Early intervention vital in controlling ocular inflammation

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EARLY intervention is vital at the first signs of inflammation in ocular surface disease in order to prevent subsequent angiogenesis and corneal opacification and a potentially irreversible vision loss, according to Reza Dana MD.

"Inflammation plays a very critical role in virtually all pathological facets of ocular surface disease, but the aggressive suppression of inflammation, in particular very early in the course of a disease, such as alkali burns or in the early postoperative period after limbal stem cell transplantation, is absolutely critical. There is also tremendous potential now for the development of new and more effective and specific strategies to control these processes," he said.

Addressing the joint meeting of the European Society of Ophthalmology (SOE) and the American Academy of Ophthalmology (AAO), Dr Dana, head of the Cornea Service at Massachusetts Eye and Ear Infirmary and a senior scientist at the Schepens Eye Research Institute, Harvard Medical School, noted that there are three principal threats to the ocular surface: inflammation, angiogenesis and cell death, all of which are highly related to one another.

Dr Dana noted that one of the most critical mechanisms in early inflammation is the recruitment of cells from the intravascular compartment.

"With corneal disease this typically occurs at the level of the limbus, where the blood vessels become engorged, and through a very well coordinated series of mechanisms these vascular changes allow cells to adhere to the vascular endothelium. When these cells leave the vascular compartment, they enter the corneal matrix and respond to specific chemokines, which provide directional information to cells. These cells then go to the site of inflammation," he said.

Dr Dana said that very mild insults to the ocular surface and cornea typically result in infiltration of a modest amount of macrophages and neutrophils, innate immunen cells that infiltrate tissue and then usually die without inducing intense inflammation.

"If the insult to the ocular surface is mild these cells normally die, and there is no induction of T cells in response to that insult, so there is no memory to the immunity that is generated. We see this in mild cases of dry eye, trauma, or minor infections as well as many other types of ocular surface insult," he said.

Dr Dana noted, however, that the picture is very different for more serious cases of inflammation such as patients with severe perennial atopic keratoconjunctivitis or severe dry eye syndromes to name a few.

"In severe and chronic inflammation, the destructive process in the corneal matrix really is induced because of the activation of T cells, which, once amplified, lead to the development of memory in the immune system and then a process begins which can sustain itself over a long period," he said.

Dr Dana emphasised that induction of T cell activity does not normally occur in the ocular surface because of the presence of many immunoregulatory molecules. However, in cases of intense inflammation, the process typically begins in the limbal and peripheral corneal areas. "In dry eye, for example, virtually all of the pathogenic T cells are in the conjunctiva, not the cornea, but they can still cause corneal damage through release of cytokines," Dr Dana said.

"Regardless, however, the most immunoreactive area of the ocular surface is the limbus. The reason why inflammation typically begins in this area is because there is a very high concentration of immune cells called antigen-presenting cells in this area that can become activated. They then infiltrate the tissue, pick up the antigen and present it to T cells that can return and cause damage in the same area," he said.

Dr Dana stressed that in order for these cells to induce T cell mediated immunity, they not only have to go into the cornea but must also leave the cornea as well.

"This concept was proven in our laboratory where we removed the lymph nodes that drain the eye and we showed that in a model of corneal transplantation if the lymph nodes are removed, those allogeneic transplants survive indefinitely. We have determined the molecular mechanisms that allow for these cells to gain access to the lymphatics is mediated by vascular endothelial growth factor receptor 3 (VEGFR-3) and various molecules that bind these cells," he said.

Turning to angiogenesis, the process of blood vessel growth, Dr Dana said that research by the team at his laboratory has helped to unlock the mystery of why the cornea maintains its avascular structure, allowing light to be focused on the retina with minimal interference.

"We have shown that the corneal epithelium has a very high expression of VEGFR-3 that acts like a sink mechanism for VEGF-C and VEGF-D, preventing their binding to vascular endothelial VEGFR-2, and thereby functioning as a critical mechanism for maintaining corneal avascularity," he said.

In intense inflammation, however, the sink mechanism can be overwhelmed leading to corneal neovascularisation, he added.

Looking to the future, Dr Dana said that new strategies such as gene therapy hold out the hope of significantly suppressing the degree of cell death often seen in autoimmune diseases such as Stevens-Johnson Syndrome and ocular cicatricial pemphigoid.

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On the way to in-vivo histology

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CONFocal in-vivo microscopy reveals corneal detail on the cellular level, enabling researchers to discover new features of corneal innervation and learn more about the cells that play a pivotal role in corneal immunity. This offers the potential to help tackle some of the complications plaguing refractive surgery today, report researchers.

"We believe that confocal in-vivo microscopy with the Rostock Cornea Module (RCM) has more clinical potential than was thought up to now. This method provides a magnification of up to 800 times actual size, allowing us to sub-divide dry eye and wound healing processes after refractive surgery, examine the different forms of filtration cushions after fistulating glaucoma surgery, as well as understand more about the neoplastic changes of the cornea, conjunctiva, and eye lids. Examinations so far verify ex-vivo histology," said Rudolf Guthoff MD, head of the Rostock University Eye Clinic in Rostock, Germany.

The Rostock Cornea Module's enormous amplification potential allows investigators to break down the corneal layers into further component parts and observe variations in keratocyte cell densities. In this way, a quantitative determination of cells in the superficial, intermediate, and basal strata of the corneal epithelium is possible. Clinical observations, such as 'dry eye' can be investigated on the microscopic level. Similarly, changes in the corneas of contact lens wearers can be explained.

The epithelial layer's topmost stratum (5µm thick), for instance, has around 1000 epithelial cells, while the basal layer has closer to 10,000 epithelial cells, which can each be individually visualised with this technology, Dr Guthoff observed in a presentation of his work at the yearly congress of the DGII (German-Speaking Society for Intraocular Lens Implantation and Refractive Surgery).

Still deeper into the cornea, at roughly 50µm, this technology illuminates the nerve cell layer, as never before seen. While scientists once believed that corneal nerves grew radially inward toward the corneal centre, investigations with the RCM revealed that they grow in a spiral-like fashion. Knowing about the anatomy of corneal nerves and their growth pattern enables us to study the changes seen with nerve regeneration in patients with keratitis and after trigeminal nerve damage. It also provides refractive surgeons with better information about the nerve plexus they are disrupting and more clues on how nerves might regenerate after surgery, Dr Guthoff explained.

The RCM makes a three-dimensional image of the corneal strata, representing approximately 250µm cuts of the cornea. In LASIK patients, it shows the precise events...
TO PIC AL drops containing tetrapeptides derived from substance P and insulin-like growth factor 1 (IGF-1) appears to be a promising therapeutic modality for promoting repair of persistent corneal epithelial defects associated with neurotrophic keratopathy, reported Teruo Nishida MD, DSc, at the 2007 Joint Congress of the European Society of Ophthalmology and American Academy of Ophthalmology.

Dr Nishida reported outcomes from an IRB-approved, open-label, uncontrolled study including 20 eyes of 19 patients with a mean age of 67 years. The investigational peptide solution was formulated with FGLM-amide (Phe-Gly-Leu-Met-amide) derived from substance P and SSR (Ser-Ser-Ser-Arg) derived from IGF-1.

Patients were instructed to use the eye drops four times a day for up to 28 days. At the end of the study, complete epithelial resurfacing was achieved in 14 (70 per cent) eyes. Mean time to healing was 10.1 days and presence of intact stem cells was associated with a favourable response. The treatment was well tolerated and there were no treatment-related adverse events.

“Currently, there are no specific treatments for persistent corneal epithelial defects in eyes with neurotrophic keratopathy. Our experience with the peptide solution is very encouraging,” and we are now planning to perform a double-masked, randomised clinical trial to better characterise the efficacy and safety of this novel treatment for persistent epithelial defects in neurotrophic keratopathy,” said Dr Nishida, professor and chairman, Yamaguchi Graduate School of Medicine, Japan.

The dual peptide solution was developed based on a series of previous studies investigating neurotrophic mediators of corneal healing. Interest in the use of substance P, which is present in the sensory nerve fibres of the cornea, derives from the observation that its level in the cornea is reduced in eyes with neurotrophic keratopathy and persistent epithelial defects.

In initial studies, Dr Nishida and colleagues reported that substance P had no effect by itself on corneal epithelial migration. However, they found substance P and IGF-1 acted synergistically to stimulate corneal epithelial migration in vitro in a rabbit cornea organ-culture system. In vivo studies, they found the combination facilitated epithelial wound closure in both an animal model and humans with neurotrophic keratopathy-related persistent corneal epithelial defects.

Subsequently, Dr Nishida and co-workers investigated the minimal amino acid sequence necessary for obtaining the desired biological effects on wound healing for each of the two polypeptides. “We found that the tetrapeptide FGLM-amide derived from the carboxyl terminus of substance P mimics the function of the entire molecule and that SSR, a tripeptide present in the C-terminal domain of IGF-1, was the minimum essential sequence for stimulating corneal epithelial migration combined with substance P. Use of these minimal amino acid sequences may be preferred as they would be expected to have little antigenicity as opposed to the full-length polypeptides and also avoid the unwanted biologic effects of the parent molecules,” he said.

The patients included in the study were all treated at Yamaguchi University Hospital between January 2004 and June 2006. Inclusion criteria required patients to have loss of corneal sensation accompanied by a persistent epithelial defect present for at least two weeks despite conventional treatment. The mean ± SD duration for presence of the epithelial defect was 3.7 ± 4.7 months. Mean ± SD corneal sensation measured with the Cochet-Bonnet esthesiometer was 1.8 ± 4.2 mm and the mean Schirmer test result was 12.6 mm per five minutes.

The peptide solution was formulated by the hospital pharmacy under sterile conditions and contained FGLM-amide 1 mg/ml and SSR 56.3 mcg/ml with phosphate buffered saline as the vehicle. Treatment response was assessed using digital analysis of the defect size identified on slit-lamp photographs after fluorescein staining. At baseline, the epithelial defect represented 13 per cent of the total area of the cornea.

Among the 14 eyes that responded, healing occurred within three to 23 days of starting treatment (median 7.1). Seven eyes (35 per cent) achieved complete resurfacing within seven days of starting the topical peptide treatment, the defect healed between days seven and 14 in four eyes (20 per cent), and the three (15 per cent) remaining responders had healed by 23 days.

Additional analyses indicated that presence of intact stem cells was significantly associated with treatment response. “Of the 14 patients who achieved complete resurfacing, only two (14 per cent) had stem cell deficiency compared with three (30 per cent) of the six patients who had not achieved healing within 28 days,” Dr Nishida reported.

Dr Guthoff believes that researchers could easily take this technology a step further to perform in-vivo biopsies, to check for corneal pathologies. Also, the ‘activated’ keratocytes that are thought to cause corneal net formation in keratoconus patients could be amplified using the RCM 3-D imaging technology to reveal and possibly explain the complex network of interacting cells.

The eye comes into direct contact with the machine, using anaesthesia, artificial tears and a small PMMA cap, which covers the objective and helps stabilise the image seen through the microscope. A newer cap design features a thin groove along the contact side, which helps reduce the application pressure and allows investigators to examine the corneal epithelium without side effects.

Dr Guthoff suggested that femtosecond laser ablation sites could be amplified using the RCM 3-D imaging technology to reveal and possibly explain the complex network of interacting cells.