye syndromes.

Recent Publications


Mechanisms Responsible for the Prevention of Blinding Inflammation in The Eye (Immunology)

A large part of our research effort has been to characterize the immunosuppressive and immunoregulating factors within immune privilege tissues. Through immunochemical and biological analysis of aqueous humor, the fluid filling the anterior chamber of the eye, we have identified several potent immunoregulating and immunosuppressing neuropeptides that 1) suppress the activation of effector Th1 cells, 2) suppress the activation and the inflammatory activity of macrophages, and 3) mediate the induction of antigen-specific CD25+ CD4+ regulatory T cells. Our research has found constitutively present neuropeptides in the immune privileged eye, including alpha-melanocyte stimulating hormone (α-MSH), vasoactive intestinal peptide, calcitonin gene related peptide, and somatostatin. Collectively, the neuropeptides in aqueous humor suppress activation of delayed type hypersensitivity of adaptive immunity, and endotoxin activation of macrophages in innate immunity. Individually, the neuropeptides target different cells and stages in the induction of an immune response. Also we have preliminary data suggesting a role for the ocular neuropeptides in the regulation of macrophage functionality in the immune privileged eye.

We are finding that within the ocular microenvironment the activation of macrophages to pathogens does not promote inflammation, but promotes suppressor functionality in the macrophages. These suppressor macrophages respond to pathogens without mediating inflammation, or activating T cells. Moreover, the suppressor macrophages produce anti-inflammatory cytokines, suppress and possibly induce apoptosis in activated T cells, and produce enzymes associated with wound repair. We have preliminary evidence that this is mediated by neurotransmitters of the sympathetic nervous system, norepinephrine and neuropeptide Y along with α-MSH and somatostatin. As we continue to examine the mechanisms of ocular immune privilege we further promote the importance of the interactions between the nervous and the immune systems and how we can use these interactions to beneficially manipulate immunity.

Relevant Publications


Therapeutic Approaches for the Treatment of Uveitis and Reestablishment of Immune Tolerance (Immunology)

Based on our current understanding of the neuropeptides mediating ocular immunosuppression, we could impose or reapply these mechanisms onto different tissue sites. One project is based on the hypothesis that the reintroduction of deficient immunosuppressive neuropeptides into eyes with autoimmune disease, will quench inflammation, and return the eye to its normal, immunosuppressive status. We have approached this project using cytokine therapy and as gene therapy. We have started with α-MSH because of its potential to suppress multiple levels of inflammation and to induce regulatory immunity, which is a focus of our research into the molecular mechanisms by which α-MSH through its melanocortin receptors on T cells and macrophages regulates immunity. Both systemic and local injection of α-MSH profoundly suppress the progression of ocular inflammation in the experimental autoimmune uveitis (EAE), and the paralysis of mice in the EAE models of autoimmune disease. In addition, the injections of α-MSH in the EAE model induced the emergence of proteolipid protein (PLP)-specific T cells with characteristics of regulatory T cells, and provided protection from a second induced episode of paralysis. In the EAU model we found also an induced population of ocular autoantigen-specific
Treg cells in the spleen, which were dependent also on the expression of melanocortin receptors. Our gene therapy approach was to inject plasmids encoding the immunosuppressive neuropeptide α-MSH. The plasmids were injected into mouse eyes that started to show symptoms of the EAU. We effectively suppressed the inflammation, minimized tissue damage, and accelerated the recovery of the ocular microenvironment from the autoimmune disease.

**Relevant Publications**


Expression of Arginase1 (green) and Nitric Oxide Synthase2 (red) in A) healthy or B) laser wounded retinas. In the healthy retina all macrophages (CD11b and CD64 positive cells) expressing NOS2 also express Arginase 1, there are non-macrophage cells expressing Arginase1 probably associated with the normal activity of matrix protein production in the retina. In the wounded retina Arginase1 and NOS2 expression is no longer in the same cell. Our results suggest that in the healthy retina there is one population of macrophages, suppressor macrophages; whereas, in the wounded retina there are at least two populations of macrophages, wound repairing macrophages and inflammatory macrophages.

Images: Taylor lab
My laboratory is directed toward repair of the mature diseased central nervous system, specifically the degeneration that occurs in the retina during disease or injury. We have focused on the use of stem or progenitor cells, which we have isolated from a number of regions of the neuraxis of several different mammalian species. During the last 5 years, work in my lab has established that neural stem or progenitor cells overcome the barrier to morphological integration present in the mature mammalian retina. We have also demonstrated that neural stem cells are an inherently immune privileged tissue, and survive in conventional sites in allogeneic recipients. We have also isolated stem cell from the mouse, pig, and human retina, and shown that such cells are capable of photoreceptor differentiation. We have now embarked upon a series of studies in the pig retina, with the goal of establishing functional connectivity between donor retinal stem cells and the mature, diseased host retina. By using large animals such as the domestic pig, we can take advantage of 1) the availability transgenic donor and hosts, so that we can graft GFP + donor cells into RP porcine recipients; 2) the large eye size that allows traditional pars plana vitrectomy and other retinal surgical techniques; and 3) the ability to evaluate the functional impact of the graft with techniques such as multifocal ERG. This approach will allow us to make important steps toward our goal of functional restoration of vision.

Recent Publications


Fluorescent green retinal stem cells express markers of photoreceptors in red (rhodopsin) and blue (recoverin). This suggests that these transplanted retinal stem cells have developed into photoreceptors with all the structures needed to capture light and translate it into chemical signals, which are then transmitted to the brain. *Images Young Laboratory*

MMP-2 expression and activation in rd1 retinas one month after retinal progenitor cell (RPC) transplantation. (a) Up-regulation of pro-MMP-2 immunostaining is seen in radial Müller cell processes (red) following RPC engraftment. Enhanced immunoreactivity was located in regions where RPCs (green) had migrated into the host retina.
Corneal Epithelial Stem Cells and Wound Repair
(Epithelial Cell Biology)
The goals of this laboratory are to understand the mechanisms involved in corneal epithelial wound repair and to determine how the epithelial stem cells are stimulated to participate in the repair process. Corneal epithelial wound healing is a complex process involving the elongation and flattening of cells during migration to cover the wound area and the proliferation of cells to repopulate the wound area. We have shown that the migratory and proliferative processes are compartmentalized, in that epithelial cells distal to the original wound exhibit an enhanced proliferative rate, while cells migrating to cover the wound do not proliferate. The corneal wound model, with its distinct compartmentalization of proliferating and non-proliferating cells, provides an excellent system to examine the basic mechanisms involved in the regulation of cell proliferation. The hypothesis we are testing is that spatial separation of migratory and proliferative responses to wounding (1) is generated through epidermal growth factor receptor activation and transforming growth factor beta mediated upregulation of cell cycle inhibitors, and (2) facilitates epithelial wound closure and restoration of the epithelial barrier. Biochemical and molecular biological techniques, including the generation of TAT-fusion proteins (technology allowing transduction of functional proteins directly into cells), are being used to test the hypothesis. These experiments will result in basic information required for developing therapeutics to be used to enhance impaired healing responses.

Recent Publications


Human Corneal Organotypic Culture (Epithelial Cell Biology)
The goal of this project is to develop an organotypic culture model for the human cornea. In this model, human corneal fibroblasts are cultured in a Vitamin C derivative, which stimulates the cells to grow, stratify, and secrete extracellular matrix materials. The fibroblast matrix serves as a scaffold for the construct. Human corneal epithelial and endothelial cells are cultured on opposite sides of the matrix. The constructs are examined using a variety of techniques including electron microscopy, Quick Freeze/Deep Etch microscopy and confocal microscopy. These experiments are being performed in collaboration with Nancy Joyce, PhD, and Jeff Ruberti, PhD. This model will allow an examination of the differentiation of human corneal epithelial cells that cannot be done in vivo. Potential it may also allow for the development of an artificial cornea.

Recent Publications


Human Corneal Fibroblasts 8 weeks in culture stimulate with Vitamin C. Blue = DAPI, a marker of all cell nuclei. Red = Phalloidin.

*Image: Audrey E.K. Hutcheon, Zieske Laboratory*