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The technique for KLAL utilizes a cadaveric cornea stored in transplant medium. The tissue is prepared by removing peripheral cornea and limbal tissue and transplanting it to the limbal area of the disease host eye. The epithelium and stem cells are very delicate and great care must be undertaken during the transplantation process. In the post-operative period, rejection of the transplanted tissue is a major clinical problem and, therefore, systemic immunosuppression is utilized for most patients.

It usually takes around three months for the new ocular surface to stabilize. Standard corneal transplantation can be undertaken once the ocular surface has stabilized. The likelihood of successful corneal transplantation is far greater after KLAL because re-establishment of the “skin” of the donor cornea by the patient is finally possible.

STATISTICS
We recently reported 25 eyes of 21 patients who underwent KLAL technique of ocular surface transplantation. Eighteen of the 25 eyes (72%) developed a stable ocular surface. Fifteen eyes (60%) demonstrated a significant improvement in visual acuity. Six of 13 eyes (46%) had successful subsequent corneal transplantation. Patients who developed a stable ocular surface had a marked reduction in pain and light sensitivity.

CONCLUSION
Patients with severe ocular surface disease are some of the most challenging in ophthalmology. Ocular surface transplantation procedures have significantly improved the success rate in managing these patients. Important issues regarding ocular surface transplantation remain to be studied. The role of systemic immunosuppression needs to be more clearly defined. In addition, the importance of tissue typing for these procedures should be studied further. Significant progress has been made in the management of severe ocular surface disease; however, continued studies are needed to improve the success rate in treating these patients.

REFERENCES

NEW LIGHT ON PTERYGLA

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SUMMARY
Pterygia are growths that start at the edge of the cornea (the clear part on the front of the eye) and slowly move onto the center of the eye. Pterygia appear to pull the conjunctiva towards the middle of the cornea. Eventually, if left untreated, the growth blocks light reaching the retina and leads to blindness. Pterygia are a very common problem in areas of the world that have a lot of intense sunlight, such as the equatorial countries, where they are found in more than 10% of the population. Pterygia affect approximately 5-9% of the people living in the southernmost parts of the United States. However, they are rare in the northernmost parts of this country except for people who work out-of-doors such as farm workers and fishermen.

Pterygia are a clinical problem described by the early Greeks (Hippocrates, Galen, Celsus, and others.) The name derives from the Greek word for wing, which describes the appearance of the tissue as it moves onto the cornea. However, as recently as 1931, Schieck wrote that it was not known whether a pterygium was a disease of the cornea or conjunctiva and, in 1961, Ida Mann stated that “this disease is still a mystery.” Until this decade, all that was known about the cause of pterygia was that its occurrence correlated with exposure of the eyes to sunlight. Major questions left unanswered were: how does sunlight cause the growth; why is it primarily found only on the nasal side of the eye; what cell is involved; and why does it recur so rapidly after it is removed?

Our current study shows pterygia occur because of a mutation (probably caused by UV light) in a gene that produces a protein involved in programmed cell death and growth control. Loss of this gene results in a cell that doesn’t die when it suffers DNA damage and doesn’t exhibit growth control. This condition allows further mutations to occur in the cell that could lead to an abnormally growing cell like those found in a pterygium. This study also shows that the cell involved in a pterygium is an epithelial cell that has a normal looking appearance. The normal appearance of the cell is probably why it is not removed during surgery and leads to recurrence of the growth. This work correlates with recent findings that light from the side of the head can be focused on the nasal side of the eye by the cornea at exactly the spot where pterygia form. This would lead to a higher rate of mutations on the nasal side of the eye and would suggest that simply wearing wrap
around sunglasses when in the sun should help to prevent pterygium.

BACKGROUND

As seen in the Illustrations A and C, pterygium grow from a region next to the cornea (called the limbus). This is an important area on the eye's surface that produces cells that are used to resurface the cornea. Cells that are capable of producing new daughter cells used for renewal are called stem cells. Work by T.T. Sun has demonstrated that such cells come from the limbal region. It was shown that these limbal epithelial stem cells would divide and produce a new cell that moves out onto the cornea and changes into a corneal epithelial cell. Dr. Sun also has obtained data that lead him to propose that stem cells tend to mutate and form abnormal growths. This means that stem cells would be a prime candidate for study as a potential cell which could result in an abnormal growth such as a pterygium.

The first question we wanted to answer was whether or not the epithelial cell (a surface cell) was the cause of a pterygium. When we began our studies, the current thinking was that the abnormal cell was a fibroblast. Fibroblasts provide the main mass of connective tissue in the body and usually lie dormant until injury occurs. In the cornea, the fibroblasts occur beneath the epithelial cells of the cornea. Fibroblasts were thought to be abnormal cells because they provide the main component of growth of the pterygium and do not grow in an ordered fashion (see insert to Illustration A) while the epithelial cells look quite normal and appear to exhibit little growth. However, due to Dr. Sun's hypothesis regarding the role of stem cells in abnormal growth, we felt it worthwhile to study the nature of the epithelial cells of a pterygium especially since this growth arises from a stem cell region of the eye. In order to do this, we looked for marker proteins (called keratins) in pterygia that allowed us to identify the origin of the different epithelial cells present in the tissue.

The second answer we sought concerned the mutations involved in this abnormal growth. Genes that cause abnormal growths such as cancers are called oncogenes. One oncogene that was a very good candidate was a gene that inhibits cell growth. The first gene discovered to produce a protein that inhibits cell growth was found by Dr. T. Dryja in studying an eye tumor called retinoblastoma. Since it was first found in a retinoblastoma, it was called the Rb gene; however, it is also found to be involved in many tumors elsewhere in the body. Shortly thereafter, another oncogene that is involved in inhibiting cell growth was found, the p53 gene. The name stands for a protein (p) that has a molecular weight of 53,000 that was found in abnormally growing cells. Not only was it later found to regulate cell growth (through interactions with the Rb gene) but, under certain circumstances, it would lead to programmed cell death (called apoptosis). If DNA of a cell is damaged, it will trigger the production of the p53 protein which produces a condition that causes the cell to die in a programmed manner. An example of this effect is seen in a sunburn. When UV light damages DNA in epithelial cells on the skin, it triggers the production of p53 which causes the cells to die and slough off. If, however, the p53 gene mutates and can not cause the cell to die, the cell will continue to grow and pass on its damaged DNA to the daughter cells (Illustration B). The p53 mutation also allows further mutations to occur in the cell and can eventually lead to a cell that grows abnormally. The fact that the p53 gene allows this sequence to occur is why mutations of the p53 gene are found in a high percentage of abnormal growths on the surface of the skin.

When the p53 gene is mutated, it also results in the buildup of defective p53 protein in the cell. Ordinarily, the concentration of p53 in a cell is very low but when it is defective, it is not destroyed as fast and is detectable in cells using specific antibodies. These p53 antibodies bind to the protein and can be detected by using immunohistochemical techniques. The presence of excess p53 protein in a cell that is not undergoing programmed cell death is found to indicate that the p53 gene is mutated in that cell. Thus, we used immunohistochemical techniques to look for the presence of p53 in pterygia.

TECHNIQUES

It has been shown by T.T. Sun and co-workers that proteins called keratin can be used as markers for the different epithelial cells found on the surface of the eye. Since a pterygium is an unusual condition where epithelial cells from the conjunctival, limbal and corneal regions are all growing on the surface of what used to be normal cornea, we looked for the presence of these keratins in the epithelial cells of pterygia using specific antibodies to the different keratins. This was done because it was also known that cells retain the presence of the keratin that indicates its tissue of origin even if it becomes cancerous. Frozen serial sections of surgically removed pterygia were used. The specimens were then treated with antibodies to the different keratins. If the antibody binds to a keratin in an epithelial cell of a pterygium, this antibody can then be detected by adding a second antibody that binds to the
first antibody and has an attached enzyme that catalyzes a reaction that causes a color change in the tissue. We found that the pterygium has epithelial cells from the limbus on its surface and is followed by tissue that has conjunctival epithelial cells. This was interesting but not unexpected. What was unexpected was that we found limbal epithelial cells rather than corneal epithelial cells on the cornea in front of the pterygia. This meant that although the cells in front of the pterygia looked like normal corneal cells they were an abnormal cell which we named the pterygium cell.

These same pterygium cells also expressed a marker for a protein (vimentin) that is only found in corneal cells under abnormal circumstances such as after a wound has occurred. This was further proof that these normal appearing epithelial cells were not normal.

The finding that these normal looking cells were really the abnormal pterygium cell meant that, although the underlying fibroblasts looked abnormal, they were not causing the pterygium since the pterygium cells were in front of the mass of proliferating fibroblasts. What we propose is that a limbal cell becomes mutated and turns into a pterygium cell. This cell then divides and starts to migrate slowly out onto the cornea. Ordinarily, when a limbal cell divides and moves over Bowman’s layer of the cornea (see Illustration A) it changes into a corneal epithelial cell. The pterygium cell over Bowman’s layer stays as a limbal type cell and leads to the dissolution of Bowman’s layer. This dissolving membrane triggers the underlying fibroblasts to start to grow, probably through the release of growth factors that are stored in Bowman’s layer. This growth then forms the mass of what is seen clinically as a pterygium.

In order to confirm this possibility, we looked for the p53 oncogene in the cells of pterygia. We found that the normal looking cells that we named the pterygium cells also contained abnormal p53 protein. The presence of abnormal p53 has now been shown in every pterygium that we have tested. Of further interest is the fact that the appearance of the abnormal p53 also occurs in the same cells (the pterygium cells) where we found the keratin for the limbal epithelial cells. Thus the pterygia cells with the mutated p53 gene precede the mass of the pterygium onto the cornea basement membrane.

When the pterygium cell divides and starts to migrate from the limbus, it moves out in all directions. However, when a pterygium is viewed clinically it only moves in one direction—towards the center of the cornea. We believe this is because it is only as the pterygium cell dissolves Bowman’s layer that the growth factors are released that stimulate the growth of the underlying fibroblasts. Pterygium cells moving in the opposite direction do not encounter a layer (like Bowman’s) that is a storage site for growth factors and thus no growing mass of cells appear. Thus, the pterygium mass is seen to move only in one direction (towards Bowman’s layer of the cornea).

However, what is still unknown is why the normally stationary limbal cells move behind the pterygium cells onto the cornea and bring the conjunctiva along behind them. At present we are exploring possible reasons for this occurrence.

**SIGNIFICANCE**

One important consequence of the finding that the pterygium cell is growing in the normal looking tissue around the mass of the pterygium is that this could explain why there is such a high recurrence rate of pterygia after they are surgically removed. Since the pterygium cells are around a pterygium and look normal, they are probably left behind after the pterygium is removed. They can continue to grow and produce a pterygium. Thus, it would be important during surgery to remove a larger margin around the pterygium than is current practice. The wider margin of removal would require scraping off the surrounding epithelial cells but would not require cutting more deeply into the cornea.

The finding of the mutation of a UV sensitive gene (Illustration B) is also consistent with the recent finding of Dr. Coroneo, in Australia, who showed that sunlight which hits the eye from the side is focused by the cornea on the limbus of the other side of the eye. He found that the cornea acts as a magnifying glass and increases the intensity 20-fold. This magnifying effect would explain why pterygia tend to form on the nasal side of the eye. Light coming from the nasal side would be blocked by the nose and would not cause a pterygium to form on the temporal side of the eye. In agreement with this hypothesis is the finding that most of the individuals who have a pterygia on the temporal side of their eye have a very low bridge to their nose.

The above results would also indicate that this same magnifying light effect might be the cause of limbal tumors. Recently we found that limbal tumors also have a p53 mutation in their epithelial cells.

**HUMAN INTEREST**

These results indicate that it is a good idea to wear sunglasses to protect your eyes from UV light, and the best type of sunglasses to wear would be those that wrap around the side of the head or that have a wide ear piece.