Inflammatory Response in the Early Stages of Wound Healing After Excimer Laser Keratectomy

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Objective: To evaluate the inflammatory response and its potential role in the early stages of corneal wound healing after excimer laser keratectomy.

Materials and Methods: Lewis rats underwent excimer keratectomy using a 193-nm excimer laser. The central corneas were ablated in 3 depths: group A, epithelium; group B, superficial stroma; or group C, deep stroma. Eyes were harvested 1, 12, 24, and 36 hours, and 1 week after the rats were killed. Immunohistochemistry was used to test frozen sections with monoclonal antibodies of various inflammatory cellular markers.

Results: Reepithelialization was observed at 12 hours in group A, and at 24 hours in groups B and C. Regenerated epithelium covered the denuded corneal surface in groups B and C after 1 week. The expression of major histocompatibility complex II antigen was detected in infiltrating cells, corneal epithelial cells, and endothelial cells 1 hour after surgery. Only a few macrophages and Langerhans cells were in the limbus at baseline. Macrophages migrated from the limbus to the corneal ablation zone and increased 2-fold after 36 hours in all 3 groups compared with baseline. Occasional lymphocytic infiltration was identified after 25 to 36 hours.

Conclusion: Macrophages play an active role in the wound healing after laser keratectomy and may contribute to transient corneal haze.


PHOTOREFRACTIVE keratectomy (PRK) alters the refractive power of the eye by reprofiling the anterior surface of the cornea. Excimer laser PRK has become a favored surgical procedure to correct refractive error including myopia, hyperopia, and astigmatism. Although the procedure is effective and safe with minimal side effects, some patients may develop a transient corneal haze and glare. Thus, it is important to investigate and understand the corneal wound healing process after excimer laser PRK.

Previous studies indicate that corneal wound healing after excimer laser PRK involves regeneration with controlled hyperplasia of the corneal epithelium and the deposition of new stromal collagen. In addition, the healing process in vivo involves interactions among various cell types within the cornea. Physical stimulation by excimer laser energy may also trigger an inflammatory response. In this study, we examined the inflammatory responses involved in the wound healing process after excimer laser PRK. Using the rat as an in vivo model system and using immunohistochemical techniques, we investigated the dynamics of macrophages, dendritic cells, and lymphocytes during the corneal healing process after excimer laser PRK.

Macrophages and dendritic cells are located in the conjunctiva, limbus, iris, and ciliary body of the eye. They belong to the mononuclear phagocyte system, function to present antigens, and act as immune effectors. These cells can release numerous cytokines and express adhesion molecules that interact with other cells of the immune system or surrounding tissues. Langerhans cells are specific dendritic cells that reside in the epidermal layer of the skin and in the limbus of the cornea. Compared with macrophages, Langerhans cells are more effective at presenting antigens, but less effective at phagocytosis.

Macrophages are classified as a heterogeneous population based on differences in their structure, enzyme activity, cell surface properties, and functional capacities. The phenotype of macrophages differs with their state of activity,
MATERIALS AND METHODS

ANIMALS

Sixty female Lewis rats (Charles River Laboratory, Raleigh, NC), each 6 to 8 weeks of age and weighing 200 g, were used in this study. They were housed in a cage, kept on a 12-hour light/dark cycle, and fed ad libitum. Institutional guidelines on animal handling and experimentation were followed.

LASER SURGERY

A 193-nm excimer laser (Twenty/Twenty Star Excimer Laser System, VISX Inc, Sunnyvale, Calif) with fluence at the cornea of 160 mJ/cm² and a pulse rate of 5 Hz was used to create a central 3-mm-diameter ablation in each of the 120 corneas. The corneas were treated with 3 different depths of ablation: group A, epithelium, 40-µm central epithelial ablation; group B, superficial stroma, 40-µm epithelial and 20-µm stromal ablation; and group C, deeper stroma, 40-µm epithelial and 40-µm stromal ablation. Normally, the average diameter of the rat cornea is 6.5 mm, and the average thickness of the rat corneal stroma is 200 µm.

Animals were killed at the following time points: 1 hour, 12 hours, 24 hours, 36 hours, and 1 week after ablation. At least 4 eyes per group were treated at each time point. Eyes were harvested and prepared for embedding in paraffin and for frozen sectioning.

HISTOLOGIC FINDINGS

The left eyes from each experimental group at each time point were immediately fixed in 4% glutaraldehyde for 30 minutes, and then transferred to 10% formaldehyde for at least 24 hours before processing. The tissue was embedded in paraffin, serially sectioned through the central vertical plane, and stained with hematoxylin-eosin. Sections of the frozen eyes from each group of rats were also stained with hematoxylin-eosin. Microscopic examination was performed by a masked observer (C.-C.C.).

IMMUNOHISTOCHEMICAL ANALYSIS

The right eyes from each experimental group were embedded in OCT compound (Miles Inc, Elkhart, Ind), snap frozen in a methylbutane and dry ice slurry, and stored at −70°C until sectioning. Eight-micrometer-thick frozen sections of the pupillary-optic nerve planes were then placed on gelatinized slides.

Immunohistochemical staining was performed using an avidin-biotin-peroxidase complex technique. Primary antibodies (Serotec Inc, Raleigh, NC) are listed in the Table. Mouse ascites IgG (2 µg/mL) was used as the control for the primary antibodies. The primary antibody was applied to the corneal frozen sections and incubated for 1 hour. Biotin-labeled goat-antimouse IgG (absorbed by rat serum, [American Qualex, San Clemente, Calif]) was used as the secondary antibody. After incubation with the avidin-biotin-peroxidase complex (Vector Laboratories, Burlingame, Calif), slides were developed in 3,3′-diaminobenzidine (Mallinckrodt Inc, Paris, Ky), dehydrated, and counterstained with 1% methyl green in methanol. Positively stained cells were then evaluated by an independent masked observer (C.-C.C.).

GRADING SYSTEM

For evaluation of the severity of cellular infiltration, we used the following grading system: 0.5+ and under, occasional positively stained cells are present in the examined area; 1+, positively stained cells are present in one fifth or less of the observation area; 2+, positively stained cells are present in one fourth to one third or less of the observation area; 3+, positively stained cells are present in one half to two thirds of the observed area; 4+, positively stained cells are present in more than four fifths of the observed area.

For evaluation of the pattern of the migrating cell infiltration, we divided the zones of observation to the limbus, peripheral cornea, and central corneal areas and graded according to this scale.

RESULTS

HISTOLOGIC FINDINGS

In group A (the epithelial ablation group), reepithelialization was observed 12 hours after treatment. A few polymorphonuclear cells and macrophages were seen in the stroma. After 36 hours, the epithelium had covered the denuded corneal ablation zone completely. After 1 week, the cornea showed mild hypercellularity in the central superficial stroma (Figure 1, A and B).

In groups B and C (the stromal ablation groups), the epithelium healed 24 hours after surgery. By week 1, epithelial hyperplasia developed in the central superficial cornea, filling in the area overlying the stromal defect in a manner corresponding with the depth of the ablation (Figure 1, C-F). In these 2 groups, mild infiltration of polymorphonuclear cells and macrophages was observed in the area surrounding or underneath the ablation site. In some cases, there were macrophages adherent to the corneal endothelium, in the anterior chamber, and within the iris.

MAJOR HISTOCOMpatibility COMPLEX H2 EXPRESSION IN THE CORNEA

Expression of major histocompatibility complex H2 membrane protein (OX6) was observed in infiltrating cells and...
corneal endothelial cells. The expression was detected 1 hour after laser treatment and lasted for 1 week (Figure 2). The peak of the expression was observed 36 hours after treatment, and lasted for at least 1 week.

INFLAMMATORY CELLS IN THE CORNEA

Macrophages

In this study, we used multiple markers to assess the presence of macrophages (Table). Although OX42, ED2, and ED3 are expressed by both macrophages and Langerhans cells, OX62 and ED1 are exclusively expressed by Langerhans cells; the subtraction of OX62 and ED1 positively stained cells from OX42 and ED2 positively stained cells should be the actual number of macrophages. In general, the predominant epitope was ED2 positively stained macrophages (Figure 2). There were no substantial differences among groups with varying ablation depths.

Langerhans Cells

The presence of Langerhans cells was assessed by 2 immunological markers: OX62 and ED1 (Figure 2). They were detected at a low level 12 or 24 hours after laser treatment and persisted for 1 week.

Lymphocytes

The presence of T cells was assessed by the immune markers OX19 and OX40. B lymphocytes were assessed by the OX33 marker. T and B lymphocytes were seen occasionally 12 to 36 hours after treatment and became undetectable 1 week after excimer laser PRK.

Endothelial Cell Response

Mild endothelial edema was observed 12 and 24 hours after surgery in all treatment groups. The structure of the
endothelial cells returned to normal after 36 hours in the epithelial ablation group, but not in the stromal ablation group. After 1 week, the endothelial cells had a normal structure.

Immunohistochemical staining showed that OX6 expression was detected in the endothelium 1 hour after laser surgery, reached a peak 12 hours after ablation, and was still present in some eyes of the stromal ablation group at week 1.

Migration of Infiltrating Cells

The migration of macrophages was noted by their kinetic distribution and density in different areas of the cornea (Figure 3 and Figure 4). At baseline, only a few macrophages and Langerhans cells were located at the limbus. One hour after laser treatment, the number of infiltrating cells at the limbus increased rapidly, peaked after 12 hours, and began to decrease after 24 hours. Macrophages increased rapidly in the peripheral cornea and persisted at high levels after 12 to 36 hours. Meanwhile, the macrophage infiltration in the central cornea increased gradually, reaching a peak after 36 hours. After 1 week, the central cornea was still inflamed, but inflammation was decreasing toward the baseline level. Analysis of the peripheral cornea indicated that the infiltrating cells migrated from the limbus to the central cornea. The infiltration of the central cornea was less in group A than in groups B and C.

Our study demonstrates that the inflammatory response associated with the corneal healing process after excimer laser PRK is characterized predominantly by macrophage infiltration. Histological examination indicates that a slower wound healing process occurs with deeper stromal ablation.

Corneal repair following surgery or laser treatment has been well studied; however, to our knowledge, the presence of a substantial number of macrophages in response to excimer laser PRK has not been documented previously. Our study clearly indicates that macrophages migrate from the limbus to the central cornea, and from superficial to deep stroma. Macrophages apparently play a major role in the wound healing process after excimer laser PRK.

Macrophages play a central role in the innate immune response by engulfing, processing, and destroying foreign invaders. Macrophages also play a crucial role in cell-mediated immune responses as antigen-presenting cells that initiate specific immune responses, as a source of various cytokines and growth factors, or as effector inflammatory cells to execute inflammatory, tumoricidal, or microbicidal activity. In addition, macrophages can secrete elastase and collagenase and ingest...
dead tissue or degenerated cells that are important for tissue repair and reorganization. Therefore, it is not surprising that macrophages are present in the cornea following excimer laser PRK. However, during the laser procedure, there are no foreign antigens or infectious factors. Thus, the macrophage may be recruited to the ablation site as an effector cell to engulf cellular debris and assist with reorganization of the laser-sculpted cornea.

Langerhans cells remained relatively stable after excimer laser PRK. This is consistent with the lack of antigen-presenting activity in the excimer laser–related corneal recovery process.

Although excimer laser PRK is generally a safe procedure, it is of serious concern that postoperative corneal haze remains a possible complication. Corneal haze is a marginal loss of corneal transparency. The structural basis and cellular events underlying the appearance of corneal haze are still under study. Keratocyte proliferation, discontinuity in interlamellar alignment, vacuoles, and new collagen are among the potential causes of corneal haze. A recent study suggested another possible cause: “the invasion of the stroma by inflammatory cells from tears.” The investigation demonstrated “a clear relationship (r = 0.72) between the total intensity measurement across the haze and the total count of fibroblast profiles in the subepithelial layer,” a poor correlation (r = 0.25) between the measurement of haze intensity and the thickness of the fresh connective tissue layer, and a significant decrease of haze intensity with corticosteroids. Although the authors did not establish a method to firmly identify fibroblastlike cells, they assumed that they were macrophages in that study. We made the same assumption in this study, and also confirmed the presence of macrophages in the corneal stroma after excimer laser PRK and suggested that the infiltrating macrophages migrate from limbus to the ablation zone. Furthermore, the mechanism by which corticosteroids substantially reduced haze intensity could be related to its effect on macrophages.

In conclusion, our study suggests that macrophage infiltration may be an important factor responsible for inducing haze after excimer laser PRK. Macrophages may promote or interfere with corneal wound healing during the early stages of recovery after excimer laser surgery. Additional studies into the later stages of wound healing are needed to elicit and/or manipulate the factors responsible for signaling the recovery process after photorefractive surgery.

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REFERENCES


A look at the past . . .

ANDOLPH gives the histories of two cases of detachment of the retina which he had examined histologically. There was widespread degeneration of the retinal layers, and especially the layer of rods and cones. The granular layers were usually preserved, and there was swelling of Müller’s fibres. But the most striking anatomic change was the fibrillary degeneration of the vitreous body, and which he considers the chief element in the pathogenesis of the disease.


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