6th International Conference on the Tear Film & Ocular Surface: Basic Science and Clinical Relevance (Florence, Italy, September 2010)

Highlights from the Platform Sessions

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The 6th International Conference on the Tear Film & Ocular Surface: Basic Science and Clinical Relevance, was held in Florence, Italy, in September 2010. This Conference was sponsored by the Tear Film and Ocular Surface Society (TFOS; www.TearFilm.org) and was designed to:

- Assess critically the current knowledge and "state of the art" research on the structure and function of tear film-producing tissues, tears and the ocular surface in both health and disease.
- Promote an international exchange of information that will be of value to basic scientists involved in eye research, to clinicians in the eye care community, and to pharmaceutical companies with an interest in the treatment of tear film or ocular surface disorders.

Numerous topics were addressed during the 3-day Conference and highlights from the platform sessions are outlined below.

SUGAR CAN BE GOOD FOR YOU: GLYCOBIOLOGY AND MUCINS

New discoveries in the area of ocular surface epitopes and their potential applications in treating ocular surface diseases were discussed in detail during the opening session of the Conference.

As reported by Michael Hollingsworth (University of Nebraska Medical Center, Omaha, NE), mucins are secreted at the surface of various epithelia, eg, the cornea and conjunctiva, where they are responsible for lubrication, forming chemical barriers, and inducing as well as modulating cellular transcription. In the cytoplasmic tail (MUC1.CT) of one of these mucins, MUC1, 18 phosphorylation motifs can be differentiated. Subsequently, the specific pattern of phosphorylation defines the interaction with several transcription factors. The phosphorylated MUC1.CT is translocated into the nucleus and associates with transcription factors, such as β-catenin, y-catenin, Era and p53, at gene regulatory elements. As such, MUC1 is a potent inducer of angiogenesis mediators and extracellular matrix component remodeling. This induction could be shown in MUC1-overexpressing cells and required a phosphorylation of MUC1.CT at distinct tyrosine motifs. These findings indicate the capacity of mucins to interact with transcription factors and modulate signaling cascades.

According to Ilene Gipson (Schepens Eye Research Institute and Harvard Medical School, Boston, MA), the mucous properties of goblet cells and their various mechanisms of action have also been an area of increased interest recently. It is widely accepted that inflammatory conditions not only increase the number and activity of goblet cells in the lungs, but also enhance goblet cell hyperplasia and overproduction of mucus. Furthermore, exposure to allergens leads to differentiation of Clara cells into goblet cells. In experiments using a transgenic mouse model conducted at Cincinnati's Children's Hospital, SAM-pointed domain-containing Ets-like factor (**SPEDF**) was able to significantly increase the number of goblet cells in airway epithelium without inducing inflammation. It also caused Clara cell differentiation into goblet cells and was up-regulated after allergen exposure. In contrast, SPEDF-deficient mice did not develop goblet cells in upper airway mucosal glands, indicating that SPEDF is an essential factor of goblet cell differentiation in chronic pulmonary diseases. Its possible function in ocular goblet cell development needs to be examined.

Scientists generally agree that a major function of mucous epithelia is its ability to form barriers. However, there remain a considerable number of differing opinions on how this is accomplished on the molecular level. Regarding one possible component of these barriers, recent data point toward the participation of O-glycans in barrier function by

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interacting with galactoside-binding lectins on the glycocalix. Using confocal microscopy and fluorometric assays, downregulation of O-glycans increased active transcellular nanoparticle delivery in human corneal epithelial cells, reported Ana Guzman-Aranguez (Complutense University, Madrid, Spain). In vivo O-glycans in mucous layers can, therefore, protect subjacent cells from nanopartical penetration. For possible treatment, transient manipulation of these proteins could potentially improve delivery of therapeutic substances through mucous layers.

Mucins are thought of as a promising target of therapeutic consideration; diquafosol for the treatment of dry eye syndrome is but one example. Diquafosol is a widely known P2Y2 agonist that is now available in Japan but still waiting for FDA approval in the U.S. Clinical studies at Santen Pharmaceuticals (Nara-Osaka), said Yuko-Takaoka Shichijo, focused on the drug's mucin secretion-enhancing properties in rabbit conjunctival tissue, specifically focused on MUC5AC. In these studies diquafosol increased secretion of mucin-like glycoproteins and MUC5AC in goblet cells, and partly increased secretion in apical parts of conjunctival epithelium via intracellular calcium pathways. BAPTA-AM, a calcium-chelating agent, was able to inhibit the secretion. Whether these new findings are scientifically strong enough to garner regulatory approval in the U.S. remains to be seen.

As this opening session showed, mucins remain an exciting fundamental and therapeutic research target in ophthalmology.

INTERNATIONAL DRY EYE WORKSHOP: UPDATES

Since publication of the 2007 Report of the International Dry Eye WorkShop (DEWS; *The Ocular Surface*, April 2007), the definition and classification of dry eye has changed little. However, as noted by Anthony Bron (University of Oxford, Oxford, UK), the means to characterize dry eye and its severity have improved and have implications for treatment selection, outcome prediction, recruitment to clinical trials, and an understanding of mixed phenotypes of dry eye. This has been made possible by better standardization and the introduction of new diagnostic techniques.

New information about the epidemiology of dry eye has also been generated during the past 3 years, according to Kelly Nichols (Columbus, OH). The global prevalence of dry eye is about 17%; several studies, however, show a higher prevalence of approximately 30% in people of Asian descent. The 10-year incidence rate in an older population was found to be 21.6%. Previous work on risk factors has been corroborated – for example, dry eye is significantly associated with age, female gender, and antidepressant and oral steroid use. In males, benign prostatic hyperplasia and its medical treatment (eg, antiandrogens), as well as hypertension, have been determined to be additional risk factors. The incidence of dry eye has been found significantly lower, however, in individuals using angiotensin-converting enzyme inhibitors or having a sedentary lifestyle. Further research is still needed, particularly in the area of the disease's natural history and the impact meibomian gland dysfunction (**MGD**) may have on disease development and/or progression.

Diagnosis and ongoing treatment strategies remain nonstandardized, but technologic advances allowing for ocular surface and tear meniscus imaging (such as infrared meibography and in vivo confocal microscopy) continue to promote minimally invasive processes.

According to Murat Dogru (Keio University School of Medicine, Tokyo, Japan), since 2007 there have been significant advances in technologies to diagnose and monitor dry eye disease. These include those related to ocular surface and tear meniscus imaging, such as infrared meibography, evaluation of lipid layer thickness and rheology, in-vivo confocal microscopy, other optical coherence tomography anterior segment imaging devices, and tear meniscus volume quantification. One of the striking developments is in assessment of tear osmolarity, which is the strongest biomarker associated with dry eye disease severity. New osmolarity measurement devices are easy to use and may advance tear osmolarity measurement as the gold standard for diagnosing dry eye and monitoring the efficacy of treatment.

No new pharmacologic treatments have been approved in either North America or Europe in the past 3 years, reported Michael Lemp. Areas of therapeutic interest include immune-modulating and anti-inflammatory agents, with several clinical trials underway. Additional treatment modalities include polymer-eluting contact lenses, agents to aid in repair of tissue damage, artificial tears with tear stabilizing and anti-evaporative effects, the controlled release of heat in MGD treatment, and the, as yet, off-label use of topical macrolide anti-microbials. As suggested by Michael Lemp and Gary Foulks, breakthroughs in clinical treatment may well await the development of better endpoints (eg, biomarkers) and metrics for assessing improvement in signs and symptoms in clinical trials.

According to Ilene Gipson, an active and energized research effort to understand the pathologic mechanism of dry eye disease has been ongoing since the 2007 DEWS Report. A consensus is beginning to be built regarding tear/epithelial surface changes in dry eye that will facilitate the development of testable hypotheses of the disease mechanism(s).

INTERNATIONAL MEIBOMIAN GLAND DYSFUNCTION WORKSHOP: REPORTS

Meibomian gland dysfunction may well be the leading cause of dry eye disease throughout the world. However, although this condition influences the health and well being of millions of people, there is no global consensus on the definition, classification, diagnosis or therapy of MGD. To achieve such a consensus, TFOS, a non-profit organization, launched the International Workshop on Meibomian Gland Dysfunction (www.tearfilm.org/mgdworkshop/index.html) in 2008. The objectives of the MGD Workshop were to: 1) conduct an evidence-based evaluation of meibomian gland structure and function in health and disease; 2) develop a contemporary understanding of the definition and classifi-

cation of MGD; 3) assess methods of diagnosis, evaluation and grading of severity of MGD; 4) develop appropriate norms of clinical trial design to evaluate pharmaceutical interventions for the treatment of MGD; 5) develop recommendations for the management and therapy of MGD; and 6) create an executive summary of recommendations for future research in MGD. The Workshop, which required 2 years to complete and finalized its report in late-2010, involved the efforts of 50 leading clinical and basic research experts from around the world. These people were assigned to subcommittees, reviewed published data, and examined the levels of supporting evidence. Subcommittee reports were circulated among all Workshop participants, presented in open forum and discussed in an interactive manner. The conclusions and recommendations of the TFOS International MGD Workshop were presented in this session.

J. Daniel Nelson (Health Partners Medical Group, Minneapolis, MN) summarized the proposed new definition and classification of MGD, and Erich Knop (Charité University, Berlin, Germany) discussed the anatomy, physiology, and pathophysiology of meibomian glands. After Ben J. Glasgow (Jules Stein Eye Institute, Los Angeles, CA) spoke about tear film lipids and lipid-protein interactions in health and disease, the latest findings in epidemiology and risk factors for MGD were presented by Debra A. Schaumberg (Harvard Medical School, Boston, MA). Additional presentations were by Alan Tomlinson (Glasgow Caledonian University, Glasgow, UK) on the evaluation, diagnosis and grading of severity of MGD, by Gerd Geerling (University of Wuerzburg, Germany) on the management and therapy of MGD, and by Penny Asbell (Mount Sinai Medical Center, New York, NY) on the design and conduct of clinical trials.

It is anticipated that the reports of the MGD Workshop will be published in early 2011.

THE TEAROME: NOT JUST HYPE

Human tear fluid is a unique composition of proteins, electrolytes, lipids, enzymes, cytokines, sugars, growth factors, immunoglobulins, and many other known substances—and probably a few that have yet to be discovered. Targeted analysis of tear components may help in diagnosing various ocular diseases, as changes in tear composition is often the cause or effect of pathologic processes.

Michal Schwartzman (New York Medical College, Valhalla, NY) presented the results of a study on the importance of eicosanoids (derivatives of arachidonic acid) as a tear film component. Eicosanoids form a considerably large group of molecules, which are synthesized via three different pathways: Prostaglandins, prostacyclin and thromboxane are generated through cyclooxygenase (**COX**); hydroxyeicosatetraenoic acids (**HETEs**), leukotrienes and lipoxins are synthesized by lipoxygenase (**LOX**); and cytochrome P450 (**CYP**) produces epoxyeicosatrienoic acids and HETEs. To evaluate the influence of the different pathways during inflammation and convalescence, the researchers analyzed tears and corneal tissue by using HPLC-mass spectrometry-based lipidomics, among other procedures. They demonstrated strong increases of COX-, LOX- and CYP-derived eicosanoids after epithelial damage. These mediators not only derived from the injured epithelium, but also from invading inflammatory cells. In human tear film, eicosanoids reach concentrations of pico- to nanograms. As the eicosanoids show both inflammatory and anti-inflammatory activities, they may play a role in almost every step of post-injury responses from inflammation to wound healing.

Harumitsu Hirata (Thomas Jefferson University, Philadelphia, PA) reported the findings of experiments designed to identify membrane receptors that mediate the corneal afferent response to tear-inducing stimuli (eg, corneal drying, tear hyperosmolarity). His results show that specific types of cold-sensitive corneal afferents, previously reported to be the afferent limb of the basal tearing reflex, contain the nerve membrane receptor, transient receptor potential cation channel subfamily M member 8 (**TRPM8**). The activation of TRPM8 may produce basal tears. Further, his results suggest that a heterogeneous pool of receptors (eg, TRPA1) also exist in these afferents. Dr. Hirata hypothesized that damage to these receptors, which may occur after ocular desiccation or tear hyperosmolarity, may lead to dry eye.

Kazuo Tsubota (Keio University School of Medicine, Tokyo, Japan) presented new anti-aging approaches for dry eye therapy. In animal models of mice and monkeys, calorie restriction and oxidative stress control altered physical appearance of older animals as well as dry eye parameters such as tear film breakup time (**TFBUT**) and fluorescein scores. Being a dry eye patient himself, Dr. Tsubota evaluated anti-aging theories for dry eye treatment in self-experiments over a 10-year period of time. Daily exercise, controlled dietary intake and "gokigen attitude" (gokigen: Japanese for "happy") with at least 7 hours of sleep not only dramatically reduced his biological age but increased his Schirmer test values from almost 0 mm in 1985-2002 to 35 mm in 2009. He emphasized the need for further research on aging mechanisms of lacrimal and meibomian glands to better adapt anti-aging therapy to dry eye causes.

Miguel C. Seabra (Imperial College, London, UK) provided an update on the regulation of membrane traffic such as granule exocytosis. His group has examined Rab GTPases, key players in membrane traffic, and their role in melanosome biology in skin melanocytes and retinal pigment epithelium. In these areas, Rab27a regulates melanosome motility. Loss-of-function mutations in Rab27a result in Griscelli syndrome, a disease defined by partial albinism and immunodeficiency due to decreased T cell killing activity. Knock-out of Rab27a leaves a related protein, Rab27b. Knock-out studies suggest Rab27b plays an essential role in platelet dense granule secretion. Some cells, eg, mast cells, even express both proteins. Surprisingly, in these cells, failure of one of the two proteins results in opposing effects, such as hypo- and hypersecretion. The researchers assume different expression patterns in different tissues, with the possibility of complementary or opposite activities, but noted more research is warranted.

LATE-BREAKING NEWS: SJOGREN SYNDROME

Sjogren syndrome (**SS**) is an autoimmune disease characterized by dryness of the eyes and mouth that can result in complaints of photophobia and lead to severe discomfort. The disease predominantly affects women (90% of those affected are female), and most are middle-aged or older. Numerous studies asking patients to describe their health-related quality of life indicate a reduced well-being in primary SS, identifying fatigue and pain as the major irritation factors. Although researchers presume a complex genetic background is causative, currently about 20 target genes are being investigated, and none has been confirmed even in studies including more than 200 cases.

The Sjogren's Genetics Network, an international collaborative group, may have achieved a breakthrough, according to research presented by Kathy L. Moser (Oklahoma Medical Research Foundation, Oklahoma City, OK). Using high-density genotyping arrays, 272 primary SS cases and 387 healthy controls underwent an initial genome-wide association scan to identify potential gene loci responsible for SS predisposition. The region associated most significantly with disease risk is the major histocompatibility complex (**MHC**), whose gene products are displayed on cell surfaces and are responsible for antigen presentation and lymphocyte recognition. Overall, 45 single nucleotide polymorphisms surpassed genome-wide significance, all of them lying within the MHC. In addition, 659 genes have been found suggestive, containing 66 not associated with MHC. One gene showing some promise is musculin (**MSC**), which codes for a transcription factor that is activated downstream of B cell receptor activation and can help cells to avoid apoptosis. An association of MSC with type 1 diabetes was also recently identified. Other genes previously linked to multiple autoimmune diseases, such as PTPN22, IRF5, STAT4, TNIP1 and others, are now correlated with SS as well. The mechanisms of action and how these genes may affect self-tolerance in SS patients still need to be determined.

Disease diagnosis and differentiation from other types of dry eye (particularly keratoconjunctivitis sicca [**KCS**]) remain problematic for those studying SS, noted Barbara Caffery (University of Waterloo, Waterloo, Canada). In a study with 25 cases each of SS, KCS, and non-dry eye controls, numerous diagnostic tools—including rose bengal staining, total tear protein, lipocalin and lysozyme concentrations, as well as MUC1 and MUC16 protein and mRNA, collected by impression cytology—were used to compare and evaluate distinct differences between the three groups. Temporal conjunctiva of SS patients showed significantly more staining than conjunctiva of KCS cases or healthy controls. Total protein and lipocalin concentrations were significantly lower in SS patients, whereas concentrations of soluble MUC1 and MUC16 were found significantly higher in the SS group than in the remaining two groups. SS may be more readily distinguished from other forms of aqueous deficient dry eye via tear composition analyses; surface cells may prove to be viable biomarkers as well. Rose bengal staining may be an easy method for eye care practitioners to quickly classify and diagnose SS patients.

Several early-stage studies are currently under way, including a completed phase 1 study on the use of Bacillus Calmette-Guérin to increase the subject's own tumor necrosis factor (**TNF**) production to selectively eradicate autoreactive T cells, according to Denise L. Faustman (Massachusetts General Hopsital & Harvard Medical School, Boston, MA). In the NOD mouse—one of the most commonly used mouse models for type 1 diabetes research—the proteasome protein LMP2 is altered, leading to increased apoptosis of autoimmune T cells in presence of higher TNF levels and subsequent regeneration of the primarily damaged organ. The same T cell error has been found in SS patients, suggesting a possible therapeutic use for TNF-inducing drugs in the future.

Troy Daniels (University of California, San Francisco, CA) noted that ongoing work of the International Sjogren's Syndrome Registry (SICCA) includes the continued development of new classification criteria and expansion of its data and specimen bank. To date, more than 1,900 participants have enrolled for baseline evaluations and over 500 for 2-year follow-up.

VISUAL AND OPTICAL EFFECTS OF TEAR FILM INSTABILITY

According to Carolyn Begley (University of Indiana School of Optometry, Bloomington, IN), patient complaints of visual disturbances such as blurry vision are a common symptom of dry eye syndrome. Two separate mechanisms are typically involved. In the first, tear instability or breakup over the pupil greatly impacts vision due to the high refractive index difference between air and tear film. In the second process, an irregular corneal surface is the cause of light scatter and subsequent micro-aberrations. Because the true underlying pathology most likely combines both tear film irregularities and surface structure changes in proportions that vary among patients, diagnostic devices are needed to determine and measure such changes in order to initiate an appropriate treatment.

Larry Thibos (Indiana University School of Optometry, Bloomington, IN) summarized existing technologies to measure the optical effects of tear film instability. Some devices, such as videokeratoscopy and interferometry, are used to detect rapid changes in the tear film surface that may be a major cause of visual impairment. However, to understand the visual disturbances associated with tear film instability and breakup requires optical measurement of the eye's entire optical system responsible for forming the retinal image. Dynamic wavefront aberrometry between blinks is the method of choice, because it provides a spatial map of changes in the refractive power of the whole eye across the pupil, including the component due to nonuniform thinning of the tear film. These spatial maps may be used to calculate the changes in retinal image quality that lead to symptoms of blurry vision. Dynamic aberration maps may also be useful for diagnosis of dry eye and for monitoring the efficacy of dry eye treatments.

Suk Kyue Choi (Inje University of Medicine in Gyeonggi, Korea) reviewed his group's research that evaluated the dynamic properties of wavefront aberrations and functional visual acuity in normal and dry eyes. Severity of dry eye was determined with Schirmer strips and TFBUT. The aberrometer was programmed to capture 10 images over the course of 10 seconds while subjects did not blink. The images were then analyzed for higher-order aberrations (**HOAs**), fluctuation index, and spherical and corneal aberrations. Dry eye patients showed significantly higher HOAs as well as fluctuations of HOAs compared to controls. As severity of aberrations correlated with dry eye status, these findings suggest wavefront aberration measurement as a suitable tool for estimating symptom severity and quality of vision.

Presuming tear film thickness and corneal surface changes can be precisely determined, outstanding questions surrounding visual impairment still remain. Yannick Nochez (Faculté de Médecine François Rabelais, Tours, France) quantified the visual impact of TFBUT in normal and dry eyes. To quantify scatter that compromises visual acuity, the Objective Scatter Index (**OSI**) was estimated by double-pass images in 10 dry eye cases and 10 healthy controls with normal TFBUT. Higher OSIs were found in patients with dry eye syndrome compared to normal eyes. He suggested the OSI be used as a progress parameter in dry eye therapy. Additionally, the OSI can evaluate the effect of eye drops on tear film quality and stability.

Directly comparing eyes with aqueous-deficiency and those with short TFBUT can help address the underlying question of how prominent a role tear film instability plays in the visual impairment of these eyes. Seika Den reported a study conducted at the Tokyo Dental College, in which 44 patients were divided into a short TFBUT (< 5s) group (**SB**), an aqueous deficient dry eye group (**AD**), and a control group. The groups were then analyzed for subjective symptoms, tear lipid layer thickness, visual acuity, tear film stability, goblet cell density, squamous metaplasia in bulbar conjunctiva, and mRNA expression of MUC5AC and MUC16. Of interest, patients in the SB group were significantly younger than those in the AD group, while subjective symptoms showed comparable scores. Visual acuity was increased similarly in both case groups compared to controls, corresponding to higher observed indices of irregular astigmatisms. Goblet cell density and mRNA expression of the two mucins showed no significant difference between cases and controls. These findings suggest that tear film instability impairs the visual function in patients with short TFBUT as much as in those with aqueous deficiency, even if epithelial damage is not as strongly present.

INFLAMMATION: A CAUSE OR CONSEQUENCE OF OCULAR SURFACE DISEASE

Numerous ocular diseases are caused by microbial infection and inflammation. Inflammatory conditions are often a consequence of pathological processes and can in turn induce subsequent alterations to the affected tissue. That series of occurrences also helps explain why the pathogenesis of some diseases is difficult to ascertain. As noted by Richard Blumberg (Brigham and Women's Hospital and Harvard Medical School, Boston, MA), it is often difficult to determine if the inflammatory process is the origin of a mucosal disease or just a consequence.

One such disease without a clearly defined pathogenesis is dry eye disease. Reza Dana (Schepens Eye Research Institute and Massachusetts Eye and Ear Infirmary, Boston, MA) discussed how different T cell subsets may contribute to dry eye development. To induce dry eye in female mice, the mice were placed in a controlled desiccating environment. The researchers activated Th1 T cells (secreting IFNγ) and Th17 T cells (secreting interleukin [**IL**]-17) in the mice and noticed a significant increase in cell numbers. To date, induction of Th2 cells has not been recordable. Under minimal Treg suppression, Th17 cells but not Th1 cells expanded, resulting in homing of pathogenic T cell subsets to the ocular surface through a coordinated expression of CC and CXC chemokines. Accordingly, both Th1 and Th17 cells may play relevant roles in dry eye induction and progression. Additional knowledge about the underlying mechanisms may lead to novel therapeutic approaches, eg, blocking of pathogenic cytokines or inducing regulatory factors.

Robert Hendricks (University of Pittsburgh, Pittsburgh, PA) noted that the recurrent inflammatory processes present in stromal keratitis and its underlying cause (herpes simplex virus [HSV]-1) result in progressive corneal scarring and reduction of visual acuity. Mouse models have been used to assign different (patho-)physiological functions to different types of T cells: While CD4+ T cells, especially Th1 and Th17, initiate the neovascularization and leukocyte infiltration that characterize this disease and lead to the described transformations of the cornea, nTreg cells have the potential to inhibit HSV keratitis (**HSK**) by blocking the activity of these effector T cells. Furthermore, when it comes to inhibiting recurrence of HSK, CD8+ T cells seem to gather in the surrounding tissue of the trigeminal ganglion and constantly monitor the affected neurons. By releasing IFN-γ and lytic granules, the CD8+ T cells are able to block HSV-1 reactivation without damaging the neuron. The commonly held belief that an unknown stimulus might trigger HSV-1 activation may not be valid; rather, the host's own immune system may be transiently weakened, allowing the recurrence. Theoretically, by augmenting CD8+ T cell function in the brain while simultaneously blocking CD4+ T cell function in the cornea could reduce the risk of HSV-1 reactivation and prevent subsequent alterations of the ocular surface.

De-Quan Li (Baylor College of Medicine, Houston, TX) reported that exaggerated immune responses to antigens can lead common allergies to cause severe tissue damage. His laboratory investigated toll-like receptor (**TLR**)-based induction of IL33 and subsequent potential allergic reactions. They were able to highly detect IL33 and its receptor ST2 in tissue specimen of patients with atopic conjunctivitis. In mice with induced allergic conjunctivitis, IL33 and ST2 transcripts were increased in corneal and conjunctival

epithelium, as well as in cervical lymph nodes. Additionally, CD11c+ dendritic cells and Th2 T cells infiltrated the conjunctiva, thereby increasing the expression of IL-4, IL-5 and IL-13. In human corneal epithelial cells confronted with various microbial components (eg, flagellin, dsRNA, and other ligands to TLR3, 5 and 2) or inflammatory cytokines (TNFα, IL-1β), IL33 was induced through TLR and NFκB pathways. IL33 itself stimulated expression of inflammatory mediators such as TNFα, IL-6, and various matrix metalloproteinases leading the researchers to conclude IL33 is a potent promoter of allergenic inflammation.

Is inflammation the cause or consequence of ocular surface disease? Laura Contreras-Ruiz (IOBA-University of Valladolid, Spain) said the answers may be found by examining the cellular contact structures. In her laboratory, a recent study tested various tight junction proteins of human corneal epithelial cells (eg, ZO-1 and 2, claudin-1 and 2, as well as occludin) for their susceptibility to IL-10, TNFα and TGFβ. After IL-10 stimulation, all tight junction proteins showed increased expression without altering the transepithelial electrical resistance of the cell layer. Conversely, although TNFα and TGFβ stimulation increased the expression of some of the proteins, their major effect was to dislocate the tight junction complex into the cytoplasm, resulting in a significant decrease of the transepithelial electrical resistance and the cell layer permeability. These data suggest inflammatory cytokine exposure results in a weakening of the barrier structures of the ocular surface.

THE BEST DEFENSE IS A GOOD OFFENSE: OCULAR SURFACE INFECTION

Microbial keratitis, an inflammatory condition of the cornea, is often accompanied by patient complaints of discomfort up to the level of moderate or intense pain, reddening of the eye, and, often, impaired eyesight. Known causes include bacteria, viruses, fungi and protozoa, all of which could benefit from new diagnostic and therapeutic strategies.

Staphylococcus, Pneumococcus and *Streptococcus* are the primary bacterial causes of keratitis. One of the specific pathologic mechanisms these bacteria use to infect host cells has now been discovered in *Streptococcus pneumoniae*. When healthy, the mucous layer on the cornea protects it from pathogen penetration. One component of this barrier function is MUC16, a membrane-tethered mucin that prevents bacterial adhesion. *S. pneumoniae* has been shown to secrete a protein that induces MUC16 shedding, allowing the bacteria to penetrate through the mucous. Sandra Spurr-Michaud et al (Schepens Eye Research Institute, Boston, MA) were able to isolate this protein—metalloproteinase ZmpC. the zmpC gene was not able to induce MUC16 shedding in cultured epithelial cells. A mutant of *S. pneumoniae* created to lack the zmpC gene was not able to induce MUC16 shedding in cultured epithelial cells. These findings are the first demonstration that bacteria can manipulate the membrane mucin barrier on wet-surfaced mucosae.

Suzanne Fleiszig (University of California, Berkeley, CA) reported that corneal epithelial defenses against bacterial traversal are separate from barrier function to fluorescein, and also from defenses against bacterial adhesion. Defenses against traversal are dependent on both calcium and MyD88. Both invasive and cytotoxic strains of *P. aeruginosa* can overcome defense against traversal given sufficient exposure time, with roles played by the bacterial type III secretion system.

Existing infections can be aggravated not only by bacterial virulence factors, but by the host's own immune system as well, researchers contend. Mihaela Gadjeva (Brigham and Women's Hospital and Harvard Medical School, Boston, MA) reported that the macrophage migration inhibitory factor (**MIF**), part of a host's innate immune response, may negatively influence the progression of infection, resulting in more severe damage and loss of visual acuity. Comparing wild-type to MIF-deficient mice both exposed to *P. aeruginosa*, the latter showed significantly increased inflammatory responses, improved bacterial clearance, and less neutrophil infiltration. Cellular responses induced by MIF led to improved bacterial invasion of epithelial cells. Furthermore, MIF induces the formation of lipid raft structures enriched with caveolin. These rafts have previously been shown to facilitate bacterial invasion. It may be presumed that MIF inhibition at the ocular surface can reduce damaging consequences of *P. aeruginosa* infection and may prove to be a beneficial therapeutic target.

Two fungi—Aspergillus and Fusarium—can cause devastating visual complications, such as corneal ulcers. Ocular trauma is a leading cause of fungal infection, but contact lens wear can also exacerbate growth of Fusarium and Aspergillus. In a recently published study at the Aravind Eye Hospital (Madurai, India), Eric Pearlman and colleagues (Case Western Reserve University, Cleveland, OH) examined tissue responses from patients with corneal ulcers caused by fungal infection. Corneal scrapings were analyzed for presence of fungal components. Additionally extracted RNA was investigated for toll-like-receptor 2 (TLR2), TLR4, Dectin-1 and 2, IL-1β, IL-8, TNFα, and IFNγ expression. TLRs and Dectins are responsible for fungal recognition and recruitment of inflammatory cells. Of the corneal ulcers tested, 64% were of fungal origin. In these ulcer samples, TLR2, TLR4, Dectin-1, IL-1β, IL-8, and TNFα expression was significantly increased compared to controls. Dectin-2 and IFNγ expression, however, were not distinctly different between the two groups, nor was there a difference between expression patterns during Fusarium or Aspergillus infection.

The most serious corneal infection is the Acanthamoeba keratitis, usually affecting contact lens wearers. Although this disease is rather rare, the severity of symptoms when it occurs underlines the need for fundamental research to fully understand the pathology of the infection and develop effective treatments, suggested Noorjahan Panjwani (Tufts University School of Medicine, Boston, MA). Dr. Panjwani discussed mechanisms of action responsible for the adherence of Acanthamoeba to the corneal surface and inducing of pathologic effects. The amoeba has been shown to

express mannose-binding protein (**MBP**), a transmembrane protein with cell surface receptor function that enables the protozoon to attach to the cell. Following this attachment, Acanthamoeba secretes a contact-dependent metalloproteinase in addition to various contact-independent serine proteinases. The inflammation of the cornea and the resulting pain are consequences of this proteinase release. The mammalian immune system, however, has developed quite efficient defense strategies: tear fluid of higher animals and humans contains anti-MBP-IgA and proteinase inhibiting factors protecting the eye from infection. Testing that theory, researchers exposed human epithelial cell cultures to Acanthamoeba. When tear fluid is added to the cell medium, it can successfully prevent cell damage. Unanswered questions remain about why the combination fails in cases of an amoeba infection. It may be possible to deliver the described defense facors in artificial tears or to deliver prophylactic therapies in corresponding contact lens solutions.

OCULAR SURFACE REGENERATION AND RECONSTRUCTION

For patients with congenital or acquired tissue deficiency or dysfunction, medical therapy alone is insufficient. Causes for often underlying limbal stem cell deficiencies may be autoimmune diseases, such as Stevens-Johnson syndrome, congenital dystrophies, or acquired corneal damage. Visual acuity is dependent upon corneal clarity – which depends on avascularity and epithelial integrity. In cases of severe corneal damage, decreased visual acuity or even loss of sight can occur. In patients who sustain chemical or thermal burns, the cornea can undergo conjunctivalization during repair, causing vessel migration and finally stroma opacification. However, in these cases, culturing the patient's own limbal stem cells may prove a viable therapeutic option, even for those who had failed earlier procedures, according to Graziella Pellegrini (Center for Regenerative Medicine, Modena, Spain) and colleagues. In a recently performed study with 112 burn victims (94 of whom had already undergone multiple unsuccessful surgeries), limbal stem cells were obtained from healthy (in unilateral burns) or spared (in bilateral lesions) limbus, cultivated and transferred to a fibrin substrate. After transplantation, 76.6% of all patients showed complete restoration of a transparent, renewing corneal epithelium and full recovery of visual acuity. Those deemed unsuccessful all failed in the first year post-operatively. Successfully treated eyes remained stable for a mean of 2.9 years (with up to 10 years of follow-up in some cases). Transplantation was considered successful when at least 3% of the total cells in the culture were p63-bright cell clones. A member of the p53 family of transcription factors, p63 presence may have the ability to predict transplant acceptance or failure.

According to Mark Rosenblatt (Weill Cornell Medical College, New York, NY), silk films may serve as improved materials for ocular surface reconstruction. Silk biomaterials are suitable for use in tissue engineering due to highly controllable material properties and lack of antigenicity. These films can act as substrates for the growth of immortalized and primary corneal epithelial cells, and surface modifications are able to direct epithelial cell movement.

If limbal stem cells cannot be obtained from the host (for example in cases of serious bilateral chemical burns), one potential solution may be so-called induced pluripotent stem cells. As reported by Shigeto Shimmura (Keio University School of Medicine, Tokyo, Japan), these are artificially derived from a non-pluripotent cell, typically an adult somatic cell, by inducing a forced expression of specific genes responsible for pluripotency (Oct4, Sox2, c-Myc, and others). The technique may allow patients lacking limbal stem cells to receive autologous cells without risking transplant rejection.

Stem cell therapy may offer benefits to those with dry eye as well. Several groups around the world are working to isolate glandular stem cells. Philipp Ackermann (Martin Luther University Halle-Wittenberg, Halle, Germany) and colleagues have been able to isolate and cultivate over the long-term murine lacrimal gland stem cells, potentially creating a model that could be transferred to human tissue as well. Masataka Ito (National Defense Medical College, Saitama, Japan) reported that progenitor cells from the ductal epithelium of the mouse lacrimal gland may permit glandular regeneration following severe ischemic atrophy. Eliminating dry eye syndrome via gland stem cell transplantation may be a possibility in the future.

Although stem cell research is not new, it is still an emerging area of study in ophthalmology. More research is needed in this comparably young but promising area, with the ongoing hope of being able to help patients in whom conventional therapy has failed.

DRUG, PRESERVATIVE AND CONTACT LENS SOLUTION INTERACTIONS WITH THE TEAR FILM AND OCULAR SURFACE

Based upon clinical and basic studies in the laboratories of Christophe Baudouin (Quinze-Vingts National Ophthalmology Hospital, Paris, France) and John Ubels (Calvin College, Grand Rapids, MI), respectively, long-term use of topical ophthalmic formulations (eg, antiglaucoma drops, contact lens solutions, artificial tears/prescription dry eye treatment) may induce harmful changes to the ocular surface. Symptoms and morphological aberrations vary, ranging from ocular discomfort and tear film instability to subconjunctival fibrosis, or epithelial apoptosis, or even an increased risk of glaucoma surgery failure. Preservatives used in the formulations—most commonly benzalkonium chloride (**BAK**)—appear primarily responsible for the damage. The side effects of these topical formulations—even if manifested in a very mild or in an unspecific matter—can be silent precursors of more severe processes, such as goblet cell loss, corneal nerve destruction, or conjunctival squamous metaplasia. Preservative-free alternatives are needed to prevent irreversible damage in patients who depend on long-term drug substitution.

The contact lens industry—one of the most rapidly

growing segments of eye care—must remain vigilant about ensuring compatibility across various material platforms and disinfectants, said Lyndon Jones (University of Waterloo, Waterloo, Canada). A good lens solution must combine high disinfection ability, end-of-day comfort for the user, and absence of long-term side effects. Traditional lens materials, such as polyHEMA in combination with other more hydrophilic monomers, tend to deposit proteins on their surfaces; the newer silicone hydrogels (SiHy), which have rapidly gained market acceptance, deposit lipids. These depositions require specific care formulations and regimens to remove the proteins and lipids. Recently, research has determined certain lens solutions, when used on particular lens materials, produce abnormally high corneal staining levels. Although there may not be any complaints of ocular discomfort, this is an unacceptable corneal response that should not be overlooked. These issues will continue to be in the forefront of future research in both contact lenses and lens care products.

As the number of contact lens users increases, so does the number of those with ocular surface diseases, such as dry eye, according to Giancarlo Montani (University of Salento Formazione Continua In Medicina, Lecce, Italy). Dr. Montani and colleagues recently evaluated the effect of different contact lens materials on tear film osmolarity in a group of patients with moderate dry eyes. The researchers showed a considerable increase in osmolarity in those who used Methafilcon A lenses, from an average of 323 mOsm/L to 340 mOsm/L after 7 hours of wearing. There was no significant increase in osmolarity with wear of Omafilcon A lenses. Thus, the measurement of tear film osmolarity can help clinicians identify materials that may exacerbate dry eye.

Treatment regimens already approved for dry eye patients need to be tested for compatibility with contact lens wear, stressed Jennifer P. Craig (University of Auckland, New Zealand). For example, a liposomal dry eye spray has been labeled suitable for use with all types of contact lenses. However, as SiHy has an increased ability for lipid uptake compared to other lens materials, there remains a question of bioavailability between these sprays and these lens types. In a recently published study performed at the University of Auckland, 31 participants wearing SiHy lenses were instructed to apply the lipid spray four times a day on one of their closed eyes. Visual acuity, noninvasive breakup time, and lipid layer quality were measured at days 0, 7, and 14; patients were asked to subjectively quantify comfort at days 7 and 14. At the final follow-up, the lenses were analyzed for lipid deposition. No significant difference in visual acuity between treated and control eyes was reported. Lipid layer thickness and tear film stability were considerably increased after lipid spray treatment; this correlated with the reported higher subjective comfort of the patient. There was no significant increase in lipid deposit into the lenses. The researchers suggest liposomal dry eye sprays may be suitable in contact lens users with mild dry eye disease.

Future challenges include development of lens care formulae that will not induce long-term damage to the ocular surface and ensuring compatibility between newer lens materials and products that are being developed to address other surface diseases.

MACRO TO MICRO: NEW IMAGING APPROACHES FOR UNDERSTANDING THE OCULAR SURFACE

Laser scanning confocal microscopy (**LSCM**) is commonly used to examine human corneas in vivo. It may also be possible to assess less transparent structures, such as the bulbar or palpebral conjunctiva. To that end, Nathan Efron and colleagues (Institute of Health and Biomedical Innovation, Queensland, Australia) used LSCM to compare morphological properties of the conjunctiva of 22 volunteers, including 11 soft contact lens wearers. The lens group had significantly thinner bulbar conjunctival epithelium compared to controls; superficial as well as basal bulbar conjunctival epithelial cell density was 91% and 79% higher in the contact lens group than in subjects not wearing contact lenses. Lens wear also increased conjunctival microcysts in number and size, while goblet and Langerhans cell density did not show significant differences. Dr. Efron suggested that LSCM may eventually be used to survey damaging effects of contact lenses, eye drops, or topical drug application.

Another diagnostic being examined as a potential tool for surface disorders is high resolution intravital microscopy. Roberto Weigert et al (National Institute for Dental and Craniofacial Research, Bethesda, MD) used high resolution microscopy to visualize complex secretory and endocytic pathways in cells of major secretory organs. Regulatory factors were analyzed in transgenic mice that expressed fluorescently labeled molecules, in order to follow the dynamics of secretory granules. The researchers found different regulation pathways than previously described in in vitro systems. Additionally, multiple functions of the actin-myosin complex activated during exocytic processes have been determined. These include the ability to 1) prevent the homotypic fusion between the granules, 2) provide a scaffold to prevent either the osmotic stress or hydrostatic imbalances from disrupting the collapse of the granules, and 3) to drive the collapse of the granules to the apical plasma membrane through the recruitment of the myosin II. Overall, this imaging approach enables one to dynamically dissect subcellular processes under physiological conditions where the three-dimensional architecture, signaling networks, and metabolic pathways are maintained and not altered, as may occur in in vitro or ex vivo models.

Ultra high resolution optical coherence tomography (**UHR-OCT**) may prove beneficial in diagnosing clinical signs of dry eye. According to Victor Perez (Bascom Palmer Eye Institute, Miami, FL), the corneal thickness of an individual varies when measured pointwise over the whole cornea. He presented the results of a study where parameters that are typically altered in dry eye patients were compared to the patient's Epithelial Irregularity Factor (**EIF**). This factor is calculated as the standard deviations of the corneal

epithelial thickness measured along the central 3 mm zone of the UHR-OCT images and provides information on how uneven a patient's cornea is on average. Parameters obtained for estimating the dry eye severity were corneal and conjunctival staining scores, TFBUT, Schirmer tests, and dry eye questionnaire scores. The dry eye questionnaire scores correlated most highly with the EIF, suggesting the EIF may be able to quantify subjective symptoms of dry eye patients and could also be used to study patients with pain and no clinical signs.

Analyzing inflammation of the ocular surface previously was limited to tissue probing or to use of artificial dyes, both considered invasive procedures. Two-photon microscopy may be able to characterize and differentiate inflammatory cells and grade the inflammatory processes without the need for invasive procedures, according to Philipp Steven (University of Lubeck, Germany). Two-photon microscopy, detecting autofluorescence and assessing fluorescence lifetime measurements (**FLIM**), was used to examine conjunctiva-associated lymphoid tissue of anesthetized BALB/c mice for the presence of T cells, B cells, macrophages, erythrocytes, and other cells. The tool successfully differentiated the cell types based on intracellular fluorophores and structural characteristics. The FLIM also allowed differentiation between adhesive and nonadhesive macrophages, and visualization of elastic fibers and collagen fibrils.

Another approach to ocular surface imaging was reported by Donald Korb (Korb Associates, Boston, MA). He described an ophthalmic imaging device that he and others invented, termed the LipiView Ocular Surface Interferometer. This device is intended for use in dry eye patients to capture, archive, manipulate, and store digital images of interferometric tear film observations.

NEW AND EMERGING DIAGNOSTICS AND TREATMENTS

Too many ophthalmic diseases lack uniform diagnoses and treatments. Franz Grus (Johannes-Gutenberg-University, Mainz, Germany) emphasized the importance of tear film proteomics in diagnostics and offered his view of how to integrate it into clinical routine. Methods such as gel-electrophoresis, microarrays, and mass spectrometry are commonly used in research centers worldwide. If they were to be integrated into ophthalmic diagnostics, they could facilitate detection of certain ocular diseases. A variety of peptide and protein biomarkers are consistently up- or down-regulated in specific ocular diseases, and these biomarkers can help distinguish between existing subgroups. Dr. Grus and colleagues have identified 10 biomarkers that may permit differentiation between aqueous and lipid-deficient dry eye (eg, calgranulin A, proline-rich proteins 3 and 4, a-anti-trypsin, human annexin). Before automated tear profiling can be used in large scale applications, much larger studies must be done to validate these early findings, a catalog of validated biomarkers must be developed, and standardizing algorithms must be established to obtain tear film. If these goals can be achieved, high throughput analyses of tear film proteins may be a useful tool for diagnosing and subcategorizing diseases such as dry eye.

It would be of considerable interest to know the relative distribution of aqueous-deficient dry eye, evaporative dry eye, or a combination of the two in a general patient population. Benjamin D. Sullivan (TearLab Corp., San Diego, CA) presented results of a prospective, multi-site clinical study with 224 patients across 11 sites in Europe and the USA that aimed to determine the frequencies of three dry eye subgroups by using Schirmer tests and MGD scores (Foulks/Bron grading system). Subjects with Schirmer scores of less than 7 mm, plus an MGD grade of 2 or less were qualified as aqueous-deficient only. Evaporative dry eye was defined as Schirmer scores of at least 7 mm coupled with an MGD grade of more than 2. The combined group comprised subjects with low Schirmer values (under 7 mm) but high MGD grades (more than 2). The more severe measurement between the two eyes was used in analysis. A total of 55% of the subjects had MGD. Fewer than 8% were quantified with aqueous-deficient dry eye alone, and 37% were categorized into the combined group. Across the entire patient population, 74% of the patients demonstrated signs of meibomian gland disease. The prevalence of MGD appears to be much higher than initially thought, and far outweighs that of pure aqueous-deficient dry eye in a general patient population. This supports the emphasis now being placed on developing better therapeutic approaches to MGD, Dr. Sullivan said.

SS has a history of being difficult to diagnose, according to Esen Akpek (Wilmer Eye Institute, Baltimore, MD). A retrospective analysis of patients who had primarily presented with dry eye symptoms and had undergone additional serologic work-up for SS found several features significantly associated with an eventual diagnosis. These were presence of dry mouth, Raynaud's disease, corneal staining, family history of systemic lupus erythematosus, diabetes mellitus type I, anti-SSA, anti-SSB, and rheumatoid factor. On the contrary, gender, age, duration of symptoms and presence of other co-morbid rheumatologic conditions did not show significant differences between the groups with and without SS. Actively using these symptoms to recommend specific serologic examinations in patients with dry eye symptoms will help to identify individuals with SS earlier in the course of their disease.

Numerous new treatments for ocular surface diseases have recently been introduced. James Chodosh (Massachusetts Eye and Ear Infirmary AND Harvard Medical School, Boston, MA) reviewed present and promising future approaches to corneal replacement. With more than 60,000 corneal transplants procedures performed worldwide every year, and a success rate of 90% at year 1 postoperatively, corneal transplants represent the most successful solid organ transplant today. However, 15 years after initial surgery, about 50% of the transplants have failed. The probability falls disproportionally with every surgery for a secondary successful transplantation after initial allograft rejection: the 5-year survival rate of a third corneal allograft is practically

zero. Newer alternatives are anterior lamellar approaches like keratolimbal auto- and allografts, Descemet's stripping endothelial keratoplasty (**DSEK**), Descemet's membrane endothelial keratoplasty (**DMEK**) and deep anterior lamellar keratoplasty (**DALK**). These reconstructive surgeries spare the patient's endothelial cells, thereby reducing graft rejection rates. Unfortunately, these procedures are contraindicated in patients with endothelial disorders. The use of keratoprotheses and bioengineered grafts has become an interesting and much-noticed procedure. The Boston keratoprothesis (also known as the KPro) is a completely artificial prosthesis that has been a therapeutic option since the 1990s; newer materials like silk are being examined for use as a transplant basis for cultured epithelial cells. The hope is that silk will further reduce graft rejection rates. Other bioengineered corneas are in the early stages of development, mostly as preclinical or phase I clinical trials.

Research on drug delivery mechanisms and devices is now examining possibilities such as nanomaterials. Alexander Kabanov (University of Nebraska Medical Center, Omaha, NE) discussed using polymer therapeutics for delivery of drugs, genes, and imaging molecules. Constructs like polymer micelles, cross-linked nanogels, and block ionomer complexes can incorporate medicine, nucleic acids, enzymes or antibodies, and may improve penetration through cellular membranes, pharmacokinetics and bioavailability, in addition to preventing early degradation. Specific nanomaterials can be "pre-loaded" into cells in the immune system, which will carry the drug to inflammation sites and release them in a controlled manner. Outside of ophthalmology, tumor therapy may benefit from these newer technologies, possibly by delivering cisplatin via folate-decorated micelles or using SP1049C, a polymeric micelle containing doxorubicin for highly resistant tumors.

MORE INFORMATION

The Abstract Book for the 6th International Conference on the Tear Film & Ocular Surface: Basic Science and Clinical Relevance includes all abstracts for the oral and poster presentations and can be found at: http://www.tearfilm.org/pdfs.

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