RESEARCH &

"Imagination is truly the key to any discovery, and science to cure blindness is no exception.It is the constant reexamination of the facts along with creative leaps of thought that give birth to innovation in every field."

— Michael Young, PhD, Associate Scientist, Schepens Eye Research Institute

DISCOVERY

This Research & Discovery section highlights some of the many scientific contributions made in recent years by the dedicated scientists in the HMS Department of Ophthalmology, whose investigations have resulted in major advancements in medical science and ophthalmic practice. Discoveries made in various fields—including genetics, immunology and ocular biology—have reshaped the foundations of ophthalmology and formed many new paradigms for the repair, regeneration, and rehabilitation of countless disorders.

Today, using a uniquely synergistic approach that combines both laboratory research and applied medicine, this pioneering team continues to advance clinical care for the eye. Fields under intensive study span several areas: physiology, ocular inflammation and immunology, neoangiogenesis optic neuropathies; ocular prostheses; new approaches to drug delivery; laser surgery, and; ocular therapeutics and surgery. Investigations have also expanded to include the specialty fields of genomics, proteomics, gene-gene and gene-environment interactions, gene therapies, and stem cell therapy.

HMS investigators also conduct preclinical investigations using a variety of disease models, and carry out small-scale clinical trials to establish a foundation for "first proof in man." In recent years, this work has led to revolutionary treatments for macular degeneration, including the development of photodynamic therapy as well as a number of anti-angiogenesis agents. Current work focuses on the development of novel pharmaceutical, biological, and gene-therapy approaches for the treatment of blinding diseases.

With ample collaborative dialogue between talented researchers and clinicians, scientific advances are being rapidly transformed into cutting-edge clinical practice.

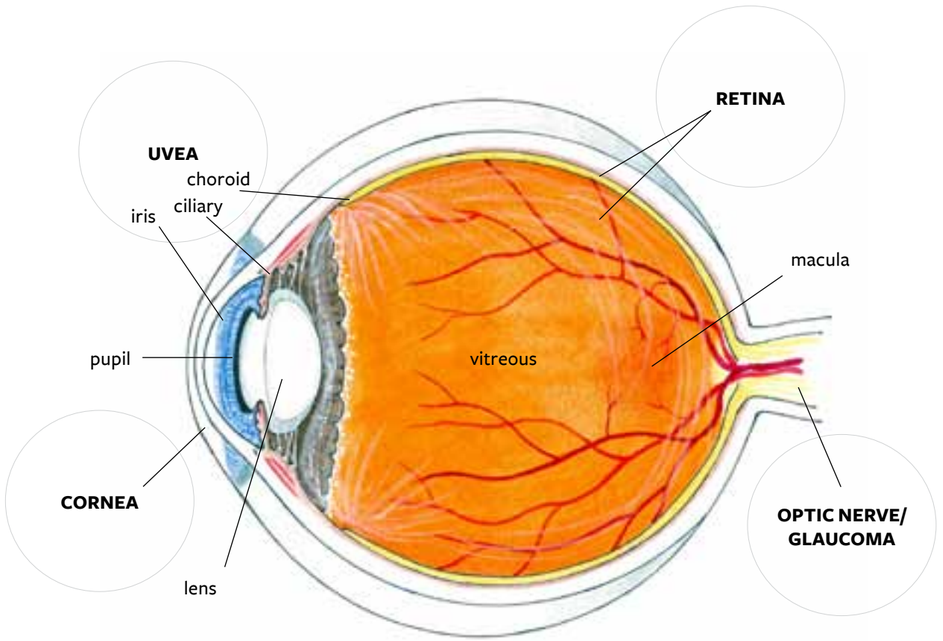
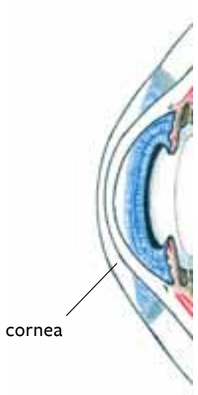


Illustration by Laurel Cook Lhowe

CORNEA

As the eye's most powerful focusing structure, the cornea is essential for acute vision. Injuries, infections, and genetic disorders can rob vision by disrupting normal corneal function. The HMS Department of Ophthalmology houses the world's largest and most esteemed group of scientists and physicians —nearly 80 MDs and PhDs in all—committed not only to understanding corneal biology, but also to treating or preventing corneal disease. With a potent arsenal of tools, technologies, and knowledge, the department is continually applying laboratory discoveries to clinical practice. With increasing success, we're treating or averting the potentially devastating effects of corneal disease, infections and injury.



CORNEAL INFECTIONS

The cornea protects the rest of the eye from injuries and microbial pathogens, such as bacteria, fungi, or viruses; however, because it is constantly exposed, the cornea itself is susceptible to infections that may cause keratitis, or corneal inflammation. Besides causing irritation, pain, and blurry vision, keratitis can damage or scar the cornea, and may lead to permanent vision loss. Several scientists in the HMS Department of Ophthalmology are conducting research and improving treatments for this potentially blinding condition.

Inflammatory responses in epidemic eye infections

Keratoconjunctivitis (commonly known as "pink eye") refers to inflammation of the mucous membranes covering the surface of the eye, including the cornea and conjunctiva. There are many causes of keratoconjunctivitis, including allergens, microbes, and chemicals; however, adenoviral keratoconjunctivitis is particularly contagious, and spreads so rapidly that it commonly causes epidemic keratoconjunctivitis (EKC). Although EKC infections generally resolve on their own, the inflammatory immune responses in the cornea may lead to corneal clouding that may linger for several weeks, months, or even years in severe cases.

James Chodosh, MD, MPH and his team generated the first mouse model of adenoviral keratitis, as well as the first whole genome sequences of EKC-causing adenoviruses. Bioinformatic analyses performed by his group provided evidence for new emergent adenoviral serotypes in EKC. His laboratory also studies how cells called keratocytes in the cornea respond to adenoviral infections, and how signals produced by these cells lead to inflammation. Because the inflammatory response is a well-conserved host defense mechanism against injury or infection, these studies are relevant to various insults to the eye beyond EKC, and may lead to novel, rationally designed therapies for numerous causes of corneal inflammation.

Treating herpes keratopathy with the Boston KPro

Varicella zoster, the virus that causes the common childhood disease known chickenpox, remains dormant in the nerves of most infected individuals. However, the virus may later reactivate and cause a painful skin rash known as herpes zoster or shingles. If the rash affects any part of the eye, it is known as herpes zoster ophthalmicus (HZO). About 10-20 percent of shingles patients develop HZO, which can cause severe corneal damage (keratopathy) and blindness. With approximately 200,000 new cases each year in the United States—and the overall number of herpes zoster cases expected to increase—HZO poses a serious public health concern.

Deborah Langston, MD, FACS, an expert in viral eye disease, recently described a patient who developed corneal ulceration and secondary bacterial and fungal infections due to HZO. Because a standard corneal transplant would have likely failed in this case, the patient received a Boston Keratoprosthesis (KPro), developed by Claes Dohlman, MD, PhD, to successfully replace the severely damaged cornea. Inflammation subsided within a week of surgery, and vision gradually improved over the next four months. This report, published in the February 2008 issue of the journal *Ophthalmology* with Dr. Dohlman as co-author, demonstrates that Boston KPro may restore vision to a great number of patients with otherwise inoperable corneal damage.

The changing landscape of atypical keratitis

Fungi and Acanthamoeba (a genus of protozoa) are relatively uncommon causes of corneal infections, yet both are difficult to treat and can be visually devastating when they occur. Kathryn Colby, MD, PhD, in collaboration with other colleagues within HMS and nationwide, has examined the changing landscape of these atypical pathogens, both at Mass. Eye and Ear, and throughout the United States. These studies demonstrated an increase in both fungal (Jurkunas, Behlau and Colby, June 2009 issue of the journal *Cornea*), and Acanthamoeba (Tanhehco and Colby, *Cornea*, September 2010) infections at Mass. Eye and Ear in recent years, paralleling nationwide trends. In addition, filamentous fungi were found to have replaced yeasts as the predominant pathogens in fungal keratitis at Mass. Eye and Ear. Soft contact lens wear was a major risk factor for developing either infection. By pinpointing the major pathogens and risk factors involved in atypical keratitis, this work may lead to improved prevention and treatment strategies for these potentially blinding infections.

CORNEAL CLARITY

The focusing power of the cornea relies on its clarity. Many conditions—from injuries to infections to dietary or genetic deficiencies—can cause the cornea to lose transparency, and thus its ability to properly refract light. Inflammatory responses to infections, injury, or even corrective surgery can also cause the cornea to become cloudy; moreover, inflammation can induce corneal neovascularization which can also impair vision. Understanding the factors that promote corneal clarity is a major area of study in the HMS Department of Ophthalmology, where scientists have defined many of the molecular and physiological mechanisms that maintain corneal transparency, as well as the pathological processes that cause corneal clouding.

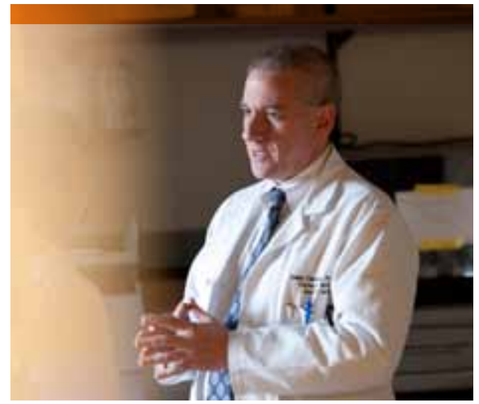
Laying the cornerstone of corneal clarity research

How the corneal matrix maintains its clarity is one of the fundamental questions in ophthalmology, and much of the current understanding of corneal clarity began with the early work of Claes Dohlman, MD, PhD. Using basic science approaches to analyze clinical samples, Dr. Dohlman helped to define the molecular and physiological mechanisms of corneal swelling and edema—major pathological processes that contribute to corneal clouding. These discoveries form the basis of many techniques currently used to restore corneal clarity and visual acuity in patients.

Investigating the mechanisms of corneal clarity

For decades, it was unclear how the cornea maintains its avascular state. To retain clarity, it must prevent the development of blood vessels. In the July 25, 2006 issue of *Proceedings of the National Academy of Sciences* (*PNAS*), a team of researchers led by Reza Dana, MD, MSc, MPH revealed a novel role for vascular endothelial growth factor receptor 3 (VEGFR3) in maintaining corneal avascularity. Prior to this study, scientists believed that only lymphatic vessels and proliferating blood vessels expressed VEGFR3; however, Dr. Dana and colleagues showed that VEGFR3 is also strongly expressed "ectopically" by normal epithelial cells in the cornea, where it acts as a "sink" for factors that induce blood vessel growth in response to inflammation. These findings presented an effective and novel mechanism for suppressing inflammation-induced CNV.

Dr. Dana is also examining the



James Chodosh, MD, MPH

Professor of Ophthalmology, Harvard Medical School

Fellowship Director, Cornea Service, Massachusetts Eye and Ear Infirmary

Dr. James Chodosh, HMS Professor of Ophthalmology and an investigator in the Howe Laboratory Viral Pathogenesis Unit, is internationally known and respected for his work on molecular virology, viral genomics, and viral epidemiology. His laboratory leads the field of ocular adenoviral pathogenesis and epidemic keratoconjunctivitis (EKC), and has contributed greatly to the prevention and treatment of vision loss due to infection, corneal inflammation, and scarring. Dr. Chodosh is also committed to promoting the use of the Boston Keratoprosthesis (KPro) worldwide, and has performed and assisted with artificial cornea implantation surgery in India, Italy, England, and Israel. Recently, he began a project to develop a $50 KPro for use in underprivileged nations. In collaboration with Claes Dohlman, MD, PhD, Dr. Chodosh is studying how to improve keratoprosthesis surgery outcomes by regulating immune responses.

Dr. Chodosh is a committed teacher and mentor, and is Fellowship Director for Mass. Eye and Ear's Cornea Service. He has authored over 110 articles and book chapters, and is a three-time recipient of awards from Research to Prevent Blindness. Having served as Chair for the Anterior Eye Disease NIH Study Section and the Department of Defense's Peer Reviewed Medical Research Program on Eye & Vision, Dr. Chodosh presently serves as a Member of the NIH National Advisory Eye Council.

Deborah P. Langston, MD, FACS

Professor of Ophthalmology, Harvard Medical School

Director of Virology Service, Massachusetts Eye and Ear Infirmary

Dr. Deborah Langston was the first woman to complete ophthalmology residency training at Harvard, and the first female fellow in Dr. Claes Dohlman's corneal fellowship program. She was also among the first to study the efficacy and toxicity profiles of antivirals in animal models, later translating these findings successfully to humans. Her expertise is sought quite prominently in national and international health policy for the treatment of ophthalmic disease, including issues of viral latency, diagnosis, public health and clinical treatment. Dr. Langston is now principally a clinician-educator, focusing on patient care, clinical research, committee work and teaching appointments. Former Chair of the FDA Ophthalmic Drug Advisory Committee, she now serves on the President's Commission on Bioterrorism Preparedness and Response Committee at the Center for Disease Control and Prevention. Dr. Langston's single-authored text, *The Manual of Ocular Diagnosis and Therapy*, comprises six editions and has been published in seven languages.

use of drugs that block blood vessel growth, such as bevacizumab (Avastin), for treating corneal neovascularization. Promising results were obtained in a recent prospective, open-label, noncomparative study using eye drops for the topical delivery of bevacizumab to treat CNV in 10 patients. This study was published in the April 2009 issue of *Archives of Ophthalmology*, with Dr. Dana as senior author. Co-authors included HMS faculty colleagues Pedram Hamrah, MD; Ula Jurkunas, MD; Roberto Pineda II, MD; and Deborah Langston, MD, FACS.

CORNEAL DYSTROPHIES

Corneal dystrophies form a diverse group of conditions that involve gradual deterioration of the cornea. Diseased corneas may become cloudy or abnormally curved, which results in impaired vision. Most corneal dystrophies are inherited; many have no symptoms for decades, and vision loss may vary widely from mild to severe. Researchers in the HMS Department of Ophthalmology are advancing therapeutic strategies for corneal dystrophies by understanding their genetic causes and developing improved treatment and surgical interventions.

Finding molecular clues in Fuchs Endothelial Corneal Dystrophy

In Fuchs' endothelial corneal dystrophy (FECD), the endothelial layer of the cornea deteriorates and eventually leads to corneal swelling and loss of vision. FECD accounts for over 10,000 corneal transplants (roughly one-third of all corneal transplantations) each year in the United States. Corneal transplantation is currently the only modality to treat FECD because the exact cause of endothelial cell degeneration is unknown. Even though FECD is an inherited condition, the genetic defects underlying this common and age-related condition have not been clearly identified.

HMS Assistant Professor Ula Jurkunas, MD, a full-time member of the Cornea Service at Mass. Eye and Ear, is spearheading efforts to understand the complex interaction between the environmental stressors and the genetic factors that, in turn, cause the development of FECD. Dr. Jurkunas is leading a laboratory effort at Schepens Eye Research Institute to evaluate the role of oxidative damage to endothelial cells as an underlying cause of FECD. They found that that reactive oxygen species are involved in the development and progression of FECD, and these novel findings were published November 2010 in the *American Journal of Pathology*. This discovery is significant because understanding the key regulators of oxidative stress-induced cellular damage may facilitate development of pharmacologic treatments for FECD patients.

Collagen cross-linking for keratoconus

The most common corneal dystrophy in the United States is keratoconus, which affects one in every 2,000 people. In keratoconus, corneal thinning causes the cornea to bulge and become uneven, which results in nearsightedness and astigmatism. Vision problems in mild or moderate keratoconus can usually be corrected with hard contact lenses, but patients with advanced keratoconus often need corneal transplantation surgery. Kathryn Colby, MD, PhD, is currently a principal investigator for a clinical trial evaluating the safety and efficacy of collagen cross-linking for preventing the progression of keratoconus. Roberto Pineda II, MD is a co-investigator for the study. This procedure aims to strengthen the cornea by applying riboflavin and ultraviolet light to the corneal surface, which introduces cross-links between the structural collagen strands. Cross-linking therapy is currently being used for the treatment of keratoconus in Europe, and the current studies at Mass. Eye and Ear are conducted in collaboration with the SUNY-Buffalo School of Medicine and the Verdier Eye Center in Grand Rapids, Michigan.

Stem cell therapy for corneal disease

In Fuchs' endothelial corneal dystrophy (FECD), the endothelial layer of the cornea deteriorates and eventually leads to corneal swelling and loss of vision. FECD accounts for over 10,000 corneal transplants (roughly one-third of all corneal transplantations) each year in the United States. Corneal transplantation is currently the only modality to treat FECD because the exact cause of endothelial cell degeneration is unknown. Even though FECD is an inherited condition, the genetic defects underlying this common and age-related condition have not been clearly identified.

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Dr. Jurkunas has received approval from PACT (Production Assistance for Cellular Therapies) for support in the manufacture of cultivated corneal and oral epithelial stem cells for corneal transplantation. Drs. Jurkunas and Dana are collaborating with researchers from Harvard's Immune Disease Institute and the Dana Farber Cancer Institute.

ULA V. JURKUNAS, MD

DRY EYE DISEASE

The triple-layered tear film, which covers the ocular surface, is a critical component of the eye and visual system; any component of the tear film may be disrupted in dry eye disease. Although it rarely leads to severe vision loss or blindness, dry eye disease can lead to extreme discomfort and significant disability. Tens of millions of people have persistent or severe dry eye disease in the United States—making it a major public health concern. In the HMS Department of Ophthalmology, the efforts of several researchers have contributed to the development of new therapies for this widespread and potentially debilitating condition.

Inflammation, immunity, and dry eye disease

For the past decade, the pathology of dry eye disease has been known to involve inflammation and immunity; however, until recently, these disease mechanisms have been

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Kathryn A. Colby, MD, PhD

Assistant Professor of Ophthalmology, Harvard Medical School

Dr. Kathryn Colby is HMS Assistant Professor of Ophthalmology and a corneal specialist at Mass. Eye and Ear and Children's Hospital, Boston. Dr. Colby's research and clinical interests involve advancing new surgical techniques for various corneal diseases. Dr. Colby was one of the first surgeons in Boston to perform selective endothelial transplantation, which replaces only the diseased endothelial cells of the cornea in conditions such as Fuchs' corneal dystrophy; she has been performing different forms of this surgery since 2002. She was the first surgeon in the area to implant the Boston Keratoprosthesis (KPro) in children, and she is currently examining novel therapies for keratoconus. Dr. Colby has been pivotal in optimizing the surgical technique for the implantable miniature telescope for restoring vision in patients with end-stage age-related macular degeneration (AMD). She has the largest ocular surface tumor practice in the New England region, and is currently evaluating the biology of conjunctival melanoma, one of the few ophthalmic conditions capable of causing death.

SPOTLIGHT:

Bridging the Gap Between Research and Clinical Application

AN INTERVIEW WITH

Reza Dana, MD, MPH, MSc

Dr. Reza Dana studies how the immune, lymphatic, and vascular systems interact during ocular inflammatory responses, and how inflammation contributes to transplant rejection, corneal neovascularization (CNV), and other pathological processes in the eye. At HMS, Dr. Dana is Professor of Ophthalmology and holds the Claes H. Dohlman Chair in Ophthalmology. He also serves as Associate Chief of Ophthalmology and Vice Chair for Academic Programs, Senior Scientist and Co-Director of Research at Schepens Eye Research Institute, Principal Investigator for the Harvard Vision Clinical Scientist Development program (K12), and Director of the Cornea and Refractive Surgery Service at Mass. Eye and Ear. With numerous ongoing projects in his laboratory and multiple collaborations with other researchers, Dr. Dana has made substantial contributions to the bodies of knowledge in both basic science and clinical research.

You are Principal Investigator of the Department's Harvard Vision Clinical Scientist Development Program, a federally funded K12 program. Explain what the K12 program is, and how it's contributing to Harvard's translational research in ophthalmology.
RD: We've made a huge effort to recruit clinician scientists to the HMS Department of Ophthalmology K12 program, which is a mentored learning and career-development program funded by the National Eye Institute (NEI) of the National Institutes of Health. It awards 4-year career development grants that provide exceptional junior faculty with financial support, mentorship and 75 percent protected research time to pursue and build independent research careers. The program has numerous benefits: it bridges the translational gap between research and clinical activities, helps us attract and retain the best and brightest talent, and enriches our clinical, teaching and research programs. For trainees, it provides an unparalleled learning and research experience not typically nurtured in an academic research institution. Straight from training, the program helps jumpstart their careers as independent, leading clinician scientists in an amazingly supportive environment.

Our K12 "alumni" have included Pedram Hamrah, Ula Jurkunas and Joseph Ciolino in cornea research, and Lucia Sobrin in retina and uveitis. Our first K12 recipient, Jennifer Sun, is conducting diabetic eye research with Lloyd P. Aiello at the Beetham Eye Institute at Joslin. This program has allowed enormous growth in our translational science program. I'm pleased to say that NEI has approved the Harvard Department of Ophthalmology's five-year grant renewal.

What is "translational research" and how is it carried out among the Harvard Department of Ophthalmology's cornea specialists?
RD: Translational research helps move a basic scientific discovery or idea from the lab to the clinic so patients benefit directly. There's a clinical side and a preclinical side, and both avenues of investigation are carried out by our HMS Ophthalmology affiliates; much of this work is complemented by the extensive preclinical laboratory work at Schepens. Combined, our prodigious team of nearly 80 HMS principal investigators and research fellows represent the world's largest group of scientists dedicated to corneal research, and they are working beyond "collaboration" in the usual sense. We've also maintained longstanding and fruitful collaborations with many of our HMS faculty who maintain private practices in the community—including Dr. Marc Abelson at Ora, Inc., and Drs. Perry Rosenthal and Deborah Jacobs at the Boston Foundation for Sight. Their work has contributed significantly to corneal translational research.

What are some of the latest developments in Cornea's infrastructure at Mass. Eye and Ear Infirmary?
RD: In the last four years, we've doubled the cornea faculty at Mass. Eye and Ear, all of whom are clinician scientists with active research programs. We've developed a corneal research infrastructure with five full-time coordinating managers and research technicians. We've also established a Cornea and Ocular Surface Imaging Center that utilizes an incredible collection of hardware and software geared toward corneal imaging, making it the leading front-of-the-eye imaging center anywhere. We're using these new technologies in cutting-edge clinical care, as well as in clinical and translational research. At the moment, corneal research at Mass. Eye and Ear involves more than 20 investigator-sponsored translational and clinical studies—a growth of more than 400 percent compared to just a handful of years ago.

What are some examples of your most novel translational Cornea research programs and initiatives?
RD: There are several active programs to highlight, including regenerative and stem cell medicine, corneal angiogenesis, corneal inflammation and dry eye, corneal transplantation, corneal imaging, drug delivery and corneal infections, and a large keratoprosthesis program.

For example, we're among the few programs that have applied for several investigational new drug applications (INDs) to the FDA to develop novel therapeutic agents. Corneal angiogenesis and inflammation are major causes of blindness worldwide, so we're looking at new ways of suppressing growth of blood vessels in cornea using topical therapies. We have active and ongoing trials related to this, and some of our findings are now published.

"We study the fundamental biological processes in the lab and define potential therapeutic targets, bringing these findings to the clinic for testing and proof of concept—it's very much a circle."
—Dr. Reza Dana

We've also made great advances in the field of imaging as well. Eight or nine years ago, using preclinical models, we identified a new class of immune cells that are present in the cornea. Now we've taken our research to the clinic. We're currently using precise high-powered confocal imaging instruments to look at the corneas of live patients. This gives us a better sense of the degree of neuropathy and immune cell activation in the cornea. The experience we gained in our earlier lab work has proven invaluable to our understanding of the clinically relevant metrics of imaging.

Another interesting development is in the field of drug delivery. Along with colleagues at MIT, we've developed an innovative design for contact lenses that elute or release drugs, representing a novel venue for sustained drug delivery. This addresses a big problem in ophthalmology, since many people can't use eye drops. Right now, we're looking at preclinical models, and the next stage will be to apply it to clinical practice.

As Director of the Cornea and Refractive Surgery Service at Mass. Eye and Ear, and Co-Director of Research at Schepens, what are your goals for the translational research program?
RD: I've tried to develop a seamless process between the two institutions—lab to clinic and clinic to lab—to understand at a cellular and molecular level what is happening to patients. We study the fundamental biological processes in the lab and define potential therapeutic targets, bringing these findings to the clinic for testing and proof of concept. In many cases, we also procure information from the clinic (for example, cells in fluid such as tears) and analyze these in the lab, so it's very much a circle. We've made significant inroads to bridge the gap between research and clinical application.

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poorly defined. In the past few years, studies led by HMS Professor Reza Dana, MD, MPH, MSc showed that autoimmune processes in dry eye result from dysregulation of certain immune cells, including regulatory T cells (Tregs) and pathogenic effector T cells. Dr. Dana and colleagues recently identified a previously unknown pathogenic T cell subset, Th17, which is associated specifically with Treg dysfunction in dry eye disease. Th17 thus represents a new therapeutic target for dry eye disease. Recent studies led by Dr. Dana have further elucidated the mechanisms underlying corneal inflammation in dry eye disease, and have identified novel therapies and dosing regimens, such as high-frequency topical cyclosporine, a blockade of specific pro-inflammatory cytokines, for this extremely prevalent condition.

Sex, steroids, and dry eye disease

David Sullivan, PhD is one of the leading ocular surface scientists in the world. Dr. Sullivan discovered that gender and sex steroid hormones are critical factors in the regulation of ocular surface tissues, as well as in the pathogenesis of dry eye disease. This disorder, which occurs predominantly in women, afflicts an estimated 30 million people in the United States alone. Dr. Sullivan has also discovered that androgens may suppress aqueous-deficient and/or evaporative dry eye, whereas that estrogens may promote the conditions. These discoveries were termed in a Castroviejo Lecture as "the most exciting development in recent years" in dry eye research. Most recently, Dr. Sullivan and colleagues have discovered boundary lubrication at the ocular surface, which may be a critical factor protecting the cornea against damaging shear forces in dry eye. Dr. Sullivan's unique and novel research findings have led to the development of various topical therapies, which may potentially treat both aqueous-deficient and evaporative dry eye disease.


DAVID A. SULLIVAN, PHD

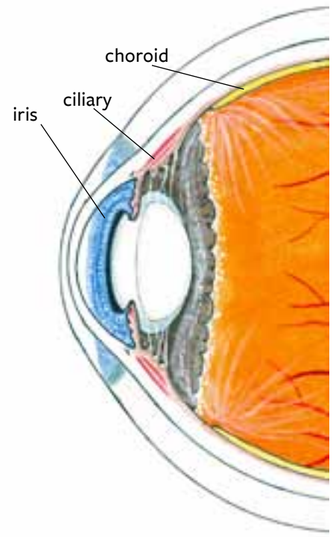

ILENE K. GIPSON, PHD

The multiple roles of mucin molecules

The ocular surface contains two types of mucins, which help protect and lubricate the cornea by holding the tear film in place. These hydrophilic molecules are either secreted into the tear film by the conjunctival goblet cells, or emanate from the membranes of the cornea and conjunctival epithelium, forming a lawn-like protective barrier on the corneal/ocular surface. In 2004, Ilene Gipson, PhD, demonstrated that membrane-tethered mucins are disrupted in both Sjögren's (autoimmune) and non-Sjögren's forms of dry eye disease. Using ocular-surface epithelial cell-culture systems that she developed, Dr. Gipson is currently identifying additional factors that regulate mucin expression, and is elucidating their roles in ocular surface biology, infectious disease, and human reproduction. Recently, researchers in Dr. Gipson's laboratory showed that pro-inflammatory molecules, particularly interferon-gamma, can alter mucin expression at the gene and protein levels, thus providing a link between inflammation and mucin behavior in dry eye syndrome. These findings were reported March 2010 in the journal *Experimental Eye Research*.

Mucins not only help keep the cornea moist, but may also form a protective barrier against bacterial infections at the ocular surface. In collaboration with Michael Gilmore, PhD, Dr. Gipson showed that MUC16 prevents the bacterium *Staphylococcus aureus* from binding corneal cells. MUC16 suppression resulted in loss of barrier function, thus allowing bacteria to bind more efficiently. These findings were reported October 2007 in the journal *Investigative Ophthalmology and Visual Science*. In a subsequent study published in the November 2008 issue of *Infection and Immunity*, Schepens Assistant Scientist Pablo Argüeso, PhD, in collaboration with Dr. Gilmore, showed that the barrier function of MUC16 was dependent on chains of carbohydrate molecules called O-glycans. These findings suggest that new strategies for preventing bacterial infections could center on improving mucin function.

UVEA



The uvea refers to the structures (iris, ciliary body and choroid) that form the middle, pigmented layer of the eye. Because uveal tissues contain many blood vessels, they are susceptible to inflammation and other immune responses from a variety of eye disorders. Inflammation of the uvea, or uveitis, can be caused by many conditions, including injuries, infections, autoimmune disorders, and systemic inflammatory diseases. If left untreated, uveitis can lead to other conditions—such as glaucoma, macular edema, and cataract—that may result in profound vision loss.

The HMS Department of Ophthalmology offers a unique combination of scientific and clinical expertise in ocular inflammatory disorders like uveitis. Multiple clinics within Mass. Eye and Ear and Massachusetts General Hospital (MGH) form the Ocular Immunology and Uveitis Service, which is one of the busiest uveitis services in the country. Outfitted with state-of-the-art diagnostic and examination equipment, the Ocular Immunology and Uveitis Service is establishing a patient information database that will allow case series, epidemiologic studies, genetic analyses, and assessments of treatment efficacy and clinical outcomes for uveitis. These combined efforts have optimized existing treatments for uveitis, and have yielded potential novel therapeutic strategies for protecting eyesight in ocular inflammatory disorders.

Expanding treatment options for ocular inflammation

Uveitis is usually treated with corticosteroids, which themselves can have serious side effects (including cataract and glaucoma). Immune-suppressing drugs, such as cyclosporine and mycophenolate mofetil, may also be used to treat some forms of uveitis; however, vision loss can still occur despite treatment with standard immunosuppressants. Thus, Dr. Sobrin and other clinician-scientists in the HMS Department of Ophthalmology are working to expand the therapeutic options for uveitis. Daclizumab and infliximab, which are immune-modulating drugs that specifically target inflammatory cytokines, have been used to effectively treat some forms of uveitis that were resistant to traditional immunosuppressant therapies. Bevacizumab (Avastin®), a prominent anti-angiogenic drug, has shown success in treating cystoid macular edema (CME) caused by posterior uveitis. Intravitreal injections of clindamycin represent a novel use of this antibiotic for treating uveitis caused by toxoplasmosis infections. Case studies conducted by Dr. Sobrin and colleagues serve as evidence-based decision support tools for uveitis, particularly in cases that are resistant to standard therapies.

A novel non-invasive treatment for anterior uveitis

Anterior uveitis, which affects the front of the eye, can cause swelling of the iris (iritis), and the painful condition known as "redeye." Like other forms of uveitis, anterior uveitis is usually treated with corticosteroids via eye drops, local injections, or systemic delivery. A potential non-invasive treatment option is the ActiPatch® device, which is based on pulsed electromagnetic field (PEMF) technology.

By emitting a low-frequency electromagnetic field, the ActiPatch® device is thought to restore the tight junctions between endothelial cells, which may in turn minimize inflammation within the eye. In an ongoing randomized, double blinded, placebo-controlled trial led by George Papaliodis, MD, patients with anterior uveitis will wear the ActiPatch® (or a placebo device) over the affected eye for eight hours per day. Inflammation, redness, and pain will then be assessed after a one-week course of treatment. This prospective trial is expected to be complete by December 2011, and may lead to larger clinical trials of PEMF therapy for uveitis. The PEMF device has great potential to reduce the dose or duration of corticosteroid treatment—thus representing a safer adjunct or alternative to standard drug therapy.

Keratoprosthesis and autoimmune disease

The Boston Keratoprosthesis (KPro) artificial cornea, developed by Claes Dohlman, MD, PhD at Mass. Eye and Ear, is highly successful in most patients—even those with failed corneal allografts. However, in patients with autoimmune disorders, current surgical treatments—including the Boston KPro—remain marred by complications and prosthetic failure. In this subset of patients, ocular inflammation and neovascularization are common concerns; the tissues adjacent to the prosthesis may also break down, which can lead to prosthetic failure.

At Mass. Eye and Ear, these issues in keratoprothesis implantation were highlighted by two recent cases of corneal blindness secondary to autoimmune disease. The team of C. Stephen Foster, MD; Jessica Ciralsky, MD; George Papaliodis, MD; Claes Dohlman, MD, PhD; and James Chodosh, MD, MPH, reviewed these cases to better understand the underlying mechanisms of keratoprosthesis failure in autoimmune patients. Because prosthetic dental and orthopedic implants have been successful in autoimmune patients, this team of clinician scientists looked to previous reports of prosthetic complications to find unifying mechanisms of prosthetic failure.

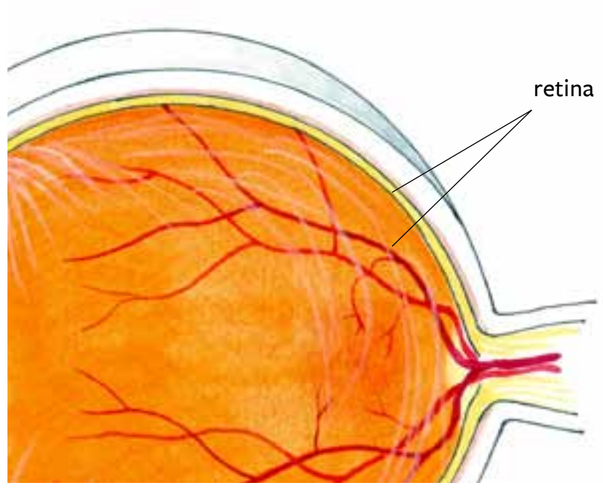
Upon systematically reviewing the published literature, the team found that prosthetic failure is clearly linked to inflammation and immune activation in autoimmune patients. Keratoprosthetic materials, such as polymethylmethacrylate (PMMA) and titanium, generally have low potential for inducing immune responses; however, the available literature suggested that in autoimmune patients, these materials themselves might stimulate inflammatory cascades that may damage recipient tissues. These findings called for further study of keratoprosthesis materials and the underlying mechanisms of autoimmunity and inflammation. Because more and more treatment options are becoming available for autoimmune diseases, ongoing efforts in the HMS Department of Ophthalmology will produce new, targeted approaches to restoring vision in autoimmune patients.

GEORGE N. PAPALIODIS, MD

RETINA

The retina, which lines the inside of the back of the eye, contains highly specialized cells that convert visual images into electrical signals. The retina then transmits the signals to the brain via the optic nerve. Genes, lifestyle, and age-related factors can all affect the retina, which is susceptible to numerous disorders. In the United States, the leading causes of adult blindness—age-related macular degeneration (AMD) and diabetic retinopathy—are diseases of the retina.

For patients with retinal disease, vision loss may be slowed or even reversed with therapies pioneered by the members of the HMS Department of Ophthalmology. Today, translational research across the HMS campus continues at an intense pace. As scientific discovery is continuously translated into clinical practice, innovative sight-saving treatments are breaking ground in vision care.



BACTERIAL INFECTIONS

Ending retinal damage in bacterial endophthalmitis

Endophthalmitis is a form of uveitis that affects much of the eye. It can rapidly damage the retina and cause loss of vision—or even loss of the entire eye. Despite advances in antibiotic therapy and ophthalmic care, infectious endophthalmitis—often caused by bacteria—remains a serious complication of eye surgeries or injuries. Although bacterial toxins cause much of the damage in this condition, the host immune responses that clear the pathogens may inadvertently damage normal host tissues.

Michael Gilmore, PhD, pioneered studies on why some causes of infectious endophthalmitis do not respond to antibiotic treatment. His lab found that if a bacterium causing endophthalmitis is producing a specific toxin, killing the bacteria with antibiotics and treating the inflammation does not limit the damage to the retina. However, if the bacteria were rendered incapable of producing the toxin, antibiotics and anti-inflammatory agents prevented this damage. His laboratory is using this scientific knowledge, as well as studies of the bacterial genome, to design new treatments to spare the retina. His studies are also identifying host components that are important for immune protection as well as damage in these infections. Unexpectedly, his lab found that a component called Fas ligand is important for activating immune cells and protecting the eye, whereas another factor in the complement system (part of the innate immune system) had little effect. Recently, he and collaborators Bruce Ksander, PhD, and Meredith Gregory-Ksander, PhD, found that the retinal protein alphaB-crystallin protects against retina cell death while the immune system clears bacteria.

Michael S. Gilmore, PhD

Sir William Osler Professor of Ophthalmology, Harvard Medical School

Member, Biological and Biomedical Sciences Program, Harvard Medical School

Member, Microbial Sciences Initiative, Harvard University

Dr. Michael Gilmore, former President and Director of Research at Schepens Eye Research Institute,and first incumbent of the Sir William Osler Professor of Ophthalmology at HMS, , joined the Howe Laboratory at Mass. Eye and Ear in July 2010 to further the development of new treatments for bacterial infection. Eye infections are potentially blinding complications of injury and surgery, and many of the causes are resistant to antibiotics. As Principal Investigator of the NIH-sponsored interdisciplinary Harvard-wide Program on Antibiotic Resistance, Dr. Gilmore is promoting collaborations between HMS, affiliate hospitals, the Broad Institute, and the pharmaceutical industry. This collaboration is identifying and validating new compounds for treating multidrug resistant staphylococcal infection, and studies of bacterial genomes are identifying new therapeutic targets.

Dr. Gilmore received his PhD in biochemistry and molecular biology, as the Colin MacLeod Fellow, from the University of Oklahoma Health Sciences Center (OUHSC). After postdoctoral training at the University of Wuerzburg in Germany and at the University of Michigan, he returned to the OUHSC to join the faculty in 1984. There he rose through the ranks in the Department of Microbiology and Immunology, and the Department of Ophthalmology, to hold the titles of George Lynn Cross Research Professor in the College of Medicine, and MG McCool Professor of Ophthalmology. From 2000–2004 he also served as OUHSC Vice President for Research. At HMS, Dr. Gilmore is an affiliate of the HMS department of Microbiology and Molecular Genetics, and is a member of the Biological and Biomedical Sciences Program. He serves on the steering committees of the Harvard Microbial Sciences Initiative, and the Broad Institute of MIT and Harvard Infectious Disease Initiative. His numerous honors include a Fogarty Senior International Fellowship at Cambridge University, an Alexander von Humboldt Fellowship, a VH Honeymon Distinguished Lecturership, and the OUHSC Regents Award for Distinguished Research.

Continuing his studies on dangerous multidrug-resistant infections that plague patients following surgery or associated with injuries, Dr. Gilmore and his former trainees Janet Manson, PhD, and Lynn Hancock, PhD, discovered a mechanism used by harmless gastrointestinal microorganisms to acquire multidrug resistance. They found that plasmids—circular pieces of DNA that replicate independently of the bacteria's chromosomal DNA—facilitated the transfer of virulence and antibiotic-resistance genes from one bacterium to another. Infections from multidrug-resistant Enterococcus are leading complications of surgeries, ranging from cataract extractions to knee replacements. Understanding the origins of these strains will help guide the judicious and effective use of antibiotics, and the development of new treatments. This groundbreaking article, published in the *Proceedings of the National Academy of Science* in 2010, was rated by Faculty of 1000 as being in the top 2% of published articles in biology and medicine.

BIOLOGY

The retinal atlas project

Specialized neurons called rods and cones are the primary light-sensing cells in the retina. These photoreceptors trigger a cascade of reactions through a complex cellular network, which recodes images into electrical impulses. Major research efforts are focused on defining the cellular events that process visual images.

Richard H. Masland, PhD, Director of the Howe Laboratory at Mass. Eye and Ear, has devoted his research career to mapping the retinal atlas, which involves identifying the cell types in the retina's complex neuronal network. The goal of this effort is to understand the fundamental mechanisms of vision, which may reveal new strategies for preventing or reversing vision loss. With contributions from several research groups worldwide, the retinal atlas is now virtually complete. This endeavor has identified approximately 60 distinct retinal cell types that are capable of an even greater number of intracellular connections.

Besides deciphering the fundamental biology of vision, the retinal atlas project may also reveal the underlying mechanisms of degenerative retinal disorders. This work, according to Dr. Masland, "extends beyond our studies in the retina, and underpins our efforts in the Howe Laboratory to treat various diseases of the eye." Now, with the detailed map of cellular architecture of the retina in hand, Dr. Masland and other Howe Laboratory researchers anticipate novel treatment strategies like directed gene and stem cell therapies. In the Howe Laboratory, researchers are currently refining preclinical discoveries for clinical evaluation. The primary goal of this ongoing translational research is to restore vision in degenerative retinal disease. "Curing blindness," says Dr. Masland, "would be the ultimate payoff for our years of research in fundamental cell biology."

Retinal patterning

Cellular diversity is not the only hallmark of retinal complexity; the cells of the retina are also arranged in defined patterns that are critical for vision. Interestingly, though retinal cell types may differ widely, many are derived from the same progenitor cells. Through various genetic, molecular, environmental, and hormonal events, the retinal progenitors produce specific cell types in specific positions in the retinal landscape.

Connie Cepko, PhD, has uncovered many mechanisms that determine the ultimate fate of retinal progenitor cells. Her laboratory has developed various lineage marking techniques, which demonstrated how progenitor cells in the retina can give rise to different cell types (such as neuronal and glial cells). These studies helped decipher the complex mechanisms of retinal cell fate determination.

Dr. Cepko's team has also uncovered many genetic factors that control various features of eye development. Her laboratory has identified many gene expression patterns within the developing eye which direct the organization of the retina and other eye structures. Using techniques to introduce gene reporters into cells (such as viral vectors and electroporation) and expression profiling techniques (such as microarrays), Dr. Cepko's laboratory is working to decipher the formation of the retinal cell types, as well as their complex circuits. They have also identified chemical factors (such as retinoic acid) and hormonal factors (such as thyroid hormone) that may help determine the spatial layout of the retina, including the formation of the macula. These studies, combined with new advances in gene therapy and stem cell technology, may lead to novel strategies for treating retinal degenerative diseases.

Rescuing photoreceptors in retinal degenerative disorders

Because genes that control development are often disrupted in disease, Dr. Cepko's research in retinal development has contributed greatly to the understanding of retinal degenerative disorders. Her laboratory mapped developmental gene expression patterns that are now used to model the molecular events that cause retinal degeneration. Having discovered several genetic factors that contribute to photoreceptor cell death, Dr. Cepko is now using gene delivery methods to help "rescue" dying rods and cones in retinal degenerative disorders. In a January 2009 report in the journal *Science*, Dr. Cepko and postdoc-

Richard H. Masland, PhD

David Glendenning Cogan Professor of Ophthalmology and Professor of Neurobiology, Harvard Medical School

Director of the Howe Laboratory of Ophthalmology and Associate Chief of Ophthalmology Research, Massachusetts Eye and Ear Infirmary

Dr. Richard Masland is an accomplished scientist in basic and translational research in the retina. He completed his undergraduate studies at Harvard College, received his PhD from McGill University, and conducted postdoctoral work at Stanford University and Harvard Medical School. Dr. Masland was an Investigator of the Howard Hughes Medical Institute from 1993–2006, and joined Mass. Eye and Ear in 2009 as Associate Chief for Ophthalmology Research and the Director of the Howe Laboratory, which houses much of the research in eye development and disease at Mass. Eye and Ear.

Dr. Masland's laboratory focuses on the neuronal diversity of the retina, its cellular interactions, and the complex photoreceptor microcircuitry that recodes visual input. Because the retina is a readily assessable extension of the central nervous system, the findings of Dr. Masland and colleagues are applicable to other neuronal processes. His ambitious and collaborative retinal atlas project is fundamental to the understanding of retinal disease, and has opened up new avenues of investigation and potential therapies for a host of degenerative retinal disorders.

For his outstanding contributions to the field of ophthalmology, Dr. Masland received the 2010 Proctor Medal, which is the highest honor bestowed by the Association for Research in Vision and Ophthalmology (ARVO). A former Howard Hughes investigator, Dr. Masland has also received Brian Boycott Prize for his retinal research. His honors for excellence in teaching include the Hoopes Prize and the Irving M. London award.

Constance L. Cepko, PhD

Professor of Genetics and Ophthalmology, Harvard Medical School

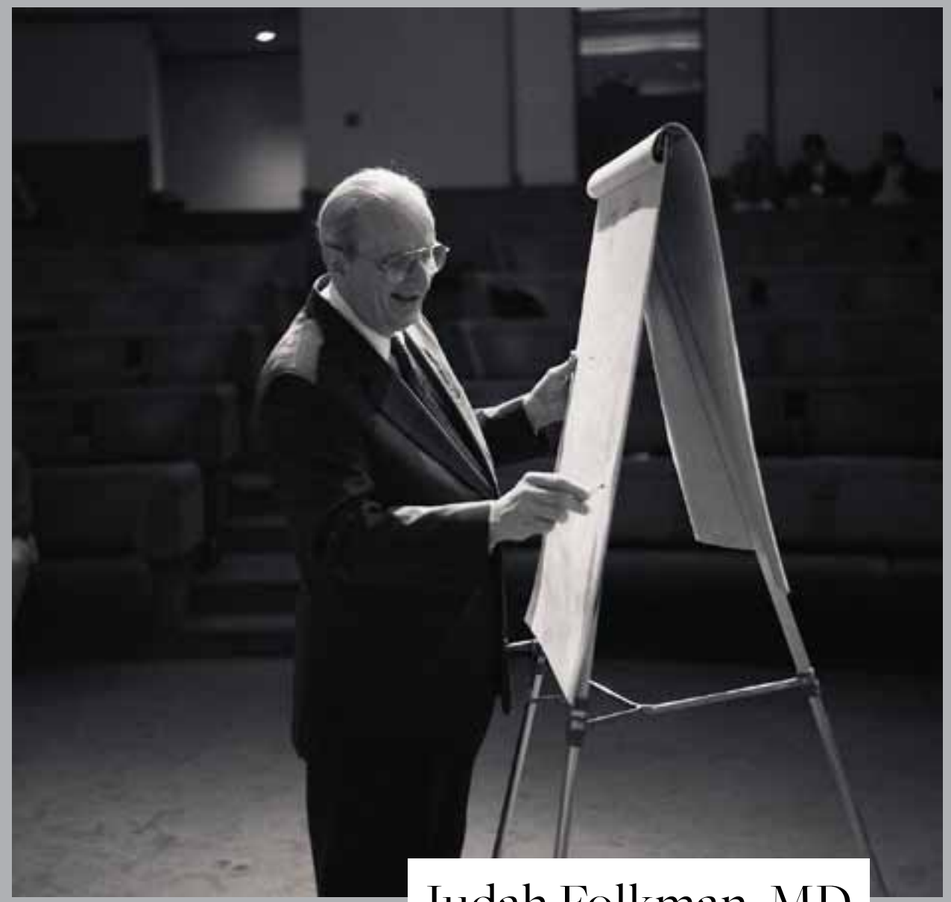
Investigator, Howard Hughes Medical Institute

Dr. Constance Cepko's scientific career began with a seventh-grade science fair project in microbiology; this led to a weekend research internship that continued through high school, followed by baccalaureate studies in biochemistry and microbiology at the University of Maryland. At MIT, under the direction of Phillip Sharp, PhD, Dr. Cepko conducted her doctoral research on adenoviral proteins. As a postdoctoral fellow in the laboratory of Richard Mulligan at MIT, Dr. Cepko helped pioneer the use of retroviruses to express transgenes in cells.

Recognizing the utility of retroviral vectors in developmental biology, Dr. Cepko used them to study retinal development when she became an independent investigator at HMS in 1985. Besides significantly advancing the basic science underlying retinal development, Dr. Cepko has applied the tools of genetics and molecular cell biology to understanding the basis of retinal disease.

Dr. Cepko has received numerous honors for vision research, including the David Cogan Outstanding Young Investigator Award in Vision Research, the Alcon Institute Research Award for Vision, and the Bressler Prize for Vision from the Jewish Guild for the Blind. Dr. Cepko has been an Investigator of the Howard Hughes Medical Institute since 1994; she was inducted into the American Academy of Arts and Sciences in 1999 and the National Academy of Sciences in 2002. She is a member of the Society for Neuroscience and the Association for Research in Vision and Ophthalmology.

toral fellow Bo Chen, PharmD, PhD, showed that histone deacetylase 4 (HDAC4), a nuclear co-repressor that regulates bone and muscle development, also regulates survival of rod photoreceptors. Because rods are the primary cells that are lost in retinitis pigmentosa, a progressive retinal degenerative disorder, this discovery is highly relevant to retinal disease. In a mouse model of retinitis pigmentosa, Drs. Cepko and Chen used electroporation to deliver HDAC4 DNA into the retina. They found that high HDAC4 expression in the mouse retina could rescue dying rod photoreceptors. This effect was due in part to the activity of hypoxia-inducible factor 1alpha (HIF1), an oxygen-sensitive transcription factor that regulates many genes involved in cell survival and function. Dr. Cepko's laboratory is currently studying how HDAC4 promotes rod survival, and is now developing gene delivery techniques that may someday be used to treat retinal degenerative disorders in humans.



Judah Folkman, MD

February 24, 1933 – January 14, 2008

The Father of Angiogenesis

As a young navy doctor in 1961, Dr. Judah Folkman noticed that tumors needed blood vessels to grow. Ten years later, Folkman published a controversial theory that is now widely accepted: targeting angiogenesis may potentially arrest cancer. Although others described tumor angiogenesis as early as 1945, Dr. Folkman's paramount achievements formed the foundation of antiangiogenic therapy, and he is unequivocally considered the "Father of Angiogenesis." By the time Dr. Folkman passed away, an estimated 1.2 million people had received antiangiogenic treatments. His scientific legacy endures through the HMSARG scientists who were mentored or otherwise influenced by this visionary of translational research.

ANGIOGENESIS

Because blood vessels play important roles in many human diseases (including cancer), they have become a major focus of translational research and drug development. Realizing that blood vessels are central to many blinding retinal diseases, scientists and clinicians of the Harvard Medical School Angiogenesis Research Group (HMSARG)[1] have focused intense scrutiny on the underlying mechanisms of blood vessel development. HMSARG researchers have individually and collaboratively discovered many mechanistic features of vascular biology, thus forming new paradigms for diseases that involve neovascularization (the abnormal formation of new blood vessels) or angiogenesis (the growth of existing vessels). Efforts of the HMSARG have translated groundbreaking scientific discoveries into innovative treatments for millions of patients with vascular disease.

Vascular endothelial growth factor (VEGF)

Antiangiogenic therapy, first advocated by Judah Folkman, MD, in 1971, targets factors in the body that regulate blood vessel growth. One molecule, first discovered in 1983, was named vascular permeability factor (VPF) for its ability to make blood vessels leaky. In 1989, researchers realized that VPF could also make blood vessels grow. Unlike other angiogenic factors that stimulate the growth of many cell types, VPF's potent effects were very specific for vascular endothelial cells. Moreover, VPF can be secreted into the bloodstream; thus, it can have effects distant from the cells of origin. Because of its specific and far-reaching effects on blood vessels, VPF was renamed vascular endothelial growth factor (VEGF), and is the primary target of current antiangiogenic therapies.

PATRICIA A. D'AMORE, PHD, MBA

The genetics of angiogenesis

An individual's genetic makeup can greatly affect many biological processes, including angiogenesis. Prompted by the fact that African-Americans rarely develop neovascular AMD or hemangiomas (benign tumors composed of endothelial cells), Robert D'Amato, MD, PhD, set out to identify genetic factors that modify the angiogenic response. In 2000, he demonstrated that genetic variations among different strains of mice may lead to differences in angiogenic response. In 2004, Dr. D'Amato mapped genetic regions that control the angiogenic response to basic fibroblast growth factor (bFGF) in mice. More recently, Dr. D'Amato's team mapped genetic regions that control the degree of choroidal neovascularization induced by laser injury. This study, reported in the July 2009 issue of *The FASEB Journal*, identified several candidate genes that may regulate angiogenesis, which presents new targets for antiangiogenic therapies. These findings may also lead to new screening tests that determine disease risk, as well as methods to predict a patient's response to specific treatments.

The benevolent side of VEGF

With the advent of antiangiogenic therapies for cancer and other vascular disorders, VEGF is often viewed in an unfavorable light. However, VEGF also has numerous important physiological roles that HMS scientists have helped to define. For example, in 1999, Ivana Kim, MD, and colleagues demonstrated that the genes for VEGF and two VEGF receptors were constantly expressed in normal eyes, indicating that these genes play active roles in normal eye physiology. While working as an investigator with Patricia D'Amore, PhD, MBA, Magali Saint-Geniez, PhD, showed that the normal adult retinal pigment epithelium (RPE) expresses VEGF abundantly. This suggested that VEGF is a survival factor for non-proliferating choroidal vessels. Dr. Saint-Geniez subsequently showed that endogenous VEGF is critical for lens development, and may even have neuroprotective effects on photoreceptors. Dr. Saint-Geniez demonstrated that abnormal VEGF expression in the RPE resulted in degeneration of the choroidal vessels, Bruch's membrane, and the RPE itself. This study was published November 2009 in *Proceedings of the National Academy of Sciences*, and was featured in the February 2010 issue of *EyeNet*, the electronic journal of the American Academy of Ophthalmology. These studies highlight the importance of endogenous VEGF in normal eye health, and emphasize the need for strategies that selectively target pathological VEGF activity without disrupting normal VEGF function when using antiangiogenic therapies.

MAGALI SAINT-GENIEZ, PHD

[1] Original group of nine HMS angiogenesis researchers who conducted pioneering bench and translational research to elucidate the role of angiogenesis in blinding ocular diseases, and subsequently developed revolutionary clinical treatments. Much of their work was initiated under the mentorship of Judah Folkman, MD. HMSARG researchers include: Anthony Adamis, MD; Lloyd P. Aiello, MD, PhD; Robert D'Amato, MD; Patricia D'Amore, PhD, MBA; Evangelos Gragoudas, MD; George King, MD, Joan Miller, MD; David Shima, PhD, and Lois Smith, MD, PhD.

Robert J. D'Amato, MD, PhD

Professor of Ophthalmology, Harvard Medical School

Judah Folkman Chair in Surgery, Children's Hospital Boston

Director, Center for Macular Degeneration Research, Children's Hospital Boston

As one of Judah Folkman's scientific trainees, Dr. Robert D'Amato has made many notable contributions to the field of angiogenesis. In 1994, as a postdoctoral fellow in the Folkman laboratory, Dr. D'Amato demonstrated the potent antiangiogenic properties of thalidomide, a sedative drug that was withdrawn in 1961 due to its devastating side effects. This discovery explained the drug's toxicity and potential to cause birth defects, and led to its current FDA-approved use in treating multiple myeloma. Dr. D'Amato completed a residency in Ophthalmology at Mass. Eye and Ear before joining Dr. Folkman's laboratory at Children's Hospital Boston, where he has been an independent investigator since 1994. He has since characterized lenalidomide (Revlimid®), an analog of thalidomide, which was approved in 2006 for treating myeloma; he also identified another potent analog Actimid®, which is currently in Phase II clinical trials.

Dr. D'Amato currently serves as Director of the Center for Macular Degeneration Research at Children's Hospital Boston, holds the Judah Folkman Chair in Surgery, and is Professor of Ophthalmology at HMS. He has studied VEGF regulation and ocular angiogenesis both independently and in collaboration with other HMSARG investigators. With a continued interest in developing new therapies for vascular disorders, Dr. D'Amato has characterized several antiangiogenic compounds in recent years. These include polymeric TNP-470 (a derivative of a fungal compound), 2-methoxyestradiol, various non-steroidal anti-inflammatory drugs (NSAIDs), and a modified form of the anthrax toxin. His recent studies demonstrated a potentially broader-spectrum antiangiogenic role for a TNP-470 polymer, Lodamin, in animal models where treatment resulted in regression of established choroidal neovascularization (CNV) lesions and a reduction of inflammatory cytokines. Dr. D'Amato envisions broader-spectrum agents such as Lodamin to have the potential to suppress a greater number of pathological disease processes, including those in AMD and cancer. Dr. D'Amato's current research focuses on the genetic factors that control angiogenesis as well as continuing work on antiangiogenic agents.

HMS ANGIOGENESIS RESEARCH Fuels a Revolution in Retinal Care

1971

Judah Folkman and colleagues at Children's Hospital and Harvard Medical School describe the isolation of a "tumor angiogenesis factor" (TAF) in the February issue of *Journal of Experimental Medicine.*

Dr. Folkman later publishes his seminal theory of tumor angiogenesis in the November issue of *New England Journal of Medicine.*

1989

George King demonstrates how protein kinase C, activated by elevated glucose levels, contributes to retinopathy and other vascular complications in diabetes. This mechanistic model of diabetic retinopathy is published in the July issue of *Proceeding of the National Academy of Sciences.*

1995

Based on the work of Joan Miller and Evangelos Gragoudas, the first AMD patient in the world is treated with Visudyne®.

1995–1997

Work from the HMSARG intensifies, and several studies led by Lloyd P. Aiello, George King, Lois Smith, Evangelos Gragoudas, Robert D'Amato, Patricia D'Amore, and Joan Miller further implicate VEGF and other angiogenic factors in vascular eye disorders. The investigators help define the role of hypoxia in regulating VEGF, and show how angiogenic inhibitors may block ocular neovascularization. These studies provide the scientific foundation for using anti-angiogenic therapies for vascular disorders of the eye.

1998

A phase IA clinical trial for pegatanib (Macugen®), a VEGF inhibitor, begins in AMD patients .

1999

Photodynamic therapy (PDT) with verteporfin (Visudyne®) shows efficacy in treating wet AMD in Phase I/II clinical studies. Joan Miller and Evangelos Gragoudas are lead investigators of these studies.

2002

The phase IA clinical trial for Macugen® shows promise for treating AMD. The results of this study are reported in the April issue of the journal *Retina.*

In studies led by Evangelos Gragoudas and Joan Miller using primate animal models, choroidal neovascularization is prevented by intravitreal injection of an anti-VEGF antibody fragment. The lead author, Magdalena G. Krzystolik, publishes the study in the *Archives of Opthalmology.*

2005–2006

In a series of studies, Evangelos Gragoudas and Joan Miller examine the safety and efficacy of combination therapies of Lucentis® and Visudyne® in preclinical models.

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1983

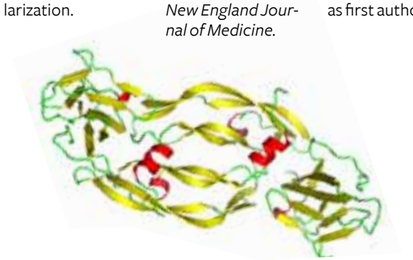
Vascular permeability factor (VPF) is discovered in the laboratory of Harold Dvorak at Harvard Medical School, and described in the journal *Science.* Dvorak's group demonstrated that this factor is 34-42,000 daltons in size and is secreted by a variety of tumors. VPF later becomes known as vascular endothelial growth factor (VEGF).

1984

The first "tumor angiogenesis factor" is isolated and identified as basic fibroblast growth factor (bFGF) by the laboratory of Michael Klagsbrun at Harvard Medical School. Judah Folkman is a co-author on the report, published in the February issue of the journal *Science.*

1994

In the journal *American Journal of Pathology*, Joan Miller, Patricia D'Amore, Judah Folkman, and colleagues correlate VEGF with ocular angiogenesis in primates. This is the first *in vivo* demonstration of VEGF's role in ocular neovascularization.



Beetham Eye Institute researchers Lloyd P. Aiello and George King also associate elevated levels of VEGF in eye fluids of patients with diabetic retinopathy and other ischemic retinal diseases. This work is published in the December issue of *New England Journal of Medicine.*

A group including Anthony Adamis, Joan Miller and Judah Folkman finds increased VEGF levels in ocular fluid of diabetic retinopathy patients; this is reported in the October issue of *American Journal of Ophthalmology,* with Tony Adamis as first author.

In the most-cited article in the journal *Investigative Ophthalmology and Visual Science,* Lois Smith and Patricia D'Amore describe a mouse model of retinopathy of prematurity (ROP) and other oxygen-induced retinal disorders.

In the July issue of *Proceeding of the National Academy of Sciences,* Robert D'Amato, working with Judah Folkman, describes how the toxicity of thalidomide is primarily due to its antiangiogenic effects. This drug is now used to treat multiple myeloma.

1997

Judah Folkman's laboratory identifies endostatin, an endogenous inhibitor of angiogenesis, and shows that it inhibits tumor growth. These findings are published in the January issue of the journal *Cell.*

1998–2004

The HMSARG maintains its dramatic pace of research, and further elucidates the molecular and genetic mechanisms of angiogenic ocular diseases. These studies serve as the foundation for further development of anti-angiogenic therapies for ocular disorders.

2000

On April 13, 2000, PDT with Visudyne® becomes the first FDA-approved drug treatment for wet AMD.

Lucentis®, a fragment of the anti-VEGF antibody bevacizumab, enters clinical testing for AMD.

2004

On December 17, Macugen® becomes the first FDA-approved anti-VEGF therapy for neovascular (wet) AMD. The approval is based on a large multi-center clinical trial published December 30 in *New England Journal of Medicine. E*vangelos Gragoudas is an investigator in this study.

2006

On June 20, Lucentis® receives FDA approval for the treatment of AMD.

2010

A landmark clinical trial shows that intraocular Lucentis® injections remarkably improve the results of standard laser photocoagulation for diabetic macular edema. Lloyd P. Aiello and Jennifer Sun are investigators of this collaborative study, conducted at 52 clinical sites within the Diabetic Retinopathy Clinical Research Network and published in the June issue of the journal *Ophthalmology.*

In July, the FDA approved Lucentis® for the treatment of diabetic macular edema following retinal vein occlusion.


JENNIFER K. SUN, MD

AGE-RELATED MACULAR DEGENERATION (AMD)

At the center of the retina is a photoreceptor-rich region known as the macula, which processes images in the middle of the visual field. The macula has a high density of cones, which are the photoreceptors that distinguish color and fine detail in bright light. The fovea, which lies at the center of the macula, is made entirely of cones, and thus provides the most acute vision. As people age, their maculas may begin to degenerate, causing progressive loss of central vision. This condition is known as age-related macular degeneration (AMD). Some AMD patients retain enough peripheral vision to continue many activities of daily life; however, as the disease progresses, it can have devastating effects on quality of life as patients lose the ability to drive, recognize faces, or read. In the United States, AMD is the leading cause of blindness in people over the age of 50.

Photodynamic therapy: a breakthrough treatment for AMD

Studies initiated in the early 1990's by Joan Miller, MD, and Evangelos Gragoudas, MD, produced a breakthrough for patients with wet AMD: photodynamic therapy (PDT) with verteporfin (Visudyne®). In this procedure, verteporfin (a light-sensitive dye) is injected systemically.; acool laser is then directed at the eye, which activates the drug specifically in the choroidal vessels. This blocks the leakiness of the immature vessels and prevents further growth. This therapy allows targeted and non-invasive treatment for patients with CNV under the fovea, which is the cone-rich central portion of the macula. Approved by the FDA for treating neovascular AMD on April 13, 2000, Visudyne® was the first pharmacologic therapy for AMD and has also been used to treat CNV caused by other ocular conditions. Photodynamic therapy also laid the foundation for a new class of vascular-targeting therapies for AMD, altering the landscape of ophthalmic care.


JOAN W. MILLER, MD

Drs. Miller and Gragoudas are actively pursuing ways to improve standard PDT. Although this therapy slows disease progression in most patients who receive it — and may even improve vision in some — PDT itself can damage the retina by causing retinal cells to undergo apoptosis (cell suicide), which is the primary cause of vision loss in both dry and wet AMD. In 2007, to better understand the mechanisms of PDT-induced photoreceptor apoptosis, Drs. Miller and Gragoudas used animal models of CNV to identify the factors that control apoptosis in photoreceptors. In 2008, using the same models, they also showed several promising ways to prevent photoreceptor apoptosis after PDT, such as injections of dexamethasone (a potent steroid) or L-NAME (a nitric oxide synthase inhibitor). These studies are helping to optimize current PDT protocols for treating CNV secondary to AMD.

Inspiring the development of VEGF inhibitors for AMD

Beginning in the early 1990s, HMSARG scientists began to reveal the role of VEGF in ocular neovascularization. In 1993, Patricia D'Amore, PhD, MBA, David Shima, PhD, Tony Adamis, MD, Judah Folkman, MD, and colleagues showed that the human retina synthesizes VEGF, and in 1995 they found that VEGF expression is induced in low-oxygen (hypoxic) conditions. Using preclinical models, Drs. Miller, Adamis and D'Amore showed that VEGF expression was virtually undetectable in healthy eyes, but dramatically increased with severity of retinal ischemia (lack of blood flow in the retina) and iris neovascularization. This 1994 discovery was the first time VEGF was implicated in ocular neovascularization. In a series of studies published between 1995 and 1996, several HMSARG members (including D'Amore, Gragoudas, Miller, Adamis, Lloyd P. Aiello, MD, PhD, George King, MD, and Lois Smith, MD, PhD) showed that VEGF inhibitors could block ocular neovascularization in preclinical models. In 1994, Drs. Adamis, Miller and Folkman showed increased VEGF in the vitreous of patients with proliferative diabetic retinopathy. This study, published October 1994 in *American Journal of Ophthalmology*, directly linked VEGF with disease. These findings were replicated in a study led by Dr. Aiello, which was published December 1994 in *the New England Journal of Medicine*. This cumulative work of the HMSARG inspired the development of anti-VEGF therapies, which have since replaced PDT as first-line treatments for wet AMD.

Macugen®

Pegaptanib (Macugen®), which consists of aptamers (short pieces of RNA) that target VEGF, was the first anti-VEGF therapy approved for treating AMD. In 2004, the VEGF Inhibition Study in Ocular Neovascularization Clinical Trial demonstrated efficacy for pegaptanib for neovascular age-related macular degeneration, and was published in the December 30th issue of the *New England Journal of Medicine*. FDA approval of Macugen®, granted on December 17, 2004, was based on this study, and opened a new era of treatment for AMD and other retinal diseases.

Lucentis®

Ranibizumab (Lucentis®), approved on June 20, 2006 for the treatment of wet AMD, was a revolutionary advance because it could improve vision in about one-third of patients treated. Ranibizumab is an anti-VEGF fragment related to bevacizumab (Avastin®), a full-length anti-VEGF antibody and the first-ever antiangiogenic drug (FDA-approved for treating colon cancer in 2004). Ranibizumab and bevacizumab were both based on the scientific principles established by Dr. Judah Folkman, and members of the HMSARG contributed to pivotal preclinical studies that laid the foundation for further development of the drug. Drs. Adamis, Shima, Gragoudas, D'Amore, Miller, and Folkman collaborated with Napoleone Ferrara, MD, of Genentech to show that an anti-VEGF antibody fragment could prevent neovascularization in a primate model. They also showed that injection of VEGF alone into the eye was sufficient to produce neovascularization, again in a primate model.

Moving from preclinical studies to clinical trials, Drs. Gragoudas and Miller led clinical safety studies of ranibizumab in 2005, and along with Ivana Kim, MD, tested its effectiveness in combination with Visudyne® PDT. Recent and ongoing studies conducted by the HMSARG are expanding the list of ocular diseases that may be treated with ranibizumab or other antiangiogenic therapies. Dr. Kim contributed to a Phase I clinical trial reported January 2011 in the journal *Ophthalmology*, demonstrating the effectiveness of ranibizumab for CNV caused by conditions other than AMD. Dr. Aiello and Jennifer Sun, MD, have also studied ranibizumab as a potential therapy for diabetic macular edema, and are participating in an ongoing, multi-center clinical trial sponsored by the Diabetic Retinopathy Clinical Research Network. Preliminary results from the trial demonstrated that anti-VEGF therapies may reduce diabetes-associated swelling in the retina. Dr. Aiello was a lead author for the study. (see profile, page 154).

Dry AMD: the unmet challenge

Dry AMD involves the degeneration of the retinal pigment epithelium (RPE), which is a thin layer of supportive cells beneath the macula. An early finding of dry AMD is the accumulation of cellular deposits under the retina. These deposits, called drusen, are associated with atrophy of the macular photoreceptors and loss of central vision. Dry AMD is the most common form of this disease; 90 percent of all AMD cases represent this subtype, and nearly one million patients per year progress from dry AMD to geographic atrophy (GA), which results in severe vision loss. Dry AMD also increases the risk of developing wet (neovascular) AMD, the more serious form of the disease, which typically progresses more rapidly and leads to profound vision loss.

HMS Ophthalmology researchers are aggressively pursuing new therapies for the dry form of AMD, which still has no FDA-approved treatments. The first step involves defining the mechanisms that underlie dry AMD and its progression to GA. Ivana Kim, MD, and Demetrios Vavvas, MD, PhD, are exploring animal models of dry AMD to better understand its pathophysiology. Currently funded efforts support the establishment and characterization of a primate model of dry AMD. In this model, experimentally induced autoimmunity is expected to induce the accumulation of drusen, a hallmark of dry AMD, as well as the neural atrophy that is typical in GA.

IVANA K. KIM, MD

By establishing preclinical models and delineating key mechanisms of vision loss in dry AMD, researchers can test novel interventions for this widespread condition.

Genes, smoking, and genetic smoking guns in AMD

AMD is a complex disease that often involves a combination of multiple genetic and environmental risk factors. Researchers in the Mass. Eye and Ear Ocular Molecular Genetics Institute seek to identify genes that contribute to AMD, and to understand how the interactions between genes and the environment may alter an individual's risk of developing AMD. By studying pairs of siblings in which one had AMD and the other did not, Margaret DeAngelis, PhD, Thaddeus Dryja, MD, and Dr. Miller identified cigarette smoking as a risk factor for wet AMD. In 2005, multiple groups identified complement factor H (CFH), a protein involved in immune responses, as a susceptibility locus for AMD. This study showed that the Y402H variant of CFH accounted for over 40 percent of AMD risk in older adults. In a 2007 study that addressed both genetic and environmental factors, Drs. DeAngelis, Dryja, Miller and Kim showed that specific variants of the CFH gene, combined with


DEMETRIOS G. VAVVAS, MD, PHD


LLOYD M. AIELLO, MD

cigarette smoking, increased AMD risk 144-fold. Using powerful genetic studies combined with gene expression microarrays and proteomic techniques, ongoing studies have identified several novel gene variants and gene-gene interactions that affect AMD risk.

Based on the genetic findings implicating the complement pathway in AMD pathogenesis, several complement-directed therapies are currently under clinical investigation. The Mass. Eye and Ear Retina Service is participating in a Phase I/II study, sponsored by Genentech, of an anti-complement factor D antibody for the treatment of advanced dry AMD or GA.

In March 2010, Alexandra Silveira, PhD, and colleagues, working with Drs. Miller, Kim and DeAngelis, also identified variants in the retinoic acid receptor-related orphan receptor alpha (*RORA*) gene that substantially contribute to an individual's risk of AMD. Because the *RORA* gene codes for a receptor that binds cholesterol and other hormones, this finding links new biological pathways to AMD, suggesting new ways to prevent or potentially treat AMD and other retinal disorders.

DIABETIC RETINOPATHY

Prior to the 1920s, type 1 diabetes was often fatal within a few years after diagnosis, usually due to ketoacidosis, kidney failure, and other life-threatening complications of uncontrolled hyperglycemia. The discovery of insulin in 1921 allowed people with this type of diabetes to live longer lives; however, the increased life expectancies also revealed long-term vascular complications of chronic hyperglycemia, such as diabetic retinopathy. By the 1950s, diabetic retinopathy had become the leading cause of blindness in the United States. Many patients with diabetes are now able to avoid blindness thanks to laser photocoagulation, a therapy developed in 1967 by researchers Lloyd M. Aiello, MD, PhD, and William P. Beetham, MD, at the Joslin Diabetes Center Despite this revolutionary treatment, diabetic retinopathy remains a major public health concern as the leading cause of blindness among working-age Americans. Researchers at the Beetham Eye Institute (BEI) at Joslin are continually advancing available treatments for diabetic retinopathy, and have also contributed greatly to the development of antiangiogenic therapy for vascular eye disorders.

Picking PKC as a target of diabetic retinopathy treatments

George King, MD, HMS Professor of Medicine at HMS and Director of Research at Joslin Diabetes Center, first detailed some of the cellular signaling pathways that underlie diabetic retinopathy over two decades ago. In his landmark report, published in July 1989 in *Proceedings of the National Academy of Sciences,* Dr. King proposed that elevated glucose levels activate protein kinase C (PKC), which in turn leads to vascular complications in diabetic retinopathy. Dr. King and his collaborator, Lloyd P. Aiello, MD, PhD, have continued to define the role of PKC in diabetic retinopathy. A report in the November 2009 issue of *Nature Medicine* showed how PKC-delta activation by hyperglycemia causes blood vessel cells to die—resulting in vascular complications in the early stages of diabetic retinopathy.

The cumulative findings of Drs. King and Aiello led to the development of ruboxistaurin (Arxxant®), a PKC-beta inhibitor, which was demonstrated to inhibit vascular disorders in preclinical models of diabetes. Results of a Phase 3 clinical trial of ruboxistaurin were reported in 2006; compared to placebo, ruboxistaurin reduced risk of sustained moderate vision loss by 40 percent in patients with moderate to severe non-proliferative diabetic retinopathy. There are still ongoing clinical trials to further test the safety and efficacy of this PKC inhibitor. Although endpoints to prevent the progression of diabetic retinopathy were not achieved in recently completed clinical trials, the secondary endpoints from these studies suggest that ruboxistaurin may still have some potential in preventing vision loss from diabetic retinopathy. More importantly, these studies have advanced the understanding of the disease processes in diabetic retinopathy, and help to guide new approaches for developing potential therapies.

VEGF as a villain of diabetic retinopathy

Since diabetic retinopathy involves leaky blood vessels and pathological neovascularization in advanced stages, it is not surprising that it involves VEGF; in fact, some of the earliest studies implicating VEGF in neovascular eye disorders were conducted in patients with diabetes. In 1994, researchers at Mass. Eye and Ear (including Drs. Miller and Adamis) and at BEI (including Drs. Aiello and King) found increased VEGF levels in the eyes of patients with diabetic retinopathy. In November of 1995, Drs. Aiello, King, and Lois Smith, MD, PhD, published the first report that VEGF inhibitors could suppress retinal neovascularization. In January of 1996, a team that included Drs. Adamis, Shima, Gragoudas, Folkman, D'Amore and Miller used a primate model to show that VEGF inhibition with a monoclonal antibody could prevent neovascularization. These studies were instrumental in the development of ranibizumab (Lucentis®), which is a fragment of a VEGF-specific antibody, for the treatment of AMD.

Although laser photocoagulation therapy, which was developed at BEI, continues to be the gold standard for treating diabetic macular edema (DME), new studies suggest that ranibizumab therapy may improve the visual outcomes of standard laser therapy for complications of diabetic retinopathy. In a multicenter clinical trial of 854 eyes in 691 patients with DME, laser combined with ranibizumab was more effective at slowing or reversing vision loss than using laser alone. At a two-year follow-up, vision was still dramatically improved in patients who received the combination treatment. This study was conducted by the Diabetic Retinopathy Clinical Research Network and published June 2010 in *Ophthalmology*. Expanded two-year results were published April 2011 in *Ophthalmology*, and confirmed the results of the previous report. Drs. Aiello and Sun were among the lead investigators of this study, which gives promising evidence that antiangiogenic therapy can greatly benefit patients with diabetic retinopathy.


GEORGE L. KING, MD

The Joslin 50-Year Medalists: holding the keys to escaping diabetic retinopathy

Because chronic hyperglycemia can have ravaging effects on blood vessels, the vast majority of people with diabetes—especially those who depend on insulin injections to control blood sugar—will eventually develop retinopathy and other vascular problems. However, some patients live with type 1 (insulin-dependent) diabetes for 50 years or longer without apparent complications. The Joslin Diabetes Center recognizes these unique individuals through its 50-Year Medalist program, and many Medalists are participating in a long-term study that aims to determine how they manage to avoid vascular complications.

Led by Dr. King, Joslin scientists discovered that only 50 percent of Medalists reported retinopathy after surviving diabetes for 50-60 years. Surprisingly, even fewer (44 percent) of the 60-year to 70-year Medalists reported retinopathy, and those who survived diabetes for over 70 years reported the lowest rates of all—only 27 percent. In contrast, over 90 percent of all patients with type 1 diabetes develop serious vascular complications. These initial findings of the 50-Year Medalist Study, which were published in the August 2007 issue of the journal Diabetes Care, suggest that certain traits make some individuals resistant to hyperglycemia-induced complications, including retinopathy. Recently, Drs. King and Aiello, along with Jennifer Sun, MD, MPH, showed that only 50 percent of Medalists have advanced retinopathy in either eye. Drs. King, Sun and Aiello are now examining these patients more closely in hopes of identifying genetic or biochemical factors that can protect people with diabetes from neovascular diseases. Results from these studies, published April 2011 in *Diabetes Care,* may lead to new strategies for improving the quality and duration of life for people with diabetes.

Genetics of diabetic retinopathy and the Jackson Heart Study

In the United States, African Americans have an increased risk of cardiovascular disease and related conditions, including diabetes. For reasons that are not entirely clear, diabetic retinopathy is not only more common in African Americans compared to other groups, but the disease also tends to progress more quickly. Because this disparity cannot be explained by differences in blood sugar control alone, researchers suspect that other reasons exist.

Lucia Sobrin, MD, MPH, believes that genetics may explain why African Americans are especially prone to diabetic retinopathy. Through a collaboration between Mass. Eye and Ear, the University of Mississippi Medical Center, and the Jackson Heart Study group, Dr. Sobrin hopes to uncover genes that may alter an individual's risk of diabetic retinopathy.

The Jackson Heart Study follows African Americans living in the greater Jackson, Mississippi area, and is the largest study in history to examine the genetics of cardiovascular disease and related conditions in African Americans. Researchers have collected DNA samples from consenting participants, and are looking for genetic clues as to why certain people are more prone than


LUCIA L. SOBRIN, MD, MPH

TERESA C. CHEN, MD, FACS

others to certain conditions.

Dr. Sobrin has carefully examined the clinical signs and genetic maps of over 500 participants thus far, and will continue to enroll participants through 2012. Genetic mapping is being done at the Broad Institute in Cambridge, Massachusetts, where Dr. Sobrin has been working with David Altshuler, MD, PhD, and Mark Daly, PhD. By identifying genes that may affect retinopathy risk and rate of progression, Dr. Sobrin hopes to improve monitoring, counseling, and therapeutic strategies for patients that may suffer vision loss from diabetic complications.

RETINAL DEGENERATIONS

Retinal degenerations are diverse genetic conditions that progressively destroy the light-sensing photoreceptors of the retina. Those that are hereditary are known collectively as retinitis pigmentosa and allied diseases, and affect approximately 100,000 people in the United States and 2 million people worldwide. Most people afflicted by this condition become night blind in adolescence, lose side vision in young adulthood, develop tunnel vision, and become blind by age 60; if untreated, some individuals become virtually blind by age 30. Though vision loss in retinitis pigmentosa is irreversible, treatment regimens developed in the HMS Department of Ophthalmology may slow the course of disease, and postpone blindness for up to 20 years. Research is ongoing with the ultimate goal of restoring vision among those with early stages of this group of diseases.

ELIOT L. BERSON, MD

Treating hereditary retinal degenerations and deciphering their genetic origins

Eliot L. Berson, MD, the William F. Chatlos Professor of Ophthalmology at HMS, has been at the forefront of research on retinal degenerations for more than four decades. In the 1960s, Dr. Berson discovered that electroretinography (ERG) could detect photoreceptor dysfunction years to a decade before vision starts to deteriorate in retinitis pigmentosa; since then, ERG has been used routinely to diagnose this condition and to estimate visual prognoses. Dr. Berson and colleagues, working in the Berman-Gund Laboratory for the Study of Retinal Degenerations, developed the first treatment regimens for retinitis pigmentosa: supplementation with vitamin A palmitate and an omega-3 rich fish diet (of which docosahexaenoic acid, or DHA is a major constituent). They have recently shown that lutein supplementation slows midperipheral visual field loss (*Archives of Ophthalmology*, 2010).

In the early 1990s, Dr. Berson and his colleague Thaddeus Dryja, MD, discovered the first gene defects associated with retinitis pigmentosa: point mutations in the rhodopsin gene, which encodes a light-sensitive pigment in photoreceptor cells. Drs. Berson and Dryja have since pinpointed identified approximately 20 genes associated with retinitis pigmentosa—revealing numerous biochemical pathways that are altered in this heterogeneous group of diseases. More recently Dr. Berson has collaborated with Carlo Rivolta, PhD, a former fellow of Dr. Dryja, to define further the mutation spectrum in these disorders. In the July 2010 issue of the journal *Human Gene Therapy*, Dr. Berson, Michael Sandberg, PhD, Basil Pawlyk and coworkers reported successful gene therapy of both rod and cone photoreceptors in an animal model of a severe form of retinitis pigmentosa (Leber congenital amaurosis) caused by with loss of the RPGRIP1 gene. These genetic studies form the foundations for new disease models and developing targeted therapies for these other forms of these debilitating disorders.

Deciphering the genetic origins of retinitis pigmentosa

The X chromosome also contains a gene that is often associated with retinitis pigmentosa; it codes for a protein called retinitis pigmentosa GTPase regulator (RPGR), which is missing or nonfunctional in about 10 percent of all cases of the degenerative retinal disease. Studies conducted in the Berman-Gund Laboratory of Retinal Degenerations have significantly advanced the understanding of how RPGR functions in the retina, and have led to promising new therapies for retinitis pigmentosa.

In the early 2000s, Dr. Tiansen Li, PhD, used a mouse model of X-linked retinitis pigmentosa to study RPGR function in photoreceptors. He showed that RPGR helps maintain proper distribution of light-sensitive opsin proteins in the cilium, a thin structure that connects the cell body with the outer segment. Dr. Li and colleagues discovered that in order for RPGR to function, it requires a cilium-specific protein called RPGR-interacting protein (RPGRIP), which anchors RPGR in the photoreceptor cilium. Moreover, they found that the gene encoding RPGRIP, located on human chromosome 14, is mutated in some patients with Leber congenital amaurosis (LCA). In this severe form of retinitis pigmentosa, lack of RPGRIP in the cilium essentially abolishes RPGR function, causing progressive vision loss that begins in early childhood.

These findings led Dr. Li's team to develop gene replacement strategies for RPGR and RPGRIP defects and for the first time utilized gene therapy delivery to photoreceptors. In a study reported in September 2005 in the journal *Investigative Ophthalmology and Visual Science*, Dr. Li and colleagues used adeno-associated virus (AAV) to express RPGRIP in a mouse model of LCA. This gene therapy approach preserved photoreceptor function as confirmed by electroretinography (ERG). In a subsequent preclinical study, reported August 2010 in the journal *Human Gene Therapy*, Drs. Li, Berson, and colleagues used a similar strategy to restore photoreceptor function in LCA mice—this time using the human RPGRIP gene. This provided proof-of-concept that gene replacement therapy is feasible for the human form of LCA, and called for clinical testing in patients with RPGRIP deficiencies. This study also suggested that gene replacement could work for other retinal degenerations caused by ciliary defects. Ongoing studies are testing AAV-mediated RPGR replacement therapy for X-linked retinitis pigmentosa.

Optical coherence tomography (OCT): a revolutionary technique in evaluating retinal disease

Optical coherence tomography (OCT) is a non-invasive technique that uses light to produce high-resolution, three-dimensional images of fine ocular structures. OCT was developed in the early 1990s at Mass. Eye and Ear in collaboration with Massachusetts Institute of Technology and Massachusetts General Hospital. OCT is now used worldwide in routine clinical practice to diagnose and monitor numerous ocular conditions. In the retina, OCT was first used to characterize morphology in the macula, and the technique expanded quickly into a role in the management of neovascular (wet) AMD.

In a case series reported June 2006 in *American Journal of Ophthalmology*, researchers at Mass. Eye and Ear (including Teresa Chen, MD, Joan Miller, MD, and Evangelos Gragoudas, MD) demonstrated for the first time that histological changes in dry AMD can be detected in time domain OCT (TD-OCT) scans of the retina. OCT is now rapidly becoming the "gold standard" for diagnosing and monitoring both wet and dry forms of AMD, and HMS researchers are continually optimizing techniques for measuring retinal changes in this increasingly prevalent condition.

TD detection is the classic and probably most widely used OCT technology. A newer form, based on spectral domain (SD) detection, has greatly improved the image quality for retinal morphology. SD-OCT has had a major impact in the diagnosis and management of patients with retinal conditions. Clinician scientists in the HMS Department of Ophthalmology continue to improve OCT techniques. Ongoing studies are using OCT to better understand the pathology of retinal disease, and to determine the effectiveness of novel therapeutic regimens.

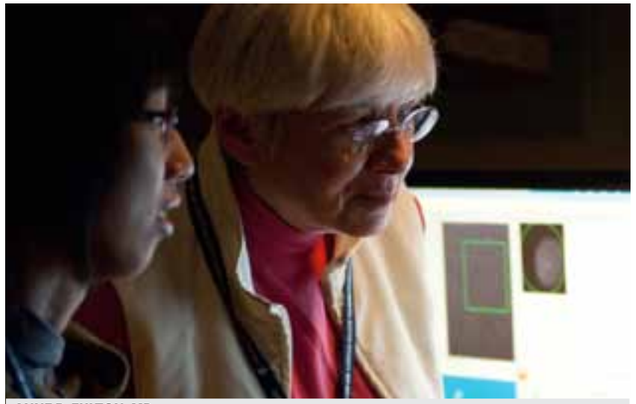
As SD-OCT has become an integral part of AMD management, so has the need to better understand the implications of OCT findings and how they relate to other clinical findings. Andrea Giani, MD, Daniel Esmaili, MD, and Dr. Miller of Mass. Eye and Ear, in collaboration with Giovanni Staurenghi, MD, and colleagues at the University of Milan, recently evaluated patients with classic CNV using SD-OCT, and correlated the findings with vessel leakage as determined by flourescein angiography (FA). The researchers found that the absolute difference between CNV material and retinal pigment epithelial reflectivity (REF), as determined by SD-OCT, was higher in untreated CNV than in previously treated (but still leaky) CNV. Moreover, the difference between CNV material and REF was higher in leaky lesions than in those without leakage. This study, slated for 2011 publication in the journal *Retina*, demonstrates that SD-OCT findings may provide important information regarding vessel leakage measurements by FA. In a related study, published August 2001 in the journal *Investigative Ophthalmology and Visual Science*, Drs. Giani, Esmaili, Miller, Staurenghi, and colleagues tested whether SD-OCT may be used to predict FA leakage in CNV. Indeed, the researchers found that SD-OCT findings were significantly correlated with FA leakage in CNV with fluid presence and with certain patterns of fluid presentation. These cumulative findings will establish the clinical relevance of OCT imaging and its use in manag-

ing AMD patients.

OCT has also proven to be useful for evaluating diabetic retinal complications and treatment regimens. In a study reported May 2010 in the journal *Ophthalmology*, Joslin researchers led by Lloyd P. Aiello, MD, PhD, used OCT to measure retinal volume in a multicenter, randomized, controlled clinical trial of focal/grid photocoagulation therapy for diabetic macular edema (DME). The researchers showed that higher baseline measurements of retinal volume were associated with worsening visual acuity two years after treatment. Overall, the study supported the use of focal/grid photocoagulation as the standard therapy for DME, and showed OCT to be a clinically useful tool in evaluating and predicting visual acuity after treatment.

For retinal degenerative disorders like as retinitis pigmentosa, OCT has proven to be extremely valuable for measuring retinal thickness and detecting complications, such as cystoid macular edema (CME) and macular cysts. In a 2005 study led by Drs. Sandberg and Berson, and published in the journal *Investigative Ophthalmology and Visual Science*, researchers showed that both retinal thinning (due to cell loss) and retinal thickening (due to presumed edema) determined by TD-OCT measurements

KIP CONNOR, PHD



ANNE B. FULTON, MD

were associated with lower acuity. More recently, in a 2010 *Investigative Ophthalmology and Visual Science* report, Dr. Sandberg and colleagues showed that macular pigment optical density (MPOD), a measure of carotenoid concentration in the fovea, was lower in eyes with higher degrees of CME as measured by TD-OCT. OCT testing is now being used by Drs. Sandberg and Berson to help identify nutritional factors that may be associated with the development of CME in patients with retinitis pigmentosa.

Research in OCT technology continues. In the October 2008 issue of *Investigative Ophthalmology and Visual Science*, HMS researchers (including Drs. Chen, John Loewenstein, MD, and Johannes de Boer, PhD) assessed the utility of optical frequency domain imaging (OFDI), a high-speed OCT system developed at Massachusetts General Hospital. The researchers used OFDI with a center wavelength of 1050 nm, previously shown to improve the imaging of deeper retinal structures (such as the choroidal vessels). OFDI was used to acquire various measures of CNV (including CNV volume, retinal thickness, subretinal fluid volume, and magnitude of photoreceptor detachment) before and after treatment with ranibizumab (Lucentis®). This study demonstrated that high-speed, three-dimensional OFDI at 1050 nm may have advantages over standard TD-OCT and current state-of-the-art SD-OCT at 850 nm for imaging neovascular (wet) AMD.

RETINOPATHY OF PREMATURITY

If a baby is born too early, the retinal vessels—which grow outward from the center of the retina—may stop growing before they reach full length. This may lead to retinopathy of prematurity (ROP), and can deprive the developing peripheral retina of oxygen and nutrients. This early stage of ROP affects about 15,000 premature infants each year in the United States; while most recover without treatment, about 10 percent of ROP cases progress to a neovascular stage that can severely damage the retina and cause vision loss. VEGF plays a major role in both stages of ROP, and its regulation by oxygen is central to the disease process. VEGF is induced by low oxygen (hypoxia) but repressed by high oxygen (hyperoxia), so when a baby is exposed to the oxygen-rich environment outside of the womb, the relative hyperoxia may halt normal retinal vessel growth by suppressing VEGF. As a result, the blood-starved peripheral retina becomes hypoxic, and produces VEGF and other angiogenic factors to induce neovascularization.

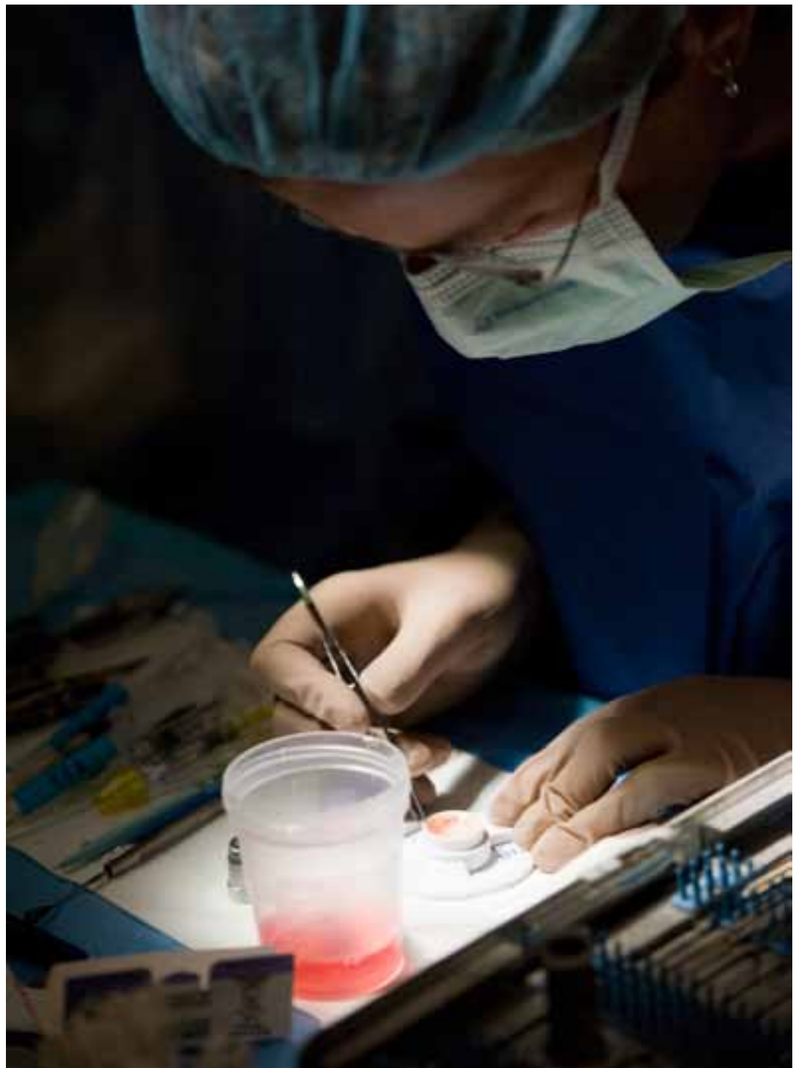
The interplay between neural and vascular networks in the developing retina

In babies with ROP, the disease starts to appear at approximately 32 weeks of gestation, or about eight weeks short of full term. This happens to be the time when the photoreceptors (the rod cells in particular) undergo rapid maturation. Because nerves and blood vessels often grow side by side, the interplay between the neural and vascular networks in the retina may be important in ROP. This neurovascular interaction is the focus of Anne Fulton, MD, HMS Professor of Ophthalmology and Senior Associate in Medicine at Children's Hospital Boston. Dr. Fulton's recent studies have explored the possibility that the rapidly maturing photoreceptors, with their increased metabolic demands, create a hypoxic environment that promotes neovascularization; thus, photoreceptor function may actually occur before vascular abnormalities appear. Using non-invasive assessment techniques such as electroretinography (ERG) in babies with or without ROP, Dr. Fulton showed that rod sensitivity could predict vascular outcome at a later age. These observations were confirmed in animal models of ROP, and suggest that rod dysfunction may have a causative role in the vascular problems of ROP. These studies establish the immature photoreceptors as potential pharmacological targets, and offer promise of very early intervention in the treatment of ROP.

Understanding unique aspects of angiogenesis in the eyes of children

By studying ROP, Lois Smith, MD, PhD, has made several notable contributions to the field of angiogenesis research. In the 1990s, Dr. Smith worked with Patricia D'Amore, PhD, MBA, and Robert D'Amato, MD, PhD, to develop a mouse model of ROP that is widely used to study VEGF regulation and oxygen-induced retinopathies. In collaboration with George King, MD and Lloyd P. Aiello, MD, PhD, Dr. Smith was among the first to implicate VEGF in retinopathy and to suppress ocular neovascularization using VEGF inhibitors. Dr. Smith has since shifted her focus to other factors that are also important in the development of ROP, and has defined novel strategies for treating and preventing this disease.

In 2007, Dr. Smith, with John Paul San Giovanni, ScD, Jing Chen, PhD, and Kip Connor, PhD, showed that omega-3 fatty acids promote normal vessel growth in the retina while reducing abnormal neovascularization. This suggested that prenatal supplementation with omega-3 fatty acids may help prevent retinopathy in newborns. In a series of studies published in the past few years, Drs. Smith and Chen demonstrated that erythropoietin (EPO) and insulin-like growth factor I (IGF-I) are both deficient in the early stages of ROP when retinal vessels are insufficient—yet both contribute to the pathological angiogenesis that occurs during neovascular ROP. These studies not only present new pharmacological targets, but also demonstrate that timing is critical for preventing and treating ROP.



RESCUING THE VISION OF NEWBORNS

As a pediatric ophthalmologist, Lois Smith, MD, PhD, has cared for many extremely premature babies—some weighing less than one pound. Not only do these vulnerable infants have life-threatening health problems, but many also suffer from retinopathy of prematurity (ROP). A number of years ago, Smith decided that she needed to find a way to help these babies preserve their vision.

Dr. Smith's quest began by looking at the first ROP risk factor: oxygen delivery. She worked with newborn mice, which, like premature babies, have only partially developed retinas. When the mice were given supplemental oxygen at high levels, their retinal blood vessels stopped growing or disappeared. Moreover, retinal levels of VEGF plummeted. But when the mice were brought back into normal room air, VEGF levels surged and blood vessels resumed growth—sometimes abnormally.

Many years of follow-up experiments revealed that VEGF is actually involved in two biochemical pathways. Targeting one pathway could enhance the normal, "good" vessel growth in the first phase of ROP without affecting the abnormal second-phase vessel growth.

To discover other critical factors acting in these pathways, Dr. Smith began to investigate growth hormone (GH), which is produced by the pituitary gland and implicated in diabetic retinopathy. Her work gradually led her to another hormone known as insulin-like growth factor I (IGF-I), which works in concert with GH and is important for growth and development of the brain, lungs, intestines and other organs. Dr. Smith conducted a series of experiments in mice that could not produce IGF-I. "We found out that IGF-I was really important for normal vessel growth," Dr. Smith says. "If IGF-I is low, vessels don't grow." The research quickly moved into humans. Dr. Smith's group found that in the third trimester of pregnancy, IGF-I levels in the fetus rise markedly because they get the factor from the mother in the womb. In very premature babies, however, this supply is cut off, and their levels of IGF-I begin to decline.

In children, retinopathy of prematurity (ROP) is a major cause of vision loss in the U.S. Each year, about 1,500 premature infants develop ROP severe enough to require medical treatment; approximately 500 children each year become legally blind from ROP.
(*National Eye Institute*)

This finding prompted Dr. Smith and Ann Hellström, MD, PhD, a collaborator in Sweden, to assess IGF-I levels in 80 babies born at 24 to 32 weeks of gestation. They found that IGF-I levels at the gestational age of 30 to 33 weeks were the most important predictor of whether a preterm baby would develop ROP. In babies who developed ROP, IGF-I levels never rose to the level they would have achieved at 30 to 33 weeks *in utero*. In contrast, premature babies who did not develop ROP managed to make enough IGF-I soon enough after birth to prevent disease.

Drs. Smith and Hellström next conducted a Phase I clinical trial in Sweden to see if supplementing IGF-I in premature newborns would prevent ROP. Starting at birth, the babies received small amounts of IGF-I intravenously until they were able to make sufficient levels of the growth factor on their own. Successful results from this trial have led to an expanded, multi-center Phase II trial in Sweden. "In science, you have to look at one thing at a time," says Dr. Smith. "You try to find pathways that you can alter to make a difference, and you just keep on pushing."

Excerpted from the Spring 2004 issue of *Dream: The Magazine of Possibilities*, a publication of Children's Hospital Boston. Original story by Nancy Fliesler. Content has been adapted and updated for this report.

FRONTIERS IN RETINAL RESEARCH: PRESERVING & RESTORING VISION

Preserving vision: new agents for neuroprotection

Though blinding retinal diseases have many different origins, they often converge on pathways that trigger the death of photoreceptors (the light-sensing neurons of the retina), resulting in vision loss. Two well-characterized biological pathways, apoptosis (cell suicide) and necrosis (cell death caused by stress and other external factors), are controlled routes of cellular destruction. Therapeutic interventions that prevent photoreceptor death by both apoptotic and necrotic pathways may potentially preserve vision for a myriad of retinal diseases. Thus, neuroprotection is an avid area of current research in the HMS Department of Ophthalmology.

In 2003, a collaboration between Cynthia Grosskreutz, MD, PhD, and Joan Miller, MD, demonstrated that apoptotic photoreceptor cell death and caspase activation occurred in an experimental model of retinal detachment. In 2008, a group led by Dr. Miller showed that a class of anti-AIDS drugs known as protease inhibitors could protect against photoreceptor death in laboratory models. In 2010, in an article published in the Proceedings of the National Academy of Science, Dr. Miller and Demetrios Vavvas, MD, PhD, reported findings that, although the caspase pathway was activated and apoptosis occurred in the model, cell death also occurs through another pathway triggered by receptor interacting protein (RIP). In this study, they demonstrated that blockage of both pathways, with a caspase inhibitor and necrostatin-1 (a RIP kinase inhibitor), was required to effectively prevent apoptosis, necrosis, and oxidative stress. Similarly, deficiency of Rip3 (a key activator of RIP1 kinase) prevented the necrotic changes. Thus, two pathways to cell death were triggered by retinal detachment in animal models, indicating that combination therapy to prevent both capase-dependent apoptosis and RIP-mediated necrosis may help preserve vision.

Retinal research at the zenith: restoring vision

The convergence of research progress in many areas has created an unrivaled opportunity in vision research. The retina, which is more accessible (and has a simpler neuronal structure) than the brain, is likely to yield the first successful neuroregenerative treatments using gene-targeting and stem cell therapies. Moreover, the retinal atlas project provides investigators with the complete cellular architecture of the retina, which complements new advances in gene therapy and stem cell research. Under current study are three distinct approaches to restoring vision: persuade healthy retinal cells to acquire photoreceptor functions, targeted gene therapies, and stem cell engraftment into the retina. These approaches are designed as revolutionary treatments for patients with vision loss, and can be applied to a multitude of ocular diseases that cause blindness.

Gene therapy with help from retinal ganglion cells

Retinal ganglion cells relay visual information, in the form of electrical impulses, from the photoreceptors to the brain. Some ganglion cells express the light-sensitive pigment melanopsin, which allows them to act as photoreceptors as well. Melanopsin is also known to impart photoreceptor-like qualities to cells that do not normally express it; thus, Richard Masland, PhD, wondered whether retinal ganglion cells, induced to express melanopsin using gene therapy techniques, could substitute for photoreceptors in eyes with retinal degeneration. In a mouse model of hereditary retinal degeneration, a team led by Dr. Masland used viral vectors to deliver the melanopsin gene to a large number of retinal ganglion cells. This treatment restored an appreciable degree of vision in mice that lacked rod and cone photoreceptors. This study, published in the October 2009 issue of *Proceedings of the National Academy of Sciences*, established melanopsin as a candidate gene therapy for retinal degenerations. More importantly, this work provides proof-of-principle that photoreceptor substitution is a viable approach for treating retinitis pigmentosa and other retinal degenerative disorders. Dr. Masland's group is currently refining this technique for potential use in patients blinded by retinal disease.

Making stem cell therapy a reality for retinal disease

Tissue and stem cell therapies hold enormous promise for repairing the retina in diseases that cause photoreceptor destruction. This is the focus of Michael Young, PhD, Associate Professor of Ophthalmology at HMS, and Director of the Minda de Gunzburg Center for Ocular Regeneration at Schepens Eye Research Institute. Studies led by Dr. Young during the past five years have significantly advanced the understanding of tissue and stem cell transplantation in the retina—particularly in terms of graft survival and the formation of functional neural connections after transplantation. Dr. Young's laboratory has developed biomaterials that can be used for delivering neuroprotective agents or stem cells to the retina; the engineered biomaterials have also been shown to promote survival and functional growth of neurons after transplantation.

In 2007, Dr. Young established a novel role for matrix metallo-

MICHAEL J. YOUNG, PHD

proteinases (MMPs) in promoting neural regrowth. Using a mouse model of retinal degeneration, Dr. Young showed that MMPs support the growth of transplanted neural progenitor cells in the retina. Subsequent studies showed that increased MMP levels create a permissive environment for neural regeneration. Recently, Dr. Young's laboratory engineered a cell delivery system consisting of a biodegradable/biocompatible polymer that provides a steady release of MMP2 to promote neural regeneration at sites of retinal injury. This technology represents a significant step toward making stem cell therapy a reality in the treatment of retinal disease.

A pharmacological approach to regenerating the retina

Another possible way to treat blinding retinal disorders is to induce the growth of new photoreceptors. So far, this strategy has remained elusive; however, in a breakthrough study published March 2008 in the journal *Investigative Ophthalmology and Visual Science*, Dong Feng Chen, MD, PhD, demonstrated a potential way to simulate photoreceptor regrowth. Dr. Chen, HMS Associate Professor of Ophthalmology and Schepens Associate Scientist, showed that non-neuronal Müller cells could become photoreceptors when stimulated with either glutamate or its analogue alpha-aminoadipate, which are both naturally occurring chemicals. Moreover, alpha-aminoadipate, which has fewer toxic effects than glutamate, also caused the newly transformed photoreceptors to migrate to appropriate places in the retina. These studies were performed in cell culture and in healthy mice; if the same approach restores visual function in preclinical models of retinal degenerations, clinical testing could soon follow. Because this pharmacological strategy targets cells that already exist in the adult retina, it could be a viable alternative to stem cell transplantation for treating hereditary retinal degenerations or vascular retinal disorders, such as age-related macular degeneration (AMD) and diabetic retinopathy.

DONG FENG CHEN, MD, PhD

From mouse to man and from man to mouse: a vision-saving discovery [3]

For the past ten years, reversing vision loss in RP has been the mission of Michael Young, PhD, an Associate Scientist who heads the Minda de Gunzburg Center for Ocular Regeneration at Schepens Eye Research Institute. Today, he is on the verge of using stem cells in clinical trials to repair human retinas damaged by this sight-robbing disease; however, his journey began far from the clinic.

Young's insight came in the form of encouragement and inspiration from one of his mentors, Fred "Rusty" Gage, PhD, who believed that the retina might be a place where stem cells could flourish and integrate to repair damaged tissue.

"We were skeptical," says Young, "that is until we witnessed it for ourselves." Young put his mentor's theory to the test almost immediately. His first subjects were rats. Dr. Young injected brain stem cells into their eyes, watched in awe as the neural stem cells transformed into retina-like cells.

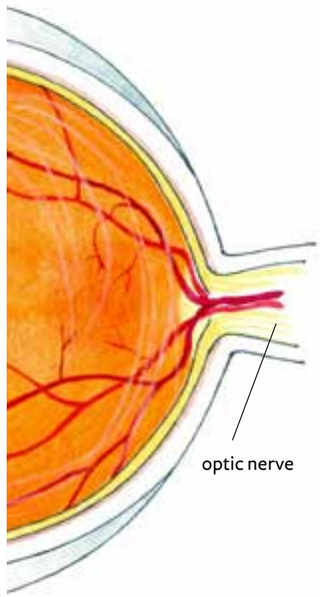
Further proof came when he transplanted retinal stem cells, which not only morphed into retinal cells in mouse eyes, but also wired themselves into the optic nerve and appeared to make the mice more light sensitive. Next, Young and his team performed the same kinds of studies in pigs who have larger, more human-like eyes, and had similar results.

Nearly a decade after these discoveries, Dr. Young is now preparing for clinical trials. To facilitate this critical step, he has enlisted the help of two new collaborators. The first is Reneuron, a company in England that can develop "immortalized" stem cells — genetically altered stem cells that reproduce indefinitely. "With just a few cells, we can produce sufficient tissue for thousands of patients," says Dr. Young.

The second new partner is the Harvard Center for Human Cell Therapy (CHCT), which will grow and store the human retinal stem cell tissue for clinical trials. The CHCT helps scientists within the Harvard community to more rapidly translate stem cell therapy to the clinic. The CHCT selects promising discoveries from many applicants, and then provides assistance from the technical level to the submission of Clinical Protocols and Investigational New Drug (IMD) applications.

Over the next three years, Dr. Young and his research team will refine their animal studies and make certain that the therapy will be safe for human beings. "We are not expecting miracles in these first trials," he says. "Our initial goal will be to regrow some of the rods in the retinas of patients with RP to increase their light perception. Our hope is that the potential of this kind of therapy will improve as we continue to refine our techniques."

[3]Excerpted with permission from the Autumn 2009 issue of *Sightings*, a publication of Schepens Eye Research Institute. The article has been adapted for this report.



OPTIC NERVE/GLAUCOMA

After photoreceptors in the retina convert visual images into electrical impulses, retinal ganglion cells transmit the impulses to the brain for processing. These neuronal cells have characteristically long axons that extend from the retina to the brain and form a cable of nerve fibers known collectively as the optic nerve. Damage to the optic nerve, or optic neuropathy, is a major cause of irreversible vision loss because it can be very difficult to diagnose or treat.

Early intervention is important for virtually any eye disorder; for optic neuropathies, it is absolutely critical because damaged optic nerve cells cannot heal or regenerate. Much effort in the HMS Department of Ophthalmology is devoted to understanding the underlying mechanisms of optic neuropathies, and developing more effective prognostic and diagnostic tools so optic nerve conditions can be treated as soon as possible.

In some optic neuropathies, both genetic and environmental triggers are likely at play; thus, research in the HMS Department of Ophthalmology aims to identify genes that modify disease risk, and to further study how these genes interact with the environment. Scientists are also developing new strategies for preventing and treating optic neuropathies—and even pursuing ways of coaxing the optic nerve to regenerate.

Preventing primary and secondary optic neuropathy

For many optic neuropathies, the lack of suitable preclinical models has hindered efforts to improve existing treatments and develop novel neuroprotective strategies. Dean Cestari, MD, a specialist in neuro-ophthalmology, is developing animal models of hereditary optic nerve disorders with the goal of preventing disease progression. Dr. Cestari also studies conditions that lead to secondary optic neuropathy, including giant cell arteritis and neurofibromatosis type I. He is particularly interested in the inflammatory and pro-angiogenic factors that mediate cell death and visual loss during ischemia (lack of blood flow) in the optic nerve. With the knowledge gained from his studies, Dr. Cestari hopes to develop novel therapies for many optic nerve conditions.

GLAUCOMA

Glaucoma encompasses several conditions that cause optic neuropathy, and affects an estimated 60 million people worldwide. Primary open-angle glaucoma (POAG) is the most common form; it is associated with increased intraocular pressure (IOP), also known as ocular hypertension, which may in turn lead to retinal ganglion cell death and optic neuropathy. Secondary glaucoma occurs as a complication of eye surgeries, injuries, or other ophthalmic conditions. Glaucoma may even occur without increased IOP in normal tension glaucoma. Many kinds of glaucoma have strong genetic and/or environmental risk factors, and any form of the disease can cause irreversible blindness if left untreated.

GENETIC SCREENING AND PROGNOSIS

The most common forms of glaucoma have complex inheritance patterns that seem to involve multiple genetic and environmental factors. By identifying genes associated with glaucoma, scientists hope to develop screening tests that allow rapid risk

LOUIS R. PASQUALE, MD

assessment and targeted treatment. The emerging importance of environmental cues may also lead to new strategies for preventing or averting this potentially blinding disease.

Nature, nurture, and glaucoma

For diseases that do not have straightforward inheritance patterns, finding a genetic basis is very much like searching for a needle in a haystack; thus, identifying glaucoma-causing genes in the human genome requires significant collaboration and the ability to search multiple genomes at once. One major collaborative project, now in its third year, is the NEIGHBOR Consortium—a multicenter cohort study organized by Janey Wiggs, MD, PhD, and Louis Pasquale, MD. This study includes case samples and controls from the Massachusetts Eye and Ear Infirmary, the Nurses' Health Study (NHS), the Health Professionals Follow-up Study (HPFS), and thousands of additional subjects from 22 other investigators at eight different institution[1], for a total of 8,000 glaucoma cases and controls.

This large, collaborative effort not only taps into the intellect and resources of multiple institutions, but also provides the large and statistically robust sample sizes needed to identify major genes that contribute to glaucoma. "Collaboration is essential for this type of study because no single hospital or glaucoma service can produce this many samples," notes Dr. Pasquale. "It's an incredibly exciting effort, and my expectation is that we'll find some major genes that contribute to glaucoma."

The NEIGHBOR Consortium and other related studies form the basis of several genetic and epidemiological projects led by Drs. Pasquale and Wiggs. The highly anticipated results of these studies may ultimately allow at-risk patients to be identified through genetic screening tests.

- A genetic epidemiology study, published in early 2010 in the journal of *Investigative Ophthalmology and Visual Science*, identified potential interactions between endothelial nitric oxide synthase 3 (eNOS3) gene variants and hormone replacement therapy in POAG. This study shows that the association between NOS3 gene variants and POAG is modified by postmenopausal hormone use. Drs. Pasquale and Wiggs are currently collaborating with researchers at MGH and Schepens to validate a mouse model of POAG that may, in turn, be used to validate these epidemiological findings.

- Genomic studies have also identified candidate genes for pseudoexfoliation syndrome, a major risk factor for glaucoma. In pseudoexfoliation, a fibrous buildup on the inner surfaces of the anterior chamber (including the lens) may exfoliate, or flake off, and block drainage of the aqueous humor. Pseudoexfoliation has been associated with LOXL1 gene variations in patients from Scandinavian countries, where the condition was first described. In 2008, researchers at Mass. Eye and Ear associated the same LOXL1 variants with pseudoexfoliation in ethnically diverse patients in the United States. This study, published in the journal *BMC Medical Genetics,* suggested that certain variations in LOXL1 confer significant risk for adult-onset glaucoma worldwide.

- The LOXL1 gene encodes an enzyme that helps build elastic fibers in numerous tissue types, and Mass. Eye and Ear researchers showed that the mutations potentially reduce LOXL1 gene expression. This study, slated for 2011 publication in *Investigative Ophthalmology and Visual Science,* increases the understanding of the pathology of exfoliation glaucoma, and reveals potential therapeutic targets for this widespread condition.

- Data from over 100,000 subjects in the NHS and HPFS cohorts revealed interesting trends for exfoliation glucoma: the disease is more common in higher geographical latitudes; risk increases with distance from the equator. When exfoliation glaucoma affects only one eye, it even appears to vary according to which side people sleep on. These results suggest that temperature and radiation exposure (such as to UV light) are factors in exfoliation glaucoma. Though not yet published, these results highlight the importance of gene-environment interactions in various forms of glaucoma.

- An NIH-funded study in India seeks to identify other genes and measurable traits (such as optic nerve size) that may affect glaucoma risk. This study focuses on certain large families with closely inter-related members, participant characteristics that often yield a wealth of genetic information. Data from these ongoing studies are currently being analyzed, and may yield new diagnostic tests for glaucoma.

CLINICAL EXAMINATION & DIAGNOSIS

Existing diagnostic tests for glaucoma often are unreliable or may catch the disease too late. For instance, IOP measurement is not a definitive test because ocular hypertension does not necessarily cause glaucoma, nor does it occur in all forms of the disease. Other diagnostic techniques, such as standard visual field testing or direct examination of the optic nerve, can only detect abnormal optic nerve function after significant numbers of retinal ganglion cells have already died. Tests that can detect optic nerve dysfunction before retinal ganglion cell death are thus urgently needed.

Improved imaging of the optic nerve

One of the most exciting areas of glaucoma research involves developing imaging techniques that allow early diagnosis and real-time monitoring of glaucomatous optic nerve damage. Although the major glaucoma imaging technologies used today include optical coherence tomography (OCT), confocal scanning laser ophthalmoscopy, and scanning laser polarimetry, only spectral domain OCT allows for ultra-high resolution, three-dimensional video imaging of the optic nerve. Because of its unprecedented ultra-high resolution and ultra-high acquisition speeds, spectral domain OCT holds the most potential for non-invasive imaging and detection of pathological changes in the optic nerve. Spectral domain OCT can also detect retinal nerve fiber layer (RNFL) thinning, which can occur before clinically-detectable, irreversible vision loss in glaucoma.

Teresa Chen, MD, FACS, Associate Professor of Ophthalmology, specializes in spectral domain OCT and was the principal clinical collaborator on the research team that first developed ultra-high resolution video-rate spectral domain OCT. Dr. Chen recently published a study which was the first comprehensive description of the full range of qualitative and quantitative changes of the optic nerve and RNFL in glaucoma. This paper also depicted the first spectral domain OCT images of an eye with glaucoma. This study was published December 2009 in the journal*Transactions of the American Ophthalmological Society.* In a subsequent study, slated for publication in a 2011 issue of Journal of Glaucoma, Dr. Chen and colleagues used spectral domain OCT to examine the eyes of 45 healthy subjects and 33 glaucoma patients. This study is the first to show that a spectral domain OCT machine with tracker has the best ability for reproducible RNFL thickness measurements. These studies provide a basis for further developing and optimizing this imaging technique in larger-scale clinical trials.

Diagnosing glaucoma using psychophysics

Peter Bex, PhD, Assistant Professor of Ophthalmology and Associate Scientist at Schepens Eye Research

PETER J. BEX, PHD

[1] Participating institutions include: University of Pittsburgh School of Medicine; Johns Hopkins University School of Medicine; Duke University School of Medicine; Miller School of Medicine, University of Miami; University of Michigan Medical School; Stanford University School of Medicine; University of California at San Diego, and; West Virginia University School of Medicine.

Janey L. Wiggs, MD, PhD

Associate Professor of Ophthalmology, Harvard Medical School

Associate Director of the Howe Laboratory and Associate Chief for Clinical Research, Massachusetts Eye and Ear Infirmary

Director, Genetic Diagnostics Section, Ocular Genomics Institute, Massachusetts Eye and Ear Infirmary

Dr. Janey Wiggs is an accomplished clinician scientist specializing in the genetics of glaucoma. Using a uniquely collaborative and multidisciplinary approach, Dr. Wiggs' overall research goal is to identify genetic factors that underlie various forms of glaucoma, including adult onset primary open angle glaucoma, pseudoexfoliation glaucoma, juvenile open angle glaucoma, and others. Her research, which has been continuously funded by the National Eye Institute for over 15 years, has provided critical information regarding the biology of the disease. Ongoing studies may greatly improve current methods of diagnosis, and lead to more effective and specific therapies.

Dr. Wiggs is the Associate Director of the Howe Laboratory, Associate Chief for Clinical Research, and co-directs the Glaucoma Center of Excellence with Louis Pasquale, MD. Together, Drs. Wiggs and Pasquale, and Howe Laboratory Director Richard Masland, PhD, have led successful efforts to cultivate interdepartmental collaboration within HMS, as well as multicenter partnerships with other universities and hospitals in the U.S. and abroad. Dr. Wiggs is also co-chair of the US INDO Joint Working Group, an international vision research partnership initiated in 2005 between the National Eye Institute and the Indian government's Department of Biotechnology. The goal of the group is to advance vision science through collaboration of ideas, expertise, and resources. In 2011, Dr. Wiggs was also tapped to lead the Genetic Diagnostics Section of the department's new Ocular Genomics Institute. In this role, she will closely collaborate with Dr. Eric Pierce, who has been recruited as Director, to develop the institute into a premier Center of Excellence for leading genomics-based advances in patient care.

Dr. Wiggs lectures nationally and internationally and is the recipient of numerous awards including, most recently, a Lew R. Wasserman Merit award from Research to Prevent Blindness.


DOUGLAS J. RHEE, MD

Institute, is studying how psychophysics—or the relationship between a physical stimulus and the subject's perception—can be used to detect the early signs of glaucoma. Using a technique known as *equivalent noise analysis*, Dr. Bex showed that motion sensitivity decreases with age and even further with POAG. This visual function test was sensitive enough to potentially distinguish unhealthy retinal ganglion cells from those that have already died—something that current tests cannot do. These findings were reported June 2007 *in Investigative Ophthalmology and Visual Science,* and Dr. Bex is currently working with Dr. Pasquale to further develop this psychophysical testing method for use in clinical settings.

PREVENTION & NEUROPROTECTION

One of the goals of optic nerve research is to develop novel therapies that ultimately protect the nerve from damage. This endeavor requires understanding the molecular, cellular, and physiological processes of optic neuropathies—including the factors that cause ocular hypertension (increased IOP), which is currently the only treatable risk factor for glaucoma. Ongoing research seeks to improve existing strategies and to find new therapeutic targets for lowering IOP. Because ocular hypertension does not occur in all forms of glaucoma, there is also a great need for additional neuroprotective approaches.


TATJANA C. JAKOBS, MD

Combating ocular hypertension: SPARC-ing interest in the extracellular matrix

In an eye with normal IOP, aqueous humor production is balanced by its drainage through the *trabecular meshwork,* a spongy tissue structure that resides at the "open angle" where the cornea and the iris meet. The trabecular meshwork is impaired in many types of glaucoma (including POAG, the most common form), and many therapeutic strategies focus on improving drainage through this tissue.

It is known that deposits of extracellular matrix material on the trabecular meshwork can block the aqueous humor outflow, and Douglas Rhee, MD, Associate Professor of Ophthalmology, is studying the role of matricellular proteins in maintaining normal IOP. Dr. Rhee previously showed that human trabecular meshwork cells normally express high levels of the matricellular protein SPARC (secreted protein, acidic and rich in cysteine), and hypothesized that this protein may somehow regulate IOP. Thus, Dr. Rhee and colleagues generated mice that are deficient for the gene that encodes the SPARC protein, and found that SPARC-null mice had significantly lower IOP than their wild-type counterparts. This suggests a pivotal role for SPARC in IOP maintenance, and establishes this protein as a novel therapeutic target in glaucoma management. Dr. Rhee is currently investigating the mechanisms SPARC and other matricellular proteins utilize to control IOP.

Protecting the optic nerve by targeting non-nerve cells

Efforts to develop neuroprotective therapies for glaucoma have focused mainly on the retinal ganglion cells that form the optic nerve. HMS Assistant Professor, Tatjana Jakobs, MD is examining how another group of cells called *astrocytes* may affect the retinal ganglion cells. She and her collaborators found that the early signs of optic nerve damage occur just where the optic nerve leaves the retina, in a region called the *optic nerve head* or the *optic disc.* This area contains many astrocytes, and Dr. Jakobs is examining how they affect optic nerve health. Dr. Jakobs and colleagues have closely examined the shape and arrangement of astrocytes around the optic nerve head, and noted that they are clearly altered wherever the optic nerve is damaged. However, it is unclear whether astrocytes serve a neuroprotective role, or if they contribute to optic nerve damage. Dr. Jakobs is currently using a mouse model of glaucoma to address the exact role of astrocytes in optic neuropathy. These studies may potentially lead to novel neuroprotective strategies that target astrocyte function.

Structural remodeling of fibrous astrocytes unveils potential new disease targets

One of the barriers in pursuing these types of studies has been the difficulty in imaging astrocytes and tracking the anatomical changes that occur associated with disease progression. It has long been known that injury to nervous tissue produces a "glial scar," but it has previously been impossible to observe the cellular events that occur during scar formation because the closely packed cells obscure each other. Daniel Sun, PhD, a fellow working with Drs. Jakobs and Masland, solved this problem by using a transgenic mouse strain in which only a few members of the resident astrocyte population express GFP so they could be observed in isolation. They studied astrocytes of the optic nerve, the corpus callosum, and the cortical gray matter. Unexpectedly, they found a multistage remodeling of the fibrous astrocytes. First, they retract and thicken their long processes. In an intermediate stage, the astrocytes appear to migrate. Finally, they re-extend long processes into an unstructured and overlapping fibrillary network, the final scar. These events occur in damaged white matter. This is different from the behavior of protoplasmic astrocytes in gray matter, previously thought to be canonical, which respect to each others' territories. These studies were published in the *Journal of Neuroscience* in 2010 and set the stage for possible points in the disease process where novel interventions can be directed. Co-authors for this study include Drs. Sun, Masland, Jakobs, and research technician, Ming Lye-Barthel.

OPTIC NERVE REGENERATION

Like most neural tissues in the adult central nervous system, the optic nerve cannot regenerate appreciably once injured. Nonetheless, because the optic nerve is readily accessible, it is one of the standard laboratory models in neuroregenerative research. One of the ultimate goals of ophthalmology—and the entire field of neurobiology—is to uncover the mechanisms of optic nerve regeneration.

Overcoming barriers to optic nerve regrowth

Axons in the central nervous system can potentially re-grow after injury, but this normally happens only in the early stages of development. Dong Feng Chen, MD, PhD, discovered that during development, optic nerve cells lose neuroregenerative abilities around the time they stop expressing Bcl-2, a regulator of cell survival. This also coincides developmentally with the maturation of astrocytes, which are cells that form scars around damaged nerves and prevent regrowth. Dr. Chen and colleagues found that by inhibiting astrocytes and inducing Bcl-2 expression, they could stimulate regrowth in severed optic nerve fibers. More recently, Dr. Chen and colleagues used a pharmaceutical approach to promote optic nerve regeneration. They tested the effects of two drugs: lithium, which stimulates Bcl-2 expression, and alpha-aminoadipate, which selectively kills astrocytes. While neither drug alone had significant effects on severed optic nerves, in combination they stimulated robust nerve regeneration in adult mice. These results present a novel therapeutic strategy for inducing neural regeneration in the central nervous system.

Ongoing studies in Dr. Chen's laboratory are targeting the remaining barricades to optic nerve regeneration—such as factors that might prevent nerves from elongating or forming functional connections once they reach their targets. Dr. Chen is also identifying novel epigenetic targets in neurodegenerative diseases, and attempting to activate resident neural stem cells in the eye to potentially restore vision after optic nerve damage.


LARRY BENOWITZ, PhD

Links between inflammation, degeneration, and regeneration in the optic nerve

In mature optic nerves, inflammation can have very divergent effects. On one hand, inflammation can lead to death of the retinal ganglion cells (RGCs) that form the optic nerve; on the other hand, inflammation can stimulate neuroregeneration to some extent. These phenomena are some of the main research interests of Larry Benowitz, PhD, Director of the Laboratories for Neuroscience Research in Neurosurgery at Children's Hospital and Professor of Surgery and Ophthalmology at HMS.

In collaboration with Toru Nakazawa, MD, PhD, and Joan Miller, MD, Dr. Benowitz showed how inflammation might mediate the harmful effects of elevated IOP in a mouse model of glaucoma. In the retinas of mice, elevated IOP induced tumor necrosis factor-alpha (TNF-alpha), a major inflammatory molecule. The elevated TNF-alpha activated immune cells called microglia, which in turn killed the oligodendrocyte cells that produce the protective myelin sheath of nerve fibers. These events resulted in the death of RGCs that form the optic nerve. Using an antibody that blocked TNF-alpha action, these investigators and colleagues were able to prevent the RGC death caused by elevated intraocular pressure. Similar protection of RGCs was seen in mice that lacked either the gene that encodes TNF alpha or one of its receptors. This study suggested that inhibition of TNF-alpha function might offer neuroprotection in elevated IOP. Because drugs that block TNF-alpha are already FDA-approved for treating other conditions, this approach presents (*continued on page 109*)


DONG FENG CHEN, MD, PhD

PROFILE SPOTLIGHT

A niche in eye-movement disorders sheds light on the developing nervous system

Dr. Elizabeth Engle, HMS Professor of Neurology and Ophthalmology, and a Howard Hughes Medical Institute Investigator, never expected to land in her current specialized research niche. But deep curiosity and a single patient encounter led her to become the world's primary researcher probing the genetics of strabismus, or misalignment of the eyes.

Her work, blending genetics with neuroscience, has defined a new category of congenital disorders that leave children unable to move their eyes in specific directions. These conditions impair vision and are often socially isolating; the eyes are fixed in abnormal positions, forcing children to hold their heads in odd positions just to see properly.

In 1992, as a neurology resident at Children's Hospital Boston, Dr. Engle met a little boy with droopy eyelids whose gaze was frozen downward. His father and 20 members of his extended family all had similar conditions. New tools had recently emerged in genetics, and Engle wondered if discovering the mutated gene behind the toddler's disorder might explain how he lost his eye control. Over tea one night in the family's home, she learned of another branch of the family with the condition and got permission to contact them. With enough family samples to do genetic studies, Engle realized she needed to make a foray into laboratory research. "I thought there was no way that I could ever run a lab," she recalls. "I didn't have a PhD and was never officially trained in the lab. I didn't even know how to make chemical solutions."

Nonetheless, Dr. Engle talked her way into a research fellowship in Children's Department of Genetics with Louis Kunkel, PhD, and Alan Beggs, PhD. Aided by their mentorship and resources, she identified the location of the gene mutated in the boy's disorder, then defined its neuropathology by conducting an autopsy of an affected family member. Eventually, she traced the boy's disorder to a single amino acid change in a protein called KIF21A, which carries specific cargo to growing nerve fibers. The subtle change apparently left the cargo stranded—leaving two of the boy's eye muscles without cranial nerve stimulation. Modeling the disorder —congenital fibrosis of the extraocular muscles type 1—in mice, Engle's lab is now studying how the mutations disrupt KIF21A's function, and will determine what its cargo is.

Since that first case, Dr. Engle has searched for other families with unusual congenital eye-movement disorders. She has built a database of more than 1,500 patients, which is large enough to pinpoint many rare genetic defects. She has developed a network of collaborators, allowing her lab to pool cases from all over the world. To date, she's discovered seven different forms of strabismus arising from a variety of genetic errors in brainstem motor neuron development.

Dr. Engle heads a National Eye Institute-designated strabismus diagnostics center at Children's. As a Howard Hughes Medical Institute investigator, she now receives steady financial support that she hopes will help her expand her research and, eventually, translate her discoveries into therapies.

Complex eye-movement disorders also make an ideal model for understanding more common central-nervous-system problems. While the brain contains millions of neurons, making mishaps difficult to identify, the eyes are relatively simple. Just six muscles move the eyeball, controlled by just three sets of cranial nerves, so the number of places things can go wrong is relatively finite.

"Think of the U.S. railroad system," Dr. Engle says. "Penn Station, with many trains and switches, is hugely complicated compared to a small Midwestern town with a single train going through daily. Yet understanding how that one train and switch work could help in figuring out Penn Station's complexities."

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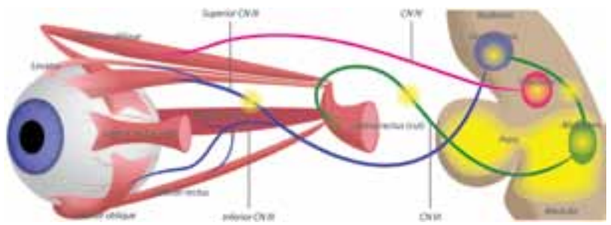

(*continued from page 107*)
a promising neuroprotective treatment for glaucoma. This study was published in 2006 in *Journal of Neuroscience.*

In 2006, Dr. Benowitz and colleagues also discovered that certain immune cells produce a calcium-binding protein called oncomodulin in response to inflammation, and that this protein could actually stimulate regeneration of optic nerves and other neurons of the mature central and peripheral nervous systems. Subsequent studies led by Dr. Benowitz confirmed that oncomodulin was indeed a link between inflammation and axon regeneration in the optic nerve. While examining the molecular mechanisms of oncomodulin in nerve regeneration, Dr. Benowitz and colleagues observed that it activated Mst3b, a signaling protein that could also stimulate regeneration in the optic nerve, as well as in other neurons in both the central and peripheral nervous systems. Because optic nerve regeneration not only requires reactivating axon growth, but also overcoming scar formation and other barriers to nerve regrowth, Dr. Benowitz and colleagues are testing these nerve regrowth factors in combination with other cellular, molecular, and pharmaceutical agents to maximize axon regrowth.